

## Natural anti-phytopathogenic fungi compound phenol, 2, 4-bis (1, 1-dimethylethyl) from *Pseudomonas fluorescens* TL-1

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Received 07 July 2018; revised 20 December 2018

A strain was isolated from tobacco phylloplane and preliminarily identified as *Pseudomonas fluorescens* TL-1, which had the visible inhibition against ten plant pathogenic fungi, viz., *Curvularia lunata*, *Bipolaris maydis*, *Valsa mali*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria alternate*, *Fusarium oxysporum* and *Verticillium dahlia* in dual culture experiments. The ethyl acetate extract of nutrient broth seeded with *Pseudomonas fluorescens* TL-1 suspension was separated into fifty-nine fractions by the Sephadex LH-20 column and the antifungal activity of each fraction was tested with paper disc diffusion method against *Curvularia lunata*. The results showed that fraction 1 to 3 had the strongest inhibitory effects on *Curvularia lunata*. Furthermore, GC/MS analysis of the constituents of fraction 1 to 59 confirmed that phenol, 2, 4-bis (1, 1-dimethylethyl) was the active compound for the antifungal activity from *Pseudomonas fluorescens* TL-1.

**Keywords:** 2, 4-bis (1, 1-dimethylethyl), Column chromatography, Gas chromatography, Mass spectrometry, Phenol, *Pseudomonas fluorescens*

Fungal plant pathogens are among the most important factors that cause serious losses to agricultural products annually<sup>1</sup>. Therefore the management of fungal plant diseases becomes a critical process for the achievement of the higher profits in agricultural production. Apart from the application of the agrochemicals in the control of fungal plant disease, more measures of biological controls are taken as an alternative to the use of agrochemicals with the advantages of greater public acceptance and reduced environmental impact<sup>2</sup>. It is well known that the bacteria are a number of biocontrol agents of plant pathogens, which include *Serratia plymuthica*<sup>3</sup>, *Paenibacillus elgii*<sup>4</sup>, *Pseudomonas fluorescens*<sup>5,6</sup>, *Bacillus amyloliquefaciens*<sup>7</sup>, *Chryseobacterium* sp.<sup>8</sup>, *Pseudochrobactrum kiredjianiae*<sup>9</sup>, *Pseudomonas azotoformans*<sup>10</sup>, *Pseudomonas putida*<sup>11</sup>, *Bacillus thuringiensis*<sup>12</sup>, *Xanthomonas sacchari*<sup>13</sup>, *Burkholderia cenocepacia*<sup>14</sup>, *Lysobacter antibiocus*<sup>15</sup>, *Bacillus cereus*<sup>16</sup>, *Brevibacillus brevis*<sup>17</sup>, *Mitsuraria* sp.<sup>18</sup> and so on from different environmental conditions. Among all of them, the genera of

*Pseudomonas* and *Bacillus* are those of currently the most widely studied for the biocontrol of plant diseases<sup>19,20</sup> and various biocontrol mechanisms involved in the biological control of plant diseases have been addressed including the production of secondary metabolites such as antibiotics, siderophores, hydrolytic enzymes, volatile extracellular metabolites, hydrogen cyanide and competition for nutrients, promotion of plant growth and induced resistance within the plants.

The compound phenol-2, 4-bis (1, 1-dimethylethyl), is a precursor to many complex compounds and widely used as antioxidants, light protection agents or UV stabilizers and chemical intermediates for the synthesis of other chemical intermediates. While as the naturally antimicrobial compound, phenol-2,4-bis (1, 1-dimethylethyl) could be achieved from plant materials<sup>21,22</sup>, animal materials<sup>23,24</sup> and the metabolites of microorganisms, such as *Streptomyces* sp.<sup>25</sup>, *Shewanella algae*<sup>26</sup> and *Pseudomonas monteili*<sup>27</sup>, *Nocardia* sp.<sup>28</sup>, *Bacillus velezensis*<sup>29</sup>, *Vibrio alginolyticus*<sup>30</sup>, *Vibrio owensii*<sup>31</sup>, *Vibrio* sp.<sup>32</sup>, *Bacillus subtilis*<sup>33</sup>, *Microbacterium mangrove*, *Sinomonas humi* and *Monashia flava*<sup>34</sup> and so on. Hitherto there have been still no reports of active compound phenol-2, 4-bis (1, 1-

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dimethylethyl) from *Pseudomonas fluorescens* against plant pathogenic fungi. This study validated the fact that phenol-2, 4—bis (1, 1-dimethylethyl) was a naturally antifungal compound from *Pseudomonas fluorescens* TL-1.

## Materials and Methods

### Isolation of strains

*Nicotiana tabacum* leaves were collected from Lingshan town, Jimo city, Shandong province, China in July 2015 and used for the isolation of epiphytic bacteria. Method for the isolation of bacteria was referred to the literature<sup>35</sup>, but only a little adjustment was done in leaf disc size. The upper and lower side of the fresh leaf disc (28.3 cm<sup>2</sup>) excised from the intact leaf was pressed on a petri dish with LB-agar (peptone 10 g, yeast extract 5 g, agar 15 g, deionized water 1000 mL) containing 50 mg actidione/L (Fluka, Neu-Ulm, Germany), in order to inhibit the growth of eukaryotic microorganisms such as yeasts and fungi. Petri dish was incubated at 28°C for several days to allow the growth of isolated bacteria. Single strains were sub cultivated on LB broth supplemented with 50 mg actidione/L. Stocks of all cultures were maintained at -80°C in LB medium containing actidione and 40% glycerol. Thirty leaf discs from the randomly intact leaf samples were used to isolate the bacteria.

