Increasing the Scale of Peroxidase Production by *Streptomyces* sp. strain BSII#1

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Abstract

Aims: To optimise peroxidase production by *Streptomyces* sp. strain BSII#1, up to 3 l culture volumes.

Methods and Results: Peroxidase production by *Streptomyces* sp. strain BSII#1 was optimised in terms of production temperature and pH and the use of lignin-based model chemical inducers. The highest peroxidase activity (1.30±0.04 U ml^{-1}) in 10 ml culture volume was achieved in a complex production medium (pH 8) at 37°C in the presence of 0.1 mmol l^{-1} veratryl alcohol, which was greater than those reported previously. Scale-up to 100

ml and 400 ml culture volumes resulted in decreased peroxidase production (0.53±0.10 U ml^{-1} and 0.26±0.08 U ml^{-1} respectively). However, increased aeration improved peroxidase production with the highest production achieved using an airlift bioreactor (4.76±0.46 U ml^{-1} in 3 l culture volume).

Conclusions: Veratryl alcohol (0.1 mmol l^{-1}) is an effective inducer of peroxidase production by *Streptomyces* sp. strain BSII#1. However, improved aeration increased peroxidase production in larger volumes without the use of an inducer, surpassing induced yields in an optimised small-scale process.

Significance and Impact of the Study: Only a limited number of reports in literature have focused on the up-scaling of bacterial peroxidase production. There remains opportunity for feasible large-scale production of bacterial peroxidases with potentially novel biocatalytic properties.

Keywords:

peroxidase, actinomycete, biocatalysis

Introduction

Peroxidases (E.C. 1.11.1.x) are versatile oxidoreductase enzymes capable of oxidising a broad range of organic substrates utilising peroxide as the electron acceptor. Different types of peroxidases occur in virtually all life-forms, from prokaryotes to higher organisms including fungi, plants and animals where they perform diverse physiological functions. In plants for example, peroxidases occur as distinctive isoenzymes which can be constitutively expressed or induced in response to external factors such as wounding, stress and attack by pathogens (Veitch 2004). In nature, white rot fungi produce various isoforms of extracellular oxidases including manganese peroxidase (MnP) and lignin peroxidases (LiP) (Wesenberg *et*

al. 2003); several reports document successful attempts to increase peroxidase yields in both native and recombinant fungal strains (Conesa *et al*. 2000; Ikehata *et al*. 2004; Tsukihara *et al*. 2006; Urek and Pazarlioglu 2007).

Peroxidases are considered high-utility enzymes as they function via a non-specific, free-radical catalysed mechanism (Burton 2003). They are capable of oxidising several classes of compounds with high chemical and structural diversity, and therefore have great potential for use in diverse applications. Peroxidases have found wide commercial application in analytical and diagnostic kits, where horseradish peroxidase (HRP) is the most commonly used enzyme. Due to the ability of peroxidase to yield chromogenic products in low concentrations with good stability, it is well-suited for the preparation of enzyme conjugated antibodies and application in diagnostic kits (Krell 1991). Similarly, peroxidases are also used as key components of biosensors and immunoassays (Valderrama *et al*. 2002).

Other applications include uses in Kraft pulp bleaching, the degradation of xenobiotics and production of pharmaceuticals, fine chemicals and optically and biologically active compounds (Valderrama *et al.* 2002). However, the utility of peroxidases is limited due to a relatively high production cost (Ferrer *et al.* 1991; Torres *et al.* 2003) and innate instability in the presence of elevated concentrations of hydrogen peroxide (de Velde *et al.* 2001; Valderrama *et al.* 2002).

Actinomycetes comprise a large and diverse group of filamentous bacteria, many of which have important ecological roles and are exploited commercially for the production of antibiotics, enzymes and other natural products (Cook and Meyer, 2000; Waksman *et al.* 2010). They represent a potential source of peroxidase for a market that is substantial and currently dominated by HRP (Mercer *et al.* 1996). Actinomycetes produce peroxidases and other oxidative enzymes which are key for the degradation of dead organic matter and the recycling of nutrients (Mason *et al.* 2001). The production and purification of peroxidases

from actinomycetes is advantageous for several reasons. Actinomycetes secrete extracellular peroxidases (Mason *et al*. 2001), which implies that the downstream purification and recovery of the enzyme may be simplified. In contrast, the recovery of peroxidase from horseradish roots for example, is a lengthy and complex procedure involving multiple precipitation, fractionation, chromatography and filtration steps, with the whole process taking days to weeks, depending on the grade of the enzyme preparation (Barnard, A., 2012). In addition, the process is inefficient and only a few grams of the highest grade peroxidase are produced from 1 tonne of roots (Barnard, A., 2012).

