Isolation and Identification of Actinomycetes from Fire Mountain, China, and the Elicitation of Antimicrobial Production

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Abstract

The increasing emergence of antimicrobial resistance alongside the decrease of novel compounds being discovered is an emerging worldwide health threat. The discovery of novel antibiotics, especially from natural products, is an important area of research for all. Natural products from secondary metabolites produced by actinomycetes are a recognised and established source of antimicrobial compounds, with many clinically relevant drugs on the market.

This thesis aimed to investigate several approaches to the discovery of novel antimicrobial compounds from putative streptomycetes, using classical methodology alongside elicitation techniques. In chapter 3, selective isolation and classical whole cell approaches were investigated to isolate putatively novel strains of *Streptomyces* spp. from a soil sample taken from Fire Mountain in China, which were subsequently tested for their ability to produce bioactive secondary metabolites. The results showed incidences of broad-spectrum activity from >7% of isolates tested. The known compounds streptothricin and wailupemyin were purified and identified from the Fire Mountain isolates, as well as a compound that inhibited the growth of multidrug resistant strains. In chapter 4, various chemical, biological, and co-culturing techniques were carried out to investigate how these methods could induce the production of antimicrobial compounds from non-active isolates. Ferrioxaime E, a siderophore commonly isolated from *Streptomyces* spp., was identified from DEM60169 and DEM60164. DEM60169 was also found to produce lipozalidinone A, a broad-spectrum antimicrobial compound, and gentamicins C1 and $C1_a$. Additionally, the use of solid scaffolds was investigated to simulate the natural soil environment that the strains were isolated from. Co-culturing techniques were employed to stimulate the production of antimicrobial secondary metabolites. Six-hundred and twenty incidences of antimicrobial action were observed using this method, and twenty strain combinations transferred to liquid media to investigate further.

It is concluded that elicitation techniques should be utilised to further investigate the production of antimicrobial compounds from *Streptomyces* spp.

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Author's Declaration

Except where acknowledgement has been given, this dissertation is the original work of the author. The material presented has never been submitted to Newcastle University or to any other educational establishment for purposes of obtaining a higher degree.

September 2018 Nicola Jane Taylor

CHAPTER 1 - INTRODUCTION

1. Introduction

1.1 The history of antibiotics

In 1909 Paul Ehrlich discovered arsphenamine, a compound used to treat syphilis, which was commercialised in 1911 under name Salvarsan. Ehrlich believed in a so called "magic bullet" for infections, which is often seen as a foresight to antimicrobials used clinically (Zaffiri et al., 2012). During the latter part of the 19th century, more and more scientists were observing changes in bacterial growth and behaviour in the presence of other organisms. Joseph Lister witnessed an altered bacterial activity when fungi were also present, and Louis Pasteur described the inhibition of *Bacillus anthracis* after inoculation with other microorganisms. It wasn't until 1927 when Gerhard Domagk developed a method to screen the efficacy of Sulfonamides in treating mice infected with *Streptococcus pyogenes*, that the testing of other compounds was introduced. This was eventually marketed as Prontosil and after Domagk successfully treated his niece's septicaemia, interest was piqued worldwide (Rubin, 2007). Following this, the famous, accidental discovery of penicillin by Sir Alexander Fleming was documented, beginning what is described as the "Golden Era" of antibiotic discovery, and one of the greatest achievements of mankind in the 20th century. Between the 1930's and 1960's, more than 20 novel classes of antimicrobial compounds were discovered (Powers, 2004). These discoveries revolutionised medical practices, and continue to be useful today. The many antibiotics that have been isolated and identified can be organised into classes, as follows (data adapted from (Coates et al., 2011):

<u>β-Lactams:</u>	-	Penicillins:	Methicillin, ampicillin, penicillin G
	-	Cepholasporins:	Cephloradine, cefotaxime
	-	Carapenems:	Imipenem, meropenem, doripenem
	-	Monobactams:	Aztreonam

β-Lactams are broad spectrum compounds which inhibit the synthesis of peptigoglycan, a main constituent of the bacterial cell wall (Fernandes et al., 2013).

Aminoglycosides: Streptomycin, kanamycin, gentamicin, spectinomycin

Aminoglycosides inhibit protein synthesis in bacterial cells, by binding to the 30S ribosomal subunit (Krause et al., 2016). The compounds are broad-spectrum acting.

Tetracyclines: Tetracycline, doxycycline, oxytetracycline

Protein synthesis is also inhibited by tetraclyclines, which also bind to the 30S subunit of the ribosome (Chopra and Roberts, 2001), and have broad spectrum activity.

Rifamycins: Rifampicin, rifabutin, rifamixin

Protein synthesis is affected by rifamycins, but by the binding to DNA-dependent RNA polymerase within the cell (Maslow and Portal-Celhay, 2015), and are broad spectrum.

Macrolides & ketolides: Erythromycin, clarithromycin, telithromycin

Macrolides and ketolides have activity against Gram-positive organisms and limited Gram-negative infections (Hof, 1994). The mode of action of these compounds is disruption of protein synthesis, by binding to the large ribosomal subunit (Gaynor and Mankin, 2003, Lonks et al., 2005).

Lincosamides: Lincomycin, clindamycin

Lincosamides disrupt protein synthesis by binding to the 50S ribosomal subunit (Spížek and Řezanka, 2017), and are most effective against Gram-positive organisms.

Glycopeptides: Vancomycin, teicoplanin

Glycopeptides are effective against Gram-positive cells, and cause a complex with lipid II in the cell wall (Zeng et al., 2016).

Lipopeptides: Daptomycin, polymyxin

Lipopeptides are compounds active against Gram-positive bacteria, and their mode of action is cell permeabilization by lipopolysaccharide binding (Meir et al., 2017).

Streptogramins: Quinupristin, dalfopristin, pristinamycin

Protein synthesis is disrupted through binding of streptogramins to the bacterial ribosome (Johnston et al., 2002). These compounds are used in pairs, as the A and B compounds are synergistic in action, and can be a hundred-fold more effective than when used singularly (Mast and Wohlleben, 2014).

Sulphonamides: Sulphanilamide, sulfadiazine

This class of antibiotic is likely the first identified, and works by inhibiting folate synthesis, which is critical for nutrition (Yun et al., 2012). These compounds are effective broad spectrum.

Oxazolidinones: Linezolid

These Gram-positive active compounds inhibit protein synthesis by interfering with the binding of tRNA to the ribosome (Shinabarger, 1999).

Quinolones: Nalidixic acid, ciproflaxin, moxifloxacin

DNA gyrase and topoisomerase IV are targeted by the broad-spectrum quinolone compounds (Aldred et al., 2014).

Others: Metronidazole, polymyxin B, colistin, trimethoprim

Despite the plethora of antimicrobial compounds over the various different classes, no new class of antibiotic has been discovered since daptomycin and linezolid in 1980s (Durand et al., 2019), and many of these drugs no longer work efficiently.

1.2 "The Urgent Need" for novel antibiotics

The need for novel antimicrobial agents has increased over the last few decades due to the increase of resistant strains of pathogens that were once controllable, ***E****nterococcus faecium*, ***S****taphylococcus aureus*, ***K****lebsiella pneumoniae*, ***A****cinetobacter baumannii*, ***P****seudomonas aeruginosa* and ***E****nterobacter spp.*, a group of infectious bacteria known as the ESKAPE pathogens (Pendleton et al., 2013). Tacconelli et al. (2008) describe a clear association between the use of antibiotics and the increase of methicillin resistant *Staphylococcus aureus* (MRSA), whereas a study analysing resistance patterns in Germany found a plateau in MRSA, but a considerable increase in vancomycin-resistant enterococci (VRE) and multidrug-resistant Gram-negative (MRGN) bacteria during the same period (Maechler et al., 2017). These examples prove that antibiotic resistance is an extremely important area to be researched to prevent further illness and mortality. These concerns over the increased incidences of antibiotic resistance pathogens has led some to believe that humankind is heading back to the pre-antibiotic era for some infections (Spellberg et al., 2008). In addition, the RAND Corporation estimated that failing to

tackle antimicrobial resistance (AMR) will see the world population between 11 and 444 million lower than it would have been if AMR were not prevalent (J. Taylor, 2014). Drug resistant strains of disease-causing bacteria are generally more severe, requiring more complex and lengthy treatment, as well as being considerably more expensive to diagnose and cure (Alanis, 2005). It has been estimated that each year 17 million deaths are caused by bacterial infections, and children and the elderly are particularly susceptible to these illnesses (Butler and Buss, 2006). There are various mechanisms of antimicrobial resistance, which are natural or acquired (Cox and Wright, 2013), and bacteria which co-exist in the same niche as organisms producing antimicrobials must evolve mechanisms to withstand the adverse effects of the compounds (Munita and Arias, 2016). As microorganisms, predominantly actinomycetes, have the ability to produce antimicrobial compounds they also must possess resistance mechanisms themselves so as not to be affected by the compounds they produce (Chien et al., 1976). D'Costa et al., (2006) proved the presence of resistance genes where there were no native antibiotics, then in the same authors identified genes resistant to β-lactams, tetracyclines, and glycopeptides from 30,000-year-old permafrost samples (D'Costa et al., 2011), proving the ancient presence of these genes. The collection of antimicrobial resistance genes was coined the "resistome" by (Wright, 2007), and includes the genes and their precursors. It is suggested that horizontal gene transfer may have occurred from the resistome to pathogens, leading to the problems we face today (Hutter et al., 2004). A map of the transfer of such genes is shown in Figure 1.1.

Gram-negative organisms are particularly efficient at up-regulating and acquiring antimicrobial resistance genes (Peleg and Hooper, 2010), as well as possessing double membranes and effective efflux pumps. Efflux pumps can be found as narrow-spectrum, single-component, or broad-spectrum, multicomponent pumps. Examples of narrow-spectrum efflux pumps are those active against chloramphenicol and tetracycline which have been found in bacteria such as *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Salmonella enterica* (Kristjansson, 1989). In contrast, broad-spectrum, multi-component efflux pumps exhibit broad specificity, and often conferring multidrug resistance. An example of this is a *P. aeruginosa* efflux pump named MexAB-OprM, which recognises and removes more than 25 substances, including

antibiotics, biocides and solvents (Turner et al., 2007). This has given rise to some cases of Gram-negative infections showing resistance to all available antimicrobial compounds, making them a particular cause for concern (Siegel, 2009).

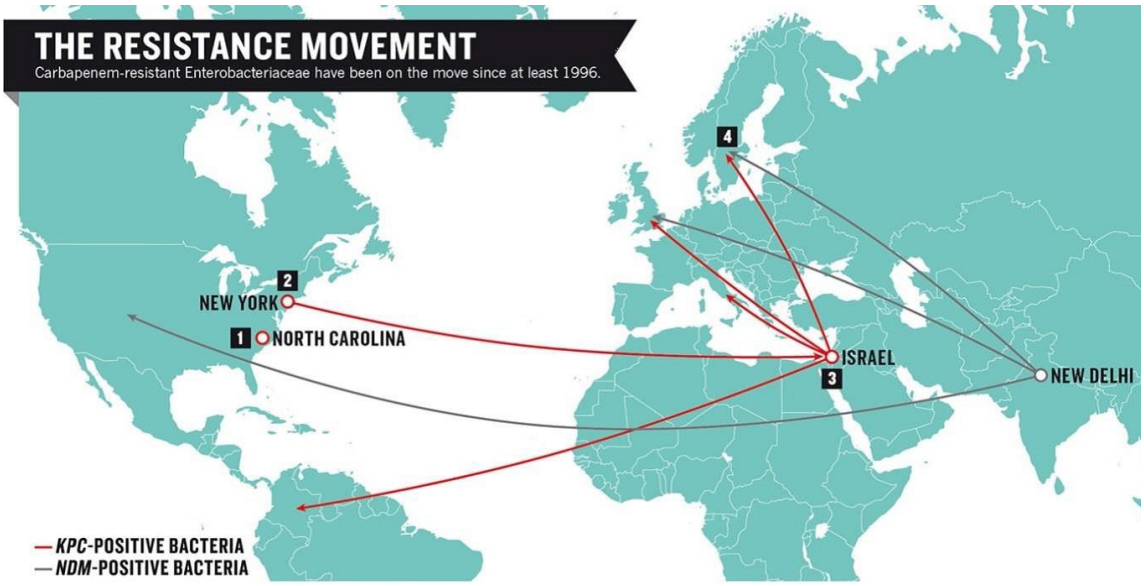


Figure 1.1 A map showing the spread of antibiotic resistance genes in Enterobacteriaceae (McKenna, 2013).

1. In 2000, an infectious *K. pneumoniae* strain is carrying a gene called KPC, conferring resistance to carbapenems **2.** Following this, in 2003, KPC-positive bacteria spreading rapidly through hospitals in New York City. By 2007 21% *Klebsiella* in the city carry the resistance gene **3.** KPC-positive bacteria found in countries such as Israel, Italy, Columbia, UK and Sweden in 2005 **4.** In 2008 a new carbapenem-resistance gene, NDM, is identified and traced back to India.

Another common mechanism of antimicrobial resistance is modification of the antimicrobial target, which decreases the affinity for the drug. For example, resistance to quinolone can be caused by mutations in DNA gyrase and topoisomerase (Martinez, 2014). Similarly, resistance to RNA polymerase binding antimicrobials such as rifampicin, are observed by the mutation of the *rpoB* gene in some *Mycobcterium* species (Ramaswamy and Musser, 1998). Another common modification is of penicillin binding

proteins, the target of β-lactam antibiotics (Frère and Page, 2014), interrupting the cross-linkage of bacterial cell wall peptidoglycan.

Inactivation or modification of the antimicrobial compound has also been observed from resistant strains (D'Costa and Wright, 2009). β-lactamase enzymes hydrolyse β-lactam antibiotics such as penicillin, causing the release of non-harmful penicilloic acid (Eun, 1996). Another similar group of enzymes are tetracycline-inactivating enzymes, which catalyse the oxidation of the antimicrobial compounds (Markley and Wencewicz, 2018).

Despite the obvious necessity, pharmaceutical companies have less incentive to spend research funds on antimicrobial compounds, as they make a better profit by focussing their efforts on chronic diseases where patients are required to take medication for prolonged periods of months or years (Projan, 2003). This means that the number of antimicrobial compounds which are approved by the FDA for clinical use is much lower than the number that are discovered (Durand et al., 2019), as seen in Figure 1.2. Throughout 2018 and 2019, from a total of 107 new drugs approved by the FDA, only nine were antimicrobial drugs (Andrei et al., 2019). A campaign was established by the British Society for Antimicrobial Chemotherapy (BSAC) called Antibiotic Action, who expressed a “grave concern” about the “relentless rise in antibiotic resistance” (Livermore, 2011). The aim of the project is to inform and educate about The Urgent Need for novel antimicrobial agents, following worrying statements from important figures, warning of a health “nightmare” and “catastrophic threat”, which are described to be as serious a risk as terrorism (Santavy et al., 1990). In March 2015, the American Presidential Advisory Council on Combating Antibiotic Resistant Bacteria was established to develop and implement strategies to tackle AMR (Ahmad and Khan, 2019). The three As have also been defined to create Awareness, encourage thought about the Availability of antibiotics, and consider Alternatives, as AMR is a problem that will not go away (Antão et al., 2018).

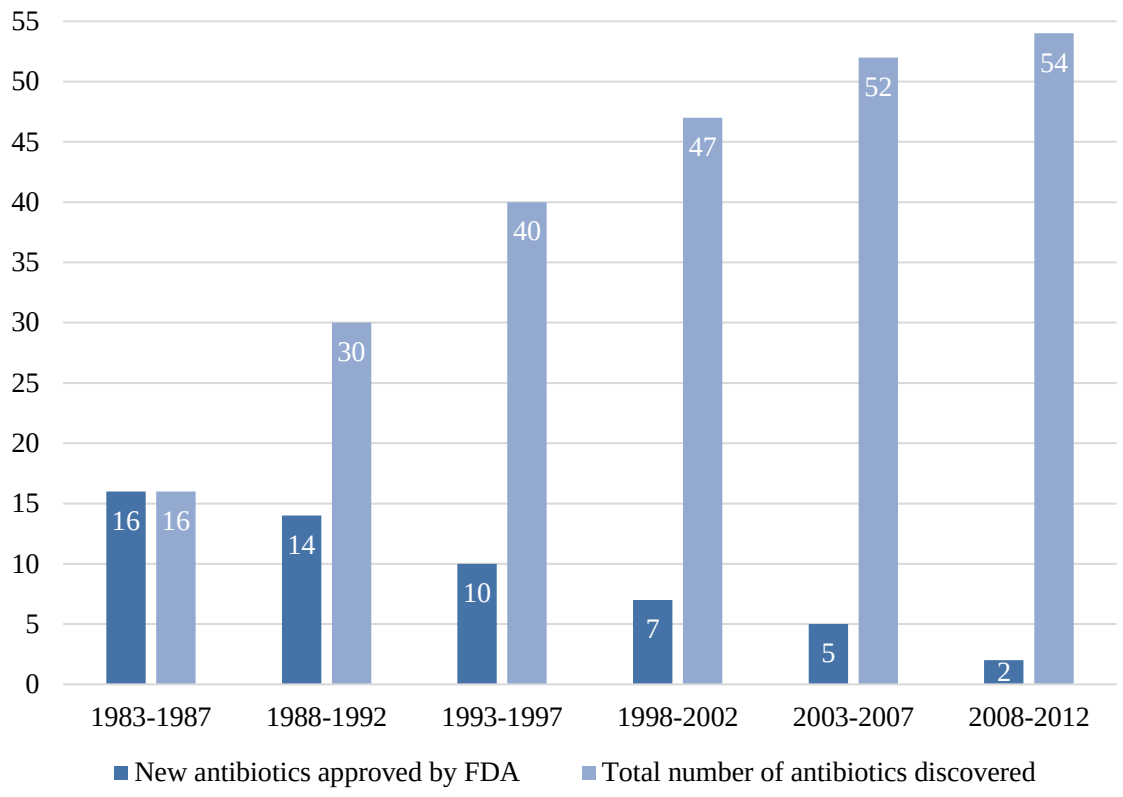


Figure 1.2. The number of antibiotics approved by the US Food and Drug Administration (FDA) compared with the total number of antimicrobial compounds discovered. This figure is adapted from Durand et al. (2019).

1.3 Sources of novel antibiotics

Since its development in the 1980's, combinatorial chemistry has been used to produce new antimicrobials using the structure of compounds already discovered. The main principle behind combinatorial chemistry is to form every possible structural variation of a compound using "building blocks", meaning that each variation has the exact same composition, but in a different order (Dibó, 2012). This means that libraries of compounds can be created fairly easily. Unfortunately, it was commonly found that analogues of an effective active compound did not share the same sort of action (Drews, 2000) hence further research into novel antimicrobial compounds is required. The techniques used in the search for novel natural products have much improved, providing around 15 new natural product derived drugs that had entered the market in the last

decade, along with around 15 more at the clinical trial stage in 2002 (Butler, 2004). These statistics suggest that exploring natural products will be rewarding. There is a much higher chemical diversity from natural products than those synthesised in the laboratory, as the producing organisms have evolved to possess this ability (Koehn and Carter, 2005). Due to this chemical diversity it can be hypothesised that novel compounds may also have modes of action that are currently unknown.

1.4 Actinobacteria as a source of novel antibiotics

Actinobacteria are a phylum of Gram-positive bacteria found in terrestrial and marine sediments have the most varied morphologies of all prokaryotes (Servin et al., 2008). These saprophytic bacteria play an integral role in the decomposition of organic matter within soil (Ataiekhorasgani et al., 2014), and can help promote the growth of plants (Da Silva Sousa et al., 2008). Members of this phylum, such as *S. coelicolor*, have been found to produce the compound geosmin, which is responsible for the characteristic smell of moist soil (Jiang et al., 2006, Jiang et al., 2007), and is thought to be how camels are able to detect water in the desert.

The genus *Streptomyces*, which are mould-like in appearance (Bobek et al., 2017), produce aerial hyphae and spores (Daza et al., 1989). Their complex life cycle is dissimilar to other prokaryotic organisms, that includes programmed cell death (PCD) phenomena and sporulation on solid media (Yagüe et al., 2012). The growth cycle of *Strepyomyces spp.* begins with germination of the spore into a network of branched substrate mycelium. After a few days, when the nutrients are becoming exhausted, aerial hyphae begin to grow upwards, away from the substrate mycelium. Following this, these aerial hyphae begin to septate to create a series of compartments which differentiate into spore chains (Yagüe et al., 2012). The aerial hyphae digests and utilises the substrate mycelium for growth, and this phase is synchronised with the production of antimicrobial compounds which protects the growing colony against other bacteria in the environment (Chater, 2006). This cycle is presented in Figure 1.3.

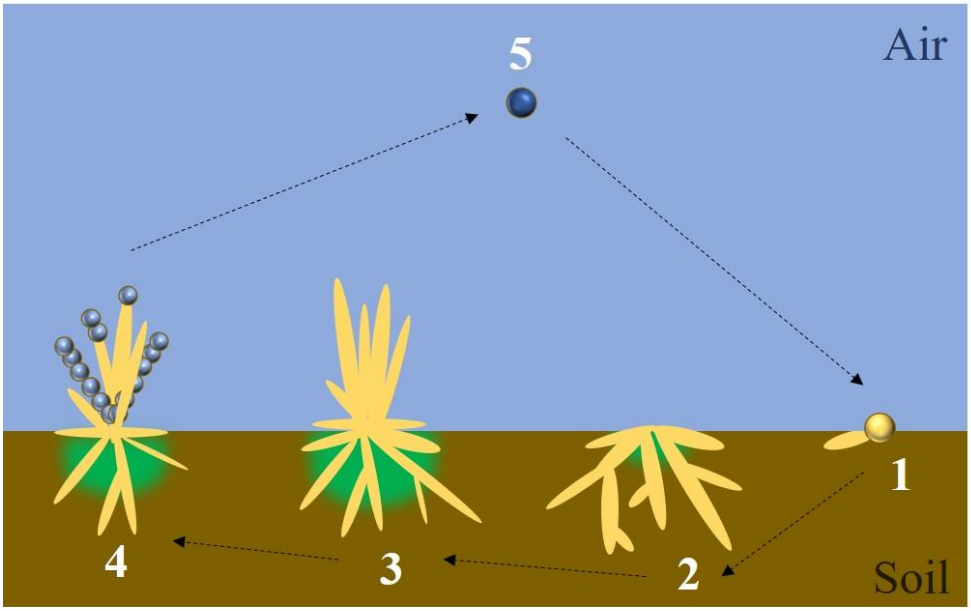


Figure 1.3. The life cycle of *Streptomyces spp.*, adapted from Urem et al. (2016). A spore begins to germinate (1) when it lands in an area with sufficient nutrients and moisture. This spore grows into a network of vegetative hyphae (2) until nutrient depletion, where this vegetative mycelium serves as a substrate for aerial hyphae (3). The production of secondary metabolites, such as antimicrobials, is often observed here (as represented in green) to protect from predatory bacteria. Finally, sporulation occurs, and chains of spores appear at the aerial mycelium (4). These spores are dispersed (5), and the cycle repeats.

Actinomycetes are found in abundance in soil and marine sediments, yet only a small proportion is thought to have been sampled (Baltz, 2008). Further studies into these organisms could reveal novel natural products with antimicrobial properties. By using specific media enrichments and selective isolation, species that have already been identified can be almost avoided. A number of studies have been carried out into under-explored habitats, including that of Kim et al. (1999) examined thermophilic actinomycetes that were isolated from areas with particularly high temperatures. The strains were grown on inorganic salt-starch agar at 45°C and grouped together on their morphological similarities. Following this, the samples were grown on different media at

different temperatures to further identify differences and similarities, followed by chemotaxonomic analyses to demonstrate differences in their biochemical compositions. Subsequently, the 16S rRNA was sequenced and compared, along with DNA-DNA hybridization, and phylogenetic trees assembled.

Similarly, Thakur and Rai (2011) took samples from the Raipur district of Chhattisgarh in India, where temperatures have been recorded up to 42°C, and selected for thermophilic *Streptomyces* using starch-casein agar at 45°C. They isolated 42 *Streptomyces* species, which were tested for antimicrobial activity against a panel of known pathogens, and almost 3% were found to be active against *E. coli*.

The focus on actinomycetes, and in particular streptomycetes, is due to their genomes, which are rich in biosynthetic gene clusters, giving them the capacity to produce antimicrobial compounds (Goodfellow and Fiedler, 2010). A number of strains, including *Streptomyces avermitilis, Streptomyces coelicolor* A3(2) and *Salinispora tropica*, have been subjected to whole-genome studies showing that each strain possessed around 20 biosynthetic gene clusters which control the production of secondary metabolites (Goodfellow and Fiedler, 2010). Further studies have also been completed to test for the presence of specific compounds often involved in the synthesis of secondary metabolites such as antibiotics. The analysis of 210 actinomycetes, covering all genera and families, found that the genes for these compounds were abundant not only in the *Streptomyces* strains, but also strains that had not been previously found to produce antimicrobials (Ayuso-Sacido and Genilloud, 2005). This study is proof and encouragement for the theory that actinomycetes, and particularly those from under-explored habitats, should be investigated further.

It has been estimated that 45% of all microbial secondary metabolites are produced by actinomycetes, and approximately 80% of these compounds are produced by the species *Streptomyces* (Kurtböke, 2012). Sequencing of *Streptomyces spp.* by Bentley et al. (2002) showed that each strain contains the necessary genetic material giving them the capacity to synthesise 20+ potential secondary metabolites, and since then even more have been identified. *S. coelicolor* A3(2) has been studied extensively, and found to produce several compounds under laboratory conditions, such as actinorhodin and red prodginine, but actually has the genetic machinery to produce 30 compounds (Nett et al., 2009).

Similarly, Becerril et al. (2018) studied *S. argillaceus* and found 31 putative biosynthetic gene clusters which were not expressed under normal laboratory conditions. These cryptic biosynthetic pathways could be the key to discovering novel antimicrobial compounds, and methods to "awaken the sleeping genes" are of great interest. Variations of media compositions and growth conditions can be utilised to activate silent gene clusters, by triggering a pleiotropic response. Unfortunately, as these methods are not focused on a single gene cluster, they lack specificity and can result in the production of more than one active compound. This complicates the subsequent purification and identification of active compounds (Rutledge and Challis, 2015). However, this method has been utilised successfully on a number of occasions, resulting in the discovery of both known and novel compounds. (2006) investigated the growth of *Aspergillus nidulans* on a rice substrate, and discovered aspoquinolones A-D which were not produced under normal lab conditions. Similarly, the addition of soil extract was successful in eliciting the production of an antimicrobial compound from *Clostridium cellulolyticum*, named closthioamide (Lincke et al., 2010), a compound which had not previously been observed under normal laboratory conditions. Additionally, there has been proof that small molecules can be employed to rouse the genes involved in secondary metabolite production (Craney et al., 2012), proving that the investigation of possible methods of elicitation could be crucial in the discovery of novel antimicrobials.

1.5 Isolation and dereplication of streptomycetes

To select for actinomycetes it is possible to use specific media, as described by (Duangmal et al., 2005). The authors developed a selective medium to isolate members of the *Streptomyces violaceoruber* 16S rRNA clade, which includes *S. coelicolor* A3(2). These organisms were selected for by growth on a solid medium formulation of dextran-histidine-sodium chloride-mineral salts, and were recognisable by the deep red colour that they produce. It has been proven that media preparations with starch or glycerol as the main carbon source, and nitrate, casein or arginine as the nitrogen source are effective in the isolation and selection of streptomycetes, such as starch-casein agar. To prevent the growth of fungi, which may overcome the target streptomycetes, it is important to add antifungal agents, such as cycloheximide and nystatin (Küster and Williams, 1964).

When carrying out selective isolation, there is the likelihood of obtaining the same isolates on multiple occasions. To prevent this, a quick comparison of the morphological features, such as size, mycelial colour, diffusible pigment colour, shape, of each strain can be carried out, and isolates appear to be identical can be discarded (Donadio et al., 2002). This is known as dereplication.

1.6 Streptomycetes from under-explored habitats

For many arid and hyper-arid areas, such as deserts, it was thought that the soil has too low a water content to support microbial growth. A number of studies have taken place on soil samples from these areas, which are surprisingly disproving this hypothesis. (Okoro et al., 2009) took soil samples from the Atacama Desert in Chile and identified a diverse range of isolates belonging to the genera *Amycolatopsis, Lechevalieria* and *Streptomyces*. Following this, the same team were able to publish results proving that at least one novel species had been discovered in the Atacama Desert soil (Santhanam et al., 2012a). The strain, *Streptomyces deserti*, was proven to be novel by chemotaxonomic and morphological scrutiny, followed by genomic DNA extraction and 16S rRNA sequencing. The subsequent phylogenetic analysis further demonstrated novelty of the species.

Among bacteria and fungi, the species *Streptomyces* has been shown to produce a profound number of antimicrobial compounds, as illustrated in Figure 1.4. A predicted 1-3% of these compounds have been discovered as yet, leaving 97-99% of these active compounds unknown. To this point in search and discovery programmes, the main attention has been focused upon mesophilic streptomycetes, for example *Streptomyces venezuelae*, the organism known to produce chloramphenicol (Bradley and Ritzi, 1968), and *Streptomyces roseosporus*, which produces daptomycin (Miao et al., 2005). Similarly to these two species, *Streptomyces griseus*, which produces streptomycin, is usually grown at 30°C. Studies on this organism showed growth up to 37°C, but the production of streptomycin was not shown above 34°C (Deeble et al., 1995). There is proof of thermophilic streptomycetes producing antimicrobial compounds, for example *Streptomyces refuineus*, the producer of anthramycin, an antimicrobial and antitumor compound (Rokem and Hurley, 1981). As thermophilic organisms are able to both grow

and produce compounds at high temperatures, they have been investigated for human use in a number of settings, for example in industry, research, and healthcare. Examples of compounds produced by thermophiles that are commonly used are Taq polymerase from *Thermus aquaticus* (Chien et al., 1976), and thermostable collagenase from *Thermoactinomyces sacchari* 21E (Petrova, 2007). With these examples in mind, it is logical to presume that thermostable antimicrobials may be produced by thermophilic streptomycetes.

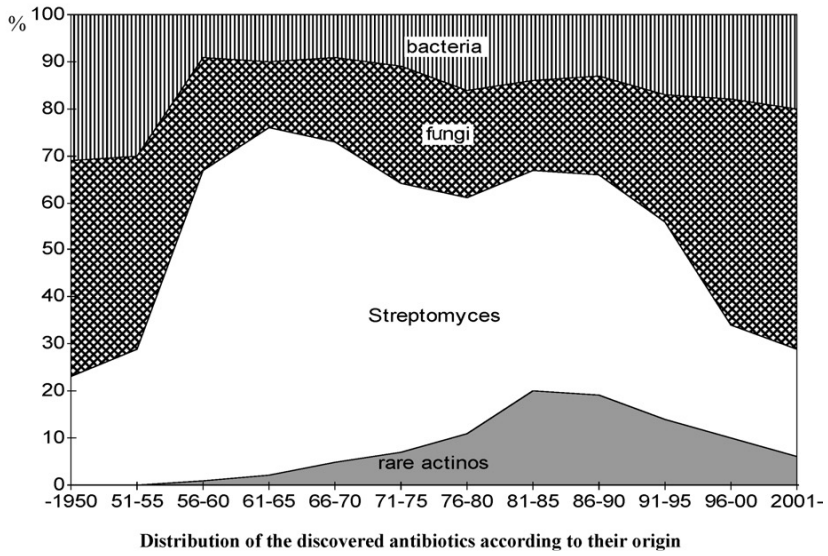


Figure 1.4 The origins of antimicrobial compounds discovered each year between 1950 and 2001 (Bérdy, 2005).

1.7 Fire Mountain, China

Found in the North Turpan Basin of Xinjiang Province, China, lies “Fire Mountain”, a stretch of land that is around 1,600 feet high, and 63 miles long. This area was recorded as the hottest place on earth by NASA in 2007, and soil temperatures can reach up to 80°C. It is named Fire Mountain, or Flaming Mountain, due to the red colour of the rocks and soil, and when the heat rises from the ground it gives the illusion that the mountain is on fire (Mildrexler et al., 2011). As with the Atacama Desert, it was unknown until recently whether bacterial life could be supported in these hyper-arid soils lacking in

nutrients and moisture, and exposed to high levels of UV, making Fire Mountain a desirable and under-explored area to pursue novel antimicrobials.

1.8 Screening for antimicrobial compounds

To screen for antimicrobial compounds produced by actinomycetes, there are a number of different strategies. Traditionally, screening for novel inhibitors of bacterial growth is carried out by streaking a panel of Gram-negative and Gram-positive organisms onto agar plates, and spotting fermentation supernatants on top. Following incubation, any activity is visible by a zone of inhibition. Similar to this method, is the use of agar plug assays. This technique uses small plugs of agar taken from a plate where prospective compound producers have been allowed to grow, placed on top of or within agar seeded with the known organism. As before, activity is shown by a zone of inhibition. These procedures allow for the quick and cheap identification of potential novel antimicrobial producers. The two main approaches for antimicrobial screening are whole-cell and target-based (Rosamond and Allsop, 2000). Whole-cell approaches, such as plug tests, identify compounds that prevent or reduce bacterial growth, and are known as the Waksman Platform after their founder, Selman Waksman (Valiquette and Laupland, 2015). Target-based approaches aim to identify the inhibitors of a specific pathway. This makes target-based screening more sensitive and accurate (Rosamond and Allsop, 2000). The main and important disadvantage to these methods is that the mechanism of action is left unknown. During these screening techniques a panel of Gram-negative and Gram-positive organisms is often used. Over 60% of antimicrobial compounds are only effective against Gram-positive organisms (Baltz, 2007), so the inclusion of a Gram-negative organism such as *Escherichia coli* ensures that focus can be aimed on less common compounds. Strains of *E. coli* K12 such as that engineered by (Baltz, 2007) containing genes encoding resistance to ampicillin, bleomycin, chloramphenicol, rifampicin, streptomycin, streptothricin, tetracycline, and multiple aminoglycosides can ensure that positive hit rates can be reduced to less than 0.1%, ensuring that common broad-spectrum compounds are not rediscovered. Once compounds that produce a zone of inhibition have been identified, it is advantageous to determine the mechanism of action. The strains carry reporter mechanisms such as β-galactosidase that are fused to promoters, and

triggered by specific antibiotic mechanisms (Fischer et al., 2004). This means that once an antibiotic activates the promoter, the reporter gene is expressed. In this case, X-gal that is added into the medium is cleaved to form galactose and 5-bromo-4-chloro-3-hydroxyindole, giving the observable blue colour to the outer zone of inhibition (Voet and Voet, 2011). A panel of *B. subtilis* reporter strains was designed by (2004) to detect compounds that inhibit cell wall, fatty acid, DNA, and protein biosynthesis pathways.