### Assay of antifungal effects of isolated bacteria

The antifungal activities of the isolated bacterial strains against the phytopathogenic fungus were investigated by a dual-culture plate method. The phytopathogenic fungi tested were *Curvularia lunata*, *Bipolaris maydis*, *Valsa mali*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria alternate*, *Fusarium oxysporum* and *Verticillium dahlia* and preserved in key lab, school of public health, Guizhou Medical University. Fungi preserved were grown on PDA (freshly peeled potato 200 g, dextrose 20 g, agar 15 g, deionized water 1000 mL) plate at 28°C for 4 days and then a small amount of mycelia from the advancing margin of plate cultures were transferred to the bilateral of other PDA plates, in the center of which the activated bacterial strains were inoculated concurrently. The plates were incubated at 28°C for 4 days and the clear zones of inhibition appeared as the criterion of antagonism. The bacterial strain of the most broad-spectrum inhibition was preliminarily identified according to the morphological and cultural characteristics, biochemical tests and 16S rRNA gene sequencing.

### Separation of antifungal substance by column chromatography and assay of its inhibitory activity

The antifungal bacterium was grown in fifteen 1000 mL triangular flasks with 600 mL nutrient broth (containing beef extract 3 g/L, peptone 10g/L, NaCl 5 g/L, pH 7.4) each for 96 h at 28°C with constant agitation (120 rpm). The culture supernatant of the antifungal bacterium was obtained by centrifugation at 6000 rpm and 4°C for 20 min and then filtered by filter-sterilizer (aperture 0.22 μM, Millipore). Ulteriorly, the culture supernatant was extracted with ethyl acetate of the equal volume and then lyophilized to powder. 2 mL methanol (chromatographical grade) was added to the lyophilized powder to solubilize the extracts and then the methanol solute was applied to a methanol pre-equilibration glass column (2.5 × 200 cm) packed with Sephadex LH-20 of 190 cm height and eluted with 1000 mL methanol. Approximately at a flow rate of a drop percent second, different 10 mL fractions of chromatography were sequentially collected in the centrifugal tubes and lyophilized in a vacuum drier and further resuspended in 1 mL 50% methanol not affecting the fungal growth for antifungal activity test. The pathogen- *Curvularia lunata* was used as the indicator and the analogous disc diffusion method was adopted<sup>36</sup> to assay the inhibitory activity of each fraction, but with a fresh colony mattress (φ 6 mm) of *Curvularia lunata* placed in the center of PDA plate instead of conidial spreading on PDA plate. Three sterile paper discs (φ 6 mm) of 3 cm distance from the center colony mattress in each PDA plate were loaded 20 μL of different fraction solutions, respectively. 50% methanol was used as a negative control (CK). Three parallel tests were performed for each fraction. A clear inhibition zone formed after incubation of 5 days at 28°C is considered to be inhibited.

### Confirmation of antifungal substance by GC/MS

The methanol solutions (1 mL) filtered with a 0.22 μm filter membrane (Agela Technologies, China) of lyophilized powders of different fractions of chromatography were subjected to GC/MS analysis. GC/MS analysis was performed using Agilent 6890 gas chromatography coupled with an Agilent 5973 mass selective detector and a HP-5 MS (5% Phenyl Methyl Siloxane) capillary column (30 m × 0.25 mm; 0.25 μm film thickness). The chromatographic conditions were as follows: column oven program, 90°C (2 min, isothermal) to 200°C (2 min, isothermal) at 10°C/min, 200 to 280°C (6 min, isothermal) at 4°C/min. The injector and detector temperatures were

280°C and 250°C, respectively. Helium was the carrier gas (flow rate 0.80 mL/min) and the ionization voltage was maintained at 70 eV. The total ion chromatogram obtained was auto integrated by Chemstation and the constituents were identified by comparison with the mass spectral database (NIST and WILEY Library, 2005).

## Results

### Inhibitory effects of isolated strains against phytopathogenic fungi

Forty six bacterial strains were isolated from *Nicotiana tabacum* leaves and their inhibitory effects with dual-culture plate method against ten phytopathogenic fungi, viz. *Curvularia lunata*, *Bipolaris maydis*, *Valsa mali*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria alternate*, *Fusarium oxysporum* and *Verticillium dahlia* showed that only one strain named TL-1 had the most obvious inhibition against all of the above-mentioned phytopathogenic fungi, while the other strains had no (93.5%) or less inhibitory effects on ten phytopathogenic fungi (4.3%).