Iqbal *et al.* (1994) studied the characteristics of peroxidases obtained from a thermophilic actinomycete, *Streptomyces thermoviolaceus.* One isoform, designated P-3, was stable at 50°C for more than 24 h and had a half-life of 70 min at 70°C. The authors suggested potential applications similar to those employing HRP. The peroxidase from *Thermomonospora fusca* BD25 has been described as a novel non-haem peroxidase showing activity at elevated temperature (50% maximum activity retained between 50 and 80°C) and pH (6.0 – 8.0) and a high stability against denaturing agents (Svistunenko *et al.* 1999). Antonopoulos and co-workers (2001) showed the suitability of extracellular peroxidase produced by *Streptomyces albus* ATCC 3005 for application in the bleaching of Kraft pulps. Advantages to the process included: (*i*) short retention times within which significant kappa number reductions occurred, (*ii*) high peroxidase activity that could be applied directly in alkaline kraft pulps, (*iii*) stability of the peroxidase with high H_2O_2 concentrations, and (*iv*) no addition of costly mediators was required (Antonopoulos *et al.* 2001). Niladevi and Prema (2005) reported a newly-isolated mangrove actinomycete, *Streptomyces psammoticus* (strain NJP49), capable of producing all three major ligninolytic enzymes (LiP, laccase and MnP). Van Bloois *et al.* (2009) characterised an extracellular haeme-containing peroxidase from

Thermobifida fusca, which was not only capable of decolourising dyes, but also catalysed enantioselective sulfoxidations.

While it is clear that actinomycetes represent a natural reserve of untapped peroxidase potential that can be exploited for biotechnological applications, there remains limited information available regarding the feasible production of actinomycete-derived peroxidases for use as an industrial biocatalyst. A previous screening programme in our research group identified *Streptomyces* sp. strain BSII#1 as a high peroxidase-producing isolate. In this study, production, on a small scale, was optimised from *Streptomyces* sp. strain BSII#1. The production scale was increased at ambient temperature and without the presence of inducers to determine if a simple large-scale bioprocess could achieve production yields similar to an optimised small-scale process.

Materials and Methods

Microorganisms and culture maintenance

Streptomyces sp. strain BSII#1 was isolated from sediment from the Bwanda hotsprings, Lochinvar National Park, Zambia. The soil samples were collected by Prof. Don Cowan and Drs Mark Taylor, Moola Mutondo and Heide Goodman on 6 June 2008. The optimal standard production medium used in this study was modified from a phenoxazinone production medium (Graf *et al*. 2007) and contained (per litre): 10 g glycerol, 10 g glucose, 10 g soy flour, 5 g yeast extract, 5 g casein hydrolysate, 4 g calcium carbonate and 1 ml trace salts solution (per litre: 1 g $FeSO_4.7H_20$; 0.9 g $ZnSO_4$; 0.2 g $MnSO_4.7H_2O$). Stock cultures were prepared in modified phenoxazinone production medium (pH 8.0) and preserved in 20% glycerol at -20°C. The stock cultures were routinely sub-cultured every 4-6 weeks. For routine inoculations the strains were first grown on starch-casein-nitrate/yeast extract

(SCN/YE) agar (per litre: 10 g starch; 0.3 g casein; 2 g KNO_3; 0.05 g $MgSO_4.7H_2O$; 0.3 g $CaCO_3$; 0.01 g $FeSO_4.7H_20$; 0.01 g yeast extract; 20 g agar and 1 ml trace salts solution) (same composition as trace salts solution used for preparation of the modified phenoxazinone production medium) for at least 5 days at 30°C. Spore/hyphae suspensions prepared in 5 ml sterile distilled water were used for further inoculations.

Peroxidase Production and Crude Extracts

Spore/hyphae suspensions (500 µl) were used to inoculate 10 ml of modified phenoxazinone medium incubated at 30°C for two days. This served as a pre-culture for subsequent inoculation in all the experimental flasks (10, 100 and 400 ml culture volumes). The inoculum size was 5% (v/v) in the initial small scale optimisation experiments in a 10 ml culture volume and 10% (v/v) for baffled flasks and the larger scale bioreactors. Flasks were incubated at the previously established optimum temperature for peroxidase production by *Streptomyces* sp. strain BSII#1 (37°C) with shaking at 160 rpm. To design a bioprocess with minimal energy input requirement, the scaled up bubble column bioreactor and airlift bioreactor experiments were performed at ambient temperature (approximately 25±2°C) without any inducers. The cultures were centrifuged at 10 000 g for 5 min at room temperature and the decanted supernatant served as the crude extracellular extract.