1.9 Aims and objectives of this thesis

Chapter 1

Review the literature concerning the subject of antimicrobial discovery, and to highlight the importance of streptomycetes in the ongoing search for novel compounds, as well as addressing the ongoing threat of antibiotic resistance.

Chapter 2

The materials and methods used throughout the experimental work are outlined in this chapter, and aim to describe the work carried out as well as allowing for reproduction if necessary.

Chapter 3

To isolate and identify putatively novel *Streptomyces* spp. from a soil sample taken from Fire Mountain, China, using selective isolation techniques. Phylogenetic analysis of these isolates to identification of closest evolutionary neighbours and their capacity to produce antimicrobial compounds. To screen these isolates for the production of antimicrobial activity, and identify these bioactive compounds following various chromatographic purification techniques, and analysis by mass spectrometry. Additionally, the scale up and optimisation of the cultivation techniques is to be investigated, using bench scale fermentation equipment.

Chapter 4

Investigate the use of chemical and biological elicitors, and physical additions to induce the production of antimicrobial compounds. Development of a co-culturing technique to investigate it as a method for elicitation of secondary metabolites. Any bioactive compounds are to be purified for identification using mass spectrometry.

Chapter 5

Summarise the discoveries from the previously described research, how these results impact the research area, and suggest further experimentation.

CHAPTER 2: MATERIALS AND METHODS

2.1 Chemicals and raw materials

All chemicals and raw materials were obtained from Sigma Aldrich, unless otherwise stated. The soil sample was collected from Fire Mountain in the Tian Shan Mountain Range, Xinjiang, China by Dr Dylan Wang (Institute of Microbiology, Chinese Academy of Sciences, Beijing) in August 2011.

2.2 Selective isolation

Selective isolation plates were prepared with 2% agar. The compositions of the different media can be found in Table 2.1. One gram of the soil sample from Fire Mountain was suspended in nine millilitres of sterile, quarter strength, Ringer's solution (Oxoid, BR0052), and vortexed to fully re-suspend. An Ultra Turrax was used to homogenise the suspension before it was serially diluted down to 10^{-2} and agitated on a rotary shaker overnight at room temperature. Following this, quadruplicate 100µl aliquots of each dilution were spread over the six different media, to select for different streptomycetes. The plates were dried for ten minutes prior to inoculation in a laminar flow cabinet, as recommended by (Vickers, 1984), and were incubated at 40°C. The numbers of presumptive actinomycetes were counted after ten days growth.

2.2.1 Colony selection

Colonies assumed to be members of the genus *Streptomyces* were chosen due to their extensively branched substrate hyphae bearing white aerial hyphae. Ninety-nine representative colonies were selected from the isolation plates, and sub cultured onto modified Bennett's agar (Iwasa et al., 1970). The strains were checked for purity by eye, and 20% (w/v) glycerol stocks prepared and stored at -80°C.

2.3 Growth and maintenance of actinomycete strains

Actinomycetes were cultivated on International *Streptomyces* Project (ISP) II agar (Shirling and Gottlieb, 1966), at a temperature of 30°C. Growth in ISPII broth was also at

30°C, with orbital agitation at 160rpm (Orbital incubator; Gallenkamp), unless otherwise stated. Growth in liquid broth was monitored using a spectrophotometer to measure the optical density (OD) at a wavelength of 450nm.

Table 2.1. Media used in the selective isolation investigations. The composition of these selective media can be found in the appendix.

Medium	Selection for	Source
Starch casein	Streptomycetes	(Küster and Williams, 1964)
Starch-Casein + Gentamycin	Rare streptomycetes	(Küster and Williams, 1964)
Humic acid-Vitamin	Streptosporangiaceae	(Hayakawa and Nonomura, 1987)
Oligotrophic	Streptomycetes	(Santavy et al., 1990)
Glucose-Yeast Extract + Rifampicin	Actinomadura	(Mossel et al., 1970)
Raffinose-Histidine	Rare streptomycetes	(Vickers, 1984)

2.3.1 Preparation of spore suspensions

SFM plates were prepared with 2% agar and inoculated with strains to be investigated. The plates were incubated at 30°C until sufficient growth as a thick sporulated lawn observed. Spore suspensions were prepared using the method described by (Hopwood et al., 2000). 20% glycerol was added to the surface of the well-grown plates, and the spores disrupted by gently agitating with an inoculation loop. The water and spore mixture was then transferred into a 10mL syringe plugged with cotton wool (sterile), which was used to filter out any mycelia. The spore suspensions were then stored at -80°C for further use.

2.4 Taxonomic investigation

2.4.1 Genomic DNA extraction

Genomic DNA was extracted from all *E. coli* active strains by suspending 4-5 loopfuls of 4-day old biomass in sterile deionised water and approximately 30µg glass beads.

The suspension was vortexed to mix before being run in the ribolyser at a speed of 5.5 for 30 seconds (s). The solutions were then cooled on ice, before being run again at 5.5 for 30s and then centrifuged at 13,000rpm for 2 minutes (min). The supernatant was then transferred to a new sterile tube, and 200µl 100% ethanol added to the remaining beads and biomass. Any precipitated DNA was then transferred to the new supernatant tube. Genomic DNA was also extracted from the same isolates using a GenElute Bacterial Genomic DNA kit, using the standard protocol. The protocol was adjusted by the addition of the homogenised supernatant being added alongside the lysis solution, to increase the yield of DNA. The eluted DNA was run on an analytical gel, using 1% agarose at 100V for 15 minutes to confirm the presence of genomic DNA.

2.4.2 16S rRNA amplification, purification and sequencing

16S rRNA PCR amplification of the extracted DNA samples was carried by out using universal bacterial 16S rRNA primers, and the reagents and amplification conditions are shown in Table 2.2. The PCR products were analysed using gel electrophoresis with a 1% agarose gel stained with GelRed (Biotium). Purification of the PCR products was carried out by adding 0.5µl exonuclease-1 and 1µl alkaline phosphatase per 10µl PCR product. The tubes were placed in the thermocycler, where they were incubated at 37°C for 15min, followed by 80°C for 15min. Sequencing was carried out by SolGent™ Sequencing.

2.4.3 Analysis of 16S rRNA genes

Sequences were returned for each of the strains and assembled using DNA Baser (DNA Baser Sequence Assembler v4.x (2014), Heracle BioSoft SRL, www.DnaBaser.com). The sequences were uploaded to EZ-Taxon (Chun et al., 2007) where the most probable nearest evolutionary neighbours were displayed, alongside their 16S rRNA sequences. Once the closest evolutionary neighbours had been identified, their 16S rRNA sequences were aligned using MUSCLE (**MU**ltiple **S**equence **C**omparison by **L**og- **E**xpectation) and curated using GBlocks by using the Phylogeny.fr form (Dereeper et al., 2008). Phylogenetic trees were then constructed using MEGA version 5 (Tamura et al., 2011) using neighbour joining algorithms.

Table 2.2. Components and steps used in PCR reaction to amplify 16S ribosomal DNA of isolates. Volumes are shown per sample.

Reagent		**Volume (µl)**	
12x Reaction Buffer		2.5	
Forward primer		0.5	
Reverse primer		0.5	
dNTPs		2	
$MgCl_2$ (50mM)		1.5	
BioTaq DNA Polymerase		0.5	
Template DNA		1	
Sterile dH_2O		Up to 25 (16.5)	
Step	**Temperature (°C)**	**Time**	**Cycles**
Initial denaturation	95	1min	1
Denaturation	95	45s	35
Annealing	62	30s	
Extension	72	30s	

2.5 Culture media

All media used for the growth of *Streptomyces spp.* are listed in Table 2.3. Solid media was prepared with 15g/L agar, unless stated otherwise. General maintenance of strains was carried out on SFM or GYM media, unless stated otherwise.

2.6 Shake flask cultivations

Unless stated otherwise, shake flask cultivations were carried out in 2L non-baffled, glass Erlenmeyer flasks sealed with foam bungs, at a working medium volume of 500mL. Flasks were inoculated using 50µL spore suspension or a loopful of densely grown spores from an agar plate. Strains were incubated at 30°C at 120rpm and tested for antimicrobial activity after 5 days of growth, unless stated otherwise.

Table 2.3. Standard Demuris production media used throughout this study and their compositions. 1.5% agar was added to give solid medium when poured into Petri dishes.

Media	Composition (/L)
A	4.0g peptone, 4.0g meat extract, 2.0g yeast extract, 2.0g soy flour, 20.0g maltose, 10.0g dextrin, pH 7
AF/MS	20.0g glucose, 2.0g yeast extract, 8.0g soy flour, 4.0g $CaCO_3$, 1.0g NaCl, pH 7.3
D Seed/Vegetative	5.0g peptone, 20.0g soluble starch, 2.0g meat extract, 3.0g yeast extract, 2.0g soy flour, 1.0g CaCO3, pH 7
GPMY	20.0g potato starch, 5.0g yeast extract, 5.0g meat extract, 20.0mL glycerol, pH 7
GYM (ISP II)	4.0g yeast extract, 10.0g malt extract, 4.0g glucose, pH 7
INA5	15.0g soy flour, 5.0g $CaCO_3$, 30.0mL glycerol, 2.0g NaCl, pH 7.3
Oatmeal (ISP III)	20.0g cooked and strained oatmeal, 1.0mL/L trace elements
RA3	2.0g peptone, 4.0g yeast extract, 10.0g malt extract, 10.0g glucose, 5.0mL glycerol, 2.0g $MgCl_2.6H_2O$, pH 7.4
SFM	20.0g soy flour, 20.0g mannitol, autoclaved twice
V	24.0g soluble starch, 1.0g glucose, 3.0g meat extract, 5.0g yeast extract, 5.0g tryptone, pH 7.2
V6	5.0g peptone, 5.0g meat extract, 5.0g yeast extract, 20.0g glucose, 3.0g casamino acids, 1.5g NaCl, pH7
Trace elements	84mg $ZnSO_4.7H_2O$, 200mg $FeCl_3.6H_2O$, 15mg $CuSO_4.5H_2O$, 10mg $MnCl_2.4H_2O$, 15mg boric acid, 10mg $(NH_4)_6Mo_7O_{24}.4H_2O$

2.7 Testing for antimicrobial activity

2.7.1 Agar plug assays

Strains isolated from Fire Mountain were grown on yeast extract-malt extract and oatmeal agars (International Streptomyces Project [ISP] 2 and 3, respectively; (Shirling and Gottlieb, 1966), prepared with 0.8% w/v agar, for 5 days at 30°C. Five days was chosen to allow for depletion of nutrients and autolysis to begin. Triplicate agar plugs were taken from each plate using a sterile P1000 pipette tip, and transferred into square 12cm Petri dishes (triplicate to allow for assay against *Escherichia coli, Bacillus subtilis,* and *Saccharomyces cerevisiae*). Broth was combined with corresponding molten agar to give a final volume sufficient to aliquot 25mL to each square Petri dish. The known organism was added to give a final OD600 of 0.05. The corresponding antibiotic and X-gal (for *E. coli* and *B. subtilis*, only) were added at a concentration of 1/1000, and the "seeded" agar poured into the Petri dishes. 20µL control antibiotic was pipetted onto a 5mm diameter disc of Whatman filter paper and placed on top of the solidified agar. The plates were then incubated at 30°C overnight and examined for antimicrobial activity. This was repeated for any strains that showed antimicrobial activity against *E. coli* but using the panel of *B. subtilis* reporter strains. Medium and antibiotic selections can be found in Table 2.4.

Plug assays were repeated in at least triplicate for confirmation of repeatability.

2.7.2 Disk diffusion assays

Mycelial water extraction

Plates with growth of active strains were crushed into Falcon tubes, using 50mL syringes. This crushed agar was then frozen overnight at -20°C and thawed the next day. The samples were then centrifuged for 2 minutes, and the supernatant transferred to a new Falcon tube. Molten agar and broth mixtures were prepared as in described in Table 2.4, aliquoted into Petri dishes, and left to solidify. 20µL of each water extract was spotted onto a 5mm diameter disc of Whatman filter paper and left to dry before being placed onto the solidified agar/broth mixture. The plates were then incubated at 30°C overnight, before being examined for antimicrobial activity. Plates that did not show activity once

crushed and the water extract tested, were transferred to glass bijou bottles and covered with 100% methanol. The mixtures were shaken and left overnight before being filtered through Whatman filter paper. The supernatant was collected and concentrated using the centrifugal evaporator, before being tested using filter paper discs, as described for water extract samples.
Water extraction assays were repeated in at least triplicate for confirmation of repeatability.

Testing of liquid cultivations

Shake flask cultivations were tested for antimicrobial activity after five days, following the same screening procedure used for water extraction. Five days was chosen to allow for depletion of nutrients and autolysis to begin. Any active broths were filtered using Whatman filter paper, followed by a 0.22μm syringe driven filter (Millex), and activity again confirmed using the same screening protocol.
Disk diffusion assays were repeated in at least triplicate for confirmation of repeatability.

2.7.3 Reporter strains

Reporter *Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus* strains were cultivated on nutrient agar (NA) at a temperature of 30°C. Lysogeny broth (LB) was generally used for growth in a liquid medium, at 30°C with an agitation of 160rpm in an orbital incubator. Growth in liquid broth was monitored using a spectrophotometer (Genesys 10VIS, Thermo Spectronic) to measure the OD at a wavelength of 600nm. The OD was monitored, and the cultures refrigerated once a reading of 1.0au was observed, for future use in assays. *Saccharomyces cerevisiae* were cultivated on yeast potato dextrose (YPD) agar and grown at a temperature of 30°C. YPD broth was used for liquid cultures at a temperature of 30°C, with orbital agitation at 160rpm. As previously, growth was monitored using a spectrophotometer to measure the optical density at 600nm. Long-term storage of strains was in their specified broth supplemented with 20% glycerol, prior to freezing at -80°C. All reporter strains, their corresponding antibiotic resistance profiles, and indicative mode of action can be found in Table 2.4.

Table 2.4. Reporter strains used in bioactivity assays alongside their control antibiotics and the pathway of action of which they report (Hutter et al., 2004).

Organism	Mode of Action Reporter	Resistance Gene	Antibiotic Control
B. subtilis yheH	Protein biosynthesis	Erythromycin	Chloramphenicol
B. subtilis yjaX	Fatty acid biosynthesis	Erythromycin	Triclosan
B. subtilis ypuA	Cell wall synthesis	Erythromycin	Cefotaxime
B. subtilis yvgS	RNA synthesis	Erythromycin	Rifampicin
B. subtilis yvqI	Lipid II	Erythromycin	Bacitracin
E. coli K12 wild type	-	-	-
E. coli Kan^R	Cell wall synthesis	Kanamycin	Ampicillin
E. coli Spc^R/Str^R	-	Spectinomycin, streptomycin	
E. coli Amp^R	-	Ampicillin	-
S. cerevisiae wild type	-	-	Nystatin

2.8 Media and temperature selection

Strains showing activity against *E. coli* and *B. subtilis* were grown at 30°C in each of the clear media (Medium B, RA3, V6, and ISP2; compositions can be found in Table A1 in Appendix A) and tested for antimicrobial activity after 5 days, following the same screening procedure used for water extraction. Any active broths were filtered using Whatman filter paper, followed by a 0.22μm syringe driven filter (Millex), and activity again confirmed using the same screening protocol. Alongside this, solid media optimisation was carried out using the full range of media shown in the appendix. After 5 days' growth, a plug test assay was carried out following the same procedure as described previously in this chapter. Any active plates were crushed and tested as described previously.

Soil extract was prepared for some of the testing in two ways: firstly, soil was washed in methanol for 30 minutes before being filtered through Whatman filter paper, and the methanol dried off. The resulting liquid was made up to 500mL with deionised water and filter sterilised. The second batch of soil extract was prepared by washing soil in water for 30 minutes before being filtered through Whatman filter paper. The resulting liquid was made up to 1L and autoclaved. Both of these preparations caused the antimicrobial activity to return, and at certain concentrations (2.5% of methanol prepared soil extract, and 5% of water prepared soil extract) the intensity of activity was increased from what has been previously observed. As the Fire Mountain strains were isolated from such an extreme environment, with soil temperatures reaching exceptionally high, the same technique as described earlier in the materials and methods, for liquid media optimisation was carried out with incubation at 40°C, and the results compared.
Activity assays were repeated in at least triplicate for confirmation of repeatability.

2.9 Toxicity and further activity testing

Active extracts from strains DEM60012, DEM60013, DEM60030, DEM60031, and DEM60073 were sent to Cubist Pharmaceuticals for testing against strains of *E. coli* and *S. aureus* which are resistant to over 20 different antimicrobials, including sideromycins, aminoglycosides, β-lactams, and tetracyclines. The MDR *S. aureus* strain was tested alongside DNA to immediately identify DNA intercalators. Cubist also carried out toxicity testing on promising strains using HK2 cells. The extracts were added to the cells in 1:10 dilution and incubated for 6 hours. The cells were tested for the concentrations of lactate dehydrogenase, which denotes membrane damage, and ATP, which represents energy perturbation. The cells were also studied under the microscope for changes in morphology.

2.10 Bench scale bioreactor cultivations

Larger scale cultivations were carried out in stirred-tank, glass bioreactor vessels (Applikon), equipped with two Rushton type impellers for dissolved oxygen control, and pH monitoring. Two vessel sizes were used, with working volumes of five or fifteen litres. Temperature control was carried out using a heated jacket and cooling coils within

the vessel. The pH and dissolved oxygen probes (Broadley James) were calibrated as per the manufacturer's instruction. The vessels were prepared with either 4.5L or 14.5L fermentation medium, 0.01% polypropylene glycol (PPG) and autoclaved for sterility. PPG was also connected to a peristaltic pump, and a foam probe calibrated to dose antifoam where necessary. The airflow rate was set up to sparge at 1vvm, and the bioreactor agitated by two Rushton impellers and one axial flow impeller at a baseling of 400rpm in the smaller vessel and 250rpm in the larger, set to increase if the dissolved oxygen level decreased to below 50%. The pH was uncontrolled in all runs, to allow for naturally occurring pH changes to take place. Seed cultures were prepared in 2L glass Erlenmeyer flasks using the fermentation medium. These flasks were inoculated with 1µL/mL spore suspension and incubated at 30°C and 120rpm for three days, or until an OD_{450} of 1 was observed. The seed culture was checked for contamination microscopically. The seed culture was transferred aseptically into the vessel, and the online pH, DO, agitation, and temperature monitored throughout the fermentation using BioXpert. Samples of 5 - 10mL fermentation broth were taken twice daily, and OD_{450}, phosphate concentration, alkaline phosphatase (APase) activity, and antimicrobial activity measured. Phosphate concentration was determined using 85µL of cell-free supernatant from each time point sample. One millilitre of ASA reagent (equal volumes of 0.75% ammonium molybdate, 0.9M sulfuric acid and 3% ascorbic acid) was added to the samples, which were incubated at 37°C for 90min. The reaction was halted by the addition of 400µL of 2M H_2SO_4. Absorbance was determined at 820nm against a reagent blank. A standard curve was calculated using phosphate (KH_2PO_4) standards of decreasing concentrations. This allowed the calculation of the concentration of phosphate in the fermentation samples. The alkaline phosphatase activity was measured by determining the breakdown, p-nitrophenyl phosphate (PNP), to a visible yellow compound, 4-nitrophenol. 40µL of each fermentation sample was added to 400µL of AP buffer (1M Tris-HCl pH8, 200 µg/mL lysozyme, 200 µg/mL chloramphenicol, and incubated at 30°C for 10 min. Following this, 300µL of pre-warmed PNP solution (1M Tris-HCl pH 8, 1mg/mL p-nitrophenyl phosphate, 0.001%SDS) was added, and incubated at 30°C for 50 min. Reactions were halted with the addition of 300µL 2M

NaOH. The samples were centrifuged, the supernatant aspirated, and the absorbance measured at 410 nm. APase activity was calculated using the equation:

$$APase = \frac{(A410 \times 235 \times V1)}{(V2 \times OD450 \times T)}$$

APase activity was expressed as nanomoles of PNP per minute per OD_{450} unit, i.e. nM min^{-1} $(OD450\ unit)^{-1}$.

Once the fermentation was complete, the bioreactor was harvested, and Amberlite XAD-16 resin beads added. Amberlite XAD-16 are polymeric resins designed to adsorb organic small to medium molecular weight substances from aqueous solutions and polar solvents. The contents were sieved, and the Amberlite beads and biomass washed with water, before being transferred into a 1L Duran bottle. A sufficient volume of 100% methanol was then added to cover the Amberlite beads and biomass to recover the compounds. Once the compounds had migrated from the Amberlite into the liquid, the mixture was then filtered into a round-bottomed flask using Whatman filter paper to remove the resin beads. Methanol was removed from the extract by rotary evaporation using a Buchi R200. Once distillation was complete, 10µl of the remaining liquid was tested for activity against *E. coli* and *B. subtilis* using the same method described earlier.

2.11 Purification of active compounds

2.11.1 Flash chromatography

Reversed phase flash chromatography was carried out using a Biotage® Isolera One system and a KP-C18-HS SNAP column. The procedure was carried out using a gradient of 100% deionised water to 100% methanol, at a flow rate suitable for the cartridge (Table 2.5). The system monitored UV absorbance at 254 and 220nm. The collected fractions were concentrated down using a rotary evaporator (Genevac) and tested against *E. coli* and *B. subtilis* using the same protocol as described previously.

2.11.2 Ion exchange chromatography

HyperSep™ anion and cation exchange 1g columns (Thermo Scientific) were used with a solid phase extraction manifold. Six millilitres of active sample was loaded onto the column, followed by 2 column volumes of water as a wash step. This was followed by 2

column volumes of 50% methanol, before 2 column volumes of 50% methanol with 1M sodium chloride. The final step was 2 column volumes of 100% methanol. All fractions were collected and concentrated, before being tested against *E. coli* and *B. subtilis.*

2.11.3 Size exclusion chromatography

Size exclusion chromatography was carried out using a bench-top, gravity-flow column, containing Sephadex LH-20 (GE Life Sciences). Fractions of 1.5mL were collected approximately every 4 minutes (depending on column size) until two column volumes had been eluted. The collected fractions were tested against *E. coli* and *B. subtilis* using the same protocol described earlier.

2.11.4 Analytical reverse phase HPLC and mass spectrometry

10µL semi-pure sample was run on the Agilent 1260 Infinity unit, using an XSelect C18 Reverse Phase column, connected to a guard pre-column. The system utilised a diode-array detector to monitor absorbance. The mobile phase was a dH_2O to acetonitrile gradient, unless otherwise stated. A single peak in the chromatogram output established purity of the final active fraction, and 20µL of this sample was sent to Pinnacle Proteomics at Newcastle University for analysis by mass spectrometry, or run by LC-MS, using an Agilent 1260 Infinity HPLC system, coupled to a Bruker micrOTOF II mass spectrometer, by Dr Stephanie Morton-Laing.

2.12 Identification of Compounds

Once results were obtained from mass spectrometry, a range of potential masses were calculated (±0.05) for each compound, and the Dictionary of Natural Products consulted to identify any known compounds that may be a match. Compounds from non-actinomycete sources were eliminated, and the spectrum of activity and other characteristics, such as MS/MS fragmentation patterns, compared to the Fire Mountain compound.

2.13 Chemical elicitation of antimicrobial compounds

2.13.1 Twenty-four well plate cultivations – Chemical elicitors

Twenty-four well plates were sterilised containing GYM medium, two glass beads per well (to avoid agglomeration of mycelia), and supplemented with a number of chemicals including sub-inhibitory concentrations of known antibiotics, biological samples, solvents, detergents, and pH alterations Table 2.5. The wells were inoculated with 5µL the spore suspensions prepared from non-active Fire Mountain isolates and incubated at 30°C for seven days. Antimicrobial activity was tested after five, six, and seven days using a disk diffusion assay, or by spotting 10µL of the cultivation broth onto seeded agar plates. The plates were incubated at 30°C overnight before being examined for inhibitory activity. These experimental techniques were repeated in triplicate.

2.13.2 Twenty-four well plate cultivations – Physical additions

Silver sand (Fisher Scientific) was used for this investigation. The approximate saturation point of the sand used was calculated as 200µL/g, and volume was used in all subsequent experimentation. Twenty-four well plates were prepared with 10g sand per well, sealed, and autoclaved. The wells were supplemented with various media, from half x to 4 x concentrations. These concentrations were chosen to take into consideration the concentration of nutrients that would be present in the same volume of 1 x medium as sand, which was calculated to be 4 x medium. Control wells supplemented with water alone were also included, and sand supplemented with each media and concentration tested as control. The wells were inoculated with the spores of inactive Fire Mountain isolates and incubated at 30°C until visible growth was observed. The sand was tested directly by placing directly onto the surface of seeded agar, and the plate incubated overnight. Additionally, a 500mg sample of sand was taken and washed with 400µL methanol, and a disk diffusion assay carried out as described previously.

100% cotton cheesecloth was chosen to consider the scalability of offering a solid scaffold for the isolates to anchor to, as it is available in a number of grades describing the density of the weave. Grades 10, 50, and 90 were chosen for the experiments, which can be seen in Figure 2.1. The cheesecloth was cut into 1cm^3 squares and transferred into twenty-four well plates for autoclaving. The flasks were then supplemented with 5mL

GYM medium and inoculated with spores of the non-active Fire Mountain isolates. The plates were placed into a static incubator for 3 days, followed by an orbital shaker for 5 days, both at 30˚C. Controls were carried out by incubating each grade of cheesecloth and medium without inoculation, as well as the strains with each cheesecloth grade and only water. Activity testing was carried out using a disk diffusion assay, as previously described.

Table 2.5. Twenty-four well plate configuration for testing with a number of chemical and biological inhibitors. pH 7 is included in the conditions as a control.

	1	**2**	**3**	**4**	**5**	**6**
A	Ampicillin (10µg/mL)	Erythromycin (10µg/mL)	Kanamycin (7.5µg/mL)	Rifampicin (1µg/mL)	Cefotaxime (2.5ug/mL)	Bacitracin (15 µg/mL)
B	Autoclaved *E. coli*	Autoclaved *S. aureus*	Autoclaved *B. subtilis*	*E. coli* Supernatant	*S. aureus* Supernatant	*B. subtilis* Supernatant
C	Sodium butyrate (25mM)	N-acetyl glucosamine (25mM)	Lanthanum Chloride (20µM)	Scandium Chloride (20µM)	Soil Extract (25µL/mL)	Medium pH 7
D	Sodium Chloride 3%	SDS 0.001%	EtOH 1%	DMSO 3%	Medium pH 3	Medium pH 10

Grade 10 Grade 50 Grade 90

Figure 2.1 The three grades of 100% cotton cheesecloth chosen for the experiments. Grade 10 is the most loosely woven grade, with grade 90 as the most tightly packed, and grade 50 lies in between.

2.12 Co-culturing to elicit antimicrobial production

2.14.1 Co-culturing Plates

Strains that had previously not exhibited the production of antimicrobial compounds were prepared as spore suspensions. All non-active Fire Mountain strains were to be tested against one another, giving >1000 possible combinations if two strains are tested adjacently at one time. This is not an efficient method to test the theory that proximity of neighbours affects the production of secondary metabolites, so a method was developed to test the greatest number of strains at one time to increase throughput. A base layer of spores from each non-active Fire Mountain strain was spread over the surface of 12x12cm SFM agar plates (20μL spores in 100μL sterile water) and allowed to dry. Following this, a multichannel pipette was used to spot 5μL of each remaining non-active strain on top. The plates were then incubated at 30°C and checked daily for growth.

2.14.2 Secondary Testing

Combinations of strains showing activity on the SFM plates were taken forward for secondary testing. The base strain from previous co-culturing plates was used as the "elicitor", and the strain spotted over the top as the "producer" and spread onto 12x12cm GYM plates. A diagram of which is shown in Figure 2.2 These plates were incubated at 30°C and checked daily for growth. When growth was observed, cross sections of the plates were taken for antimicrobial testing against *E. coli* and *B. subtilis*, as described previously.

2.14.3 Twenty-four Well Plate Testing

Combinations of inducing and producing strains were grown together in twenty-four well plates and tested for antimicrobial activity using a disk diffusion assay. Active combinations were scaled up into 2L non-baffled Erlenmeyer flasks in 500mL GYM medium.

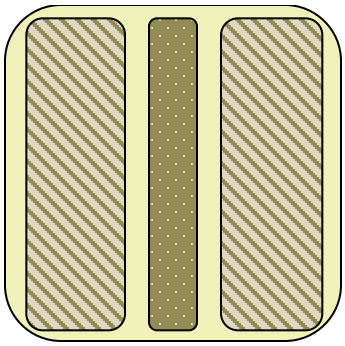


Figure 2.2. The producer strain was streaked on the outer edges of the plate (lined areas) and the inducer strain streaked between these two areas (spotted area). Horizontal cross sections were taken and tested against reporter strains for antimicrobial activity.

CHAPTER 3: ISOLATION AND IDENTIFICATION OF NOVEL ANTIMICROBIAL PRODUCING STREPTOMYCETES FROM FIRE MOUNTAIN, CHINA

3.1 Introduction

Actinomycetes are generally found in soil and marine sediments, and there have been specific methods developed to sequester and purify them for further investigation. Selective isolation is a method that often utilises specifically designed media for targeted species of bacteria. A number of methods can be used to ensure that only actinomycetes are cultured, such as the use of porous cellulose over the agar plate, and the diluted, mixed sample grown over the top. This allows for the filamentous mycelia of the actinomycetes to penetrate the filter, and anchor into the medium, whereas other bacterial species are restricted to the filter surface. Once the filter is removed, so are the non-actinomycetes (Hirsch and Christensen, 1983). As well as physical methods, the use of antibiotics within the selection medium can also select for growth of actinomycetes, as described by (Williams and Davies, 1965). The authors tested a number of antibiotics and media, concluding that a mixture of starch casein agar with two antifungals (nystatin and actidione) and two antibiotics (sodium penicillin and polymyxin B sulphate) was most suitable for the isolation of actinomycete colonies.

These methods have been used for decades to isolate strains that produce useful secondary metabolites; therefore, it is important to consider ways that avoid re-isolation of the same species producing known compounds. One method to improve the likelihood of isolating novel antimicrobial producing strains is the addition of rarely expressed antibiotics into the isolation medium, with the premise that strains with the capacity to produce similar compounds will possess resistance genes, therefore giving them the ability to grow in the presence of the compound.

Whilst selective isolation is a technique that has lasted the test of time, there is the chance that the same isolates will be obtained on multiple occasions. To prevent this, a quick comparison of the morphological features, such as size, mycelial colour, diffusible pigment colour, shape, of each strain can be carried out, and isolates appearing to be identical to known isolated can be

discarded (Donadio et al., 2002). It is important to focus on the discovery of novel species of Streptomycetes, as the screening of known actinobacteria has previously lead to the rediscovery of the same compounds (Bérdy, 2012). It is hypothesised that previously undiscovered strains may harbour the ability to produce novel bioactive compounds (Bull, 2011).

Found in the North Turpan Basin of Xinjiang Province, China, lies "Fire Mountain", a stretch of land that is around 1,600 feet high, and 63 miles long. This area was recorded as the hottest place on earth by NASA in 2007, and soil temperatures can reach up to 80°C. It is named Fire Mountain, or Flaming Mountain, due to the red colour of the rocks and soil (Figure 3.1), and when the heat rises from the ground it gives the illusion that the mountain is on fire.

As with the Atacama Desert, it was unknown until recently whether bacterial life could be supported in these hyper-arid soils lacking in nutrients and moisture, and exposed to high levels of UV, making Fire Mountain a desirable and under-explored area to pursue novel antimicrobials.

a. 

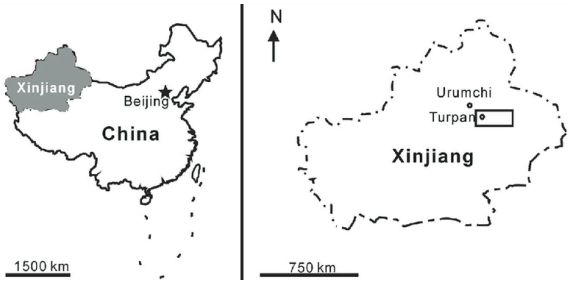
b. 

Figure 3.1 The red rocks and soil of Fire Mountain (a) and where the mountain is situated in the North Turpan Basin (b). Map from Yao et al. (2012).

Over the last few decades there has been an upsurge of antimicrobial resistant strains of pathogenic bacteria, particularly ESKAPE pathogens described by (Rice, 2008) as those most likely to "escape" the effects of antimicrobial compounds. The ESKAPE pathogens are: *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa*, and *Enterobacter* species. Because of these drug resistant pathogens, there are now infections that occur which cannot be treated effectively by any antimicrobial drug (Boucher et al., 2009). Figure 3.2 shows the antibiotic resistance timeline compiled by (Clatworthy et al., 2007), which describes when an antibiotic was deployed in comparison to

where antibiotic resistance was observed. In most cases, the timescale between these two events is less than a decade, with many only a couple of years. These sorts of observations highlight the importance of research into novel antimicrobial compounds, if we do not wish to decline into an era of medicine where once again people die from seemingly non-severe infections such as UTIs, chest infections, and so on.