### Identification of strain TL-1

Strain TL-1 is Gram-negative, rod-shaped (0.4-0.7 × 0.7-2.0 μm) and motile. The colony on LB-agar appears brown, dry, flat and irregular, with lobate margins. The biochemical tests of strain TL-1

showed that positive results were catalase, hydrolysis of urea, Voges-Proskauer, tryptophan deaminase, oxidase, reduction of nitrate to nitrite, hydrolysis of esculin, gelatin liquefaction starch hydrolysis, acid production from fructose; while negative results for indole production, 3-Ketolactose production, malonate utilization, H<sub>2</sub>S production, phenylalanine deaminase, utilization of citrate as a source of carbon, acid production from dulcitol, mannitol, rhamnose, lactose, maltose, arabinose, inulin, dextrin, xylose, sorbitol. The 16S rRNA gene sequence of strain TL-1 had a 99% identity with the type strain of *Pseudomonas fluorescens*, available in the public domain. The phylogenetic tree (Fig. 1) was constructed using 16S rRNA gene sequences and a close cluster was formed between strain TL-1 and the type strain *Pseudomonas fluorescens* (HQ420253.1). According to the morphological and cultural characteristics, biochemical tests and 16S rRNA gene analysis, the bacterial strain TL-1 was identified preliminarily as *Pseudomonas fluorescens*.

### Inhibitory effects of chromatography fractions on *Curvularia lunata*

Approximately 1 g of yellow lyophilized powder was obtained from the extract of ethyl acetate. Fifty-nine fractions were collected, lyophilized and solubilized with 50% methanol for the assay of inhibitory effects with the analogous disc diffusion method. The results showed that only fractions 1<sup>st</sup>-3<sup>rd</sup> had strong inhibitory

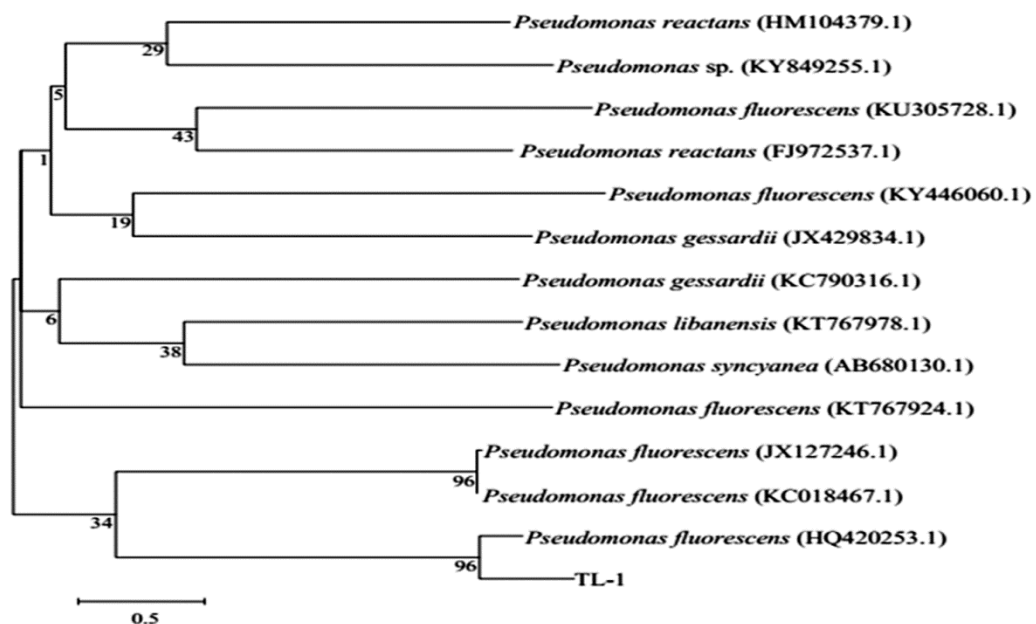


Fig. 1 — Neighbor-joining phylogenetic tree including stains of TL-1 and other related strains downloaded from GenBank based on 16S rDNA sequence (Bootstrap values n = 1000)

effects against *Curvularia lunata* compared with other fractions (Fig. 2A). Therefore, there was a need to achieve the chemical information about the antifungal substance from *Pseudomonas fluorescens* TL-1.

#### Confirmation of antifungal compound from *Pseudomonas fluorescens* TL-1

The metabolites of *Pseudomonas fluorescens* TL-1 were extracted, separated and concentrated and the analytes of all the fractions were then analyzed by GC/MS. The compounds from *Pseudomonas*

*fluorescens* TL-1 in fractions 1<sup>st</sup>-6<sup>th</sup>, 58<sup>th</sup>-59<sup>th</sup> were identified and listed in table 1. Based on the corresponding information of chemical constituent (Table 1) and antifungal activity (Fig. 2B) of different fractions, especially for fraction 3, we draw the conclusion that phenol, 2,4-bis(1, 1-dimethylethyl) was really natural antifungal compound from *Pseudomonas fluorescens* TL-1, while the nearly ubiquitous p-xylene, o-xylene or m-xylene in all of the other fractions ranged from 1.2 to 100% did not display any inhibitory effects