Peroxidase Assay

Peroxidase activity was measured using the 2,4-dichlorophenol (2,4-DCP) assay (Antonopoulos *et al*. 2001). The reaction mix contained equal volumes of 100 mmol l^{-1} Tris-HCl buffer (pH 8.0), 16 mmol l^{-1} 4-aminoantipyrine, 50 mmol l^{-1} H_2O_2, 25 mmol l^{-1} 2,4-DCP and the enzyme extract. The reaction was initiated by the addition of H_2O_2 and the increase in A_{510} monitored for 5 min on an Anthos Zenyth 1100 microtiter plate reader. One unit (U) of peroxidase activity was defined as the amount of enzyme required for the

oxidation of 1 µmol of substrate (2,4-DCP) in 1 min. In all cases, dilute commercial HRP (0.002mg/ml) was used as a positive control and water was used as a negative control. All experiments also had uninoculated medium as further negative controls (i.e. containing all the medium components for that experiment).

Small-scale Optimisation Studies

Small-scale optimisation experiments were carried out in 10 ml culture volume, inoculated with 500 µl of two-day old *Streptomyces* sp. strain BSII#1 pre-cultures.

Optimal temperature: Peroxidase production was assessed when *Streptomyces* sp. strain BSII#1 was grown at room temperature, 30°C, 37°C and 45°C in modified phenoxazinone production medium (pH 7).

Optimal initial pH: The effect of the initial medium pH on peroxidase production by *Streptomyces* sp. strain BSII#1 in modified phenoxazinone production medium was assessed over the pH range 5.0-10.0 and at the noted temperature of optimal peroxidase production.

Effect of lignin model compounds: Streptomyces sp. strain BSII#1 was grown in modified phenoxazinone production medium at the optimum temperature (37°C) and initial pH (pH 8) of peroxidase production and in the presence of the following chemical inducers: veratric acid, anisaldehyde, veratryl alcohol, pyrogallol or guaiacol (each at 0.1 mmol l^{-1}, 0.5 mmol l^{-1} and 1 mmol l^{-1} concentration). The effect of indulin (0.01%, 0.05% and 0.1%, w/v) on the production of peroxidase was also assessed.

Effect of natural lignocellulosic compounds: The effect of different natural lignocellulose-containing materials on peroxidase production by *Streptomyces* sp. strain BSII #1 was assessed. These materials included grape seeds, sorghum malt, banana peel, orange peel, wheat bran and toothpick shavings. The materials were added to the modified phenoxazinone medium (pH 8) at 1% (w/v) concentrations (unless stated otherwise). The effect of sugarcane bagasse and wheat bran (0.5%, 1% and 1.5%, w/v) was also assessed.

Scale-up of peroxidase production: Erlenmeyer vs Baffled flasks

Peroxidase production was assessed in 100 ml and 400 ml culture volumes. The effects of flat-bottomed (Erlenmeyer) vs baffled flasks on peroxidase production by *Streptomyces* sp. strain BSII #1 in modified phenoxazinone medium (pH 8) were assessed.

Bubble Column Bioreactor

Two-day old pre-cultures were used to inoculate 350 ml of modified phenoxazinone production medium (pH 8; 10% (v/v) inoculum) in 1 l bubble reactor vessels. Air input was facilitated by the use of fish tank air pumps set at maximum feed rate (Regent air pump; Model 9500; aeration capacity range 0.3-1.4 l min^{-1}). pH was not monitored or maintained during the incubation.

Airlift Bioreactor

Two-day old pre-cultures served as inoculum to seed 3 l modified phenoxazinone production medium (pH 8; 10% (v/v) inoculum). Air input was facilitated via the use of an air compressor, and the aeration rate was gradually increased from 2 l hr^{-1} to 18 l hr^{-1} as fermentation proceeded to ensure adequate mixing and aeration of the culture. Peroxidase activity was assessed at 12 h intervals. pH was not monitored or maintained during the incubation.

Statistical Analyses

Univariate analysis of variance followed by a Bonferroni post hoc test were used to determine significance of results. P-values ≤ 0.05 were defined as significant.