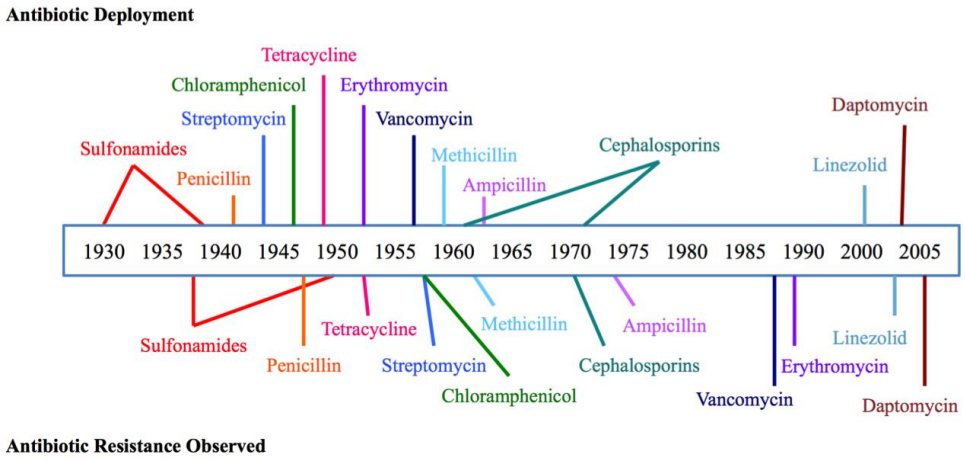


Figure 3.2 The year that the antibiotic was first deployed is shown at the top of the timeline, in comparison to when resistance to the antibiotic was observed along the bottom of the timeline. Adapted from (Clatworthy et al., 2007).

Species of *Streptomyces* are continuously being investigated for novel antimicrobial compounds, due to their genomes in which biosynthetic gene clusters are found in abundance, giving them the capacity to produce novel antimicrobial compounds (Goodfellow and Fiedler, 2010). Proving novelty is one of the most important aspects of antimicrobial discovery, alongside adequate testing for toxicity against eukaryotic cells. Lead compounds must be purified using a number of chromatographic techniques such as high performance liquid chromatography (HPLC) (Selsted, 1997), thin layer chromatography (TLC) (Zhao et al., 2012), ion exchange chromatography (IEX) (Pingitore et al., 2007), before being subjected to mass spectrometry (Hsieh and A. Korfmacher, 2006) and nuclear magnetic resonance (NMR) (Ramamoorthy, 2009).
This chapter will explore how these traditional approaches can be utilised to identify putatively novel antimicrobial compounds from Streptomycetes isolated from extreme environments.

3.2 Results

3.2.1 Selective Isolation and Colony Selection

Colonies presumptively identified as *Streptomyces* were distinguished from other species based on their morphology, namely extensively branched substrate hyphae and white aerial hyphae. These colonies were observed on all selective media, and the highest colony count was found on humic acid-vitamin agar. Figure 3.3 shows representative isolation plates at a dilution of 10^{-1}. Over 100 presumptive streptomycetes of different morphologies were sub-cultured onto modified Bennett's agar, followed by oatmeal agar. Representative strains showing the great diversity of Fire Mountain isolates can be found in Figure 3.4. These plates show a range of aerial hyphae, diffusible pigments, and levels of sporulation.

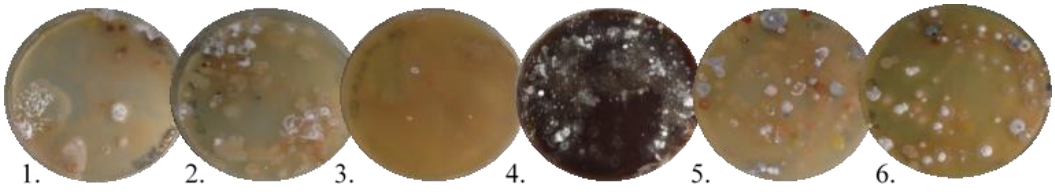


Figure 3.3 Representative 10-1 Isolation Plates: 1. Starch-casein agar + gentamicin; 2. Oligotrophic agar; 3. Raffinose-histidine agar; 4. Humic acid-vitamin agar; 5. Starch-casein agar; 6. Glucose-yeast extract agar.

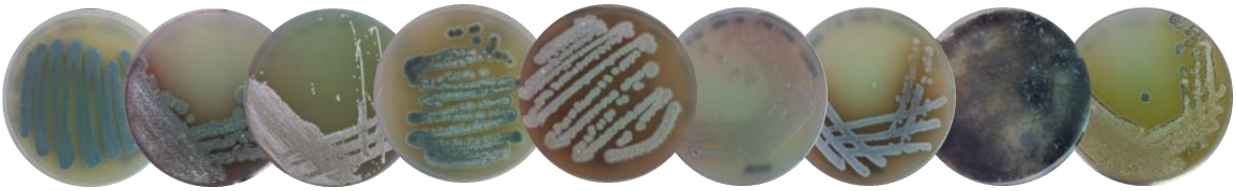


Figure 3.4 Representative isolates from Fire Mountain soil, showing the diverse range of phenotypes and brightly coloured secondary metabolites which are diffused into the solid agar.

3.2.2 Phylogenetic Analysis

Genomic DNA was extracted from 31 isolates using a GenElute™ Bacterial Genomic DNA Kit, and the DNA subjected to PCR. 16S rRNA amplification was carried out using 27F (forward) and 1525R (reverse) primers, which are standard for 16S rRNA sequencing. A single band was observed at around 1,500bp for each strain, as anticipated for 16S rRNA PCR products. Sequences were returned for each of the strains, and all but one assigned to the genus *Streptomyces*. The returned sequences can be found in Appendix A, alongside those of the closest evolutionary neighbours. Once the sequences had been returned and assembled using DNA Baser, they were grouped next to their closest neighbours as can be seen in the phylogenetic tree (Figure 3.4). Strain DEM60027 was found to be a *Nocardiopsis sp.*, another family of soil actinomycetes. *Nocardiopsis spp.* are morphologically similar to *Streptomyces spp.,* with aerial spores and filamentous hyphae (Bennur et al., 2015). Its closest neighbour was *N. dassonvillei subsp. albirubida*, and these strains served as a useful outgroup for the phylogenetic comparisons. (Labeda et al., 2012) carried out phylogenetic analysis of almost all described species within the *Streptomycetaceae* family based on their 16S rRNA sequences, and assigned them to statistically supported clades. Each of the Fire Mountain isolates have therefore been designated a putative clade based on their closest neighbours, as described in the work by Labeda et al. (2012), the results of which can be seen in Table 3.1, where any bioactive compounds produced by closest neighbours are also detailed.

Clade 41

Isolates DEM60068, DEM60073, DEM60134, DEM60136, DEM60153, DEM60158, and DEM60175 share identical 16S rRNA gene sequences with one another, as well as with the type strains of *S. enissocaesilis, S. plicatus* and *S. rochei*.

Clade 85

The closest evolutionary neighbour to isolate DEM60061 is *S. fradiae*. They share 99.57% sequence similarity, with a difference of 6 nucleotides in 1385. The next closest neighbour is *S. coeruleoprunus*, which has 10 out of 1385 nucleotides different.

Clade 88

Isolate DEM60071 has 11 nucleotides of 1429 different to its closest neighbour, *S. levis*, giving 99.23% similarity. The next closest neighbour is *S. carpinensis* with 98.89% similarity, equivalent to 16 nucleotides different of 1442.

Clade 94

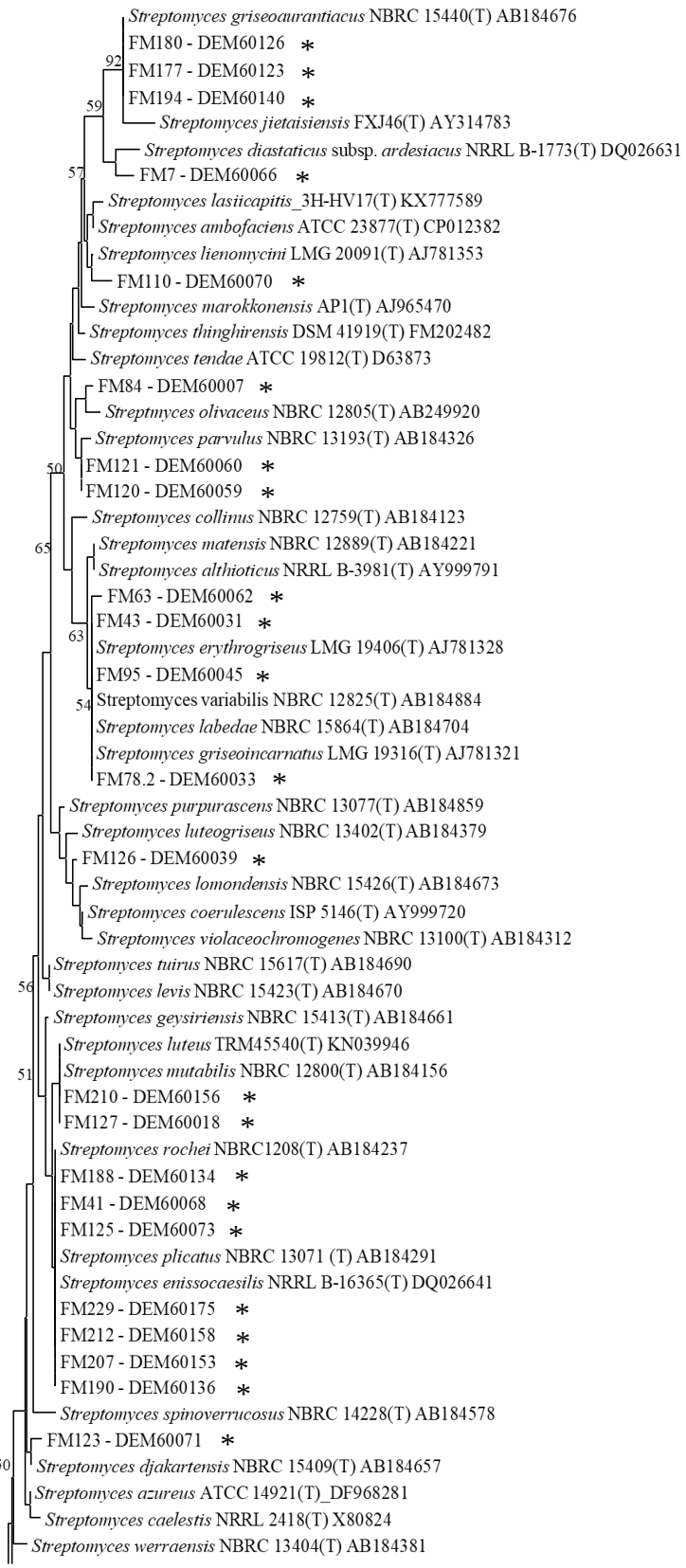
Three isolates, DEM60123, DEM60126, and DEM60140, share 100% sequence similarity to their closest neighbour, *S. griseoaurantiacus*. The closest evolutionary neighbours following this, are *S. jietaisiensis* with 99.79% sequence similarity or 3 of 1396 nucleotides different, and *S. lasiicaptis* with 98.23% sequence similarity or 25 nucleotides different of 1409, for each Fire Mountain isolate.

Clade 96

The closest evolutionary neighbour to isolate DEM60070 is *S. ambofaciens*, with 99.46% sequence similarity, equal to 7 nucleotides different in 1285. *S. violaceochromogenes* is the next closest neighbour with 11 of 1276 nucleotides different, or 99.14% sequence similarity.

Clade 100

DEM60045 and DEM60033 share 100% sequence similarity to *S. griseoincarnatus, S. variabilis, S. erythrogriseus,* and *S. labedae*. Similarly, DEM60031 shares 99.93% sequence similarity to these four type strains, with only 1 out of 1371 nucleotides different each. Also included in this clade is isolate DEM60057, which has 99.26% sequence similarity to *S. matensis* and *S. althioticus,* or 10 of 1352 nucleotides different. The next closest evolutionary neighbour is *S. erythrogriseus,* with 99.04% similarity and 13 of 1359 nucleotides different. The final isolate in this group is FM63, which has 99.77% sequence similarity to *S. erythrogriseus*, *S. griseoincarnatus, S. variabilis,* and *S. lebadae,* or 3 of 1290 nucleotides different. The next closest evolutionary neighbour is *S. griseorubens*, with 2 of 1290 nucleotides different, or 99.46% sequence similarity.


Streptomyces griseoaurantiacus NBRC 15440(T) AB184676
FM180 - DEM60126 *
92
FM177 - DEM60123 *
FM194 - DEM60140 *
59
Streptomyces jietaisiensis FXJ46(T) AY314783
Streptomyces diastaticus subsp. *ardesiacus* NRRL B-1773(T) DQ026631
57
FM7 - DEM60066 *
*Streptomyces lasiicapitis*_3H-HV17(T) KX777589
Streptomyces ambofaciens ATCC 23877(T) CP012382
Streptomyces lienomycini LMG 20091(T) AJ781353
FM110 - DEM60070 *
Streptomyces marokkonensis AP1(T) AJ965470
Streptomyces thinghirensis DSM 41919(T) FM202482
Streptomyces tendae ATCC 19812(T) D63873
FM84 - DEM60007 *
Streptmyces olivaceus NBRC 12805(T) AB249920
Streptomyces parvulus NBRC 13193(T) AB184326
50
FM121 - DEM60060 *
FM120 - DEM60059 *
Streptomyces collinus NBRC 12759(T) AB184123
65
Streptomyces matensis NBRC 12889(T) AB184221
Streptomyces althioticus NRRL B-3981(T) AY999791
FM63 - DEM60062 *
FM43 - DEM60031 *
63
Streptomyces erythrogriseus LMG 19406(T) AJ781328
FM95 - DEM60045 *
54
Streptomyces variabilis NBRC 12825(T) AB184884
Streptomyces labedae NBRC 15864(T) AB184704
Streptomyces griseoincarnatus LMG 19316(T) AJ781321
FM78.2 - DEM60033 *
Streptomyces purpurascens NBRC 13077(T) AB184859
Streptomyces luteogriseus NBRC 13402(T) AB184379
FM126 - DEM60039 *
Streptomyces lomondensis NBRC 15426(T) AB184673
Streptomyces coerulescens ISP 5146(T) AY999720
Streptomyces violaceochromogenes NBRC 13100(T) AB184312
Streptomyces tuirus NBRC 15617(T) AB184690
56
Streptomyces levis NBRC 15423(T) AB184670
Streptomyces geysiriensis NBRC 15413(T) AB184661
Streptomyces luteus TRM45540(T) KN039946
51
Streptomyces mutabilis NBRC 12800(T) AB184156
FM210 - DEM60156 *
FM127 - DEM60018 *
Streptomyces rochei NBRC1208(T) AB184237
FM188 - DEM60134 *
FM41 - DEM60068 *
FM125 - DEM60073 *
Streptomyces plicatus NBRC 13071 (T) AB184291
Streptomyces enissocaesilis NRRL B-16365(T) DQ026641
FM229 - DEM60175 *
FM212 - DEM60158 *
FM207 - DEM60153 *
FM190 - DEM60136 *
Streptomyces spinoverrucosus NBRC 14228(T) AB184578
FM123 - DEM60071 *
50
Streptomyces djakartensis NBRC 15409(T) AB184657
Streptomyces azureus ATCC 14921(T)_DF968281
Streptomyces caelestis NRRL 2418(T) X80824
Streptomyces werraensis NBRC 13404(T) AB184381

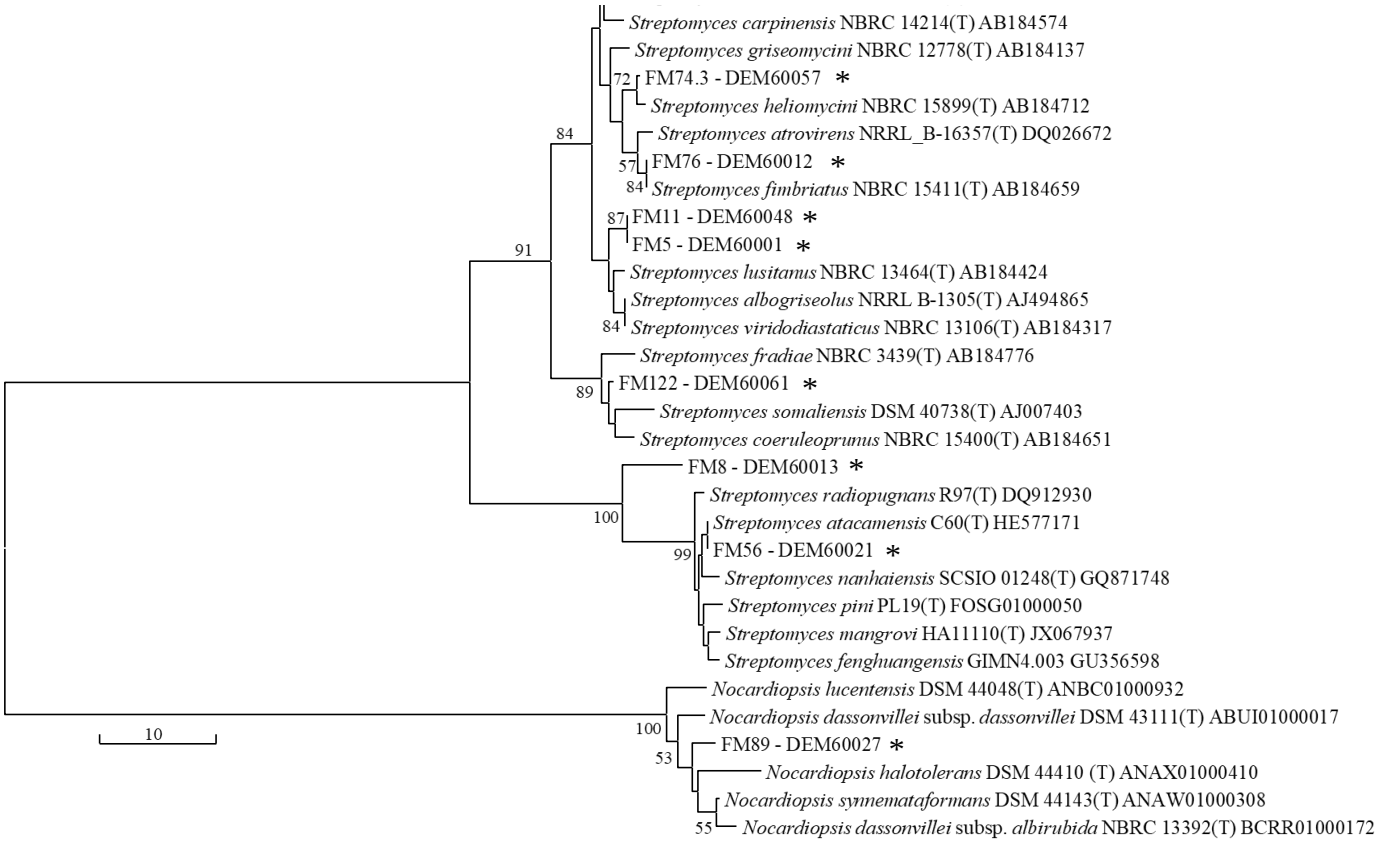


Figure 3.4 A phylogenetic tree showing the evolutionary relationships between all Fire Mountain strains (marked with an asterisk) subjected to 16S rRNA sequencing, and their closest neighbours. The numerical value given next to some nodes is the bootstrap percentage, indicating the level of support for this branch. The scale bar indicates 10 substitutions per nucleotide position.

Clade 102

A single isolate, DEM60066, is placed into clade 102. It shares 99.23% sequence similarity to its closest neighbour, *S. heliomycini*¸ or 11 out of 1423 nucleotides different. The next closest neighbour is *S. diastaticus subsp. ardesiacus,* with 13 of 1426 nucleotides different, equating to 99.09% sequence similarity.

Clade 103

DEM60039 is the only isolate grouped within clade 103. The closest evolutionary neighbours are *S. tuirus* and *S. luteogriseus,* with 11 of 1369 nucleotides different, or 99.2% sequence similarity. Second to these strains is *S. parvulus,* with 99.12% sequence similarity, or 12 of 1369 nucleotides different.

Clade 104

Isolate DEM60012 is the only strain assigned to this clade, and it shares 100% similarity to *S. fimbriatus,* but only 98.8% similarity to its next closest neighbour *S. werraensis,* with 17 differences at 1425 locations.

Clade 109

A single Fire Mountain isolate, DEM60059, is assigned to this clade. Its closest evolutionary neighbour is *S. spinoverrucosus,* with 6 of 1407 nucleotides different and 99.57% sequence similarity. The next closest neighbour is *S. purpurescens,* with a sequence similarity of 99.43% and 8 of 1409 nucleotides different. As neither of these type strains were featured in the clades defined by Labeda et al., the next closest evolutionary neighbour was chosen to assign this clade. This strain is *S. lusitanus,* which also has a similarity of 99.43%.

Clade 110

Isolates DEM60001 and DEM60048 each share a 99.65% similar 16S rRNA sequence to *S. atrovirens, S. albogriseolus,* and *S. viridodiasticus,* with 5/1425, 5/1425, and 5/1423bp differences. A strain with similar evolutionary data is DEM60007, which has a 16S rRNA gene similarity of 99.3% to the type strain of *S. albogriseolus,* a result equivalent to 9 nucleotide differences at 1,434 sites. However, the next two closest evolutionary neighbours to this strain

are *S. viridodiasticus* (99.36% similarity, with 9 of 1416 nucleotides different) and *S. olivaceus* (99.08% similarity, 13 of 1420 nucleotides different).

Clade 114

DEM60060 was assigned to this clade alone, with its closest neighbour *S. spinoverrucosus*. The sequence similarity is 99.57%, or 6 of 1406 nucleotides different. As with DEM60059, the next closest neighbour was used to assign the clade as *S. spinoverrucosus* did not feature in the previously described paper (Labeda et al., 2012). This is *S. coerulescens*, which has 7 of 1407 nucleotides different, and a sequence similarity of 99.5%.

Clade 119

Assigned to this clade are isolates DEM60018 and DEM60156, both of which have 100% sequence similarities with *S. luteus* and *S. mutabilis.* The next closest evolutionary neighbours are *S. enissocaesilis* and *S. plicatus*, with sequence similarities of 99.7% and 4 of 1397 or 1412, respectively.

Clade 127

DEM60013 forms a distinct phyletic line and is most closely related to *S. pini*. The two strains share a 16S rRNA gene similarity of 98.95%, a value equivalent to 15 nucleotide differences at 1427 nucleotide locations. Its next closest neighbours are *S. mangrovi* (98.91% similarity, 15 of 1372 nucleotides different) and *S. atacamensis* (98.24% similarity, 25 of 1421 nucleotides different). DEM60021 is also allocated to this clade, as its closest neighbour is *S. radipugnans*. The sequence similarity is 99.72% with 4 out of 1430 nucleotides different. The next closest evolutionary neighbour is *S. atacamensis*, with 8 nucleotides different of 1446, equalling 99.45% sequence similarity.

Table 3.1 The thirty-one sequenced Fire Mountain strains alongside their closest neighbours, with the percentage similarity, nucleotide difference of the 16S rRNA gene, and any bioactive compounds produced by the closest evolutionary neighbours. An asterisk signifies putatively novel strains, of which there are eleven.

Strain	Closest Neighbour	% Similarity	Nucleotides Different	Clade	Bioactive compounds from closest neighbour	Putatively Novel
DEM60001	*S. atrovirens*	99.65	5/1425	110	-	
DEM60007	*S. albogriseolus*	99.37	9/1418	110	Vineomycin Neomycins	*
DEM60012	*S. fimbriatus*	100	0/1420	104	Cephamycin Septacidin	
DEM60013	*S. pini*	98.95	15/1427	127	Siderophore	*
DEM60018	*S. luteus*	100	0/1399	119	-	
DEM60021	*S. radiopugnans*	99.72	4/1430	127	-	
DEM60027	*N. dassonvillei subsp. albirubida*	98.87	16/1420	-	-	
DEM60031	*S. griseoincarnatus*	99.93	1/1371	100	Antitumor	
DEM60033	*S. griseoincarnatus*	100	0/1400	100	Antitumor	
DEM60039	*S. tuirus*	99.2	11/1369	103	Tuoromycin	*
DEM60045	*S. griseoincarnatus*	100	0/1419	100	Antitumor	
DEM60048	*S. atrovirens*	99.65	5/1425	110	-	*
DEM60057	*S. matensis*	99.26	10/1352	100	Matamycin (Altheomycin)	*
DEM60059	*S. spinoverrucosus*	99.57	6/1407	109	-	*
DEM60060	*S. spinoverrucosus*	99.57	6/1406	114	-	*

DEM60061	*S. fradiae*	99.57	6/1385	85	Neomycin Fosfomycin Tylosin	*
DEM60062	*S. erythrogriseus*	99.77	3/1290	100	-	
DEM60066	*S. heliomycini*	99.23	11/1423	102	Heliomycin	*
DEM60068	*S. enissocaesilis*	100	0/1417	119	-	
DEM60070	*S. ambofaciens*	99.46	7/1285	96	Ambobactin	*
DEM60071	*S. levis*	99.23	11/1429	88	Antifungal	*
DEM60073	*S. enissocaesilis*	100	0/1417	119	-	
DEM60123	*S. griseoaurantiacus*	100	0/1408	94	Manumycin Chinikomycin Diperamycin Griseolic acid	
DEM60126	*S. griseoaurantiacus*	100	0/1407	94	As above	
DEM60134	*S. enissocaesilis*	100	0/1418	41	-	
DEM60136	*S. enissocaesilis*	100	0/1415	41	-	
DEM60140	*S. griseoaurantiacus*	100	0/1408	94	As above	
DEM60153	*S. enissocaesilis*	100	0/1417	41	-	
DEM60156	*S. luteus*	100	0/1424	119	-	
DEM60158	*S. enissocaesilis*	100	0/1424	41	-	
DEM60175	*S. enissocaesilis*	100	0/1419	41	-	

3.2.3 Screening for the production of antimicrobial compounds

Altogether, 169 strains (including 86 that were isolated in a previous investigation; Taylor, 2012) were screened against *E. coli, B. subtilis* and *S. cerevisiae*, using agar plug assays. A total of 29 strains showed activity against *S. cerevisiae*, 62 showed activity against *B. subtilis*, and 16 showed activity against *E. coli*. Any strains that were active against *S. cerevisiae* were rejected immediately, as yeast is used in this purpose as a model organism for human cells, leaving a total

of 33 strains active against *B. subtilis* and 12 active against *E. coli.* All strains that were active against *E. coli* were found to also be active against *B. subtilis.*

Activity against the *B. subtilis* reporter strains was infrequent, with only seven Fire Mountain strains triggering a response from the *yvqI* mutant, which reports for cell envelope, specifically lipid II, interruption. These strains are: DEM60121, DEM60156, DEM60133, and DEM60164 which also displayed activity against eukaryotic test organisms; DEM60132 which displayed broad spectrum activity, but did not affect eukaryotic cells; DEM60157 and DEM60161 which were active against Gram-positive organisms, only.

All *E. coli* positive plates were crushed and frozen, and the mycelial extract collected. Mycelial extracts from strains DEM600012, DEM60130, DEM60131, and DEM60139 were active, so filtered and taken forward for further purification. Methanol extracts from strains DEM60068, DEM60007 and DEM60045 were active, so filtered and taken forward for further purification. Strains DEM60013, DEM60068, DEM60012, DEM60007, DEM60045, DEM60073, DEM60030, DEM60031, DEM60138, DEM60139, and DEM60162 were grown on a range of solid media including oatmeal agar, ISP2, medium A, medium B, medium C, medium V, D/seed vegetative agar, INA5, RA3, GPMY, and V6, and the results compared. The medium that gave the largest zone of inhibition was therefore chosen as the optimal medium (Figure 3.5).

3.2.4 Media and temperature selection

Strains DEM60013, DEM60068, DEM60012, DEM60007, DEM60045, and DEM60073 were grown in a range of different clear, liquid media and tested for activity. Strains DEM60068, DEM60007, DEM60045, and DEM60073 were found to be active from the supernatant (see Table 3.2 for further details; Figure 3.5 shows representative active samples). Twenty millilitres of active material were centrifuged and the supernatant filter sterilised for further purification. Clear differences in the activity levels can be seen between the different media formulations, and those giving the largest zone of inhibition were chosen for further work.

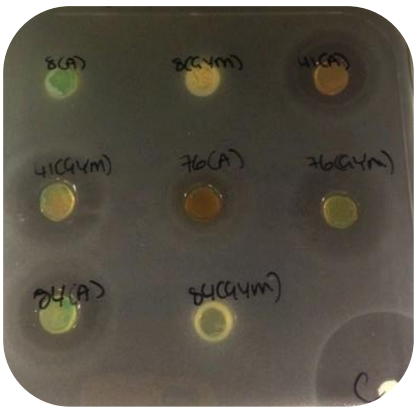
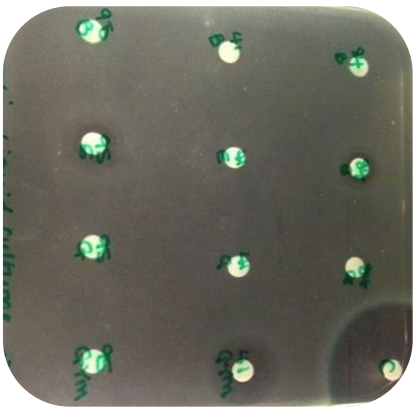
a.  b. 

Figure 3.5 Representative screening plates showing a. solid media optimisation and b. liquid media optimisation. Control antibiotics are featured in the bottom right hand side of each image, with a large zone of inhibition of the *B. subtilis* impregnated within the agar.

A selection of the Fire Mountain strains producing broad spectrum compounds were grown at both 30°C and 40°C. For four out of the five strains tested (DEM60042, DEM60007, DEM60073, and DEM60030), activity was observed at 40°C as well as 30°C, when grown in Medium B (composition can be found in the appendix). DEM60031 is the only strain tested that did not produce bioactive compounds at 40°C. The inhibitions observed from growth at 30°C and 40°C are similar, with differences of less than 10% of the activity compared to the control, in strains DEM60042, DEM60007, and DEM60073. For strain DEM60030, at 30°C in Medium B the zone of inhibition of *E. coli* was 69% of the ampicillin control, and at 40°C in Medium B the inhibition was 50%.

3.2.5 Toxicity and further activity testing

Active extracts from DEM60012, DEM60013, DEM60030, DEM60031, and DEM60073 were sent to Cubist Pharmaceuticals (early 2013) for testing against their MDR *E. coli* and *S. aureus* strains, as well as controls for each. Extract from DEM60013 had the ability to inhibit the growth of both the *S. aureus* and *E. coli* controls, as well as the multi-drug resistant mutants. The *S. aureus* strain was also incubated with DNA to identify any DNA intercalating molecules, as these are not suitable for clinical use, due to their toxicity in eukaryotic cells (Snyder and Arnone, 2002). Extracts from DEM60012 and DEM60031 returned negative results when incubated with

DNA, suggesting the possibility that they could be DNA intercalating molecules. The cut off points for growth inhibition were 95% cut off for *S. aureus* and 50% cut off for *E. coli*. These results can be found in Table 3.3. Cubist Pharmaceuticals also tested the toxicity of DEM60013 active extract, by incubating with HK2 cells for 6 hours. The levels of lactate dehydrogenase (LDH) and ATP (both signs of cell distress) were acceptable, and the cells appeared normal when examined microscopically. These results are shown in Table 4.4.

The results from these tests identified DEM60013 as the most promising of the Fire Mountain strains. DEM60007 was not sent to Cubist Pharmaceuticals, but the in-house toxicity tests (carried out by Dr Bernhard Kepplinger) showed promising results. This, alongside a fairly distinct phyletic line, meant that this strain was also chosen for further investigation.

Table 3.2. Broad-spectrum activity observed from Fire Mountain Isolates, and the medium giving the largest zone of inhibition. All values are a percentage of the control, in this case ampicillin. Entries given as "-" signify that a result was not obtained or that no activity was observed.

Isolate	Liquid Medium		Solid Medium	
DEM num.	Medium Type	Zone of Inhibition (mm)	Medium Type	Zone of Inhibition (mm)
DEM60013	GYM+Soil Ex	40	Oatmeal	44
DEM60068	GYM	72	GYM	76
DEM60012	V	44	GYM	92
DEM60041	RA3	60	Oatmeal	64
DEM60007	RA3	36	A	84
DEM60045	RA3	48	A	88
DEM60073	GYM	68	GPMY	124
DEM60130	-	-	GYM	72
DEM60131	-	-	Oatmeal	52
DEM60138	-	-	GYM	68
DEM60139	-	-	GYM	68
DEM60162	-	-	GYM	64

Table 3.3 Screening results returned from Cubist Pharmaceuticals against MDR strains with more than 20 resistance genes, alongside their wild type counterparts. The row in red denotes strain DEM60013, which showed almost complete killing percentages in all but one of the strains.

Strain ID	*S. aureus* control	MDR *S. aureus* + DNA	*E. coli* control	MDR *E. coli*	Primary Spectrum
DEM60012	98	-21	99	0	All
DEM60013	100	98	100	100	All
DEM60030	97	29	-25	2	G+ Only
DEM60031	73	-14	98	3	G- Only
DEM60073	91	29	99	-19	G- Only

Table 3.4. Results of the toxicity testing carried out by Cubist Pharmaceuticals of active extract taken from DEM60013. An increase of lactate dehydrogenase (LDH) and decrease in ATP suggest cell distress, which was not observed.

Strain	LDH % Killing	ATP % Killing	Visual Comments
DEM60013	1.95	-8.94	Healthy

3.2.6 Bench scale bioreactor cultivations

Cultivation of DEM60013 was carried out in a glass 5L vessel (Applikon) using GYM + soil extract, which exhibited preferable activity in previous tests. The online data (Figure 3.6) shows a rise from pH 7 to ~8.4 at around 60 hours, followed by a drop back down to around neutral at around 100 hours, and then a slow rise to pH 8.7 as the fermentation came to end at 191 hours. Although the dissolved oxygen decreased during the cultivation, it did not drop below 50%, and the agitation remained at the 400rpm set point. Samples were taken several times daily, and tested for a number of components, which can be found in Figure 3.7. Activity against *E. coli* was observed after 60 hours, and further activity was not observed after 145 hours. It is suggested that the end of trophophase and primary metabolism is at around this time, and secondary metabolism, or idiophase, is observed from then on, coinciding with antimicrobial activity.