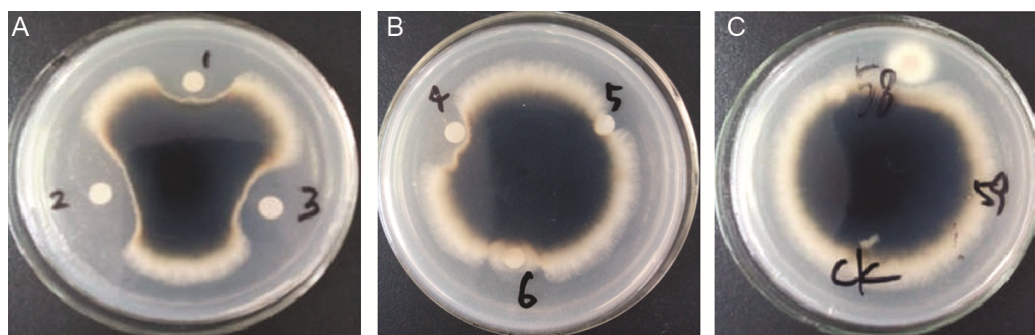


Fig. 2 — (A-C) Test on antifungal activity of fractions against *Curvularia lunata* (Number denoted the sequence of chromatographic solution collected from the beginning (1) to the end (59); CK (control))

Table 1 — Chemical constituents identified in the 1<sup>st</sup>-6<sup>th</sup>, 58<sup>th</sup>-59<sup>th</sup> fractions by GC/MS showing their RT, % of total and quality of compounds

Fraction number	Peak	Retention time	Identified compound	% of total	Quality
1	1	6.281	O-xylene	12.1	97
	2	8.044	Phenol	4.4	96
	3	14.216	Indole	3.3	95
	4	15.210	Phenol,2,4-bis(1, 1-dimethylethyl)	3.8	96
	5	25.714	L-leucyl-D-leucine	52.3	50
	6	25.978	2,5-piperazinedione,3,6-bis(2-methylpropyl)	19.8	43
2	1	6.284	O-xylene	28.9	94
	2	14.463	4-(3,4-dimethoxybenzylidene)-1-(4-nitrophenyl)-3-phenyl-2-pyrazolin-5-one	6.8	42
	3	15.210	Phenol,2,4-bis(1, 1-dimethylethyl)	4.9	87
	4	25.673	L-leucyl-D-leucine	8.0	38
3	1	6.284	P-xylene	14.8	97
	2	15.209	Phenol,2,4-bis(1, 1-dimethylethyl)	2.0	90
4	1	6.284	P-xylene	19.2	94
	2	15.211	Phenol,2,4-bis(1, 1-dimethylethyl)	1.2	87
	3	19.426	Carbamic acid,(4-chlorophenyl)-,1-methylethyl ester	2.9	27
5	1	6.280	O-xylene	14.8	97
	2	19.425	n-(2,7-dioxooctyl)acetamide	3.1	22
	3	25.952	2,5-piperazinedione,3,6-bis(2-methylpropyl)-	3.3	64
	4	35.483	1,2-benzenedicarboxylic acid,mono(2-ethylhexyl)ester	11.7	87
6	1	6.283	O-xylene	6.0	97
	2	25.968	2,5-piperazinedione,3,6-bis(2-methylpropyl)-	8.4	43
	3	35.489	1,2-benzenedicarboxylic acid,mono(2-ethylhexyl)ester	85.6	97
58	1	6.278	P-xylene	21.1	97
59	1	6.280	m-xylene	100	97

on *Curvularia lunata*, such as 21.1% p-xylene in fraction 58 and 100% m-xylene in fraction 59 (Fig. 2C).

### Discussion

Plant pathogenic fungi are major threats to crops and plant production. Compared with the application of agrochemicals for the control of fungal diseases, that of *Pseudomonas* sp. represents another safe and environmentally friendly method for plant protection in agriculture. Nowadays, several commercial products based on various *Pseudomonas* species such as *P. syringae*<sup>37</sup>, *P. chlororaphis*, *P. aureofaciens*<sup>37</sup>, *P. fluorescens*<sup>37</sup> have been marketed as bio fungicides and most of the biocontrol efficacies are attributed to the active metabolites secreted by various *Pseudomonas* species, which include siderophores from *Pseudomonas* sp.<sup>38</sup> and *Pseudomonas fluorescens*<sup>39</sup>; phenazines from *Pseudomonas fluorescens*<sup>40</sup> and *P. chlororaphis*<sup>41,42</sup>; pyoluteorin from *Pseudomonas fluorescens*<sup>43</sup>; pyrrolnitrin from *Pseudomonas fluorescens*<sup>44</sup>; 2-hexyl, 5-propyl resorcinol from *P. chlororaphis*<sup>45</sup>; nunamycin and nunapeptin from *P. fluorescens*<sup>46</sup>; pyocyanin from *Pseudomonas fluorescens*<sup>47</sup>; 2,4-diacetylphloroglucinol (2,4-DAPG) from *Pseudomonas fluorescens*<sup>48</sup>; hydrogen cyanide (HCN) from *Pseudomonas fluorescens*<sup>49</sup> and *Pseudomonas chlororaphis*<sup>50</sup>; gluconic acid from *Pseudomonas* sp.<sup>51</sup>; rhamnolipid from *Pseudomonas aeruginosa*<sup>52</sup>; lipopeptides from *Pseudomonas* sp.<sup>53-56</sup>; piliferolide A from *Pseudomonas brassicacearum*<sup>57</sup>; cellulose and protease from *P. chlororaphis*<sup>58</sup> and so on. Besides its noble antagonistic property and diverse active metabolites against a wide variety of phytopathogenic fungi and bacteria, *Pseudomonas fluorescens* also shows the great potential application as biofertilizer in agriculture<sup>59</sup>. In the present study, *P. fluorescens* TL-1 from tobacco leaves had the strong inhibition against many plant pathogenic fungi and its antifungal substance was confirmed as 2, 4-bis (1, 1-dimethylethyl) with chromatographic separation, disc diffusion and GC/MS analysis. This is the first report of active compound phenol, 2, 4-bis (1, 1-dimethylethyl) deriving from *P. fluorescens* against phytopathogenic fungi.

As a natural compound, phenol, 2, 4-bis (1, 1-dimethylethyl) has been reported to have many functions for medicine, food and agriculture. In medicine, it has the antioxidant<sup>60,61</sup>, anticancer<sup>62</sup>, antifungal<sup>63</sup>, antibacterial<sup>21</sup> properties and the protection against trimethyltin (TMT) -induced cognitive dysfunction<sup>64</sup>. In food, it has been proposed to prevent browning

in fresh apple juices<sup>65</sup> and the growth of *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium chrysogenum* on wheat grains<sup>66</sup>. In agriculture, phenol, 2, 4-bis (1, 1-dimethylethyl) extracted from the rhizome of cogon grass (*Imperata cylindrica*) was found to have allelopathic effects on germination and seedling growth of weedy plants under soilless conditions<sup>67</sup> and it has also been reported as the defense compound of avocado root which prevents the root rot caused by *Phytophthora cinnamomi*<sup>22</sup>. However in this pioneer study of *P. fluorescens* TL-1 producing the antifungal compound 2, 4-bis (1, 1-dimethylethyl), phenol, 2, 4-bis (1, 1-dimethylethyl) was expected to develop the green fungicides for the protection of fungal plant diseases in agriculture.

### Acknowledgment

The authors acknowledge the Natural Science Foundation of Guizhou Province, China (NO. 2013 [2057]) and the First-Class Discipline Construction Project in Guizhou Province-Public Health and Preventive Medicine (NO.2017[85]) for their financial support

### References

- Ekundayo EA, Adetuyi FC & Ekundayo FO, *In vitro* antifungal activities of bacteria associated with maize husks and cobs. *Res J Microbiol*, 6 (2011) 418.
- Reino JL, Guerro RF, Hernández-Galán R & Collado IG, Secondary metabolites from species of the biocontrol agent *Trichoderma*. *Phytochem Rev*, 7 (2008) 89.
- Shen SS, Choi OH, Park SH, Kim CG & Park CS, Root colonizing and biocontrol competency of *Serratia plymuthica* A21-4 against *Phytophthora* blight of pepper. *Plant Pathol J*, 21 (2005) 64.
- Kim CR, Choi SJ, Kim JK, Park CK, Gim MC, Kim YJ, Park GG & Shin DH, 2, 4-Bis (1, 1-dimethylethyl)phenol from *Cinnamomum loureirii* improves cognitive deficit, cholinergic dysfunction and oxidative damage in TMT-treated mice. *Biol Pharm Bull*, 40 (2017) 932.
- Habibi R, Tarighi S, Behravan J, Taheri P, Kjølner AH, Brejnrod A, Madsen JS & Sørensen SJ, Whole-genome sequence of *Pseudomonas fluorescens* EK007-RG4, a promising biocontrol agent against a broad range of bacteria, including the fire blight bacterium *Erwinia amylovora*. *Genome Announc*, 5 (2017) e00026.
- Cabanás CG, Schilirò E, Valverde-Corredor A & Mercado-Blanco J, The biocontrol endophytic bacterium *Pseudomonas fluorescens* PICF7 induces systemic defense responses in aerial tissues upon colonization of olive roots. *Front Microbiol*, 5 (2014) 427.
- Zhang S, Jiang W, Li J, Meng L, Cao X, Hu J, Liu Y, Chen J & Sha C, Whole genome shotgun sequence of *Bacillus amyloliquefaciens* TF28, a biocontrol entophytic bacterium. *Stand Genomic Sci*, 11(2016) 73.