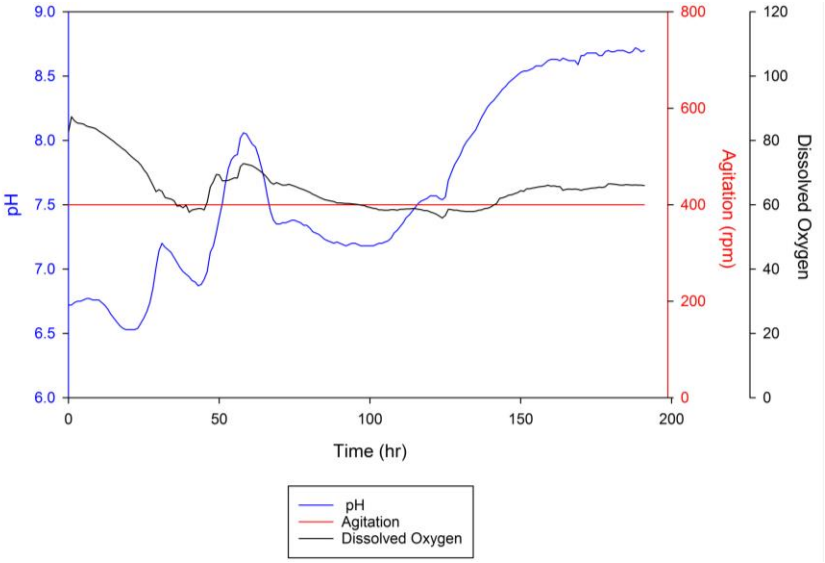


Figure 3.6. Online data for the 5L cultivation of DEM60013 in GYM + soil extract. pH was uncontrolled in this cultivation, and the dissolved oxygen controlled by a cascade. When the dissolved oxygen dropped in the fermenter, the agitation increased to a maximum of 650rpm to counteract this.

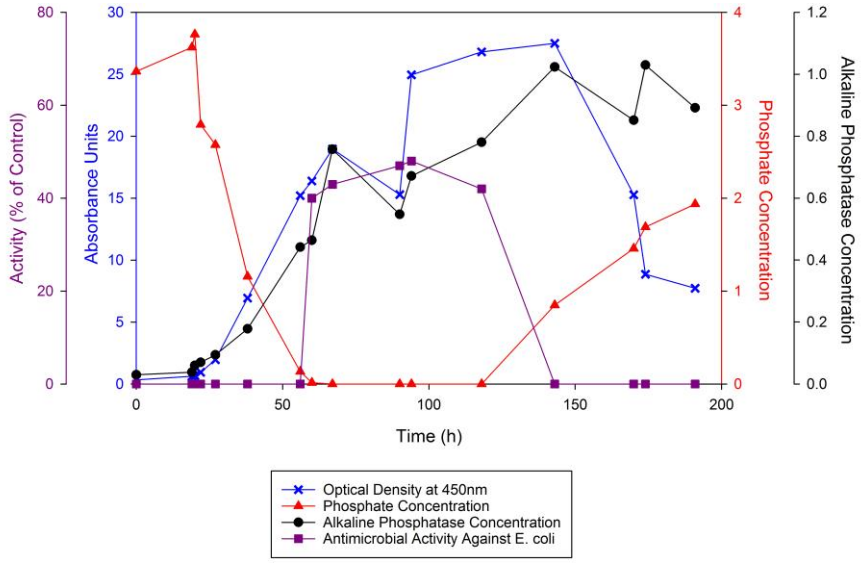


Figure 3.7 Online data for the 5L cultivation of DEM60013, in GYM + soil extract. The phosphate concentration is measured in mM, and the alkaline phosphatase concentration in nM min^{-1}. Secondary metabolism likely begins around 60 hours, coinciding with antimicrobial activity.

This coincides with a reduction in phosphate concentration, and subsequent rise in alkaline phosphatase activity. The activity was observed when the optical density reached a plateau and was not observed again before the OD dropped. Subsequent runs were stopped at around 60-70 hours to obtain active material.

Cultivation of DEM60007 was primarily run in the 5L vessel with RA3 medium, and the run was terminated as soon as activity was observed. The online data (Figure 3.8) showed an abrupt drop in pH from around 18 hours until 20 hours, when it increased sharply up to pH 8.7 at 50 hours and began to drop again. The dissolved oxygen decreased from 100% down to 30% within the first 20 hours, where the agitation was increased to a maximum of 650rpm to counteract it. At ~24 hours, there was a sharp decrease in dissolved oxygen, resulting in a spike in agitation. The samples taken from the reactor were tested using a disk diffusion assay, and showed an initial decrease in phosphate concentration between the start of the fermentation and around 23 hours, followed by a sharp increase at 27 hours, and another decline to the end of the run. The increase at 27 hours coincided with an increase in alkaline phosphatase activity, which rose to around 48 hours before declining. Antimicrobial activity was observed at around 50 hours, and the vessel was harvested at 58 hours. The optical density hit a plateau from 30 hours, which coincides with where the second drop in phosphate concentration begins to be observed. This data is shown in Figure 3.9.

Following the 5L run, DEM60007 was also grown at 15L scale, under the same conditions. The online data can be seen in Figure 3.10, and as with the 5L cultivation there is a drop in pH followed by a steep rise up to ~pH 8.3. Similarly, the abrupt drop in dissolved oxygen was also observed, following a steady decrease. The parameters tested for the offline data (Figure 3.11) also follow a similar pattern to those observed in the 5L fermentation. The phosphate concentration dropped, before increasing slightly as the alkaline phosphatase activity increased. The phosphate concentration decreased until 140h, where an increase was subsequently observed again. Antimicrobial activity was not observed until around 90 hours, and similarly to the 5L cultivation, this is when the OD began to plateau. A visible colour change was observed in both 5L and 20L cultivations of DEM60007, which is exemplified in Figure 3.12.

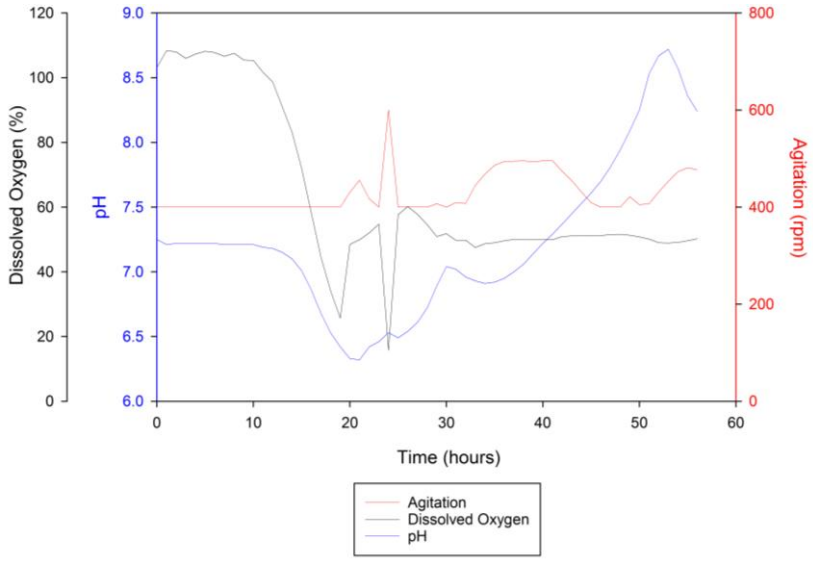


Figure 3.8 Online data collected from strain DEM60007 run in a 5L vessel in RA3 medium. pH was uncontrolled in this cultivation, and the dissolved oxygen controlled by a cascade. When the dissolved oxygen dropped in the fermenter, the agitation increased to a maximum of 650rpm to counteract this.

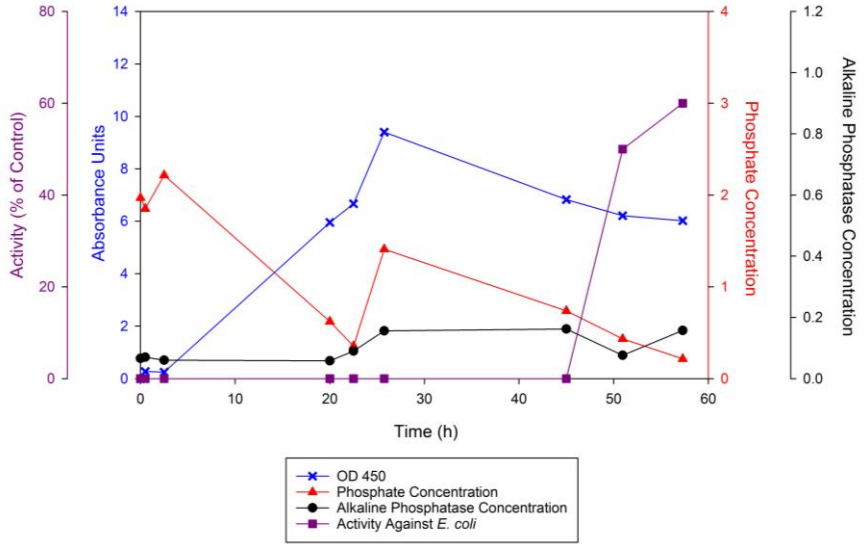


Figure 3.9 Offline data collected from strain DEM60007 run in a 5L vessel in RA3 medium. The phosphate concentration is measured in mM, and the alkaline phosphatase concentration in nM min^{-1}.

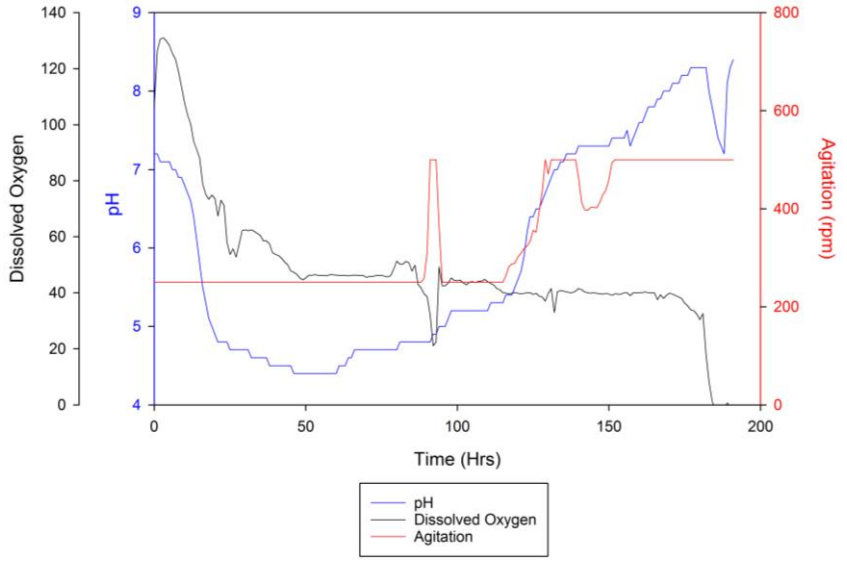


Figure 3.10 The online data collected for strain DEM60007 in RA3 medium, at 2L scale. pH was uncontrolled in this cultivation, and the dissolved oxygen controlled by a cascade. When the dissolved oxygen dropped in the fermenter, the agitation increased to a maximum of 650rpm to counteract this.

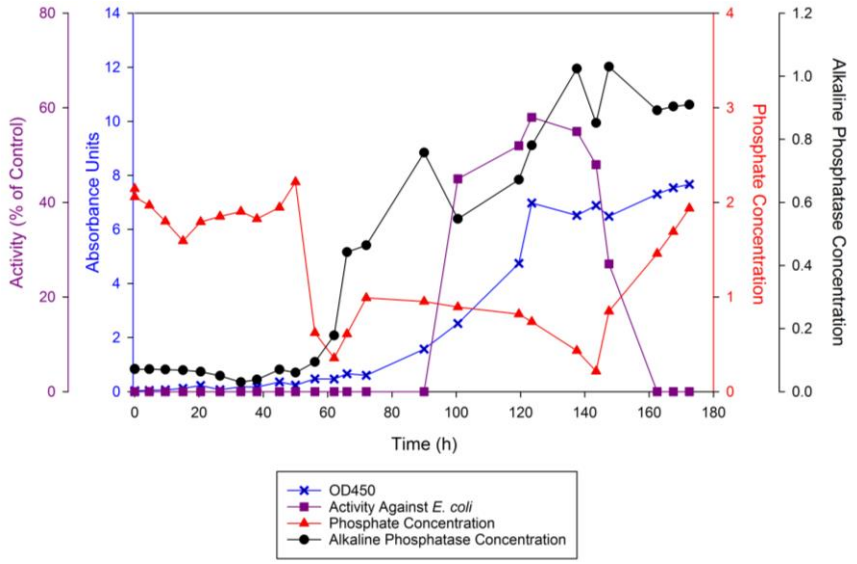


Figure 3.11 Offline data for strain DEM60007 grown in RA3 medium, at 20L scale. The phosphate concentration is measured in mM, and the alkaline phosphatase concentration in nM min-1.

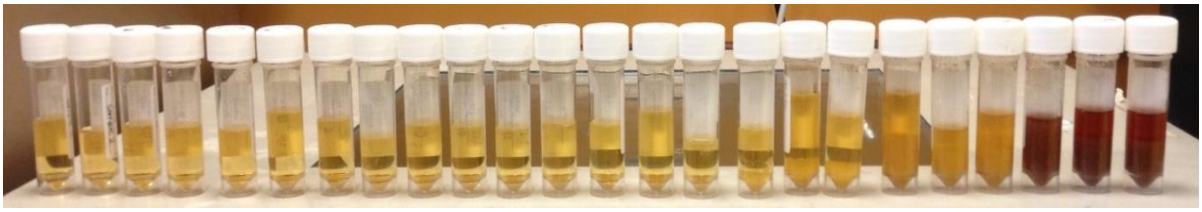


Figure 3.12 Samples taken from the 20L cultivation of DEM60007 in RA3 medium. A drastic change in colour can be seen in the last few samples, which correspond to around 140 hours, and signifies when the cultivation is to be terminated and harvested.

The data from these bench scale bioreactor runs follow similar patterns in primary and secondary metabolism, as expected. For example, the levels of alkaline phosphatase increase as the levels of phosphate decrease, in an attempt at renewal. The reducing phosphate levels are a symptom of nutrient depletion, which coincides with an increase in biomass, and subsequent decrease in dissolved oxygen (higher biomass has higher oxygen demand and utilises more nutrients). As discussed previously, *Streptomyces spp.* begin to self-digest when nutrients deplete, and this occurs in parallel with antimicrobial activity. These trends are observed during all of the cultivations in the bench scale bioreactors.

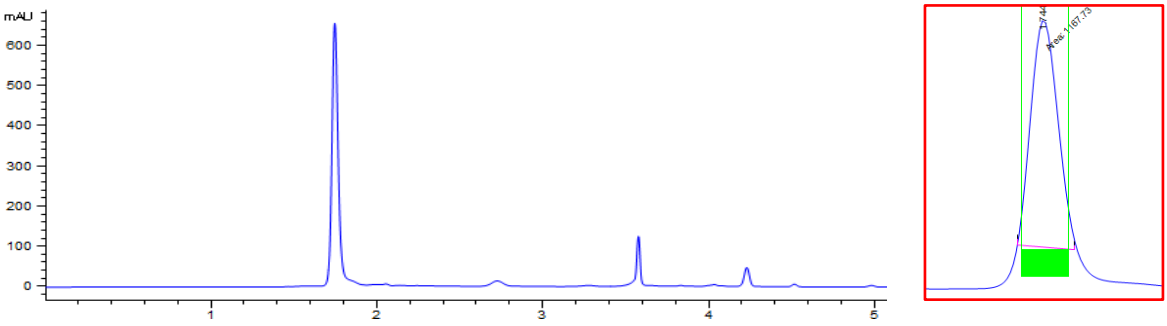
3.2.7 Purification of Active Compounds

DEM60013

Active material obtained from a culture of DEM60013 in GYM + soil extract was purified primarily by organic extraction by ethyl acetate. The three samples of the compound were taken forward, and the pH was adjusted to pH 3, pH 7, and pH 10, before being shaken with ethyl acetate. The activity was lost at pH 3, but activity was observed at pH 7 and pH 10. The ethyl acetate extraction was repeated at pH 7 using the remainder of the active sample. The active organic phase was then subjected to Biotage® Flash Chromatography with a SNAP 25g column, and two areas of activity were observed at fractions 5 and 6 (compound A) and 12 to 16 (compound B). Compound A was analysed by HPLC and appeared to be clean, and the peak subsequently picked off manually. The purity of the sample was confirmed by spectral analysis by the HPLC software and deemed pure enough for mass spectrometry (Figure 3.13).
The protonated mass of the compound was measured as 545.2671, and calculated as 544.25928 with the mass of the hydrogen removed, as can be seen in Figure 4.9. The Dictionary of Natural

Products was consulted, and the compound hypothesised to be N-acetyl streptothricin F, which has a mass of 544.260527. The structure of this molecule can be found in Figure 3.14.

a.



b.

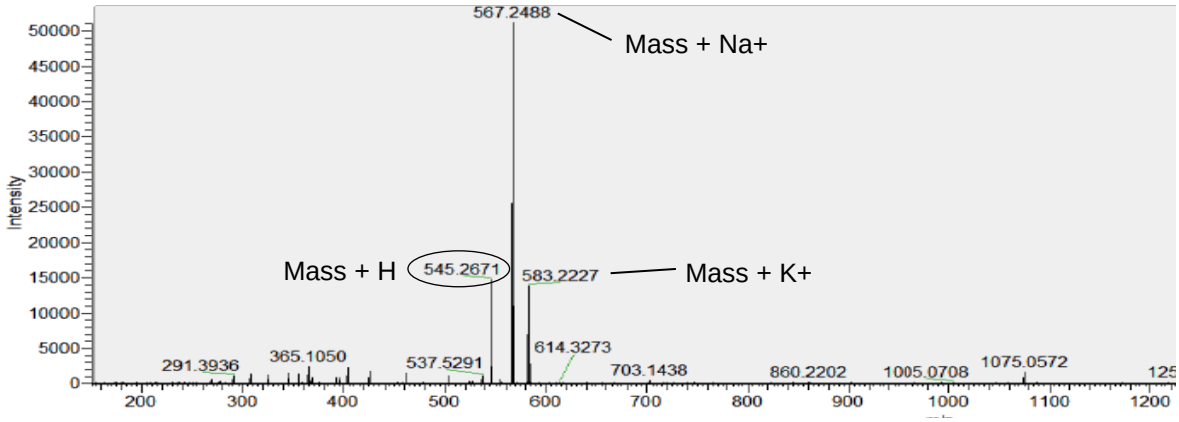


Figure 3.13 The HPLC chromatogram of DEM60013 compound A (a). The largest peak was manually picked off, and tested for purity. The green area signifies that the peak is pure, based on spectral analysis. The mass spectrum returned from Pinnacle Proteomics for compound A produced by DEM60013 (b). The actual mass was found to be 544.25928, which corresponds to the molecule N-acetyl streptothricin F.

The fractions containing DEM60013 compound B were analysed by HPLC to confirm that they were the same compound and combined for further work. The fractions were freeze dried and run through a size exclusion chromatography column, using methanol. The active compound was found in fraction 6, but was not pure enough to send for mass spectrometry. HPLC was carried out on the semi-pure material, and the fractions collected and tested for bioactivity, of which none was observed. The run was repeated using a more concentrated sample, but again the results were negative. It was hypothesised that the formic acid, used as an ion-pairing agent in the water and acetonitrile, could cause degradation of the compound, so the HPLC was repeated using

solvents without formic acid, and the fractions collected. As before, no activity was observed against Gram-positive or Gram-negative organisms. The remaining active material was subjected to cation and anion exchange chromatography, and the eluted material dried down before testing against *B. subtilis* and *E. coli*, but no activity was observed. As the active material containing compound B was depleted, DEM60013 was grown in a number of different media, and activity observed from RA3 + scandium chloride. The active material was again subjected to flash chromatography, but only compound A was present.

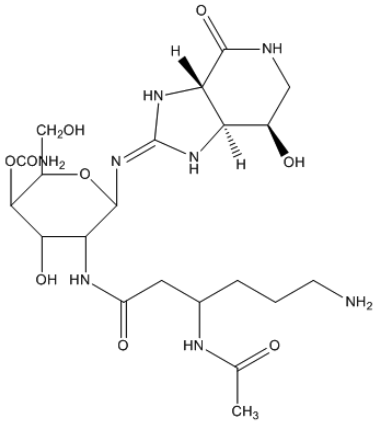


Figure 3.14 The structure of N-acetyl streptothricin F, the compound hypothesised to be produced by DEM60013.

DEM60007

The fermentation of DEM60007 yielded ~500mL active material, extracted from the Amberlite beads. An ethyl acetate extraction was carried out at pH 3, pH 7 and pH 10, but the active compounds did not move into the organic phase, even with the addition of sodium chloride. The active material was subjected to reverse phase flash chromatography using the Biotage® system, and the fractions tested for activity following drying in the GeneVac centrifugal evaporator. Fraction 3 was active against both *B. subtilis* and *E. coli* and was taken forward for further purification. Following this, cation and anion exchange chromatography was carried out, and activity observed on the sodium chloride wash step during cation exchange, leading to the conclusion that the molecule is charged. The active eluate was finally subjected to size exclusion chromatography, using water as the solvent (previous experimentation showed that the compound

precipitates in methanol, possibly due hydrophilic nature). Activity was observed following size exclusion, and the active fraction analysed by HPLC. Despite three purification steps, three peaks were still visible on the chromatogram, so preparatory HPLC was carried out to further isolate the active compound. The fractions were tested against *B. subtilis* and *E. coli*, but no activity was recorded, despite visible peaks on the HPLC chromatogram. Corresponding fractions to where peaks were observed on the chromatogram were analysed by HPLC, but immediate elution occurred, suggesting that the compounds had degraded, as with DEM60013.

Thin layer chromatography was carried out using 50% acetonitrile, to see if crude extract alongside semi-pure material could migrate up the TLC plate. A control of 50% methanol was also run for comparison. Once the solvent reached 1cm from the top of the plate, it was removed from the solvent, and the silica allowed to air dry. Once dry, the plates were placed face down on a *B. subtilis* seeded nutrient agar plate and left for diffusion of the compounds into the agar, for 10-15 minutes. The plates were incubated overnight and examined for activity. Clear areas were observed on the 50% methanol plates, in comparison to a full bacterial lawn where the 50% acetonitrile TLC plate had been placed.

Flash chromatography was repeated using a gradient of 0 – 20% methanol, and fraction 3 showed broad-spectrum activity. Cation exchange, followed by size exclusion chromatography, was performed and then analysed by HPLC. A single peak on the HPLC was observed, and the software analysed the spectral data to confirm purity of the peak, confirming it clean for mass spectrometry. The returned mass spectra were inconclusive, due to a high level of sodium chloride. The semi-pure fraction obtained from the flash chromatography was subjected to LC-MS (carried out by Dr Stephanie Morton-Laing), and the results analysed using Compass Data Analysis, Version 4.2. A number of masses were observed on the mass spectrum (Figure 4.14), with 365.1068 at the active peak, or an actual mass of 364.09968. This mass is likely equivalent to that of Wailupemycin F, a compound with similar bioactivity, which has a mass of 364.0947. The structure of this compound can be found in Figure 3.15.

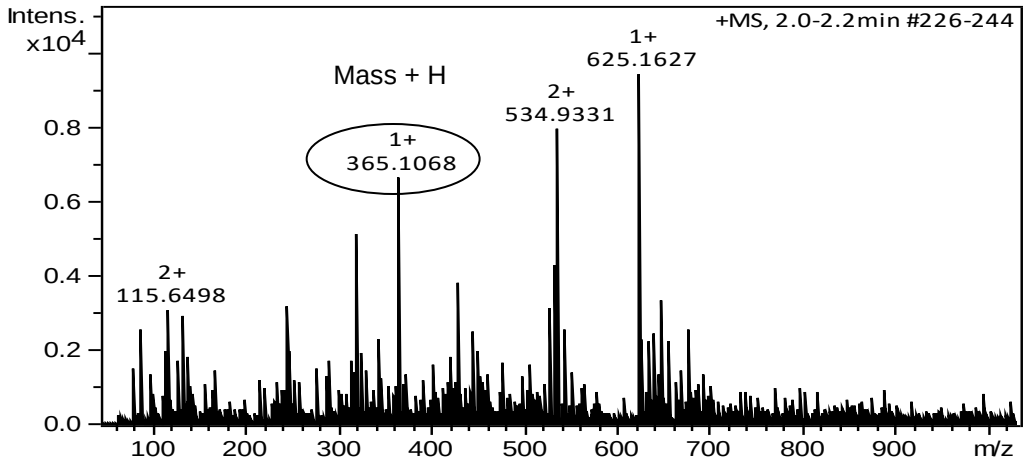


Figure 3.14 Mass spectrum of active compound from DEM60007. The mass circled is thought to be the one which corresponds to the active compound produced by DEM60007, as it is eluted at a similar point to the active compound. This mass is protonated, making the exact mass of the compound 364.09968.

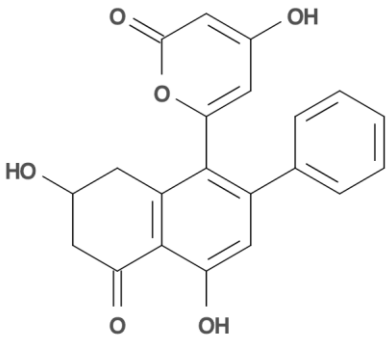


Figure 3.15. The structure of Wailupemycin F, a compound with a mass of 364.0947, and therefore likely to be produced by DEM60007.

3.3 Discussion

The theory of investigating underexplored habitats to discover novel strains and therefore novel antimicrobial compounds was employed in the planning of this project. The taxonomic diversity of a soil sample taken from Fire Mountain was explored using selective isolation techniques, and over 100 thermophilic strains able to grow at 40°C were identified. In a previous study (Taylor,

2012), several hundred strains were also identified, and these groups were combined for this work. The 16S rRNA genes were documented, and the sequenced strains placed into the 16S rRNA gene tree. All but one of the Fire Mountain strains that were sequenced were found to be of the genus *Streptomyces*, and 35% of these are proposed to be novel species, with five or more nucleotides different to the closest neighbour, and these were prioritised when adhering to the hypothesis that novel biology will present novel chemistry of bioactive compounds. The first of these strains is DEM60013, whose closest neighbour was found to be *S. pini*, which was isolated from pine leaves in India. Following further investigation, *S. pini* was found to produce a siderophore compound, but no other bioactive molecules (Madhaiyan et al., 2016). The next closest neighbour to DEM60013 is *S. atacamenis*, a strain isolated from hyper-arid soil from the Atacama desert, by (Santhanam et al., 2012b), another strain that has not been reported to produce any compounds with antimicrobial activity.

DEM60061 is another isolate from Fire Mountain soil that is thought to be novel, with the closest evolutionary neighbour *S. fradiae*, a strain first described by Waksman and Curtis (1916). *S. fradiae* has been reported to produce a number of bioactive compounds, including neomycin (Waksman et al., 1950), fosfomycin (Rogers and Birnbaum, 1974), tylosin (Stratigopoulos et al., 2002), fracidin (Swart et al., 1950), and streptothricin (Umezawa et al., 1950). Its next closest neighbour is *S. coeruleoprunus*, with 10 base pairs different. This strain has been shown to produce the bioactive compound neomycin B (Vardanyan and Hruby, 2006).

Another Fire Mountain isolate that is likely to be novel is DEM60007, whose closest neighbour is *S. albogriseolus*, a strain first described by (Benedict et al., 1954). However, it has been isolated many times since, and from numerous locations, including mangrove sediments (Li et al., 2010) and marine sediments (Cui et al., 2007). Li et al. (2010) and Cui et al. (2007) describe some bioactive compounds being produced, such as neomycin and vineomycin, as well as a nematocidal compound reported by (Zeng et al., 2013).

DEM60001 and DEM60048 are both strains that are putatively novel, and share the same closest evolutionary neighbour, *S. atrovirens*, a strain isolated from soil in Egypt (Preobrazhenskaya and Terekhova (1986). Both of these Fire Mountain isolates also share 100% similarity to *S. albogriseolus*, as described earlier, and *S. viridodiasticus*. The latter strain was isolated from Egyptian soil and was reported to produce antimicrobial compounds (El-Nasser et al., 2009). It is possible that these strains are the same species that have been isolated independently, due to their matching 16S sequences, and further research would be required to clarify this.

Similarly, DEM60059 and DEM60060 share the same evolutionary neighbour, *S. spinoverrucosus*, a strain isolated from the air in Kuwait (Diab and Al-Gounaim, 1982). Despite sharing the same closest evolutionary neighbour, the strains do not have the second or third closest neighbours in common.

Despite the other four having 100% similarity to the closest neighbours, this does not mean that they are the same strain. This is not an unusual phenomenon, and is described by Vogler et al., (2016). This review highlights that *Yersinia pestis* has 100% identical 16S rRNA sequence to *Y. pseudotuberculosis*, despite them being distinctly different species. There are a few instances of this within the Fire Mountain strains, such as isolate DEM60073 which has 100% 16S rRNA similarity to *S. enissocaesilis, S. plicatus,* and *S. rochei*. However, these three known strains have been investigated and either do not produce any compounds that have been published (*S. enissocaesilis*) or produce compounds that exhibit different activities (*S. plicatus* produces norplicacetin, which is active against Gram-positive bacteria, and *S. rochei* produces lankacidin, an anti-tumour compound). In addition to DEM60073, DEM60068 is also 100% similar to *S. enissocaesilis, S. plicatus,* and *S. rochei*, as well as having 100% 16S rRNA similarity to DEM60073. It is suggested that they are not the same strain, however, as they are morphologically different to one another. Similarly, DEM60012 is 100% similar to *S. fimbriatus*, but exhibits different antimicrobial activity: DEM60012 produces a broad-spectrum compound, whereas *S. fimbriatus* produces an antifungal compound. The second closest neighbour, *S. werraensis* has 17 base pairs different to DEM60012, and has been reported to produce werramycin (Rusanova et al., 2000), a compound with weak activity against Gram-positive organisms. This again differs to the activity shown from DEM60012, suggesting novelty.

DEM60045 is another of the Fire Mountain strains that was shown to have 100% 16S rRNA similarity to its closest evolutionary neighbours, *S. griseoincarnatus, S. variabilis*, *S. erythrogriseus,* and *S. labedae*. As with other strains with 100% similarity to those isolated from Fire Mountain soil, those most similar to DEM60045 either produce anti-tumour compounds or have not been reported to produce any bioactive compounds.

The isolated strains from Fire Mountain all lie within clades 85 to 114 in the (Labeda et al., 2012) 16S rRNA gene tree. This suggests that *Streptomyces* species which have the ability to grow in high temperatures, low moisture and high UV radiation environments are more closely related to one another than with other species of *Streptomyces.*

It was calculated in the previous study (Taylor, 2012) that over 800 colonies were observed growing on the selective isolation plates. Although every strain on the selective isolation plates could not be sub-cultured, it can be hypothesised that if each of them had been subjected to 16S rRNA sequencing then a considerable number of novel species may have been identified. If these estimations are correct it is highly likely that useful secondary metabolites could be sourced from the strains dwelling in Fire Mountain soil, since *Streptomyces* has been described as the genus that is so far the highest antibiotic producer in the microbial world (Rodríguez et al., 2013, Watve et al., 2001).

There have been a number of investigations into 16S rRNA sequencing, which have concluded that it cannot be used alone as a final tool for concluding the similarities and differences between species (Fox et al., 1992, Ashelford et al., 2005). An alternative method for investigating the novelty of organisms is DNA-DNA hybridisation, as described by (Sibley and Ahlquist, 1984). This method involves two genomic DNA samples to be compared, both of which are sheared by sonication into single stranded fragments, before being allowed to re-associate. The remaining single stranded fragments are radioactively labelled and separated using chromatography. The radioactivity is then counted, and this value can be used to compare the similarities between the DNA-DNA hybrids, and the single stranded copies. By using this method it is possible to determine if a strain is novel or not using fragments of genomic DNA and DNA microarrays (Cho and Tiedje, 2001). In addition to this, morphological and phenotypic tests could be carried out to distinguish the Fire Mountain strains from their nearest taxonomic neighbours (Weisburg et al., 1991). 16S rRNA gene sequence is regarded as an excellent approach in placing an uncharacterized strain in an approximate location in onto the “phylogenetic framework of all microbes”, but can be too conserved to distinguish between two closely related (Chun and Rainey, 2014). The application of whole genome sequencing (WGS) offers an alternative manner of strain identification, which would allow the resolution of closely related species. A study by Ranjan et al., (2016) demonstrated that shotgun WGS has a number of advantages compared with the classic 16S rRNA method. These advantages include superior detection of bacterial species and increased detection of diversity. Due to the increased length of the reads, resulting from the method itself or the assembly of contigs, improved accuracy of species detection was observed. The investigation of soil taken from Fire Mountain in China yielded many putative Streptomycetes. It is proposed that 35% of these isolates may be novel species or type strains, and their closest evolutionary neighbours have shown the ability to produce a variety of

antimicrobial compounds. Further work was carried out to investigate whether the Fire Mountain isolates are capable of producing novel antimicrobial compounds.

As Fire Mountain has such an extreme environment, it was unknown until recently if life could be supported in its hyper arid soils, and in such elevated temperatures. Because of these factors, it was hypothesized that many of the strains would be novel, and would produce putatively novel antimicrobial compounds, that could potentially be stable at high temperatures.

The Fire Mountain strains were tested for activity against *E. coli*, *B. subtilis*, and *S. cerevisiae* to begin with (full data shown in appendix), and strains showing broad-spectrum activity were subsequently tested against a panel of *B. subtilis* reporter strains to help establish the mechanism of action. Out of the 169 strains isolated and tested against these organisms, 12 were found to exhibit broad-spectrum activity, without affecting yeast. This is important, as yeast is used in this instance as a model for toxicity against human cells.