- 8 Jeong J, Park BH, Park H, Chol I & Kim KD, Draft genome sequence of *Chryseobacterium* sp. strain GSE06, a biocontrol endophytic bacterium isolated from cucumber (*Cucumis sativus*). *Genome Announc*, 4 (2016) e00577.
- 9 Qin Y, Fu Y, Kang W, Li H, Gao H, Vitalievitch KS & Liu H, Isolation and identification of a cold-adapted bacterium and its characterization for biocontrol and plant growth-promoting activity. *Ecol Eng*, 105 (2017) 362.
- 10 Fang Y, Wu L, Chen G & Feng G, Complete genome sequence of *Pseudomonas azotoformans* S4, a potential biocontrol bacterium. *J Biotechnol*, 227 (2016) 25.
- 11 Park JY, Han SH, Lee JH, Han YS, Lee YS, Rong X, Gardener BBM, Park HS & Kim Y C, Draft genome sequence of the biocontrol bacterium *Pseudomonas putida* B001, an oligotrophic bacterium that induces systemic resistance to plant diseases. *J Bacteriol*, 193 (2011) 6795.
- 12 Jeong H, Jo SH, Hong CE & Park JM, Genome sequence of the endophytic bacterium *Bacillus thuringiensis* strain KB1, a potential biocontrol agent against phytopathogens. *Genome Announc*, 4 (2016) e00279-16.
- 13 Fang Y, Lin H, Wu L, Ren D, Ye W, Dong G, Zhu L & Guo L, Genome sequence of *Xanthomonas sacchari* R1, a biocontrol bacterium isolated from the rice seed. *J Biotechnol*, 206 (2015) 77.
- 14 Ho YN, Chiang HM, Chao CP, Su CC, Hsu HF, Guo CT, Hsieh JL & Huang CC, In planta biocontrol of soilborne Fusarium wilt of banana through a plant endophytic bacterium, *Burkholderia cenocepacia* 869T2. *Plant Soil*, 387 (2015) 295.
- 15 Gardener BBM, Kim IS, Kim KY & Kim YC, Draft genome sequence of a chitinase-producing biocontrol bacterium, *Lysobacter antibioticus* HS124. *Res Plant Dis*, 20 (2014) 216.
- 16 Xu YB, Chen M, Zhang Y, Wang M, Wang Y, Huang QB, Wang X & Wang G, The phosphotransferase system gene *ptsI* in the endophytic bacterium *Bacillus cereus* is required for biofilm formation, colonization and biocontrol against wheat sharp eyespot. *FEMS Microbiol Lett*, 354 (2014) 142.
- 17 Che J, Liu B, Lin Y, Tang W & Tang J, Draft genome sequence of biocontrol bacterium *Brevibacillus brevis* strain FJAT-0809-GLX. *Genome Announc*, 1 (2013) e00160-13.
- 18 Rong X, Gurel FB, Meulia T & Gardener BBM, Draft genome sequences of the biocontrol bacterium *Mitsuaria* sp. strain H24L5A. *J Bacteriol*, 194 (2012) 734.
- 19 Shanahan P, O'Sullivan DJ, Glennon JD & O'Gara F, Isolation and characterization of an antibiotic-like compound from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. *Appl Environ Microbiol*, 58 (1992)353.
- 20 Nielsen TH, Thrane C, Christophersen C, Anthoni U & Sorensen J, Structure, production, characteristics and fungal antagonism of tensin- a new antifungal cyclic lipopeptide from *Pseudomonas fluorescens* strain 96.578. *J Appl Microbiol*, 89 (2000) 992.
- 21 Abdullah ASH, Mirghani MES & Jamal P, Antibacterial activity of Malaysian mango kernel. *Afr J Biotechnol*, 10 (2011) 18739.
- 22 Rangel-Sánchez G, Castro-Mercado E & García-Pineda E, Avocado roots treated with salicylic acid produce phenol-2, 4-bis(1, 1-dimethylethyl), a compound with antifungal activity. *J Plant Physiol*, 171 (2014) 189.
- 23 Janaki M, Santhi V & Kannagi A, Bioactive potential of *Fusinus nicobaricus* from gulf of mannar. *Int J Pharm Res Bio-Sci*, 4 (2015) 262.
- 24 Yoon MA, Jeong TS, Park DS, Xu MZ, Oh HW, Song KB, Lee WS & Park HY, Antioxidant effects of quinoline alkaloids and 2, 4-Di-tert-butylphenol isolated from *Scolopendra subspinipes*. *Biol Pharm Bull*, 29 (2006) 735.
- 25 Kumar PS, Duraipandiyar V & Lgnacimuthu S, Isolation, screening and partial purification of antimicrobial antibiotics from soil *Streptomyces* sp. SCA7. *Kaohsiung J Med Sci*, 30 (2014) 435.
- 26 Gong AD, Li HP, Shen L, Zhang JB, Wu AB, He WJ, Yuan QS, He JD & Liao YC, The *Shewanella algae* strain YM8 produces volatiles with strong inhibition activity against *Aspergillus* pathogens and aflatoxins. *Front Microbiol*, 6 (2015) 1091.
- 27 Dharni S, Sanchita, Maurya A, Samad A, Srivastava SK, Sharma A & Patra DD, Purification, characterization and *in vitro* activity of 2,4-Di-tert-butylphenol from *Pseudomonas monteilii* PsF84:conformational and molecular docking studies. *J Agr Food Chem*, 62 (2014) 6138.
- 28 Sabu R, Soumya KR & Radhakrishnan EK, Endophytic *Nocardioopsis* sp. from *Zingiber officinale* with both antiphytopathogenic mechanisms and antibiofilm activity against clinical isolates. *3 Biotech*, 7 (2017) 115.
- 29 Gao Z, Zhang B, Liu H, Han J & Zhang Y, Identification of endophytic *Bacillus velezensis* ZSY-1 strain and antifungal activity of its volatile compounds against *Alternaria solani* and *Botrytis cinerea*. *Biol Control*, 105 (2017) 27.
- 30 Padmavathi AR, Abinaya B & Pandian SK, Phenol, 2,4-bis(1, 1-dimethylethyl) of marine bacterial origin inhibits quorum sensing mediated biofilm formation in the uropathogen *Serratia marcescens*. *Biofouling*, 30 (2014) 1111.
- 31 Karthick P & Mohanraju R, Antimicrobial potential of epiphytic bacteria associated with seaweeds of Little Andaman, India. *Front Microbiol*, 9 (2018) 611.
- 32 Pawar R, Mohandass C, Dastager SG, Kolekar YM & Malwankar R, Antioxidative metabolites synthesized by marine pigmented *Vibrio* sp. and its protection on oxidative deterioration of membrane lipids. *Appl Biochem Biotechnol*, 179 (2016) 155.
- 33 Gao H, Li P, Xu X, Zeng Q & Guan W, Research on volatile organic compounds from *Bacillus subtilis* CF-3: biocontrol effects on fruit fungal pathogens and dynamic changes during fermentation. *Front Microbiol*, 9 (2018) 456.
- 34 Azman A-S, Othman I, Fang C-M, Chan K-G, Goh B-H & Lee L-H, Antibacterial, anticancer and neuroprotective activities of rare actinobacteria from mangrove forest soils. *Indian J Microbiol*, 57 (2017) 177.
- 35 Schreiber L, Krimm U, Knoll D, Sayed M, Auling G & Kroppenstedt RM, Plant-microbe interactions: identification of epiphytic bacteria and their ability to alter leaf surface permeability. *New Phytol*, 166 (2005) 589.
- 36 Nweze EI, Mukherjee PK & Ghannoum MA, Agar-based disk diffusion assay for susceptibility testing of dermatophytes. *J Clin Microbiol*, 48 (2010) 3750.
- 37 Stockwell VO & Stack JP, Using *Pseudomonas* spp. for integrated biological control. *Phytopathol*, 97 (2007) 244.
- 38 Rani CU, Priyanka & Rao AS, Antifungal properties exhibited by bacteria isolated from agriculturally cultivable soils and their antagonistic nature towards fungal phytopathogen suppression. *Agric Sci Dig*, 36 (2016) 17.