At Demuris, the percentage of strains producing broad-spectrum compounds is under 2%, and the percentage of Fire Mountain strains producing broad-spectrum compounds is over 7%. This is further evidence proving that the investigation of under-explored areas is likely to yield clinically significant compounds. Following the testing the *B. subtilis* reporter strains, only seven of the active extracts triggered the β-galactosidase response to lipid II interruption from *yvqI* reporter. The remaining compounds exhibited antimicrobial activity but there were no blue halos signifying the mode of action as RNA polymerase disruption from *yvgS*, lipid II interruption from *yvqI*, cell wall synthesis disruption from *ypuA*, the obstruction of fatty acid synthesis from *yjaX*, protein synthesis interruption from *yheH*, and DNA synthesis disturbance from *phi*105. This is not a discouraging result, as although a compound may be novel, if it has the same or a similar mechanism to an established bioactive compound it may be quickly disabled by strains possessing antibiotic resistance genes.

Once strains producing putatively novel compounds have been identified, it is important to establish an optimal medium for each of them. The differing results between both the solid and liquid media optimisations and between strains show that it is impossible to come up with a formulation to meet the requirements of every producing strain. Comparing the sizes of the zone of inhibition chooses the best medium, and the medium showing the largest zone is taken forward as the optimum. As yeast and other complex media components lack homogeneity between batches, only defined liquid media was chosen for media optimisation. Strain DEM60013 was found to produce two broad-spectrum antimicrobial compounds quite unreliably on both solid

and liquid media. As this strain is the most diverse evolutionally and thus most likely to be producing a novel compound, it was important to ensure that the activity was restored, and so it was included in further elicitation experiments.

Alongside the optimal medium preparation, elevated temperature was investigated due to the climate that the strains were endogenous to. The results found that all but one strain tested at 40°C was able to produce broad-spectrum antimicrobial compounds at the higher temperature. The size in the zones of inhibitions between 30°C and 40°C were not significantly different and were equal in some cases, suggesting that the compounds produced at the two temperature levels may be the same. If this statement is correct, it is fair to presume that the compounds are slightly more thermostable, which has great clinical significance. For further evidence, this testing should be repeated on another couple of occasions, and statistical analysis carried out.

The preliminary toxicology testing has led to the conclusion that of a number of the strains may produce clinically relevant compounds, as they exhibited activity against resistant mutants able to withstand all classes of antimicrobial (Cubist strains). The data returned from Cubist Pharmaceuticals was compared with in house data, and a list of prioritised strains was compiled. As Cubist were able to test the extracts against multi-drug resistant Gram-positive and Gram-negative strains, it was possible to exclude any strains that were likely producing known compounds. DEM60013 was the only Fire Mountain strain producing a compound able to eradicate both the Gram-positive and Gram-negative MDR strains, further adding to the conclusion that it is producing a novel compound.

DEM60007 showed promising activity during the in-house toxicity testing, as well as being evolutionarily diverse, and so work was also continued on this strain. To obtain a large enough volume of active material, cultivations at a larger scale were carried out on DEM60013 and DEM60007, using both 5L and 20L fermenters. Due to the physical characteristics of *Streptomyces spp.* in liquid cultures, it is important to ensure that the agitation and sparging of air into the vessels is not too strong, as this hydromechanical stress would cause shear of the flocs (Olmos et al., 2013). These physical features have been proven to effect the production of antimicrobials, and the breakup of flocs or clumps may actually have an adverse effect on secondary metabolite production (Pamboukian and Facciotti, 2005).

DEM60013 was cultivated in the 5L vessel only, using GYM + soil extract, and activity was observed after 60 hours, following a rise in pH and decrease in dissolved oxygen. The decrease in dissolved oxygen can be attributed to the increase in cell density, and it should be noted that

around this time is where the optical density no longer rises, signifying stationary phase, and the beginning of secondary metabolism. Phosphate levels were also extremely low at this point, which is often linked to the production of antimicrobial compounds (Martín, 2004), due to nutrient depletion.

The purification of DEM60013 Compound A following fermentation was successful, and it was identified as N-acetyl streptothricin F. However, some differences were observed in the activity of DEM60013 A and N-acetyl streptothricin F. For example, streptothricins are toxic compounds (Heilman, 1945), but both in-house and Cubist testing found active extract from DEM60013 to be non-toxic. There could be differences in the concentrations used for these investigations, and when the MS-MS data was compared with published data for N-acetyl streptothricin F, there is an obvious similarity (Ji et al., 2008), suggesting that the compounds are the same. In addition, when the pH of extract from DEM60013 was dropped to pH 3 there was no activity observed, which corresponds with how streptothricins behave (Donovick et al., 1948). In addition, the active compound was tested against a Streptothricin resistant strain of *E. coli*, and activity was not observed, offering more evidence that it is Streptothricin F.

DEM60007 was cultivated at both 5L and 20L scale in RA3 medium, and the online and offline data were comparable between the two. An abrupt drop was seen in pH once the cell density was at a level to suggest that the stationary phase has begun, and the pH was seen to then sharply increase. Around this time of pH escalation, the activity was observed on each run. Due to this distinctive and repeatable pH profile, it was not necessary to test for activity daily. Instead, once the pH was observed to rise again to around 6.8 – 7, the vessel was harvested, and active material yielded. Another interesting and valuable trait of DEM60007 is the drastic change in colour that was observed, which coincides with the drop off in activity. These two characteristics mean that the strain can be monitored visually for activity and harvested at the right time, without the need for bioassays. It was also possible to drain the vessel, leaving 2L at the bottom, and replenish with fresh medium to run the cultivation again, without having to clean the vessel in between. A large volume (~1L) crude active material was yielded this way.

The purification of the active compound was successful, and LC-MS analysis suggests that this compound is Wailupemycin F. Wailupemycin F is a broad-spectrum polyketide, with the same activity profile as the compound produced by DEM60007. There were a number of other masses observed in the mass spectrum for the semi-pure material run through LC-MS, however the mass of 365.1068 was concluded to be that of the active compound as it is present in the active peak.

Whole genome sequencing was carried out (by Dr Adam Hopkins) on Fire Mountain strains DEM60013, DEM60068, DEM60012, DEM60045, DEM60073, DEM60130, DEM60031, and DEM60162, and the resulting sequences analysed with antiSMASH (Medema et al., 2011). The sequences for numerous streptothricin compounds were identified in each of the strains' genomic data, and so further work was not carried out.

CHAPTER 4: ANTIMICROBIAL COMPOUND ELICITATION USING VARIOUS TECHNIQUES

4.1 Introduction

As discussed previously, there are many *Streptomyces* spp. that have been tested for antimicrobial activity and do not produce antimicrobial compounds under laboratory conditions. Since sequencing of *Streptomyces* spp. has indicated that many strains contain the necessary genetic material giving them the capacity to synthesise potential secondary metabolites (Bentley et al., 2002), it is important to investigate how these strains can be provoked to express these critically important compounds. Chemical elicitation has been investigated with both *Streptomyces* spp. as well as numerous other species of bacteria, with a range of results.

N-acetylglucosamine is the most abundant carbon-nitrogen bio-compound on earth, and is an important source of nutrients for soil bacteria (Bertram et al., 2011). Streptomycetes are saprophytic soil bacteria, so they metabolise naturally occurring polysaccharides found in the soil, such as cellulose, chitin, and xylan as carbon sources. N-acetylglucosamine is a monomer of chitin, and constituent of bacterial peptidoglycan, and suitable carbon source for streptomycetes (Świątek et al., 2012), and is preferred by streptomycetes over glucose (Nothaft et al., 2003). *S. coelicolor* was grown on minimal medium containing only N-acetylglucosamine as the carbon source, resulting in accelerated and enhanced antibiotic production. This result was also observed for a number of other actinomycetes (Zhu et al., 2014a). Similarly, the presence of chitin was found to stimulate the differential expression of up to 700 genes in *S. coelicolor* (Nazari et al., 2013).

Rare earth elements (REE's) have recently been shown to induce the overproduction of antibiotics, as well as the activation of silent or poorly expressed genes in bacteria. REE's are scandium, yttrium, and the lanthanides. Microorganisms have a great capacity to absorb REEs, and siderophores may play a role in their accumulation (Moriwaki et al., 2013). Low concentrations (100-100uM) of scandium chloride improved the production of antimicrobial compounds by two to twenty-five-fold in *S. coelicolor* (actinorhodin), *S. antibioticus* (actinomycin) and *S. griseus* (streptomycin) (Kawai et al., 2007). In addition to this, low concentrations of lanthanum or scandium activated the expression of nine genes 2.5 to 12-fold, belonging to nine secondary metabolite coding gene clusters in *S. coelicolor* (Tanaka et al., 2010). HPLC analysis indicated that several compounds were only detected in culture that had

been treated with REE's. The effects of REE's have not only been observed in actinomycetes. The addition of scandium to the growth medium of *B. subtilis* stimulated the production of a-amylase and bacilysin (Inaoka and Ochi, 2011).

Sodium butyrate is a histone deacetylase (HDAC) inhibitor, which affects histone acetylation and modifies the structure of chromatin. This is shown to activate biosynthetic clusters for natural products in fungi (Bok et al., 2009). HDAC proteins are widespread and many are found in bacteria. Three HDAC-like proteins have been identified in *S. coelicolor* alone (Leipe and Landsman, 1997, Moore et al., 2012), indicating that this compound may have positive effects on antimicrobial production from other streptomycetes. Minimal medium supplemented with sodium butyrate caused the enhanced production of actinorhodin in *S. coelicolor*.

Solvents such as dimethyl sulfoxide (DMSO) and ethanol have also been used in the investigation of secondary metabolites. (Chen et al., 2000) looked at the effect of low levels of DMSO on secondary metabolite production, and found significant increases in chloramphenicol, thiostrepton, and tetranomycin in streptomycetes, and similar results were observed in the antimicrobial producing bacillus, *B. circulans*. Similarly to this, the production of jadomycin B by *S. venezuaelae* was observed following exposure to ethanol (Doull et al., 1994).

As soil extract media has been used widely in the isolation of actinomycetes (Lebeau et al., 2002, Hamaki et al., 2005), it was decided to include soil extract. These results suggest that certain components of the soil biome may be imperative for antimicrobial production, and lead to the conclusion that other strains which did not show activity to begin with may be spurred into action by the presence of soil extract.

As well as chemical additions, biological samples such as autoclaved bacteria and filtered supernatant from bacterial cultivations have been proven to induce the production of antimicrobial compounds. (Luti and Mavituna, 2011a) used heat killed *B. subtilis* and *S. aureus* cells to increase the production of undecylprodigiosin by *S. coelicolor* and found that the concentration was higher with *S. aureus*.

Since antimicrobial production has been linked to cell stress (Janek et al., 2016), SDS was included in these experiments. SDS is a detergent which has been reported to disrupt the cell membrane (Woldringh and van Iterson, 1972, Walton et al., 2008).

Sub-inhibitory concentrations of antibiotics have also been investigated to induce the production of bioactive compounds, both in this study, and in others. (Imai et al., 2015) found that the presence of lincomyin at low levels increased the production of actinorhodin in *S. coelicolor*, as

well as several antimicrobial compounds from *S. lividans* which were not present in lincomycin free extracts. (Goh et al., 2002) describe how low levels of antimicrobials such as rifampicin can alter the transcription levels in bacteria, suggesting that the presence of sub-inhibitory concentrations of antibiotics could awaken cryptic gene clusters in non-active Fire Mountain isolates. It is thought that the responses of bacteria to antimicrobial compounds are dependent on the concentrations in which the compounds are found. At high concentrations, the bioactive compounds have toxic effects on susceptible cells, whereas sub-inhibitory concentrations induce genetic and cellular responses, as highlighted in Figure 4.1.

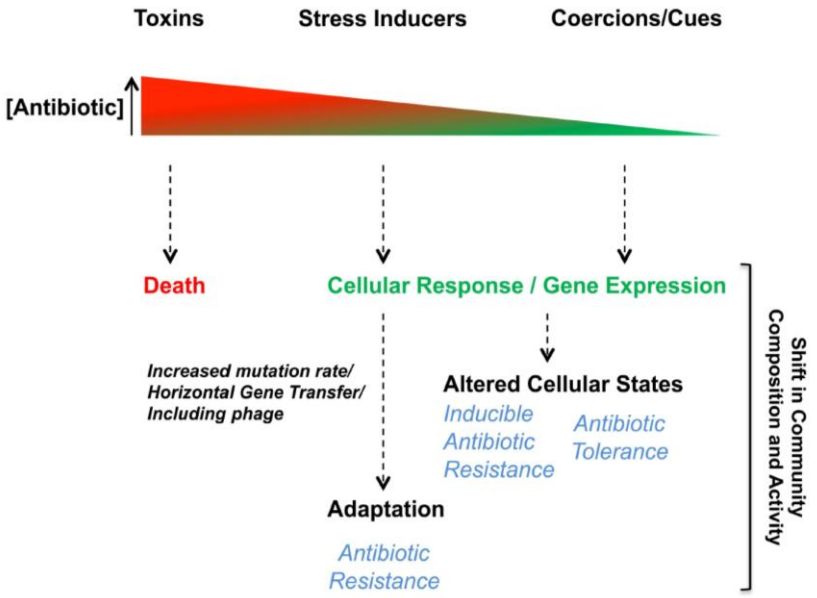


Figure 4.1 The responses to antimicrobial compounds vary depending on the concentration of the bioactive compound. The toxic action occurs when the concentration is high, leading to the arrest in cell growth or death of the susceptible species. Alternatively, lower concentrations of the same compound can act as cues or inducers to cells, resulting in the expression of secondary metabolites such as antimicrobial compounds. This could be due to competition within the environment. Figure from (2013).

Streptomyces spp. are sporulating bacteria that grow with aerial hyphae and branching substrate mycelia (Manteca and Sanchez, 2009), as can be seen in Figure 4.2. It is widely accepted that the morphology of the streptomycete is correlated with secondary metabolism, and has been suggested that the formation of clumps or flocs is essential for the production of bioactive

secondary metabolites (Pamboukian and Facciotti, 2005) for instance, retamycin in the case of *S. olindensis* (Hobbs et al., 1989) and nikkomycin from *S. tendae* (Vecht-Lifshitz et al., 1992). *Streptomyces spp.* are soil dwelling bacteria, and the diminishing of antimicrobial production in liquid medium may be due to their environment in these lab settings. The use of solid scaffolds has been investigated to determine whether imitating the natural environment of these bacteria will induce the production of antimicrobial compounds, due to the encouragement of clumps and flocs.

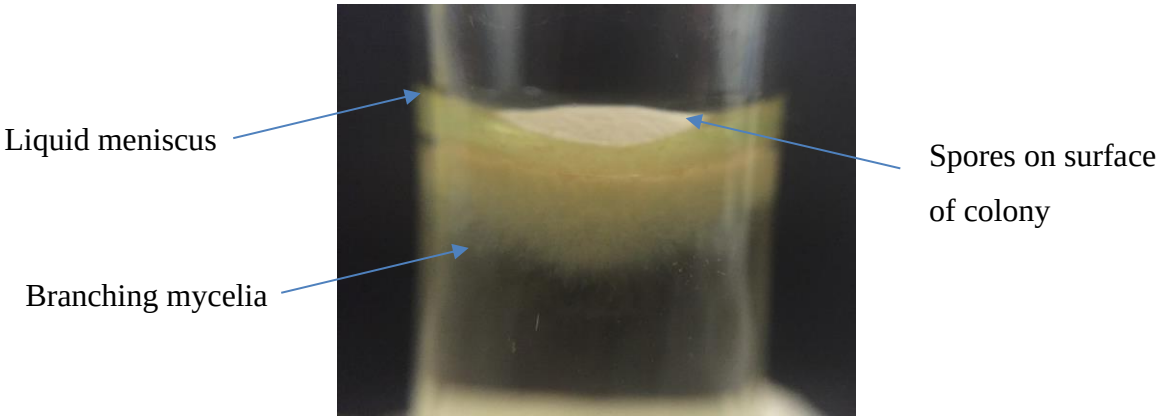


Figure 4.2. The growth of *Streptomyces sp*. on the surface of a liquid medium. Spores are seen on the surface of the colony, with visible substrate mycelia seen branching down into the liquid.

In nature, microorganisms live in environments where numerous difference species are present. It is hypothesised that antimicrobial compounds are produced either as signalling molecules or as biological weapons in the evolutionary arms race, due to this co-existence, meaning that the co-cultivation of strains to elicit the production of antimicrobial compounds is a logical step to the discovery of novel compounds. Several studies have shown the benefit of mixed cultures in laboratory settings, including the production of antimicrobial compounds. For example, the marine sponge actinomycete *Salinispora arenicola* was co-cultured with an *Emericella* sp. and three molecules not produced in monoculture were detected (Oh et al., 2007). Similarly, when *S. coelicolor* was grown alongside *E. coli* by (Luti and Mavituna, 2011b) a six-fold increase in the yield of undecylprodigiosin was observed, compared to the monoculture of the streptomycete. (Luti and Mavituna, 2011a) also used heat killed *B. subtilis* and *S. aureus* cells to increase the production of undecylprodigiosin by *S. coelicolor* and found that the concentration was higher

with *S. aureus.* It is hypothesized that this result was observed due to the more foreign nature of *S. aureus*, as *B. subtilis* is also a soil bacterium. A number of marine species were co-cultured with *S. tenjimariensis* in liquid medium (Slattery et al., 2001), a marine actinomycete known for producing the antibiotic istamycin. The researchers reported a two-fold increase in production of the active compound, in comparison to the monoculture. In a study carried out by (Kurosawa et al., 2008), *Streptomyces padanus* and *Rhodococcus fascians* were grown together in co-culture, and the novel compounds rhodostreptomycins A and B were discovered. What is most interesting about the findings from this study is that DNA from *S. padanus* appeared to have been passed onto *R. fascians* by horizontal gene transfer. Not only is this method interesting for the investigation of antimicrobial compounds for clinical use, but for bioremediation efforts. The activity of actinomycete isolates from Manipur were investigated against rice fungal pathogens, and fungicidal activity was observed (Ningthoujam et al., 2009), showing the range of activity that actinomycetes have.

Whilst these studies prove the importance of co-culturing to increase and induce the production of antimicrobial compounds, they focus on very difference species. If it is known that *Streptomyces spp.,* are important producers of natural products, then the co-culture of these strains should be investigated. The Fire Mountain strains were all isolated from the same soil sample, meaning it is possible that they may have evolved mechanisms to compete with one another.

Following initial antimicrobial screening, 63 strains did not exhibit any antimicrobial activity against *E. coli, B. subtilis*, or *S. cerevisiae*. These strains were used in the succeeding experiments, to test whether antimicrobial activity can be elicited using chemical and physical modifications to the growth medium, as well as co-culturing methods.

1.2 Results

4.2.1 Twenty-four well plate cultivations – Chemical elicitors

Of the 63 strains tested, 23 exhibited antimicrobial activity against the test organisms, after incubation with a chemical elicitor, 10 of which were broad spectrum active compounds. Some conditions tested did not induce the production of previously unseen compounds, such as the pH alterations and autoclaved *B. subtilis*. Activity was not observed with plain GYM medium, offering evidence that it is in fact the presence of the eliciting compound which triggers production of antimicrobial compounds, in these cases. These results can be found in Figure 4.2.

Of the 87 incidences of antimicrobial activity observed in the chemical elicitation experiments, ~57% of these were triggered by the presence of sub-inhibitory concentrations of antibiotics, with six instances of broad-spectrum activity. Chemical additions triggered ~30% of the instances, with ten of these broad-spectrum activities. Finally, bacterial additions caused antimicrobial activity on ~14% of the incidences, with three broad-spectrum incidences. The majority of broad-spectrum activities were triggered by chemical additions (ten of nineteen incidences), and five of these were triggered by SDS. Interestingly, only 12% of the activities induced by antimicrobials were broad-spectrum, five of which were in the presence of cefotaxime, a third-generation cephalosporin.

4.2.2 Twenty-four well plate cultivations – Physical additions

Isolates DEM60013, DEM60039, DEM60018, DEM60176, and DEM60130 were grown in sand supplemented with RA3 culture medium, and tested for antimicrobial activity against the panel of test organisms. Broad-spectrum activity was observed from DEM60013 and Gram-positive activity from DEM60039 following testing on agar plates. Representative screening plates can be seen in Figure 4.3. Antimicrobial activity was observed more frequently from direct testing of the sand, which may have been due to bacteria in the sample producing the compounds when encountering the test organisms.

All non-active Fire Mountain isolates were incubated in twenty-four well plates with cheesecloth, and broad-spectrum activity was observed from DEM60018 and DEM60142, and Gram-positive activity observed from DEM60016, DEM60144, and DEM60167. These incidences of activity were mainly observed from Grade 50 cheesecloth, although activity was observed from DEM60142 with Grade 50 and Grade 90, and activity was observed from DEM60144 with Grade 10 cheesecloth only. A summary of these results can be found in Table 4.1, and representative disk diffusion assays in Figure 4.4.

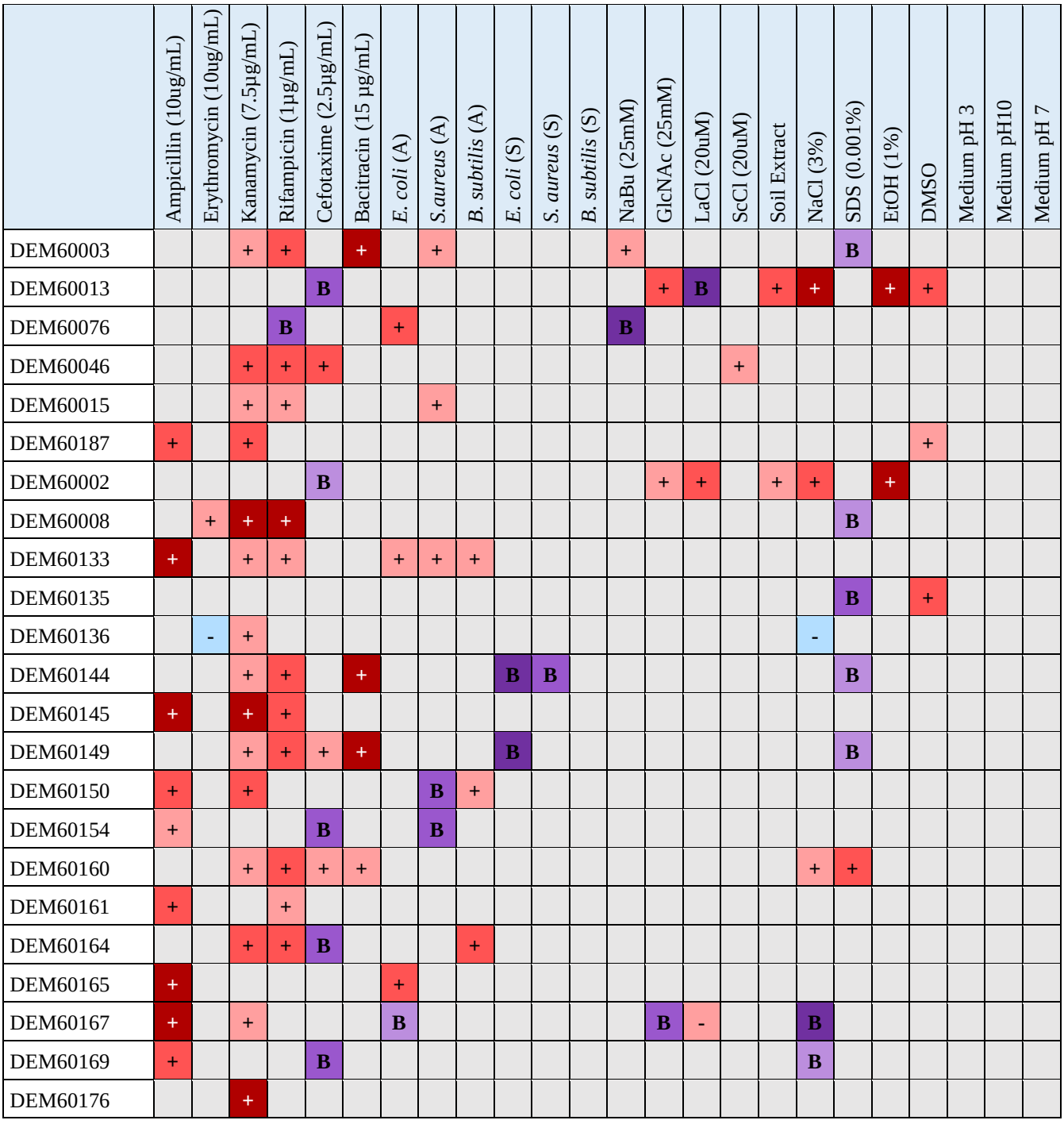


Figure 4.2. Positive results obtained from chemical elicitation experiments. Strains also tested in these investigations are DEM60007, DEM60060, DEM60072, DEM60039, DEM60018, DEM60016, DEM60120, DEM60121, DEM60129, DEM60137, DEM60138, DEM60140, DEM60141, DEM60142, DEM60148, DEM60151, DEM60152, DEM60153, DEM60155 DEM60156, DEM60157, DEM60159, DEM60166, DEM60168, DEM60180, and DEM60186.

4.2.3 Shake Flask Cultivations and Identification of Putatively Novel Compounds

The following isolates were scaled up into 2L flasks, and their active extracts subjected to partial purification by reversed phase Flash Chromatography: DEM60013 + Cefotaxime, DEM60076 + Rifampicin, DEM60135 + SDS, DEM60169 + Cefotaxime, DEM60164 + Cefotaxime, and DEM60013 + Cefotaxime. In addition, DEM60018, DEM60016, DEM60142, DEM60144, and DEM60167 were grown in 2L flasks with 5cm^2 pieces of cheesecloth, and the active extracts subjected to partial purification using reversed phase flash chromatography or solid phase extraction.

Figure 4.3 Representative plates from the testing of direct sand and methanol washes for DEM60013. *E. coli* is pictured on the left and *B. subtilis* on the right. The direct sand can be seen as irregular brown shapes, and the methanol washes on white discs of Whatman filter paper.

Table 4.1 Details of activities observed with the addition of cheesecloth in the medium. Most activity was observed with Grade 50 cheesecloth, and only one incidence of activity was observed with either Grade 10 or Grade 90 cheesecloth.

Strain	Cheesecloth Grade	Spectrum of Activity
DEM60018	50	Broad
DEM60016	50	G+
DEM60142	50 & 90	Broad
DEM60144	10	G+
DEM60167	50	G+

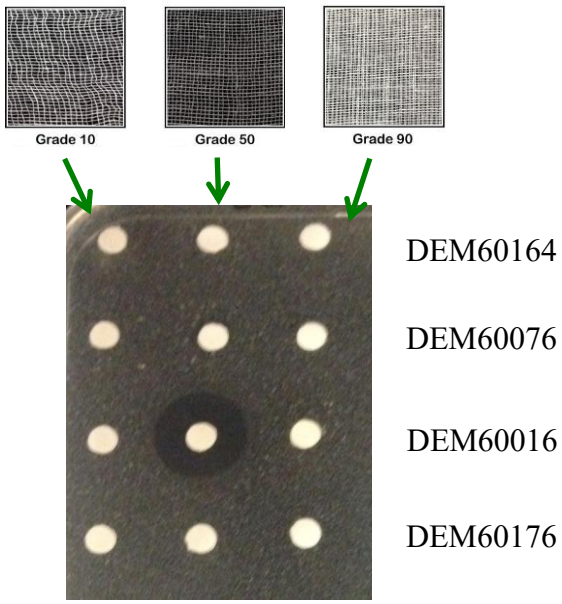


Figure 4.4. Representative disk diffusion assay showing the differences in activity from the same strain with different cheesecloth grades.

4.2.4 Identification of putatively novel compounds

DEM60169 + Cefotaxime

Active supernatant was subjected to reversed phase flash chromatography, and two compounds identified. Compound A was observed in the mid-phase of the 0 – 100% methanol gradient, and Compound B towards the end, showing hydrophobic properties. The compounds were analysed by HPLC to determine their purity, and submitted for LC-MS. The mass of Compound A was calculated as 653.2681, which is illustrated in Figure 5.6. The Dictionary of Natural Products was consulted, and the compound hypothesised to be Ferrioxamine E, the structure of which can be seen in Figure 5.7. The results of Compound B were inconclusive, and this material should be subjected to further purification.

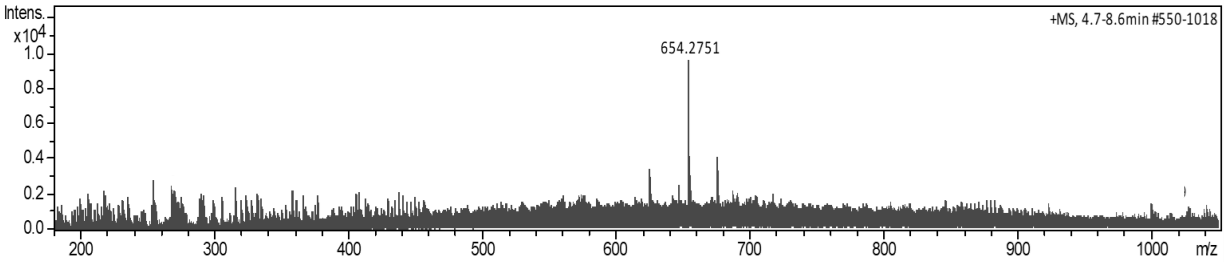


Figure 4.5 The mass spectrum of active compound A from DEM60169 + cefotaxime. The mass shown is protonated, making the exact mass of the compound 653.2681. This mass is similar to that of Ferrioxamine E, which has a mass of 653.2597.

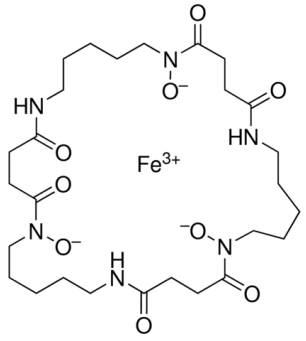


Figure 4.6 The structure of Ferrioxamine E, a siderophore produced by DEM60164 and DEM60169 when in the presence of sub-inhibitory concentrations of cefotaxime. This structure shows the compound in complex with iron.

DEM60164 + Cefotaxime

Active supernatant was subjected to reversed phase flash chromatography, and two compounds identified. Compound A was observed at the very beginning of the 0 – 100% methanol gradient, and Compound B in the middle. The active fractions were subjected to HPLC analysis to determine their purity, before being subjected to LC-MS. The material submitted for Compound A returned a number of masses, some of which correspond to Gentamicin family compounds, and can be seen in Figure 4.7. The first mass, 321.1868, corresponds to the compound Lipoxazolidinone A (Figure 4.8), which has a mass of 321.2304. Secondly, the masses of 477.3140 and 449.2810 correspond to Gentamicin C_1 & C_{1a} (Figure 4.9). The mass of Gentamicin

C_1 is 477.3162, and the mass of Gentamicin C_{1a} is 449.2849. The material submitted for Compound B returned a mass of 653.2637, illustrated in Figure 5.11, which is hypothesised to be Ferrioxamine E (Figure 4.6), which is described previously.

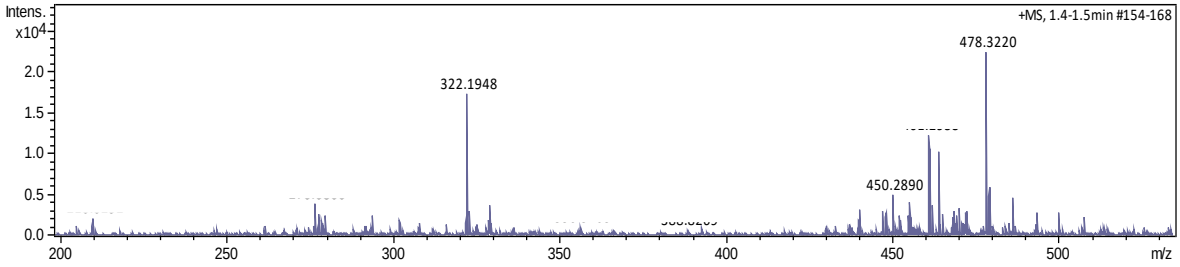


Figure 4.7. The mass spectrum returned for the first active fractions purified from DEM60164 + cefotaxime. The masses shown are protonated, making the exact masses 321.1868, 449.2810, and 477.3140. These masses are thought to be the compounds Lipoxazolidinone A, Gentamicin C_1 & C_{1a}.

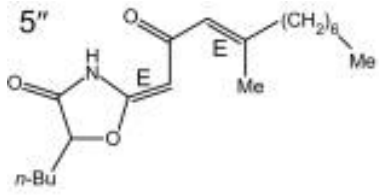


Figure 4.8. The structure of lipoxazolidinone A, which has a mass of 321.2304. It is thought that this compound is included in the first active fraction of DEM60164.

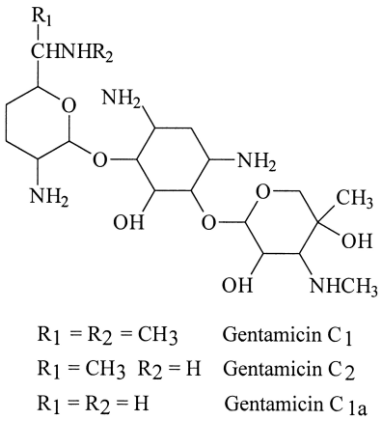


Figure 4.9 The structure of the gentamicins (Isoherranen and Soback, 2000), found in the first active fraction of DEM60164.

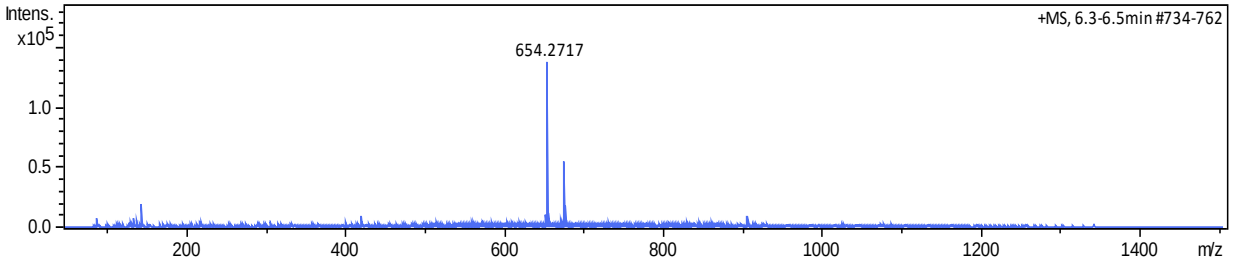


Figure 4.10 The mass spectrum returned for the second active fraction purified from DEM60164 + cefotaxime. The mass shown is protonated, making the exact mass 653.2637, and this compound is thought to be Ferrioxamine E.

4.2.5 Co-Culturing Plates

54 non-active isolates were tested against one another on SFM medium, and 620 incidences of activity were observed, as can be seen in Figure 4.11. Representative co-culturing plates can be seen in Figure 4.12. Due to the high incidence of activity observed, the strains taken forward for further testing were decided on how frequently the isolates showed the production of antimicrobial activity (>50% and <10%), how frequently the isolates' growth was inhibited by their neighbour (<10%), and those which elicited a change in pigmentation or sporulation of their neighbour. Altogether 62 strain combinations were investigated further (Table 4.2).

4.2.6 Secondary Testing

Of the 62 combinations taken forward for further testing, 42 continued to exhibit broad spectrum antimicrobial activity when tested again. Representative testing plates can be seen in Figure 4.13, with clear areas of inhibition around the producer strain, and little to no activity around the inducer strain. These results are detailed in Table 4.2.

4.2.7 Twenty-four well plate testing

Twenty combinations of strains were taken forward for further testing in liquid medium which can be found in Table 4.3. Only one combination of isolates, DEM60167 and DEM60176, was observed to produce broad-spectrum active compounds in triplicate (Figure 4.14). This combination was taken forward into larger scale cultivations, but it was not possible to identify the compounds of interest.

Table 4.2. Results of co-culturing experiments carried out on strains showing antimicrobial activity against their neighbouring strains (>50% and <10%), how frequently the isolates' growth was inhibited by their neighbour (<10%), and those which elicited a change in pigmentation or sporulation of their neighbour.

Inhibits >50%		
Active	**Base**	**Activity**
DEM60013	DEM60169	Broad
DEM60013	DEM60151	Broad
DEM60144	DEM60161	Broad
DEM60144	DEM60186	Broad
DEM60144	DEM60169	Broad
DEM60144	DEM60136	Broad
DEM60169	DEM60176	G+ve
DEM60169	DEM60120	G+ve
DEM60013	DEM60120	Broad
DEM60013	DEM60165	Broad
DEM60003	DEM60019	Broad
DEM60003	DEM60151	Broad
DEM60003	DEM60155	Broad
DEM60003	DEM60120	Broad
DEM60169	DEM60154	G+ve
DEM60169	DEM60136	G+ve
DEM60169	DEM60150	Broad
DEM60150	DEM60145	Broad
DEM60150	DEM60168	Broad
DEM60150	DEM60169	Broad
DEM60150	DEM60151	Broad
DEM60003	DEM60039	Broad

Inhibited by < 10%		
Active	**Base**	**Activity**
DEM60145	DEM60013	Broad
DEM60003	DEM60039	Broad
DEM60003	DEM60169	Broad
DEM60153	DEM60003	Broad
DEM60094	DEM60176	Broad
DEM60133	DEM60013	Broad
DEM60186	DEM60133	G+ve
DEM60072	DEM60003	G+ve
DEM60167	DEM60039	Broad
DEM60167	DEM60169	Broad
DEM60129	DEM60133	Broad
DEM60150	DEM60002	None

Inhibits <10%		
Active	**Base**	**Activity**
DEM60076	DEM60169	Broad
DEM60046	DEM60161	G+ve
DEM60166	DEM60015	None
DEM60140	DEM60161	G+ve
DEM60176	DEM60120	G+ve
DEM60015	DEM60169	G+ve
DEM60120	DEM60155	None
DEM60141	DEM60151	G+ve
DEM60188	DEM60120	Broad
DEM60060	DEM60155	Broad
DEM60151	DEM60161	G+ve
DEM60020	DEM60120	Broad
DEM60001	DEM60039	Broad

Pigment/Sporulation Changes		
Active	**Base**	**Activity**
DEM60019	DEM60039	Broad
DEM60155	DEM60135	Broad
DEM60136	DEM60188	Broad
DEM60169	DEM60155	Broad
DEM60159	DEM60176	G+ve
DEM60166	DEM60060	G-ve
DEM60169	DEM60155	Broad
DEM60121	DEM60180	None
DEM60015	DEM60149	Broad
DEM60137	DEM60153	Broad
DEM60039	DEM60072	G+ve
DEM60094	DEM60145	Broad
DEM60149	DEM60060	Broad
DEM60144	DEM60159	Broad
DEM60136	DEM60180	Broad

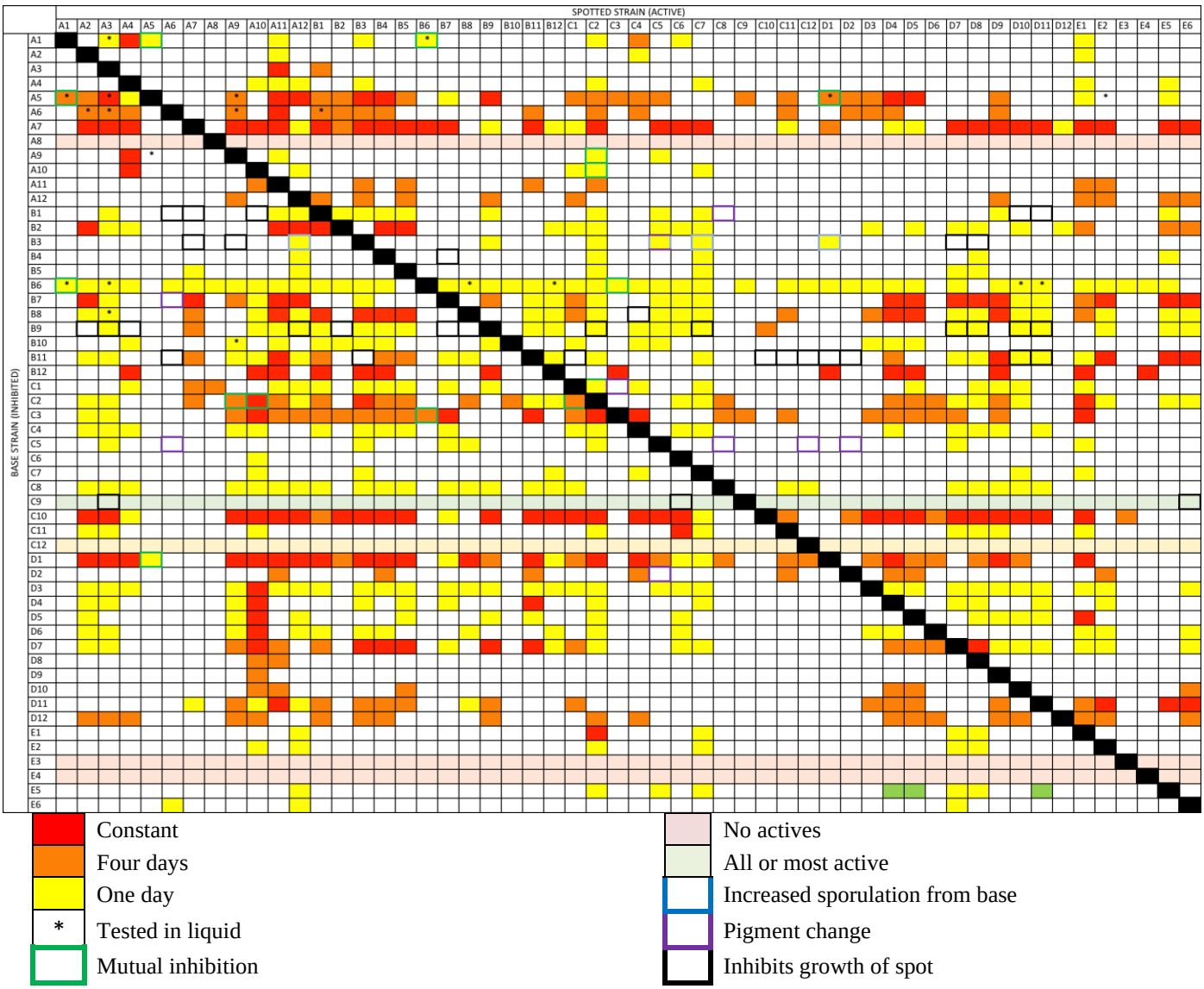


Figure 4.11 Results of the co-culturing carried out on solid media. Each strain is listed down each side of the grid, and coloured squares indicate the result observed, as described in the legend.

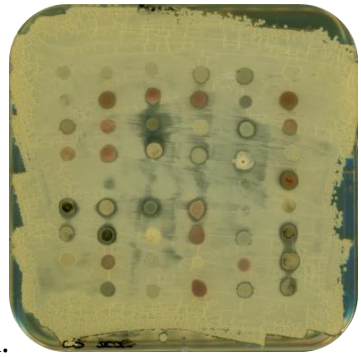
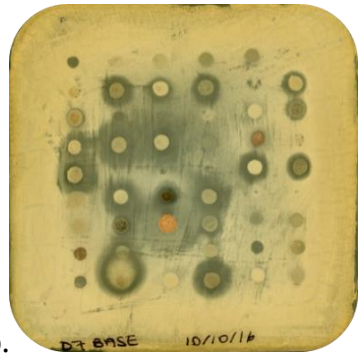
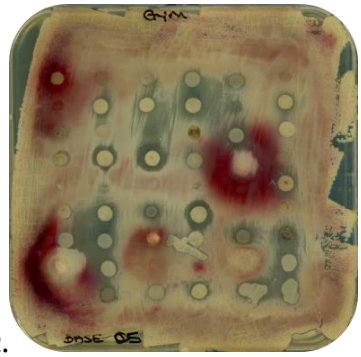
a.  b.  c. 

Figure 4.12 Representative co-culturing plates, showing a base lawn with zones of inhibition where bioactive compounds have been produced by the spotted strains. The right-hand image shows clear areas where the pigmentation of the lawn has changed due to the presence of the spotted isolate, likely due to the activation of secondary metabolism. Image A shows Fire Mountain active strains on a lawn of DEM60155, image B depicts a lawn of DEM60121, and image C shows a lawn of DEM60159.

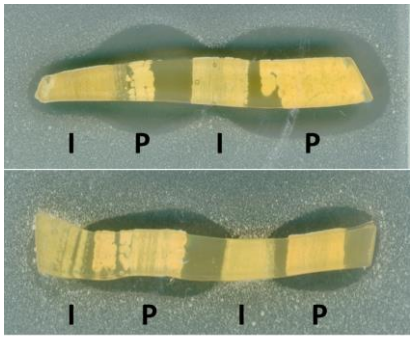
a.  b. 

Figure 4.13 Secondary testing of antimicrobial compounds produced by Fire Mountain isolates when in co-culture. Image A shows agar plug tests carried out on 5-day old agar plates streaked with a combination of Fire Mountain isolates. The test organism used is B. subtilis. Image B shows inducer (I) and producer (P) isolates streaked in close proximity to one another. These cross sections were placed on top of B. subtilis and incubated overnight to test for antimicrobial production.

Table 4.3. Isolate combinations taken forward for liquid cultivation into 24 well plates. These combinations were chosen as they exhibited antimicrobial activity in triplicate, on solid media.

Active Strain	Inhibited Strain
DEM60120	DEM60165
DEM60133	DEM60120
	DEM60157
	DEM60161
	DEM60165
	DEM60176
DEM60145	DEM60176
DEM60157	DEM60120
DEM60161	DEM60165
	DEM60167
DEM60165	DEM60161
DEM60167	DEM60161
	DEM60176
	DEM60180
DEM60019	DEM60161
DEM60142	DEM60176
DEM60072	DEM60120
DEM60151	DEM60161
DEM60152	DEM60120
DEM60168	DEM60120

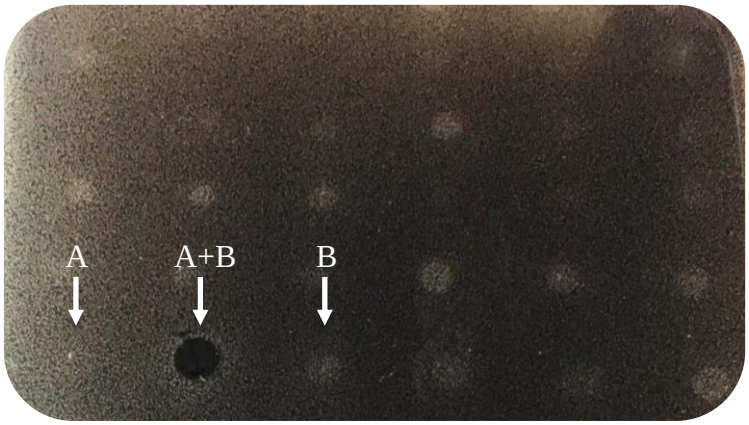


Figure 4.14. A screening plate showing the activity of DEM60167 (A) and DEM60176 (B) in co-culture, flanked by the non-active spots of broth taken from each isolate in monoculture. The activity was only observed when both isolates were grown together.

The complete results for chemical, physical, and co-culturing elicitation techniques are displayed in Table 4.4. Co-culturing had the highest incidences of antimicrobial elicitation of the different techniques, when tested using solid media. This is likely due to the plethora of secondary metabolites that are produced *in vivo* by the Fire Mountain strains. This active response likely sees communication and hence competition between the strains, giving the elevated number of antimicrobial activities observed.

Table 4.4 The complete elicitation results from this thesis. Chemical and biological elicitors for each strain are noted, as well as the appropriate cheesecloth grade. The number incidences of activity during co-culturing is displayed in the far-right column. Grey cells are incidences in which antimicrobial activity was not observed.

Isolate	Chemical & Biological Additions			Cheesecloth Grade	Co-culturing
DEM60003	Km, Rif, Bac	*S. aureus* (A)	SDS		1
DEM60019					1
DEM60013	Cef	GlcNAc	LaCl, EtOH		9
DEM60076	Rif	*E. coli* (A)			
DEM60076					4
DEM60046					2
DEM60072					1
DEM60039					9
DEM60018				50	2
DEM60189	Km, Rif	*S. aureus (A)*			
DEM60187					1
DEM60188	Amp, Km		SDS		
DEM60002	Cef	GlcNAc, Soil	Lacl, NaCl, EtOH		3
DEM60016				50	2
DEM60008	Em, Km, Rif		SDS		4
DEM60120					1
DEM60121					1
DEM60133	Am, Km, Rif	Bacteria (A)			5
DEM60135			SDS, DMSO		3
DEM60136	Em, Km		NaCl		4
DEM60138				50, 90	1
DEM60142	Km, Rif	*E. coli* (S)	SDS	10	6
DEM60144	Am, Km, Rif				1
DEM60145	Km, Cef				5

DEM60148					5
DEM60149	Km, Rif, Cef	*E. coli* (S)	SDS		4
DEM60150	Am, Km	*S. aureus* (A)			1
DEM60151					1
DEM60152					2
DEM60153					5
DEM60154	Am, Cef	*S. aureus* (A)			2
DEM60155					1
DEM60157					3
DEM60159					1
DEM60160	Km, Rif, Cef, Bac		NaCl, SDS		2
DEM60161	Am, Rif				2
DEM60164	Am, Rif, Cef	*B. subtilis* (A)			
DEM60165	Am	*E. coli* (A)			
DEM60166					1
DEM60167	Am, Km	GlcNAc, *E. coli* (A)	NaCl	50	3
DEM60168					1
DEM60176	Km				1
DEM60169	Am, Cef		NaCl		5
DEM60196	Km				
DEM60186					3

1.3 Discussion

Chemical elicitation of active compounds has been detailed in a number of studies (Zhu et al., 2014a, Zhu et al., 2014b, Abdelmohsen et al., 2015, Pettit, 2011), proving its importance in the discovery of novel antimicrobial compounds.

During this investigation, almost 40% of the isolates tested with chemical and biological elicitors showed bioactivity against the screening panel. Conditions that did not elicit activity were the pH alterations (pH3 and pH10), autoclaved *B. subtilis*, and plain medium, which was included as a control. Conversely, the most effective additions were sub-inhibitory concentrations of known antibiotics, eliciting activity in all but one of the newly active isolates. The majority of these combinations were not followed up, as the active compounds were Gram-positive acting, or did not continue to exhibit antimicrobial production after testing in triplicate. The compound eliciting the most incidences of broad-spectrum antimicrobial activity was cefotaxime, which was included at a concentration of 2.5µg/mL. Cefotaxime is a third-generation cephalosporin, which shows broad-spectrum activity against Gram-positive and Gram-negative organisms. A similar

result was reported by Seyedsayamdost (2014) when they tested 640 small molecule compounds for their ability to elicit secondary metabolite production. The authors found nine elicitors, all of which were clinical antibiotics, and one of which was cefotaxime. The mass of cefotaxime (477.038884, protonated 478.046709) was searched for using DataAnalysis 4.2 (Bruker) in the chromatogram data returned for any active samples, and was not found, proving that the activity observed was not due to the presence of cefotaxime. Additionally, 2.5µg/mL cefotaxime was tested for activity against *E. coli* and *B. subtilis*, and the results were negative, offering further proof that cefotaxime is not the cause of the antimicrobial activity observed. The two Fire Mountain isolates that exhibited antimicrobial activity in the presence of cefotaxime are DEM60169 and DEM60164. DEM60169 produced a number of compounds, which were identified as lipoxazolidinone A, gentamicin C_1, gentamicin C_{1a}, and ferrioxamine E. Lipoxazolidinone A was first discovered by (Macherla et al., 2007) from a marine *Marinispora* sp. from a sediment sample collected in Cocos Lagoon, Guam. The compound exhibits broad-spectrum activity, matching the activity profile of the compound produced by DEM60169, offering further evidence that this match is correct. The two aminoglycoside gentamicin compounds were first discovered by (Weinstein et al., 1963), isolated from *Micromonospora purpurea*. These compounds are part of the gentamicin complex, which usually includes gentamicin C_2, but show antimicrobial activity individually (Weinstein et al., 1967). DEM60169 was first thought to be a *Streptomyces* sp. due to its phenotypic traits, but further work should be carried out to confirm this following the isolation of these molecules. Both DEM60169 and DEM60164 were observed to produce the compound ferrioxamine E, a siderophore which has been isolated from numerous *Streptomyces* spp., such as *S. griseus* (Yamanaka et al., 2005), *S. parvulus* (GállTamás et al., 2016), and *S. ambofaciens* (Imbert et al., 1995). Ferrioxamine E is a high-affinity iron-chelating compound, utilised by bacterial strains to scavenge and transport iron into the cells (Imbert et al., 1995). It is likely that the areas of inhibition observed on disk diffusion assays was likely due to iron chelation rather than true antibiotic activity. It should be noted that the sandy soil of Fire Mountain has been proven to contain the iron oxides hematite and goethite (Zhang et al., 1992), from which iron can be requisitioned as a nutrient source in the low-nutrient soil.

Using sand as a growth medium for *Streptomyces spp.* was successful in some cases (DEM60013 and DEM60039), however the scalability of this method was questioned. Primarily it was noted that bacterial growth was mainly observed on the surface of the sand, despite the sand being

mixed thoroughly and altering the method to inoculate the media supplementation with spores prior to addition to the sand, in an attempt to ensure uniform distribution. This may be due to oxygen limitation within the confines of the 24 well plate, which was sealed with microplate foil to ensure sterility. The wells were mixed throughout the incubation period to aerate the sand, but this did not appear to promote better growth than wells left untouched, and also introduced the increased risk of contamination. It was deduced that this would be a problem when developing the process for large scale cultivation using this method. In addition to this, the preparation of the sand and medium environment, and further purification steps is likely to be difficult if not impossible to set up at a scale larger than 1L, therefore the use of a woven scaffold in liquid medium was investigated.

Cheesecloth submerged into liquid media proved a successful method for inducing antimicrobial activity, with 8% of isolates tested showing activity against the test strains, after testing in triplicate. Two isolates, DEM60018 and DEM60142, produced broad-spectrum active compounds. DEM60018 exhibited this activity when incubated with grade 50 cheesecloth, and DEM60142 with grade 50 and grade 90 cheesecloth. The purification of these active compounds was unsuccessful, with activity not observed following flash chromatography. The grade of cheesecloth that was most successful was grade 50, the midpoint of the different weaves. Growth was localised to the cheesecloth for the majority of strains incubated alongside it, rather than sediments being observed on the side of the shake flask glass, as with standard cultivations. It is possible that due to the branching nature of the strains this may be preferable for their growth, and the stimulation of secondary metabolite production following auto-digestion. The suitability for woven material to be used in larger scale cultivations, such as stirred tank reactors, is unknown. It is possible that the material would get stuck to the impellers and cause a mechanical fault, as well as the disruption to dissolved oxygen and pH probes if the cloth became entangled. It is also possible that cleaning would be more difficult, due to fragments of material becoming lodged in small gaps. This method is something that requires further research to determine its suitability for large scale fermentation.

The hypotheses are that antimicrobial compounds are produced either as signalling molecules or as biological weapons in the evolutionary arms race (Abdelmohsen et al., 2015). Either way, it is expected that the presence of other bacteria in close proximity would elicit a biological and chemical response. The results of this investigation seem to contribute evidence for this theory, due to the number of activity incidences observed. Observations were made that some isolates

inhibited the growth of a neighbour when spotted on a lawn but were inhibited by that same neighbour as a spore spot. This is likely due to the density of the cells when spotted using a pipette in comparison to those spread over the entire plate. It is possible in these instances that incubating the lawn for a day or so before applying the spots may be a better way to fairly observe the true relationship between these isolates. Similarly, some strains (DEM60076, DEM1044, DEM60186) were unable to grow in the presence of the spotted spores at all, with growth only observed at the outer edges of the plate. Allowing the germination of the base spores for a couple of days before the spots are added should also be investigated for these more susceptible strains. A number of isolates produced coloured compounds around neighbouring strains, and sporulation was increased specifically next to others. It is possible that this is due to signalling molecules being secreted from certain isolates in close proximity (Vaz Jauri et al., 2013), and in the case of sporulation could be a defence mechanism to avoid cell death.

Secondary testing was carried out on the isolates to determine which should be taken forward for further work. The strains were streaked onto agar plates, and their activity against *E. coli* and *B. subtilis* tested. Those producing broad-spectrum compounds were then streaked on each side of a 12 x 12cm agar plate, and cross sections removed and tested against the reporter panel. For the majority of the combinations tested, there was a clear area of reporter microbial growth where the assumed inducer strain was growing. These results give further evidence that the presence of a competing strain does induce the production of antimicrobial compounds. The use of technologies such as the iChip have utilised co-culturing methods successfully, which even led to the discovery of a novel compound, teixobactin (Ling et al., 2015).

Unfortunately, the same frequency of antimicrobial production was not observed between solid and liquid media, with only one set of isolates continuing to exhibit antimicrobial activity in liquid cultivations. The combination of co-culturing and other eliciting methods, such as a solid anchor or chemical additions, could be investigated to follow up on these promising leads. Another possibility is to grow the two isolates separately for a fixed period, before combining them in a larger vessel. This reduces the chance that a more dominant strain will out compete the other when inoculated at the same time and increases the chance of secondary metabolites being produced.

Further work into the co-culturing of previously inactive Fire Mountain isolates should investigate the growth profiles of strains in liquid media. Fermentation profiles of separate strains should be compared to that of the co-culture. Work was carried out to determine if either

DEM60167 or DEM60176 was resistant to a panel of antimicrobial compounds, and it was discovered that DEM60176 was resistant to oxytetracycline, actinomycin D, and rifampicin whereas DEM60167 was susceptible. It is therefore possible to determine the approximate ratio of each strain within the fermentation broth at each sample point. This would give insight into the growth rate of each isolate, and whether one outcompeted the other. In addition to this, it could be investigated whether the inducing strain could be immobilised within the vessel, or if a perfusion style setup could be used.

CHAPTER 5: FINAL DISCUSSION AND CONCLUSIONS

The issue of antimicrobial resistance has dramatically increased over the last decade, with the proportion and number of resistant strains growing. Multidrug resistant bacteria are recognised as a global disease and a significant public health threat (Roca et al., 2015). The incidences of resistance to common bacterial infections have been reported more frequently, indicating that many of the treatment options for these diseases are becoming ineffective (WHO, 2014). These antimicrobial resistant infections often have major complications, contribute to longer hospital stays and increased mortality (Llor and Bjerrum, 2014). Although the occurrence of antibiotic resistance in microorganisms happens naturally, resistance selection has been boosted by overexposure of antimicrobials in healthcare, agriculture, and the environment (Holmes et al., 2016). Since the "Golden Era" of antibiotic discovery in the 1940s to the 1960s, it has become more and more difficult to find novel and useful antibiotics, proven by a decrease in clinical success rate (Gilbert and Brown, 2018). Because of this, the importance of discovering new bioactive compounds is important, and due to the proven success of natural products (Moloney, 2016), especially those isolated from *Streptomyces* spp. (Barka et al., 2016), this is a logical place to start. Traditional approaches to antimicrobial discovery such as whole cell screening and dereplication were once very successful, but in recent years have returned known compounds (Baltz, 2008). Despite this, due to advances in high throughput sequencing, studies over the last few years have proven the presence of hundreds of silent biosynthetic gene clusters lying dormant in the genomes of Actinomycetes (Katz and Baltz, 2016). It is thought that novel approaches to antimicrobial discovery, using novel strains, may harbour a wealth of novel compounds. The methods investigated in this study are traditional strategies, including whole cell screening and activity guided discovery of antimicrobial compounds, and a number of pleiotropic approaches including the variations of growth conditions, both physical and chemical, and competition-based approaches, such as co-culturing.

Chapter 3 of this work investigated traditional approaches to antimicrobial discovery, with selective isolation carried out on a soil sample taken from Fire Mountain in China, one of the hottest places on earth (Mildrexler et al., 2011). The aim of this experimentation was to isolate and identify novel microbes from the soil sample, and to identify any bioactive compounds. Hundreds of isolates were discovered from this soil sample, many of which are hypothesised to

be previously undocumented. This was an unexpected result, due to the arid environment the soil was sampled from, and it was unknown if life could be supported in such extreme environments. These results provide further evidence to confirm that life in under-explored habitats is extensive, as also reported by Okoro et al. (2009) and Santhanam et al. (2012a), who studied the Atacama Desert. Further to this, traditional screening approaches were investigated, with the addition of the *B. subtilis* reporter strains and *E. coli* drug resistant mutants. In addition, the collaboration with Cubist Pharmaceuticals allowed screening against multidrug resistant bacteria. These approaches improved the traditional approaches, reducing the likelihood to rediscovering 'low hanging fruit' (Flemming, 2013). There were many challenges when carrying out this research, namely the difficulty in purifying and identifying Fire Mountain compounds, which often degraded throughout the purification process. Additionally, the lack of synchronicity between the antimicrobial expression in solid and liquid media contributed to these challenges, but led to the development of the elicitation experiments, in particular the addition of cheesecloth to liquid cultures. Despite the deficiencies, the traditional screening approaches did show the promise of harbouring novel compounds, and the results obtained from Cubist Pharmaceuticals in regard to DEM60013, which showed promising activity against their multidrug resistant panel of bacteria, with a low level of toxicity against mammalian cells. Additionally, the incidences of broad-spectrum activity observed were much higher in the Fire Mountain isolates (>7%) than in the Demuris library (<2%), giving evidence that the investigation of under explored environments is a valuable source of antimicrobial compounds. These findings align with other literature looking into under explored habitats, such as deep-sea sediments. Tortorella et al. (2018) review a variety of papers which describe the discovery of bioactive compounds such as marthapeptide A and desotamide B from *S. drozdowiczii.* As with Fire Mountain, marine sediments have been untouched by humans, albeit the anaerobic nature of the sea floor introduces further possible difficulties in culturing these putatively novel species.

The following chapter 4, aimed to identify further methods to induce the activity of novel or unusual antimicrobial compounds from Fire Mountain isolates that did not initially exhibit the capability to produce bioactive secondary metabolites. Later in this chapter, focus was on the use of chemical and biological elicitors to trigger the expression of biosynthetic gene clusters that were silent under normal lab conditions. There is a plethora of studies in the literature describing the use of chemical additions to culture media in the hopes of stimulating the production of bioactive compounds (Kawai et al., 2007, Wang et al., 2008, Tanaka et al., 2010, Ochi and

Okamoto, 2012, Ochi et al., 2014, Imai et al., 2015), but there does not seem to be a conclusion on which method is most successful. In this set of experiments on Fire Mountain isolates, the majority of the elicitors used were successful in this goal, albeit the compounds produced being primarily Gram-positive. There were several exceptions, however, mainly the presence of sub inhibitory concentrations of known antibiotics. The most effective was cefotaxime, which triggered the production of ferrioxamine E in isolates DEM60169 and DEM60164, and lipoxazolidinone A, gentamicin C1 and $C1_a$ in DEM60169. The latter compounds are of particular interest, as the isolate DEM60169 was hypothesised to be a *Streptomyces* sp. due to its phenotypic traits, yet these compounds have been previously identified from *Micromonospora* spp. and *Marinispora* spp. It is possible that horizontal gene transfer of the biosynthetic gene clusters responsible for these compounds is an explanation for this, which has been widely observed in nature (Jensen, 2016). Further work into the identification of DEM60169 should be carried out to confirm its species. Also, in this chapter, additional experimentation was carried out to investigate the use of solid scaffolds as a way to simulate the natural environment of the isolates and reduce the drop of antimicrobial activity observed when switching from solid to liquid media types. Firstly, the use of media supplemented sand was examined, and gave some positive results. This method has been described as far back as the 1950s (SKINNER, 1956) and the scalability of this method was questioned, and further work discontinued due to this. Alternatively, woven material submerged into liquid media was studied as a method to emulate the natural environment of *Streptomyces* spp., with varying results. The majority of activity was observed in the presence of the mid weaved cheesecloth, grade 50, but broad-spectrum activity was observed from only two strains, DEM60018 and DEM60142. The purification of these active compounds was unsuccessful, due to their degradation in the process.

The research looked into the co-culturing of previously inactive Fire Mountain isolates to induce the production of antimicrobial compounds. The method finally employed for this research was developed by firstly using a pin-spotting tool to transfer spores from a 96 well plate to production GYM agar plates. A number of challenges were faced with this technique, firstly the non-uniform transfer from each pin, which did not provide truly comparable results. Secondly, there were difficulties in maintaining the sterility of the pin spotting tool, with contaminants often observed. It was also impossible to test each of the 63 strains against one another effectively due to the inflexibility of the tool, and the close proximity in which they were applied to the agar. The option of spotting each strain on top of one another was also considered, however due to the

time-consuming nature of this method, it was concluded to be unachievable. The final method of streaking a lawn of each strain with the remaining isolates spotted on top was adopted to carry out this research and proved to be successful. Six-hundred and twenty incidences of previously unobserved antimicrobial activity were observed, with most strains exhibiting the capacity to produce bioactive secondary metabolites in co-culturing conditions.

Of the 20 strain combinations, the two isolates DEM60167 and DEM60176 showed further activity in liquid media. Again, a large number of active combinations did not appear to produce antimicrobial activity in liquid media. Future work should investigate the production of seed cultures to inoculate the co-culturing production vessel, to observe if this aids in the production of bioactive compounds, as it is possible that one of the isolates outgrows the other when inoculated from spores. These experiments showed a huge increase in bioactivity from strains that were not observed to produce active compounds in monoculture, highlighting the potential of these methods to stimulate the expression of silent biosynthetic gene clusters.

Proposed further work

The work described in this thesis has opened many doors of opportunity for Demuris Ltd to further investigate their extensive library of streptomycetes, collected over the years by Professor Michael Goodfellow. The first area that should be investigated is the use of whole genome sequencing and DNA-DNA hybridisation to confirm the identity of the Fire Mountain strains. This is particularly interesting in the case of DEM60619, which was found to produce lipozalidinone A and two gentamicin compounds typically expressed by *Micromonospora spp.*

In addition to this, it would be beneficial to investigate further into extreme temperatures. This study only extended to 40°C, but the likelihood is that some of the Fire Mountain strains would tolerate much higher, due to the habitat they were sampled from. Growth at this temperature would also inhibit the growth of mesophilic strains which may have been isolated previously. In a similar thought, testing the strains under anaerobic or anoxic conditions which may better mimic the soil environment may also uncover rarer and undiscovered species.

During the cultivation in bench scale bioreactors it is suggested that the carbon source be monitored throughout, allowing the initiation of secondary metabolism to be pin pointed to when nutrient depletion is observed. Additionally, a fed batch cultivation should be investigated for increased biomass production prior to the initiation of secondary metabolism. The control of pH

within the bioreactor would also allow for much better bioprocess control overall, and should be attempted and compared to the data presented in this thesis.

Finally, the methods described for elicitation successfully increased the incidences of antimicrobial activity. It is therefore concluded that this work should be carried out on the entire Demuris strain collection, to elicit putatively novel antimicrobial compounds that may be clinically significant.

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APPENDIX

Table A1. Media used in the selective isolation investigations, and the compositions.

Medium	Selection for	Source	Composition (/L)
Starch casein	streptomycetes	Küster and Williams (1964)	10.0g Soluble starch 0.3g Casein (Vitamin Free) 2.0g KNO_3 0.05g $MgSO_4$.7H2O 2.0g K_2HPO_4 2.0g NaCl 0.02g $CaCO_3$ 0.01g $FeSO_4$.7H2O
Starch-Casein + Gentamycin	Rare streptomycetes	Küster and Williams (1964)	As above + 15 µg/ml gentamicin
Humic acid-Vitamin	Strepto-sporangiaceae	Hayakawa and Nonomura (1987)	1.0g Humic acid (dissolved in 0.2M NaOH) 0.5g $NaHPO_4$ 1.71g KCl 0.05g $MgSO_4.7H_2O$ 0.01g $FeSO_4.7H_2O$ 0.02g $CaCO_3$ B-vitamins (0.5mg each of thiamine-HCl, riboflavin, niacin, pyridoxin-HC1, inositol, Ca-pantothenate, paminobenzoic acid, and 0.25mg biotin)
Oligotrophic	streptomycetes	Santavy et al. (1990)	0.05g yeast extract 0.5g tryptone 0.1g sodium glycerophosphate
Glucose-Yeast Extract + Rifampicin	*Actinomadura*	Mossel et al. (1970)	20.0g Glucose 10.0g Yeast extract 20.0g $CaCO_3$ 0.0001g Biotin
Raffinose-Histidine	Rare streptomycetes	Vickers (1984)	10.0g Raffinose 1.0g L-histidine 0.5g $MgSO_4 \cdot 7H_2O$ 0.01g $FeSO_4 \cdot 7H_2O$ 1.0g K_2HPO_4

Table A2. 16S rRNA sequences for Fire Mountain strains and their closest evolutionary neighbours. This data was used to compile a phylogenetic tree, using a neighbour joining algorithm, to show the evolutionary relationship between the Fire Mountain strains and other genetically similar Streptomycetes.