- 39 Solanki MK, Singh RK, Srivastava S, Kumar S, Kashyap PL, Srivastava AK & Arora DK, Isolation and characterization of siderophore producing antagonistic rhizobacteria against *Rhizoctonia solani*. *J Basic Microb*, 54 (2014) 585.
- 40 Thomashow LS & Weller DM, Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. *J Bacteriol*, 170 (1988) 3499.
- 41 Liu H, He Y, Jiang H, Peng H, Huang X, Zhang X, Thomashow LS & Xu Y, Characterization of a phenazine-producing strain *Pseudomonas chlororaphis* GP72 with broad-spectrum antifungal activity from green pepper rhizosphere. *Curr Microbiol*, 54 (2007) 302.
- 42 Jain R & Pandey A, A phenazine-1-carboxylic acid producing polyextremophilic *Pseudomonas chlororaphis* (MCC2693) strain, isolated from mountain ecosystem, possesses biocontrol and plant growth promotion abilities. *Microbiol Res*, 190 (2016) 63.
- 43 Giralda M, Perotto S, Moenne-Loccoz Y, Bergero R, Lazzari A, Defago G, Bonfante P & Luppi AM, Impact of biocontrol *Pseudomonas fluorescens* CHA0 and a genetically modified derivative on the diversity of culturable fungi in the cucumber rhizosphere. *Appl Environ Microbiol*, 67 (2001) 1851.
- 44 Kirner S, Hammer PE, Hill DS, Altmann A, Fischer I, Weislo LJ, Lanahan M, Heinz van Pee K & Ligon JM, Functions encoded by pyrrolnitrin biosynthetic genes from *Pseudomonas fluorescens*. *J Bacteriol*, 180 (1998) 1939.
- 45 Calderón CE, Ramos C, de Vicente A & Cazorla FM, Comparative genomic analysis of *Pseudomonas chlororaphis* PCL1606 reveals new insight into antifungal compounds involved in biocontrol. *Mol Plant Microbe Interact*, 28 (2015) 249.
- 46 Michelsen CF, Watrous J, Glaring MA, Kersten R, Koyama N, Dorrestein PC & Stougaard P, Nonribosomal peptides, key biocontrol components for *Pseudomonas fluorescens* In5, isolated from a greenlandic suppressive soil. *MBio*, 6 (2015) e00079-15.
- 47 Dahiya JS, Woods DL & Tewari JP, Control of *Rhizoctonia solani*, casual agent of brown girdling root rot of rapeseed, by *Pseudomonas fluorescens*. *Bot Bull Acad Sinica*, 29 (1988) 135.
- 48 Kwak YS, Bonsall RF, Okubara PA, Paulitz TC, Thomashow LS & Weller DM, Factors impacting the activity of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* against take-all of wheat. *Soil Biol Biochem*, 54 (2012) 48.
- 49 Voisard C, Keel C, Haas D & Defago G, Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. *EMBO J*, 8 (1989) 351.
- 50 Nandi M, Selin C, Brawerman G, Fernando WGD & de Kievit T, Hydrogen cyanide, which contributes to *Pseudomonas chlororaphis* strain PA23 biocontrol, is upregulated in the presence of glycine. *Biol Control*, 108 (2017) 47.
- 51 Kaur R, Macleod J, Foley W & Nayudu M, Gluconic acid: an antifungal agent produced by *Pseudomonas* species in biological control of take-all. *Phytochem*, 67 (2006) 595.
- 52 Borah SN, Goswami D, Lahkar J, Sarma HK, Khan MR & Deka S, Rhamnolipid produced by *Pseudomonas aeruginosa* SS14 causes complete suppression of wilt by *Fusarium oxysporum* f. sp. *pisi* in *Pisum sativum*. *Biol Control*, 60 (2015) 375.
- 53 Nielsen TH, Sorensen D, Tobiassen C, Andersen JB, Christophersen C, Givskov MC & Sorensen J, Antibiotic and biosurfactant properties of cyclic lipopeptides produced by fluorescent *Pseudomonas* spp. from the sugar beet rhizosphere. *Appl Environ Microbiol*, 68 (2002) 3416.
- 54 Tran H, Ficke A, Asiimwe T, Höfte M & Raaijmakers JM, Role of the cyclic lipopeptide massetolide A in biological control of *Phytophthora infestans* and in colonization of tomato plants by *Pseudomonas fluorescens*. *New Phytol*, 175 (2007) 731.
- 55 Zachow C, Jahanshah G, de Bruijn I, Song C, Ianni F, Pataj Z, Gerhardt H, Pianet I, Laemmerhofer M, Berg G, Gross H & Raaijmakers JM, The novel lipopeptide poaeamide of the endophyte *Pseudomonas poae* RE\*1-1-14 is involved in pathogen suppression and root colonization. *Mol Plant Microbe Interact*, 28 (2015) 800.
- 56 Ma Z, Geudens N, Kieu NP, Sinnaeve D, Ongena M, Martins JC, Hofte M, Plasencia J & Riely BK, Biosynthesis, chemical structure and structure-activity relationship of orfamide lipopeptides produced by *Pseudomonas protegens* and related species. *Front Microbiol*, 7 (2016) 382.
- 57 Andersson PF, Levenfors J & Broberg A, Metabolites from *Pseudomonas brassicacearum* with activity against the pink snow mould causing pathogen *Microdochium nivale*. *Biol Control*, 57 (2012) 463.
- 58 Alström S, Characteristics of bacteria from oilseed rape in relation to their biocontrol activity against *Verticillium dahliae*. *J Phytopathol*, 149 (2001) 57.
- 59 Saxena J, Saini A, Kushwaha K & Ariño A, Synergistic effect of plant growth promoting bacterium *Pseudomonas fluorescens* and phosphate solubilizing fungus *Aspergillus awamori* for growth enhancement of chickpea. *Indian J Biochem Biophys*, 53 (2016) 135.
- 60 Choi Y & Lee J, Antioxidant and antiproliferative properties of a tocotrienol-rich fraction from grape seeds. *Food Chem*, 14 (2009) 1386.
- 61 Kadoma Y, Ito S, Atsumi T & Fujisawa S, Mechanisms of cytotoxicity of 2- or 2, 6-di-tert-butylphenols and 2-methoxyphenols in terms of inhibition rate constant and a theoretical parameter. *Chemosphere*, 74 (2009) 626.
- 62 Malek SNA, Shin SK, Wahab NA & Yaacob H, Cytotoxic components of *Pereskia bleo* (Kunth) DC. (Cactaceae) leaves. *Molecules*, 14 (2009) 1713.
- 63 Zhou BL, Chen ZX, Du L, Xie YH, Zhang Q & Ye XL, Allelopathy of root exudates from different resistant eggplants to *Verticillium dahliae* and the identification of allelochemicals. *Afr J Biotechnol*, 10 (2011) 8284.
- 64 Kim YH, Park SK, Hur JY & Kim YC, Purification and characterization of a major extracellular chitinase from a biocontrol bacterium, *Paenibacillus elgii* HOA73. *Plant Pathol J*, 33 (2017) 318.
- 65 Suh HJ, Park S & Park S, Inhibition of browning on fresh apple juices by natural phytochemicals from *Rumex crispus* L. seed. *J Korean Soc Appl Biol Chem*, 54 (2011) 524.
- 66 Varsha KK, Devendra L, Shilpa G, Priya S, Pandey A & Nampootheri KM, 2, 4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated *Lactococcus* sp. *Int J Food Microbiol*, 211 (2015) 44.
- 67 Zhang XH, Zhang EH & Lang DY, Autotoxic compounds from rhizosphere soil of *Humulus lupulus* L. extracts: identification and biological activity. *Agron J*, 103 (2011) 695.