Strain name	16S rRNA sequence
DEM60001	AGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCG GTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGG GACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCGCTTGGGCATCCAAGC GGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAG GTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACA CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA CAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTT GTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGC CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAA TTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGG CTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGG AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAG GCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGG ATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATT CCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCG CAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGG CTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGTC TGGAGACAGGCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCT CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTG CCAGCAAGCCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGG AGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGT GCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAA GCCGGTCTCAGTTCGGATTGGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCT AGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCG CCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGG AGGGAGCTGTCGA

DEM60007	TTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACG ATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTG CCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCGCT TGGGCATCCAAGCGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCA GCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGG GCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGT GGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGA CGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTG CAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAA GCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTG TGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGG TAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAAC ACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTG GGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAG GTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCT GGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC GGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACAT ACACCGGAAACGGCCAGAGATGGTCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCAT GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC CTTGTCCCGTGTTGCCAGCAAGCCCCTTCGGGGGTGTTGGGGACTCACGGGAGACCG CCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTC TTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGG AGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATG AAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGG CCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCC CAACCCCTTGTGGGAGGGA
DEM60012	TTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGA TGAACCGCCTTCGGGCGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCT GCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACAACCACTT CGGGCATCCGATGGTGGTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATC AGCTTGTTGGTGAGGTAACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAG GGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAG TGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATG ACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCT GCAGAAGAAGCGCCGGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGC GAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGT TGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTC GGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGA ACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCG TGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACT AGGTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGC CTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAA GCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGAC ATACACCGGAAAACCCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGC ATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAA CCCTTGTCCCGTGTTGCCAGCAAGCCCCTTCGGGGGTGTTGGGGACTCACGGGAGAC CGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATG TCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGT GGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCA TGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCG GGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGG CCCAACCCCTTGTGGGAGGGAGCTGTCGA

DEM60013	TTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGA TGAACCTCCTTCGGGAGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCT GCCCTGCACTCTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATACGACCACCG CGGGCATCCGTGGTGGTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCA GCTTGTTGGTGGGGTGATGGCCCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGG GCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGT GGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGA CGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTG CAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGA GCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGATG TGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTTCGG TAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAAC ACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTG GGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTAG GTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTTCCCCGCCT GGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC GGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACAT ACACCGGAAACGGCCAGAGATGGTCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCAT GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC CTTGTTCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCACAGGAGACTGCC GGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTT GGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATGCCGTGAGGTGGA GCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGA AGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGC CTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCC AACCCCTTGTGGGAGGGAATCGTCGA
DEM60018	TCGACGATGAACCACCTTCGGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTG GGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAC TGACCCTCGCAGGCATCTGCGAGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGC GGCCTATCAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGC CTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG GCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTG AGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGA CGGTACCTGCAGAAGAAGCGGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACG TAGGGCGCAAGCGTTGTCCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGT CACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGG CTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATAT CAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAG CGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGG TGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAG TGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGC CCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCA AGGCTTGACATACACCGGAAAACCCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTAC AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA CGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACG GGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCC CCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACC GCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTC GACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATAC GTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAG CCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGG

DEM60021	CCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAC CACTTCGGTGGGGAAGAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTG CACTCTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATACGACTCTCCAGGGCA TCCTGGAGGGTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTGGT TGGTGGGGTAACGGCCCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACC GGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAA TATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTT CGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGCAGAAG AAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGT CCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCCTGTCGCGTCGGATGTGAAAGC CCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCAGGCTAGAGTTCGGTAGGGGA GATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTG GCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCG AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGCACTAGGTGTGGGC GGCATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTA CGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGC ATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGA AACGGCCAGAGATGGTCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCG TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTG TGTTGCCAGCGTGCCTTTCGGGGTGACGGGGACTCACAGGAGACTGCCGGGGTCAAC TCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCAC ACGTGCTACAATGGCCGGTACAATGAGCTGCGATGCCGTGAGGTGGAGCGAATCTCA AAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTC GCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACA CCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCCTTG TGGGGAGGGAATCGTCGAATGTGGGACTGGCGATTGGGACGAAGT
DEM60027	GCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGGCCCT TTCGGGGTACACGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACT CTGGGATAAGCGGTGGAAACGCCGTCTAATACCGGATACGACCCTCCGTCTCATGAC GGTGGGTGGAAAGTTTTTCGGTCAGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTG GGGTAACGGCCTACCAAGGCGATTACGGGTAGCCGGCCTGAGAGGGCGACCGGCCA CACTGGGACTGAGACACGGCCCAGACTCCTGCGGGAGGCAGCAGTGGGGAATATTG CGCAATGGGCGAAAGCCTGACGCAGCGACGCCGCGTGGGGGATGACGGCCTTCGGG TTGTAAACCTCTTTTACCACCAACGCAGGCCCGGGGTTCTCTCCGGGTTGACGGTAGG TGGGGAATAAGGACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTCC GAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCGTGTCGCGTCTGC TGTGAAAGACCGGGGCTTAACTCCGGTTCTGCAGTGGATACGGGCATGCTAGAGGTA GGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGA ACACCGGTGGCGAAGGCGGGTCTCTGGGCCTTACCTGACGCTGAGGAGCGAAAGCA TGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTA GGTGTGGGGACTTTCCACGGTTTCCGCGCCGTAGCTAACGCATTAAGCGCCCCGCCT GGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC GGCGGAGCATGTTGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGTTTGACAT CACCCGTGGACCTGCAGAGATGTGGGGTCATTTAGTTGGCGGGTGACAGGTGGTGCA TGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC CCTTGTTCCATGTTGCCAGCACGTAGTGGTGGGGACTCATGGGAGACTGCCGGGGTC AACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTG CAAACATGCTACAATGGCCGGTACAATGGGCGTGCGATGCCGTAAGGTGGAGCGAA TCCCTAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGGTG GAGTCGCTAGTAATCGCGGATCAGCAACGCCGCGGTGAATACGTTCCCGGGCCTTGT ACACACCGCCCGTCACGTCATGAAAGTCGGCAACACCCGAAACTTGCGGCCTAACCC CTTACGGGA

DEM60031	TCGACGATGAACCTCCTTCGGGAGGGGATTAGTGGCGAACGGGTGAGTAACACGTG GGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAC TGACCCGCTTGGGCATCCAAGCGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGC GGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGC CTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG GCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTG AGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGA CGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT AGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCA CGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCT AGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCA GGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCG AAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTG GGCACTAGGTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTG CCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCC GCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAG GCTTGACATACACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGGTGTACAG GTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG AGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGG AGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCC TTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGC GAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGA CCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGT TCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCAAAGCC GGTGGCCCAACCC
DEM60033	GATGAACCTCCTTCGGGAGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAA TCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACC CGCTTGGGCATCCAAGCGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCT ATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAG AGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC AGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGA TGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTAC CTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCG CGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGG TTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTT CGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGG AACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGC GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCAC TAGGTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCG CCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACA AGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGA CATACACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTG CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA ACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCG CCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTC TTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGG AGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATG AAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGG CCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCC CAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGC

DEM60039	CGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGG CAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTG ACCTTCACAGGCATCTGTGAGAGTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGG CCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCT GAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC AGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAG GGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACG GTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAG GGCGCGAGCGTTGTCCGGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCAC GTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTA GAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAG GAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGA AAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGG GCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGC CCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCC GCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAG GCTTGACATACACCGGAAAGGTTTAGAGACAGTCCCCCCCTTGTGGTCGGTGTACAG GTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG AGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGG AGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCC TTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGC GAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGA CCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGT TCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCC GGTGGCCCAACCC
DEM60045	TTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACG ATGAACCTCCTTCGGGAGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAAT CTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCC GCTTGGGCATCCAAGCGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTA TCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGA GGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA GTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGAT GACGGCCTTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTAC CTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCG CGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGG TTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTT CGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGG AACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGC GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCAC TAGGTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCG CCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACA AGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGA CATACACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTG CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA ACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCG CCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTC TTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGG AGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATG AAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGG CCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCC CAACCCCTTGTGGGAGGGA

DEM60048	AGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCG GTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGG GACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCGCTTGGGCATCCAAGC GGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAG GTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACA CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA CAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTT GTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGC CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAA TTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGG CTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGG AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAG GCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGG ATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATT CCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCG CAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGG CTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGTC TGGAGACAGGCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCT CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTG CCAGCAAGCCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGG AGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGT GCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAA GCCGGTCTCAGTTCGGATTGGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCT AGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCG CCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGG AGGGAGCTGTCGA
DEM60057	TCATTCGGGAGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTG CACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCATCTTGGGC ATCCTTGGTGATCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTG TTGGTGAGGTAACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGAC CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGA ATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCC TTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGCAGAA GAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTT GTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAA GCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGG GAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGG TGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAG CGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGG GCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAG TACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGA GCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCG GAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGT CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTC CCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCA ACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGC ACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCT CAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAG TCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACA CACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCC

DEM60059	TGCAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACA CGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGG ATACGACCATCTTGGGCATCCTTGATGGTGGAAAGCTCCGGCGGTGCAGGATGAGCC CGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCC GGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG GAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGC GTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGT GACGGTACCTGCAGAAGAAGCGCCGGCTTAACTACGTGCCAGCAGCCGCGGTAATA CGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTG TCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAG GCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATA TCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGA GCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG GTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAA GTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGG CCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCA AGGCTTGACATACACCGGAAACGTCCAGAGATGGGCGCCCCCTTGTGGTCGGTGTAC AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA CGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACG GGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCC CCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACC GCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTC GACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATAC GTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAG CCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCG
DEM60060	GCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACA CGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGG ATACGACCATCTTGGGCATCCTTGATGGTGGAAAGCTCCGGCGGTGCAGGATGAGCC CGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCC GGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG GAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGC GTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGT GACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATAC GTAGGGCGCAAGCGTTGTCCGGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTG TCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAG GCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATA TCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGA GCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG GTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAA GTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGG CCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCA AGGCTTGACATACACCGGAAACGTCCAGAGATGGGCGCCCCCTTGTGGTCGGTGTAC AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA CGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACG GGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCC CCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACC GCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTC GACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATAC GTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAG CCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGC

DEM60061	TCGAACGATGAACCCACTTCGGTGGGGGATTAGTGGCGAACGGGTGAGTAACACGT GGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATA CGACCACTTCAGGCATCTGATGGTGGTGGAAAGCTCCGGCGGTGCAGGATGAGCCCG CGGCCTATCAGCTTGTTGGTGAGGTAACGGCTCACCAAGGCGACGACGGGTAGCCGG CCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGA GGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGT GAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTG ACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACG TAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTC GCGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCAGGC TAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATC AGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGC GAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTT GGGAACTAGGTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGT TCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCC CGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAA GGCTTGACATACACCGGAAACACCCAGAGATGGGTGCCCCCTTGTGGTCGGTGTACA GGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC GAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGG GAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCC CTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAAAGAGCTGCGATACCG CGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCG ACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACG TTCCCGGGCCTTGTACACACCGCCCGTTACGTCACGAAAGTCGGTAACACCCGAAGC CGGTGGCCCAACCCCTTGCGGGAGGGA
DEM60062	TCATTCGGGAGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTG CACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCGCTTGGGC ATCCAAGCGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTG TTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGAC CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGA ATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCC TTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAA GAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTT GTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAA GCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGG GAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGG TGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAG CGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGG GCGACATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAG TACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGA GCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCG GAAACCATCAGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGT CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTC CCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCA ACTCGGAAGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGC ACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCT CAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGAGT CGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCC

DEM60066	GGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCCGCTTCGG TGGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGG GACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCGGCCTGGGCATCCAGG CGGGTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGA GGTAACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCAC ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGC ACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGT TGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCG CCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGA ATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGG GCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCG GAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAA GGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAG GATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACAT TCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCC GCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTG GCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCA TCAGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGC TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTG CCAGCAACTCTTCGGAGGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAG GAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGC TACAATGGCCGGTACAATGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAG CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAG TAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAG GGAGCTGTCGA
DEM60068	TTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACG ATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTG CCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCTCG CAGGCATCTGCGAGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCA GCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGG GCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGT GGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGA CGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTG CAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAA GCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTG TGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGG TAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAAC ACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTG GGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAG GTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCT GGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC GGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACAT ACACCGGAAAACCCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCAT GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC CTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCG GGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTG GGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAG CGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAA GTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCC TTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCA ACCCCTTGTGGGAGGGA

DEM60070	AGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCG GTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGG GACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCTGTCTGGGCATCCAGGC GGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAG GTAGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACA CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA CAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTT GTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGC CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAA TTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGG CTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGG AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAG GCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGG ATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATT CCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCG CAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGG CTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCAT CAGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCT CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTG CCAGCAAGCCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGG AGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGT GCTACAATGGGCCGGGTACAATGAGCTGCGATACCGCAAGGTGGAGCGAATCTCAA AAAGCCGGGTCTCAGTTCGGGATTGGGGTCTGCAACTCGACCCCATG
DEM60071	ACGGAACTGTCTGCGTGCTTACCATGCAAGTCGAACGATGAACCACTTCGGTGGGGA TTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGC CCTGGAAACGGGGTCTAATACCGGATATGACCGTCTTGGGCATCCTTGATGGTGTAA AGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAACGGC TCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACT GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGC GAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTC TTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACT ACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGC GTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTAACCCC GGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGG TGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTC TGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATAC CCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGT CCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAA AACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCG ACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTGGAGACA GGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTG AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGC CCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGG GGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGG CCGGTACAATGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCA GTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAG ATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTC ACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCCTGTGCGAGGCAGCTGTC CGAAGGTGGGACTGGCGACAAGACGAAGTCGTATCAAGGTAGCAAAGGGG

DEM60073	TTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGA TGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGC CCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCTCGC AGGCATCTGCGAGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAG CTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGG CGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTG GGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGAC GGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGC AGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAG CGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGT GAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGT AGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACA CCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGG GGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGG TGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTG GGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCG GCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATA CACCGGAAAACCCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATG GCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC TTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGG GGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGG GCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGC GAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAG TCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCT TGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAA CCCCTTGTGGGAGGGA
DEM60123	TATAGTTTGATCATGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTC GAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGC AATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACAAC CACTGACCGCATGGTCGGGTGGTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGG CCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCT GAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC AGCAGTGGGGAATATTGCACAATGGGCGGAAAGCCTGATGCAGCGACGCCGCGTGA GGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGAC GGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTA GGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCAC GTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTA GAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAG GAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGA AAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGG GCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGC CCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCG CACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGG CTTGACATACACCGGAAAGCATTAGAGATAGTGCCCCCCTTGTGGTCGGTGTACAGG TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA GCGCAACCCTTGTCCCGTGTTGCCAGCAACTCTTCGGAGGTTGGGGACTCACGGGAG ACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTA TGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGTGAG GTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCC CATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCC CGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCCGT GGCCCAACCCGCAAGG

DEM60126	CGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGG GATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAA GCCCTGGAAACGGGGTCTAATACCGGATACAACCACTGACCGCATGGTCGGGTGGTG GAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGT GGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGG ACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATG GGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAA CCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCT AACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATT GGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAA CCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTC CTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGA TCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAG ATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACG TTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGG CTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAA TTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATTAGA GATAGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGT CGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGC AACTCTTCGGAGGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGG TGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAA TGGCCGGTACAATGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAGCCGGT CTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATC GCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA CGTCACGAAAGTCGGTAACACCCGAAGCCCGTGGCCCAACCCGCAAGG
DEM60134	GCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACT TCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTC TGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCTCGCAGGCATCTG CGAGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGT GAGGTAACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCC ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT GCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGG GTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAG CGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCG GAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCG GGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGAT CGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCG AAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAAC AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAAC ATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGG CCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATG TGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAA CCCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCA GCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTG TTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCG GAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACG TGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAA AAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGC TAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACC GCCCGTCACGTCACGAAAGTCGGTAACACCCNAAGCCGGTGGCCCAACCCCTTGTGG GAGGGAGC

DEM60136	CAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCG GTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGG GACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCTCGCAGGCATCTGCGA GGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAG GTAACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACA CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA CAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTT GTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGC CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAA TTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGG CTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGG AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAG GCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGG ATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATT CCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCG CAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGG CTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCC TGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCT CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTG CCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGA GGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTG CTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAA GCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTA GTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGC CCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGA GG
DEM60140	TCCTTGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAA CCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTG CACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACAACCACTGACCGCA TGGTCGGGTGGTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTG TTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGAC CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGA ATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCC TTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAA GAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTT GTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAA GCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGG GAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGG TGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAG CGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGG GCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAG TACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGA GCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCG GAAAGCATTAGAGATAGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGT CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTC CCGTGTTGCCAGCAACTCTTCGGAGGTTGGGGACTCACGGGAGACCGCCGGGGTCAA CTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCA CACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGTGAGGTGGAGCGAATCTC AAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGT CGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACAC ACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCCGTGGCCCAACCCGCAA GG

DEM60153	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCTCGCAGGCATCTGCGAGGT TCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTA ACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA TGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGG CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTA TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTT AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCC ACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTG GAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCC AGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGG AAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCT ACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGC CGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGT AATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGG GA
DEM60156	CAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACCTTC GGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCT GGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCTCGCAGGCATCTGC GAGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTG AGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCA CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG CACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGG TTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGC GCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGG AATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGG GGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATC GGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGA AGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACA GGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACA TTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGC CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGT GGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAAC CCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAG CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTT GCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGG AGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGT GCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAA AGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCT AGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCG CCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGG A

DEM60158	GCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACT TCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTC TGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCTCGCAGGCATCTG CGAGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGT GAGGTAACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCC ACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT GCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGG GTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAG CGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCG GGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCC GGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAG ATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGG CGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGA ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCA ACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTAC GGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCA TGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAA AACCCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGT CAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCG TGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACT CGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACA CGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAA AAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCG CTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACAC CGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTG GGAGGGAGCNTCGAA
DEM60175	AGTTTTGAATCATGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCG AACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCA ATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGAT CCTCGCAGGCATCTGCGAGGTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCC TATCAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGA GAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG CAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGG ATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTA CCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGC GCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCG GTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGT TCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAG GAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAG CGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCA CTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCC GCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCAC AAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTG ACATACACCGGAAAACCCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGT GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC AACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACC GCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGT CTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTG GAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCAT GAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGG GCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGTAAGCCGGTGG CCCAACCCCTTGTGGGAGGGAGC

Streptomyces levis NBRC 15423(T) AB184670	CGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAA CGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGG GGTCTAATACCGGATATGACCATCTTGGGCATCCTTGATGGTGTAAAGCTCCGGCGG TGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCG ACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCC CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGAT GCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGG AAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCA GCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTC GTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGT CGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTG AAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATA CTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTC CACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCA GCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAG GAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGC GAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTGGAGACAGGGTCCCCCT TGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGG GTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGT GCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGT CAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAAT GAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTG GGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTG CTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCG GTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGG ACTGGCGATTGGGACG
Streptomyces carpinensis NBRC 14214(T) AB184574	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGG GGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACA AGCCCTGGAAACGGGGTCTAATACCGGATATGACCGTCTTGGGCATCCTTGATGGTG TAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAAC GGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGG ACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATG GGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAA CCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGGCT AACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATT GGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTAA CCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTC CTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGA TCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAG ATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCCACG TCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGG CTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAA TTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTGGA GACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTG TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAG CAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAA GGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTAC AATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCG GTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAA TCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGT CACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGG AGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces djakartensis NBRC 15409(T) AB184657	CGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGC GAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAA ACGGGGTCTAATACCGGATACTGACCCTCACGGGCATCTGTGAGGTTCGAAAGCTCC GGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAACGGCTCACCA AGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACA CGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGC CTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAG CAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC CAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAG AGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCT GCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGC GGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCC GATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGT AGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGC CGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAA CGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTGGAGACAGGGTCC CCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGT TGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGT GGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGA CGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTAC AATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGG ATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGC ATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAA GTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGT GGGACTGGCGATTGGGAC
Streptomyces azureus ATCC 14921(T) DF968281	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCATCTTGGGCATCCAAGGTGT TCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTA ATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA TGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGG CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTA TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTT AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCC ACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTG GAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCC AGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGG AAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCT ACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGC CGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGT AATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCCTTGTGGGGA GGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces enissocaesilis NRRL B-16365 (T) DQ026641	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCTCGCAGGCATCTGCGAGGT TCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTA ACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA TGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGG CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTA TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTT AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCC ACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTG GAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCC AGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGG AAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCT ACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGC CGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGT AATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGG GAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces plicatus NBRC 13071 (T) AB184291	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGG GGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACA AGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCTCGCAGGCATCTGCGAGGTT CGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTAA CGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT GGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAA ACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGC TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTAT TGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTA ACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATT CCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGG ATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTA GATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCAC GTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAG GCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTA ATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTGG AGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCA GCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGA AGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA CAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCC GGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTA ATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGG AGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces rochei NBRC1208(T) AB184237	ACGAACGCTGGCGNCGTGCTTAACNCATGCAAGTCGAACGATGAACCACTTCGGTGG GGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACA AGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCTCGCAGGCATCTGCGAGGTT CGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTAA CGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT GGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAA ACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGC TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTAT TGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTA ACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATT CCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGG ATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTA GATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCAC GTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAG GCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTA ATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTGG AGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCA GCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGA AGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA CAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCC GGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTA ATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGG AGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces geysiriensis NBRC 15413(T) AB184661	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGG GGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACA AGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCTCGCAGGCATCTGCGAGGTT CGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAA CGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT GGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAA ACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGC TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTAT TGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTA ACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATT CCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGG ATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTA GATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCAC GTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAG GCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTA ATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTGG AGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCA GCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGA AGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA CAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCC GGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTA ATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGG AGCTGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTA

Streptomyces luteus TRM45540 (T) KN039946	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACCTTCGGG TGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGG ACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCTCGCAGGCATCTGCGAG GTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGG TAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACAC TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC AATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTG TAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCC GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAAT TATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGC TTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGA ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGG CGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTC CACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGC AAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGC TTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCT GGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGC CAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAG GAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGC TACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAG CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAG TAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAG GGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces mutabilis NBRC 12800 (T) AB184156	CGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACCTTCGGGTGGGGA TTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGC CCTGGAAACGGGGTCTAATACCGGATACTGACCCTCGCAGGCATCTGCGAGGTTCGA AAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTAATGG CTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGAC TGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGG CGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCT CTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAAC TACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGG CGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCC CGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTG GTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT CTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATA CCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTG TCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTA AAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTC GACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTGGAGAC AGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGT GAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGG CCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTG GGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATG GCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCT CAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGC AGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACG TCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCT GTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces griseoaurantiacus NBRC 15440(T) AB184676	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGG GGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACA AGCCCTGGAAACGGGGTCTAATACCGGATACAACCACTGACCGCATGGTCGGGTGGT GGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAG TGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT GGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAA ACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGC TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTAT TGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTA ACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATT CCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGG ATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTA GATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCAC GTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAG GCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTA ATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATTAG AGATAGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCA GCAACTCTTCGGAGGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAA GGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTAC AATGGCCGGTACAATGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAGCCG GTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAA TCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGT CACGTCACGAAAGTCGGTAACACCCGAAGCCCGTGGCCCAACCCGCAAGGGAGGGA GCGGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces jietaisiensis FXJ46(T) AY314783	CGGCGTGCTTAACACATGCAAGTCGAACGATGAACCAGCTTCGGTGGGGATTTGTGG CGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAA ACGGGGTCTAATACCGGATACAACCACTGACCGCATGGTCGGGTGGTGGAAAGCTCC GGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCA AGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACA CGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGC CTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAG CAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC CAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCGGAATTATTGGGCGTAAAG AGCTCGTAGACGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCT GCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGC GGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCC GATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGT AGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGC CGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAA CGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATTAGAGATAGTGCCCC CCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTAAGATGTT GGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAACTCTTCGGA GGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACG TCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAA TGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATT GGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATT GCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTC GGTAACACCCGAAGCCCGTGGCCCAACCCGCAAGGGAGGGAGCGGTCGAAGGTGGG ACTGGCG

Streptomyces lasiicapitis 3H-HV17(T) KX777589	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCCTTCGGG GTGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGA CAAGCCCTGGAAACGGGGTCTAATACCGGATATGACACGGGATCGCATGATCTTCGT GTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGT AGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACT GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA ATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGT AAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATT ATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCT TAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGA ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGG CGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTC CACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGC AAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGC TTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATC AGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGC CAGCAAGCCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGA GGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTG CTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAA GCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTA GTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGC CCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCCTTGCGGG GAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces marokkonensis AP1(T) AJ965470	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCATCACGGGCATCTGTGATGG TCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTA ATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA TGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGG CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTA TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTT AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCC ACGTTGTCCGCGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATCA GAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCC AGCAAGCCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAG GAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGC TACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAG CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAG TAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAG GGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces variabilis NBRC 12825 (T) AB184884	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCTCCTTCGGGA GGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGA CAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCGCTTGGGCATCCAAGCGG TTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGT AATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACT GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA ATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGT AAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATT ATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCT TAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGA ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGG CGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTC CACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGC AAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGC TTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATC AGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGC CAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAG GAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGC TACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAG CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAG TAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAG GGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces labedae NBRC 15864 (T) AB184704	GCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCTCCTTCGGGAGGGGATTAGT GGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGG AAACGGGGTCTAATACCGGATACTGACCCGCTTGGGCATCCAAGCGGTTCGAAAGCT CCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCAC CAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGA CACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAA GCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTC AGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGT GCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAA AGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGT CTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTA GCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGG CCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTG GTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCCACGTCGTCCGT GCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACT CAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGC AACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATCAGAGATGGTGC CCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTT GTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGAC GACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGT ACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCG GATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAG CATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAA AGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAG GTGGGACTGGCGATTGGGACG

Streptomyces erythrogriseus LMG 19406 (T) AJ781328	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATRAACCTCCTTCGGG AGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGG ACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCGCTTGGGCATCCAAGCG GTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGG TAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACAC TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC AATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTG TAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCC GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAAT TATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGC TTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGA ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGG CGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTC CACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGC AAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGC TTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATC AGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGC CAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAG GAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGC TACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAG CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAG TAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAG GGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces ambofaciens ATCC 23877(T) CP012382	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGA CAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCGCTTGGGCATCCAGGCGG TTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGT AGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACT GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA ATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGT AAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATT ATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCT TAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGA ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGG CGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTC CACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGC AAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGC TTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATT AGAGATAGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGC CAGCAAGCCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGA GGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTG CTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAA GCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTA GTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGC CCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGA GGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces violaceochromogenes NBRC 13100(T) AB184312	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGG GGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACA AGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCGTCTGGGCATCCAGATGGTT CGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAG TGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT GGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAA ACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGC TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTAT TGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTA ACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATT CCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGG ATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTA GATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCAC GTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAG GCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTA ATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAANNTNNAG AGATNGNCNCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCA GCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGA AGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA CAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCC GGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTA ATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGG AGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces lienomycini LMG 20091(T) AJ781353	GCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGG CGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAA ACGGGGTCTAATACCGGATACTGATCCTCGCGGGCATCTGCGAGGTTCGAAAGCTCC GGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCA AGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACA CGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGC CTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAG CAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC CAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAG AGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCT GCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGC GGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCC GATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGT AGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGC CGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAA CGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATCAGAGATGGTGCCC CCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGT TGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAAGCCCTTCG GGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACG ACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTA CAATGAGCTGCGATACCGCAAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGG ATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGC ATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAA GTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGT GGGACTGGCGATTGGGACG

Streptomyces collinus NBRC 12759(T) AB184123	GGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTG GCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGA AACGGGGTCTAATACCGGATACTGATCCGTCTGGGCATCCAGATGGTTCGAAAGCTC CGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACC AAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGAC ACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAG CCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCA GCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTG CCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAA GAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTC TGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAG CGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGC CGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGG TAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTG CCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTC AAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCA ACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATTAGAGATAGTGCC CCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATG TTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGT GGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGA CGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTAC AATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGG ATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGC ATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAA GTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGT GGGACTGGCGATTGGGACG
Streptomyces heliomycini NBRC 15899(T) AB184712	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGG GGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACA AGCCCTGGAAACGGGGTCTAATACCGGATACTGATCCGCCTGGGCATCCAGGCGGTT CGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAA CGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT GGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAA ACCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGGC TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTAT TGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTA ACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATT CCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGG ATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTA GATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCCAC GTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAG GCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTA ATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATCAG AGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCA GCAAGCCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGG AAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCT ACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGC CGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGT AATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGG GAGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces diastaticus subsp. *ardesiacus* NRRL B-1773(T) DQ026631	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCGCCTTCGGG TGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGG ACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCTGCCGAGGCATCTCGGCG GGTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGG TAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACAC TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC AATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGT AAACCTCTTTCAGCAGGGAAGAAGCGAGAGTGACGGTACCTGCAGAAGAAGCGCCG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATT ATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCT TAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGA ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGG CGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTC CACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGC AAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGC TTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATC AGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGC CAGCAACTCTTCGGAGGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGG AAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCT ACAATGGCCGGTACAATGAGCTGCGATACCGCAAGGTGGAGCGAATCTCAAAAAGC CGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGT AATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGG GAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces thinghirensis DSM 41919(T) FM202482	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCATCTTGGGCATCTTTGATGG TCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTA ATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA TGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGG CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTA TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTT AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCC ACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATCA GAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCC AGCAAGCCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAG GAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGC TACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAG CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAG TAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAG GGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces fradiae NBRC 3439(T) AB184776	CTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCCACTTCGGTGGGGGATT AGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCC TGGAAACGGGGTCTAATACCGGATACGACCACTTCAGGCATCTGATGGTGGTGGAAA GCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTAACGGCT CACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTG AGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCG AAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCT TTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTA CGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCG TAAAGAGCTCGTAGGCGGCCTGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCCG GGTCTGCATTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGT GTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCT GGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC CTGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTGGGCGACATTCCACGTCGTC CGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAA ACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGA CGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACACCCAGAGATGG GTGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGA GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCC CTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGG GACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGC CGGTACAAAGAGCTGCGATACCGCAAGGTGGAGCGAATCTCAAAAAGCCGGTCTCA GTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAG ATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTC ACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGT CGAAGGTGGGACTGGCGATTGGGACG
Streptomyces coeruleoprunus NBRC 15400(T) AB184651	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCTCCTTCGGGA GGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGA CAAGCCCTGGAAACGGGGTCTAATACCGGATATGACCACTTCAGGCATCTGATGGTG GTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGT AACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACT GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA ATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGT AAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATT ATTGGGCGTAAAGAGCTCGTAGGCGGCCTGTCGCGTCGGATGTGAAAGCCCGGGGCT TAACCCCGGGTCTGCATTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTGGGCGACATTCC ACGTCGTCCGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACACTCA GAGATGGGTGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCC AGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGG AAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCT ACAATGGCCGGTACAAAGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAGC CGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGT AATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGG GAGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces somaliensis DSM 40738(T) AJ007403	GCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCCGCTTCGGTGGGGGAT TAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCC CTGGAAACGGGGTCTAATACCGGATACGACCGCTTCGGGCATCCGATGGCGGTGGAA AGCTCCGGCGGTGCAGGATGGGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAACGGC TCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACT GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGC GCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTC TTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACT ACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGC GTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGATGTGAAAGCCCGGGGCTTAACCCC GGGTCTGCATTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGG TGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTC TGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATAC CCTGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTGGGCGACATTCCACGTCGT CCGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAA AACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCG ACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACATCCAGAGATG GGTGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTG AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGC CCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGG GGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGG CCGGTACAAAGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTC AGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCA GATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGT CACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTG TCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces lomondensis NBRC 15426(T) AB184673	GCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGG CGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAA ACGGGGTCTAATACCGGATACTGATCANCGCAGGCATCTGTGGTGTTCGAAAGCTCC GGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCA AGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACA CGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGC CTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAG CAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGC CAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAG AGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCT GCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGC GGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCC GATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGT AGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGC CGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCA AAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAA CGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGTCCAGAGATGGGCGCC CCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGT TGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGT GGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGA CGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTAC AATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGG ATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGC ATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAA GTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGT GGGACTGGCGATTGGGACG

Streptomyces spinoverrucosus NBRC 14228(T) AB184578	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATACGACCATCTTGNGCATCCTTGATGNT GGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAA CGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT GGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAA ACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGC TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTAT TGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTA ACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATT CCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGG ATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTA GATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCAC GTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAG GCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTA ATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGGCCGG AGACGGTCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCA GCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGA AGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA CAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCC GGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTA ATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGG AGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces coerulescens ISP 5146(T) AY999720	CAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACAC GTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGA TACTGACCACCTTGGGCATCCAAGGTGTTCGAAAGCTCCGGCGGTGCAGGATGAGCC CGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCC GGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG GAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGC GTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGT GACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATAC GTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGT CACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGG CTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATAT CAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAG CGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGG TGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATTAAG TGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGC CCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCA AGGCTTGACATACACCGGAAACGTCCAGAGATGGGCGCCCCCTTGTGGTCGGTGTAC AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA CGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACG GGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCC CCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACC GCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTC GACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATAC GTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAG CCGGTGGCCCAACCCCCTTGTGGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGG GACG

Streptomyces purpurascens NBRC 13077(T) AB184859	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGG GGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACA AGCCCTGGAAACGGGGTCTAATACCGGATATTGACCATCTTGGGCATCTTTGATGGT GGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTA ATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA TGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGG CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTA TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTT AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCC ACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGTCTG GAGACAGGCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCC AGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGG AAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCT ACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGC CGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGT AATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGG GAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces lusitanus NBRC 13464 (T) AB184424	TAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGT GAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCT AATACCGGATACTGATCATCTTGGGCATCCTTGGTGATCGAAAGCTCCGGCGGTGCA GGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGA CGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGA CTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAG CGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGA AGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCG CGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAG GCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGAT ACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAAT GCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGA CGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACG CCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCCACGTCGTCCGTGCCGCAGCTA ACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAAT TGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAG AACCTTACCAAGGCTTGACATACACCGGAAACGTCCAGAGATGGGCGCCCCCTTGTG GTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA AGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTG GGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAG TCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGC TGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGT CTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGC GGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAA CACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTG GCGATTGGGACG

Streptomyces matensis NBRC 12889(T) AB184221	CTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAG TGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTG GAAACGGGGTCTAATACCGGATACTGATCNTCTTNGGCATCNNRGNTGNTCGAAAGC TCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGGTAATGGCTCA CCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAG ACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAA AGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTT CAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACG TGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTA AAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGG TCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGT AGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGG GCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCT GGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCCACGTCGTCCG TGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAAC TCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACG CAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATCAGAGATGGTG CCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGA TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTT GTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGAC GACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGT ACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCG GATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAG CATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAA AGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAG GTGGGACTGGCGATTGGGACG
Streptomyces althioticus NRRL B-3981(T) AY999791	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCMTCTTGGGCATCYWRGMKG WTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTAGTTGGTGAGG TAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACAC TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC AATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTG TAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCC GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAAT TATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGC TTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGA ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGG CGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTC CACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGC AAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGC TTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATC AGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGC CAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAG GAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGC TACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAG CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAG TAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAG GGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces griseoincarnatus LMG 19316 (T) AJ781321	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCTCCTTCGGG AGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGG ACAAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCGCTTGGGCATCCAAGCG GTTCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGG TAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACAC TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC AATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTG TAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCC GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAAT TATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGC TTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGA ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGG CGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTC CACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGC AAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGC TTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATC AGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGC CAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAG GAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGC TACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAG CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAG TAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAG GGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces atrovirens NRRL B-16357(T) DQ026672	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCGCCTGGGCATCCAGGCGGT TCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTA ACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA TGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAA ACCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGGC TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTAT TGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTA ACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATT CCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGG ATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTA GATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCCAC GTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAG GCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTA ATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGTCTGGA GACAGGCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTG TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAG CAAGCCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGA AGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA CAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCC GGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTA ATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGG AGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces albogriseolus NRRL B-1305(T) AJ494865	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCGCTTGGGCATCCAAGCGGT TCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTA ATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA TGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGG CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTA TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTT AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCC ACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGTCCA GAGATGGGCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCC AGCAAGCCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAG GAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGC TACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAG CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAG TAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAG GGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces viridodiastaticus NBRC 13106(T) AB184317	CGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGG GATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAA GCCCTGGAAACGGGGTCTAATACCGGATACTGACCCGCTTGGGCATCCAAGCGGTTC GAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAAT GGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGG ACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATG GGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAA CCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCT AACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATT GGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAA CCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTC CTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGA TCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAG ATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCCACG TCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGG CTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAA TTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGTCCAGA GATGGGCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTG TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAG CAAGCCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGA AGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA CAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCC GGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTA ATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGG AGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces caelestis NRRL 2418(T) X80824	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCATCTTGGGCATCCAAGGTGT TCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTA ATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA TGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGG CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTA TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTT AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCC ACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTG GAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCC AGCAAGCCTTCGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGG AAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCT ACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGC CGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGT AATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGG GAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptmyces olivaceus NBRC 12805T (AB249920)	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATATTGACCTTCACGGGCATCTGTGAGGT TCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTA ATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA TGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGG CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTA TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTT AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCC ACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGGCC AGAGATGGTCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGC CAGCAAGCTCCTTCGGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGG AGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGT GCTACAATGGCCGGTACAATGAGCTGCGATACCGCAAGGTGGAGCGAATCTCAAAA AGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCT AGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCG CCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGG AGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAAG

Streptomyces tuirus NBRC 15617 (T) AB184690	GGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTG GCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGA AACGGGGTCTAATACCGGATACTGACCATCGCAGGCATCTGTGAGGGTCGAAAGCTC CGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACC AAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGAC ACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAG CCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCA GCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTG CCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAA GAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTC TGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAG CGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGC CGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGG TAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTG CCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTC AAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCA ACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTGGAGACAGGGTC CCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATG TTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGT GGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGA CGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTAC AATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGG ATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGC ATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAA GTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCCGTCGAAGG TGGGACTGGCGATTGGGACG
Streptomyces luteogriseus NBRC 13402 (T) AB184379	ACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAACGGGTGA GTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAA TACCGGATACTGACCATTGCAGGCATCTGTGATGGTCGAAAGCTCCGGCGGTGCAGG ATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACG GGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACT CCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCG ACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAG CGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCG GTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGC GGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATAC GGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGC GCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACG CTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCC GTAAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAAC GCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTG ACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAA CCTTACCAAGGCTTGACATACACCGGAAACGTCTGGAGACAGGCGCCCCCTTGTGGT CGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAG TCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGG GACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTC ATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCT GCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTC TGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCG GTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAAC ACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGG CGATTGGGACG

Streptomyces parvulus NBRC 13193 (T) AB184326	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGG GGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACA AGCCCTGGAAACGGGGTCTAATACCGGATACTGACCTTCACGGGCATCTGTGAAGGT CGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAA TGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT GGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAA ACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGC TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTAT TGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTA ACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATT CCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGG ATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTA GATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCCAC GTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAG GCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTA ATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGTCCAG AGATGGGCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGT GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCA GCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGA AGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTA CAATGGCCGGTACAATGAGCTGCGATACCGCAAGGTGGAGCGAATCTCAAAAAGCC GGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTA ATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGG AGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces tendae ATCC 19812 (T) D63873	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTG GGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGAC AAGCCCTGGAAACGGGGTCTAATACCGGATACTGACCCTCGCAGGCATCTGCGAGGT TCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTA ATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAA TGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGG CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTA TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTT AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCC ACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCATCA GAGATGGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCC AGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGG AAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCT ACAATGGCCGGTACAATGAGCTGCGATACCGCAAGGTGGAGCGAATCTCAAAAAGC CGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGT AATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGG GAGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Nocardiopsis dassonvillei subsp. *albirubida* NBRC 13392 (T) BCRR01000172	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGGCCCTTCGGG GTACACGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACTCCGGG ATAAGCGGTGGAAACGCCGTCTAATACCGGATACGACCCGCCACCTCATGGTGGAGG GTGGAAAGTTTTTCGGTCAGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTA ACGGCCTACCAAGGCGATTACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTGCGGGAGGCAGCAGTGGGGAATATTGCGCAA TGGGCGAAAGCCTGACGCAGCGACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTA AACCTCTTTTACCACCAACGCAGGCCCGGAGTTCTCTTCGGGTTGACGGTAGGTGGG GAATAAGGACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTCCGAGC GTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCGTGTCGCGTCTGCTGTG AAAGACCGGGGCTTAACTCCGGTTCTGCAGTGGATACGGGCATGCTAGAGGTAGGTA GGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACAC CGGTGGCGAAGGCGGGTCTCTGGGCCTTACCTGACGCTGAGGAGCGAAAGCATGGG GAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTG TGGGGACTTTCCACGGTTTCCGCGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGG AGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCG GAGCATGTTGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGTTTGACATCACC CGTGGACTCGCAGAGATGTGAGGTCATTTAGTTGGCGGGTGACAGGTGGTGCATGGC TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT GTTCCATGTTGCCAGCACGTAATGGTGGGGACTCATGGGAGACTGCCGGGGTCAACT CGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCAAA CATGCTACAATGGCCGGTACAATGGGCGTGCGATACCGTAAGGTGGAGCGAATCCCT AAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGGTGGAGTC GCTAGTAATCGCGGATCAGCAACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACA CCGCCCGTCACGTCATGAAAGTCGGCAACACCCGAAACTTGCGGCCTAACCCCTTGT GGGAGGGAGTGAGTGAAGGTGGGGCTGGCGATTGGGACG
Nocardiopsis lucentensis DSM 44048 (T) ANBC01000932	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGGCCCTTCGGG GTACACGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACTCCGGG ATAAGCGGTGGAAACGCCGTCTAATACCGGATACGACCCACCACCTCATGGTGGAGG GTGGAAAGTTTTTCGGTCAGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTA ACGGCCTACCAAGGCGATTACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTGCGGGAGGCAGCAGTGGGGAATATTGCGCAA TGGGCGAAAGCCTGACGCAGCGACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTA AACCTCTTTTACCACCAACGCAGGCTCCGGGTTCTCCCGGGGTTGACGGTAGGTGGG GAATAAGGACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTCCGAGC GTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCGTGTCGCGTCTGCTGTG AAAGACCGGGGCTTAACTCCGGTTCTGCAGTGGATACGGGCATGCTAGAGGTAGGTA GGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACAC CGGTGGCGAAGGCGGGTCTCTGGGCCTTACCTGACGCTGAGGAGCGAAAGCATGGG GAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTG TGGGGACTTTCCACGGTTTCCGCGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGG AGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCG GAGCATGTTGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGTTTGACATCACC CGTGGACCTGCAGAGATGTGGGGTCATTTAGTTGGTGGGTGACAGGTGGTGCATGGC TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT GTTCCATGTTGCCAGCACGTAATGGTGGGGACTCATGGGAGACTGCCGGGGTCAACT CGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCAAA CATGCTACAATGGCCGGTACAATGGGCGTGCGATACCGTGAGGTGGAGCGAATCCCT AAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGGTGGAGTC GCTAGTAATCGCGGATCAGCAACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACA CCGCCCGTCACGTCATGAAAGTCGGCAACACCCGAAACTTGTGGCCCAACCCCTTGT GGGAGGGAATGAGTGAAGGTGGGGCTGGCGATTGGGACG

Nocardiopsis synnemataformans DSM 44143 (T) ANAW01000308	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGGCCCTTCGGG GTACACGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACTCTGGG ATAAGCGGTGGAAACGCCGTCTAATACCGGATACGACCTGCCACCTCATGGTGGAGG GTGGAAAGTTTTTCGGTCAGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTA ACGGCCTACCAAGGCGATTACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTGCGGGAGGCAGCAGTGGGGAATATTGCGCAA TGGGCGAAAGCCTGACGCAGCGACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTA AACCTCTTTTACCACCAACGCAGGCTCGAAGTTTTCTTCGGGTTGACGGTAGGTGGG GAATAAGGACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTCCGAGC GTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCGTGTCGCGTCTGCTGTG AAAGACCGGGGCTTAACTCCGGTTCTGCAGTGGATACGGGCATGCTAGAGGTAGGTA GGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACAC CGGTGGCGAAGGCGGGTCTCTGGGCCTTACCTGACGCTGAGGAGCGAAAGCATGGG GAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTG TGGGGACTTTCCACGGTTTCCGCGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGG AGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCG GAGCATGTTGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGTTTGACATCACC CGTGGACTCGCAGAGATGTGAGGTCATTTAGTTGGCGGGTGACAGGTGGTGCATGGC TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT GTTCCATGTTGCCAGCACGTAATGGTGGGGACTCATGGGAGACTGCCGGGGTCAACT CGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCAAA CATGCTACAATGGCCGGTACAATGGGCGTGCGATACCGCAAGGTGGAGCGAATCCCT AAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGGTGGAGTC GCTAGTAATCGCGGATCAGCAACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACA CCGCCCGTCACGTCATGAAAGTCGGCAACACCCGAAACTTGCGGCCTAACCCCTTGT GGGAGGGAGTGAGTGAAGGTGGGGCTGGCGATTGGGACG
Nocardiopsis dassonvillei subsp. *dassonvillei* DSM 43111 (T) ABUI01000017	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGGCCCTTCGGG GTACACGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACTCTGGG ATAAGCGGTGGAAACGCCGTCTAATACCGGATACGACCCGCCACCTCATGGTGGAGG GTGGAAAGTTTTTCGGTCAGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTA ACGGCCTACCAAGGCGATTACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTGCGGGAGGCAGCAGTGGGGAATATTGCGCAA TGGGCGAAAGCCTGACGCAGCGACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTA AACCTCTTTTACCACCAACGCAGGCTTCCAGTTCTCTGGAGGTTGACGGTAGGTGGG GAATAAGGACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTCCGAGC GTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCGTGTCGCGTCTGCTGTG AAAGACCGGGGCTTAACTCCGGTTCTGCAGTGGATACGGGCATGCTAGAGGTAGGTA GGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACAC CGGTGGCGAAGGCGGGTCTCTGGGCCTTACCTGACGCTGAGGAGCGAAAGCATGGG GAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTG TGGGGACTTTCCACGGTTTCCGCGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGG AGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCG GAGCATGTTGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGTTTGACATCACC CGTGGACTCGCAGAGATGTGAGGTCATTTAGTTGGCGGGTGACAGGTGGTGCATGGC TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT GTTCCATGTTGCCAGCACGTAATGGTGGGGACTCATGGGAGACTGCCGGGGTCAACT CGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCAAA CATGCTACAATGGCCGGTACAATGGGCGTGCGATACCGTAAGGTGGAGCGAATCCCT AAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGGTGGAGTC GCTAGTAATCGCGGATCAGCAACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACA CCGCCCGTCACGTCATGAAAGTCGGCAACACCCGAAACTTGCGGCCTAACCCCTTGT GGGAGGGAGTGAGTGAAGGTGGGGCTGGCGATTGGGACG

Nocardiopsis halotolerans DSM 44410 (T) ANAX01000410	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGTAAGGCCCTTCGGG GTACACGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACTCCGGG ATAAGCGGTGGAAACGCCGTCTAATACCGGATACGACCCTTGGCCTCCTGGCCGGGG GTGGAAAGTTTTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTA ACGGCCTACCAAGGCGATTACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG GGACTGAGACACGGCCCAGACTCCTGCGGGAGGCAGCAGTGGGGAATATTGCGCAA TGGGCGAAAGCCTGACGCAGCGACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTA AACCTCTTTTACCACCAACGCAGGCTCGGAGTTTTCTTCGGGTTGACGGTAGGTGGG GAATAAGGACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTCCGAGC GTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCGTGTCGCGTCTGCTGTG AAAGACCGGGGCTTAACTCCGGTTTGGCAGTGGATACGGGCATGCTAGAGGTAGGTA GGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACAC CGGTGGCGAAGGCGGGTCTCTGGGCCTTACCTGACGCTGAGGAGCGAAAGCATGGG GAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTG TGGGGACTTTCCACGGTTTCCGCGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGG AGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCG GAGCATGTTGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGTTTGACATCACC CGTGGACTCGCAGAGATGTGAGGTCATTTGGTTGGCGGGTGACAGGTGGTGCATGGC TGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTT GTTCCATGTTGCCAGCACGTAGTGGTGGGGACTCATGGGAGACTGCCGGGGTCAACT CGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCAAA CATGCTACAATGGCCGGTACAATGGGCGTGCGATGCCGTAAGGTGGAGCGAATCCCT AAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGGTGGAGTC GCTAGTAATCGCGGATCAGCAACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACA CCGCCCGTCACGTCATGAAAGTCGGCAACACCCGAAACTTGCGGCCTAACCCCTTGT GGGAGGGAGTGAGTGAAGGTGGGGCTGGCGATTGGGACG
Streptomyces radiopugnans R97 (T) DQ912930	TGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGAAGAGTGGCGAAC GGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGATAACTCCGGGAAACCGG AGCTAATACCGGATACGACTCTCCAGGGCATCCTGGGGGGTGGAAAGCTCCGGCGGT GCAGGATGAGCCCGCGGCCTATCAGCTGGTTGGTGGGGTAATGGCCCACCAAGGCG ACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCC CAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGAT GCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGG AAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGCCAGCA GCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTC GTAGGCGGCCTGTCGCGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATT CGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTG AAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATA CTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTC CACGCCGTAAACGTTGGGCACTAGGTGTGGGCGGCATTCCACGTCGTCCGTGCCGCA GCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAG GAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGC GAAGAACCTTACCAAGGCTTGACATACACCGGAAACGGCCAGAGATGGTCGCCCCCT TGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGG GTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCGTGCCTTTCGGGGT GACGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGT CAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAAT GAGCTGCGATGCCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTG GGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATTG CTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCG GTAACACCCGAAGCCGGTGGCCCAACCCCTTGGGGAGGGAATCGTCGAAGGTGGGA CTGGCGATTGGGACG

Streptomyces atacamensis C60 (T) HE577171	AACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGCTGAACCACTTCGGTGGGGA AGAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGATAACT CCGGGAAACCGGAGCTAATACCGGATACGACTCCACGGGGCATCTCGTGGGGGTGG AAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTGGTTGGTGGGGTAACG GCCCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGA CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG GCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACC TCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCACCGGCTAA CTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTATTGG GCGTAAAGAGCTCGTAGGCGGCCTGTCGCGTCGGATGTGAAAGCCCGGGGCTTAACC CCGGGTCTGCATTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCT GGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATC TCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGAT ACCCTGGTAGTCCACGCCGTAAACGTTGGGCACTAGGTGTGGGCGGCATTCCACGTC GTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCT AAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATT CGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGGCCAGAGA TGGTCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCG TGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCGT GCCTTTCGGGGTGACGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGG TGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAA TGGCCGGTACAATGAGCTGCGATGCCGTGAGGTGGAGCGAATCTCAAAAAGCCGGT CTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATC GCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA CGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCCTTGTGGGGAGGG AATCGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces fenghuangensis GIMN4.003 GU356598	CATGCAGTCGAACGCCGAACCGCCTTCGGGCGGGGAGGAGTGGCGAACGGGTGAGT AACACGTGGGCAATCTGCCCTGCACTCTGGGATAACTCCGGGAAACCGGAGCTAATA CCGGATACGACCTTCCAGGGCATCCTGGGGGGTGGAAAGCTCCGGCGGTGCAGGAT GAGCCCGCGGCCTATCAGCTGGTTGGTGGGGTGACGGCCCACCAAGGCGACGACGG GTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTC CTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGA CGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGC GCAAGTGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGG TAATACGTAGGGTGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG GCCTGTCGCGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACG GGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCG CAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGC TGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCG TAAACGTTGGGCACTAGGTGTGGGCGGCATTCCACGTCGTCCGTGCCGCAGCTAACG CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGA CGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAAC CTTACCAAGGCTTGACATACACCGGAAACGGCCAGAGATGGTCGCCCCCTTGTGGTC GGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT CCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCGTGCCTTTCGGGGTGACGGG GACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTC ATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCT GCGATGCCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTC TGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCACTGCTGCG GTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAAC ACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAATCGTCGAAGGTGGGACTGG CGATTGGGACG

Streptomyces nanhaiensis SCSIO 01248 (T) GQ871748	CGTGCTTACACATGCAAGTCGAACGCTGCCCCTTCTTCGGGAGGGGCGGAGTGGCGA ACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGATAACTCCGGGAAACC GGAGCTAATACCGGATACGATCCTCCAGGGCATCCTGGGGGGTGGAAAGCTCCGGC GGTGCAGGATGAGCCCGCGGCCTATCAGCTGGTTGGTGGGGTAACGGCCCACCAAG GCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACG GCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCT GATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCA GGGAAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGCCA GCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAG CTCGTAGGCGGCCTGTCGCGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGC ATTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGG TGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGA TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAG TCCACGCCGTAAACGTTGGGCACTAGGTGTGGGCGGCATTCCACGTCGTCCGTGCCG CAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAA AGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAAC GCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGGCCAGAGATGGTCGCCC CCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTT GGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCGTGCCTTTCGGG GTGACGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGAC GTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACA ATGAGCTGCGATGCCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGAT TGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAC TGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGT CGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAATCGTCGAAGGTG GGACTGGCGATTGGGACG
Streptomyces pini PL19 (T) FOSG01000050	GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCTCCTTCGGG AGGGGAAGAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGG GATAACTCCGGGAAACCGGAGCTAATACCGGATACGACCACCGCGGGCATCCGTGG TGGTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTGGTTGGTGGG GTGACGGCCCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACA CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA CAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTT GTAAACCTCTTTCAGCAGGGAAGAAGCGCGAGTGACGGTACCTGCAGAAGAAGCAC CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAA TTATTGGGCGTAAAGAGCTCGTAGGCGGCCTGTCGCGTCGGATGTGAAAGCCCGGGG CTTAACCCCGGGTCTGCATTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGG AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAG GCGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGG ATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGCACTAGGTGTGGGCGGCATT CCACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCG CAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGG CTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAACGGC CAGAGATGGTCGCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCT CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGC CAGCGTGCCCTTCGGGGTGACGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGA GGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTG CTACAATGGCCGGTACAATGAGCTGCGATGCCGCGAGGTGGAGCGAATCTCAAAAA GCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTA GTAATCGCAGATCAGCACTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGC CCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGA GGGAATCGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces mangrovi HA11110 (T) JX067937	AGTCGACGATGAACCTCCTTCGGGAGGGGAAGAGTGGCGAACGGGTGAGTAACACG TGGGCAATCTGCCCTGCACTCTGGGATAACTCCGGGAAACCGGAGCTAATACCGGAT ACGACCACCGCGGGCATCCGTGGTGGTGGAAAGCTCCGGCGGTGCAGGATGAGCCC GCGGCCTATCAGCTGGTTGGTGGGGTGACGGCCCACCAAGGCGACGACGGGTAGCC GGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG GAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGC GTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGTAAGT GACGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATAC GTAGGGTGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCCTGT CGCGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCAGG CTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATAT CAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGACGCTGAGGAG CGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGT TGGGCACTAGGTGTGGGCGGCATTCCACGTCGTCCGTGCCGCAGCTAACGCATTAAG TGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGC CCGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCA AGGCTTGACATACACCGGAAACGGCCAGAGATGGTCGCCCCCTTGTGGTCGGTGTAC AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA CGAGCGCAACCCTTGTTCTGTGTTGCCAGCGTGCCTTTCGGGGTGACGGGGACTCAC AGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGC CCCTTATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAATGAGCTGCGATGC CGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACT CGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCACTGCTGCGGTGAATA CGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAA GCCGGTGGCCCAACC
Streptomyces fimbriatus NBRC 15411 (T) AB184659	GGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCGCCTTCGGGCGGGGATTAG TGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTG GAAACGGGGTCTAATACCGGATACAACCACTTCGGGCATCCGATGGTGGTGGAAAG CTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAACGGCTC ACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGA GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGC AAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTT TCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTAC GTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGT AAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGG GTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTG TAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTG GGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC TGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTCCACGTCGTCC GTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAA ACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTTAATTCGA CGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCTGGAGACAG GGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGA GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAAGCC CCTTCGGGGGTGTTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGT GGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAAT GGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGCCGGTC TCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCG CAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAC GTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGC TGTCGAAGGTGGGACTGGCGATTGGGACG

Streptomyces werraensis NBRC 13404 (T) AB184381	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCTCCTTCGGGA GGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGA CAAGCCCTGGAAACGGGGTCTAATACCGGATACTGATCATCTTGGGCATCCTTGGTG ATCGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGT AATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACT GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA ATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGT AAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATT ATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCT TAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGA ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGG CGGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGA TTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCGACATTC CACGTCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGC AAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGC TTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAACCCT GGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGC CAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAG GAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGC TACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAG CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAG TAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAG GGAGCTGTCGAAGGTGGGACTGGCGATTGGGACG
Streptomyces griseomycini NBRC 12778 (T) AB184137	ACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCTCCTTCGGGA GGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGA CAAGCCCTGGAAACGGGGTCTAATACCGGATACGACATCCTCGGGCATCCGATGGAT GTGGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGT AACGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACT GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA ATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTA AACCTCTTTCAGCAGGGAAGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGG CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTA TTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTT AACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAA TTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGC GGATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT TAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTGGGCAACATTCC ACGTTGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCA AGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGTGGCT TAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATACACCGGAAAGCTCTG GAGACAGAGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCC AGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGGTCAACTCGGAGG AAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCT ACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGC CGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGT AATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGG GAGCTGTCGAAGGTGGGACTGGCGATTGGGACG

Table A3. Full, primary, agar plug screening of Fire Mountain strains against *B. subtilis*, *E. coli* and *S. cerevisiae.* 'Yes' signifies that activity was observed, and 'No' that activity was not observed. The medium in which activity was observed is either Oatmeal Agar or ISP2/GYM Agar.

Strain	Medium	B. subtilis	E. coli	Yeast
DEM60001	ISP2/OA	No	No	No
DEM60002	ISP2/OA	No	No	No
DEM60003	ISP2/OA	No	No	No
DEM60004	ISP2/OA	No	No	No
DEM60005	ISP2/OA	No	No	No
DEM60006	ISP2/OA	No	No	No
DEM60007	ISP2/OA	Yes	Yes	No
DEM60008	ISP2/OA	No	No	No
DEM60009	ISP2/OA	No	No	No
DEM60010	ISP2/OA	No	No	No
DEM60011	ISP2/OA	No	No	No
DEM60012	OA	Yes	Yes	No
DEM60013	OA	Yes	Yes	No
DEM60014	ISP2/OA	No	No	No
DEM60015	ISP2/OA	No	No	No
DEM60016	ISP2/OA	No	No	No
DEM60017	ISP2/OA	Yes	No	No
DEM60018	ISP2/OA	No	No	No
DEM60019	ISP2/OA	No	No	No
DEM60020	ISP2/OA	No	No	No
DEM60021	ISP2/OA	No	No	No
DEM60022	ISP2/OA	No	No	No
DEM60023	ISP2/OA	No	No	No
DEM60024	ISP2/OA	No	No	No
DEM60025	ISP2/OA	No	No	No
DEM60026	ISP2/OA	No	No	No
DEM60027	ISP2/OA	No	No	No
DEM60028	ISP2/OA	No	No	No
DEM60029	ISP2/OA	No	No	No
DEM60030	ISP2/OA	No	No	No
DEM60031	ISP2/OA	Yes	No	No
DEM60032	ISP2/OA	No	No	No
DEM60033	ISP2	Yes	No	No
DEM60034	ISP2/OA	No	No	No
DEM60035	ISP2/OA	No	No	No
DEM60036	ISP2/OA	No	No	No
DEM60037	ISP2/OA	No	No	No
DEM60038	ISP2/OA	Yes	No	No

DEM60039	ISP2/OA	No	No	No
DEM60040	OA	Yes	No	No
DEM60041	OA	Yes	Yes	No
DEM60042	ISP2/OA	No	No	No
DEM60043	ISP2/OA	No	No	No
DEM60044	ISP2/OA	No	No	No
DEM60045	OA	Yes	Yes	No
DEM60046	ISP2/OA	No	No	No
DEM60047	ISP2/OA	No	No	No
DEM60048	ISP2/OA	No	No	No
DEM60049	ISP2/OA	No	No	No
DEM60050	ISP2/OA	No	No	No
DEM60051	ISP2/OA	No	No	No
DEM60052	ISP2/OA	No	No	No
DEM60053	ISP2/OA	No	No	No
DEM60054	ISP2/OA	Yes	No	No
DEM60055	ISP2	Yes	No	No
DEM60056	ISP2/OA	No	No	No
DEM60057	OA	Yes	No	No
DEM60058	ISP2/OA	No	No	No
DEM60059	ISP2/OA	No	No	No
DEM60060	ISP2/OA	No	No	No
DEM60061	ISP2/OA	No	No	No
DEM60062	ISP2/OA	No	No	No
DEM60063	ISP2/OA	No	No	No
DEM60064	ISP2/OA	No	No	No
DEM60065	ISP2/OA	No	No	No
DEM60066	ISP2/OA	No	No	No
DEM60067	ISP2/OA	No	No	No
DEM60068	OA	Yes	Yes	No
DEM60069	ISP2/OA	No	No	No
DEM60070	ISP2/OA	No	No	No
DEM60071	ISP2/OA	No	No	No
DEM60072	ISP2/OA	No	No	No
DEM60073	ISP2	Yes	Yes	No
DEM60074	ISP2	Yes	No	No
DEM60075	ISP2/OA	No	No	No
DEM60076	ISP2/OA	No	No	No
DEM60077	MED V	No	No	No
DEM60078	ISP2/OA	No	No	No
DEM60079	ISP2/OA	No	No	No
DEM60080	ISP2/OA	No	No	No
DEM60081	ISP2/OA	No	No	No
DEM60082	ISP2/OA	No	No	No
DEM60083	ISP2/OA	No	No	No

DEM60084	ISP2/OA	No	No	No
DEM60085	ISP2/OA	No	No	No
DEM60086	OA	Yes	No	No
DEM60087	OA	Yes	No	No
DEM60088	OA	Yes	Yes	Yes
DEM60089	OA	No	No	Yes
DEM60090	OA	No	No	Yes
DEM60091	OA	Yes	No	Yes
DEM60092	OA	Yes	No	No
DEM60093	OA	Yes	No	Yes
DEM60094	OA	No	No	No
DEM60095	OA	Yes	No	No
DEM60096	OA	Yes	No	Yes
DEM60097	OA	Yes	No	Yes
DEM60098	OA	Yes	No	Yes
DEM60099	OA	Yes	No	Yes
DEM60100	OA	Yes	No	Yes
DEM60101	OA	No	No	No
DEM60102	OA	No	No	No
DEM60103	OA	Yes	No	Yes
DEM60104	OA	Yes	No	Yes
DEM60105	OA	No	No	No
DEM60106	OA	Yes	No	No
DEM60107	OA	Yes	No	Yes
DEM60108	OA	Yes	No	Yes
DEM60109	OA	Yes	No	Yes
DEM60110	OA	No	No	Yes
DEM60111	OA	No	No	Yes
DEM60112	OA	Yes	No	Yes
DEM60113	OA	Yes	No	Yes
DEM60114	OA	Yes	No	Yes
DEM60115	OA	Yes	No	No
DEM60116	OA	No	No	No
DEM60117	OA	Yes	No	Yes
DEM60118	OA	Yes	No	Yes
DEM60119	OA	Yes	No	No
DEM60120	OA	No	No	No
DEM60121	OA	No	No	Yes
DEM60122	OA	Yes	No	No
DEM60123	OA	Yes	No	Yes
DEM60124	OA	No	No	No
DEM60125	OA	Yes	No	No
DEM60126	OA	Yes	No	Yes
DEM60127	OA	Yes	No	No
DEM60128	OA	Yes	No	No

DEM60129	OA	No	No	No
DEM60130	OA	Yes	Yes	No
DEM60131	OA	Yes	Yes	No
DEM60132	OA	Yes	No	No
DEM60133	OA	No	No	Yes
DEM60134	OA	Yes	No	Yes
DEM60135	OA	No	No	Yes
DEM60136	OA	No	No	Yes
DEM60137	OA	No	No	No
DEM60138	OA	No	Yes	No
DEM60139	OA	Yes	Yes	No
DEM60140	OA	No	No	No
DEM60141	OA	No	No	No
DEM60142	OA	No	No	No
DEM60143	OA	No	No	No
DEM60144	OA	No	No	No
DEM60145	OA	No	No	No
DEM60146	OA	No	No	No
DEM60147	OA	No	No	No
DEM60148	OA	No	No	No
DEM60149	OA	No	No	No
DEM60150	OA	No	No	No
DEM60151	OA	No	No	No
DEM60152	OA	No	No	No
DEM60153	OA	No	No	Yes
DEM60154	OA	No	No	No
DEM60155	OA	No	No	No
DEM60156	OA	No	No	Yes
DEM60157	OA	No	No	No
DEM60158	OA	Yes	No	Yes
DEM60159	OA	No	No	No
DEM60160	OA	No	No	No
DEM60161	OA	No	No	No
DEM60162	OA	Yes	Yes	No
DEM60163	OA	No	No	No
DEM60164	OA	No	No	Yes
DEM60165	OA	No	No	No
DEM60166	OA	No	No	No
DEM60167	ISP2	No	No	No
DEM60168	ISP2	No	No	No
DEM60169	OA	No	No	No
DEM60170	OA	Yes	No	No
DEM60171	OA	Yes	No	No
DEM60172	OA	Yes	No	No
DEM60173	OA	Yes	No	No

DEM60174	OA	Yes	No	Yes
DEM60175	OA	Yes	No	Yes
DEM60176	OA	No	No	No
DEM60177	OA	Yes	No	Yes
DEM60178	OA	Yes	No	No
DEM60179	OA	Yes	No	No
DEM60180	OA	No	No	Yes
DEM60181	OA	Yes	No	No
DEM60182	OA	No	No	No
DEM60183	OA	No	No	No
DEM60184	OA	No	No	No
DEM60185	OA	No	No	No
DEM60186	OA	No	No	No

Table A4. Organisation of the non-active Fire Mountain strains used in elicitation studies.

	1	2	3	4	5	6	7	8	9	10	11	12
A	60165	60145	60133	60039	60161	60176	60015	60076	60167	60003	60013	60002
B	60149	60008	60144	60007	60160	60120	60156	60157	60186	60180	60142	60168
C	60169	60148	60155	60154	60159	60164	60018	60060	60046	60015	60016	60141
D	60151	60140	60138	60136	60135	60137	60121	60129	60153	60072	60152	60166
E	60150	60019	60188	60020	60001	60094						