Results

Small-scale Optimization studies

Temperature

At each of the different temperatures tested, maximum peroxidase production typically occurred on either day 4 or day 5 (Figure 1). Maximum peroxidase production (0.48±0.06 U ml^{-1}) was observed when *Streptomyces* sp. strain BSII#1 was incubated at 37°C (Figure 1). No observable growth of the microorganism occurred at 45°C.

pH

Maximum peroxidase production (0.59±0.01 U ml^{-1}) was observed on day 5 at initial pH 8.0 and 37°C (Figure 2). The most extreme pH values tested (pH 5.0 and pH 10.0) resulted in the lowest peroxidase production. On day 5, there was no significant difference in peroxidase production at pH 7, 8 or 9 ($p>0.05$).

Effect of lignin model compounds

The inclusion of 0.1 mmol l^{-1} veratryl alcohol or 0.1 mmol l^{-1} pyrogallol in the growth medium both resulted in similar, significant increases in peroxidase production on day 5 (1.30±0.04 U ml^{-1} and 1.26±0.06 U ml^{-1}, respectively, $p<0.01$) (Figure 3). Increasing the concentration of either inducer to 0.5 mmol l^{-1} did not improve peroxidase production and a further increase in inducer concentration to 1 mmol l^{-1} resulted in complete inhibition of the growth of *Streptomyces* sp. strain BSII#1 and no peroxidase activity was detected. Indulin was also tested as a potential inducer of peroxidase production at low concentrations. However, at all concentrations tested (0.01-0.1% indulin) there was no significant inductive effect ($p>0.05$) when compared to the control on the day of maximum peroxidase production.

Effect of natural lignocellulosic compounds

Aside from wheat bran, the natural lignocellulose-containing materials tested (grape seeds, sorghum powder, orange peel, banana peel, sugar cane bagasse and birchwood shavings) showed zero to no significant induction ($p>0.05$) of peroxidase production in *Streptomyces* sp. strain BSII#1 when compared to the control on the day of maximum peroxidase production (data not shown). When considering the day of maximum peroxidase production (day 4 for no wheat bran and day 6 for all concentrations of wheat bran tested), there was only a significant improvement in peroxidase production in the presence of 1.5% (w/v) wheat bran ($p<0.05$; Figure 4).

Scale-up

Upon scale-up to 100 ml culture volume in flat-bottomed Erlenmeyer flasks (no inducer), it was noted that there was a significant decrease ($p<0.05$) in maximum peroxidase production, from 0.83±0.06 U ml^{-1} in 10 ml culture volumes to 0.068±0.004 U ml^{-1} in 100 ml culture volumes (i.e. >90% decrease in maximum production). However, maximum production was significantly increased ($p<0.05$) in 100 ml culture volume when baffled flasks were used for all inoculum sizes tested (Figure 5). The highest peroxidase activity (0.53±0.1 U ml^{-1}) was observed using a 10% (v/v) inoculum and baffled flasks after 4 days incubation. Further scale-up to 400 ml culture volume in a baffled flask resulted in both a decrease in the maximum peroxidase production (0.26±0.08 U ml^{-1}) and a delay in retention time required for maximum peroxidase production (6 days compared to 4 days; Figure 6).

Bubble Column Bioreactor

As aeration appeared to improve or restore maximum peroxidase production by Streptomyces sp. strain BSII#1 on scale-up, a bubble column reactor (using fish tank pumps for aeration) was used to further improve peroxidase production in larger culture volumes. Aeration

through bubbling was an effective method of enhancing peroxidase production in 500 ml culture volumes (up to 2.10±0.56 U ml^{-1} on day 7; Figure 6). While the peroxidase concentration continued increasing, it was not feasible to continue the process for longer than 7 days as the culture medium became thick and viscous which resulted in a decrease in the volume of crude extracellular fraction that could be successfully extracted after centrifugation.

Airlift Bioreactor

A further scale increase was achieved using an airlift bioreactor format. On this scale, with improved aeration rates due to the use of compressed air, the retention time for high peroxidase production was dramatically shortened (to within 36 h of inoculation; Figure 7). Furthermore, the peroxidase production continued to increase up to 4.76±0.46 U ml^{-1} at 60 h. At this point, cultures were halted as the biomass growth was excessive (50 mg dry wt/ml culture) and any further retention in the reactor resulted in reduced yields in terms of the volume of crude extract that could be removed after centrifugation.

Discussion

In this study we report the production of relatively high amounts of peroxidase by *Streptomyces* sp. strain BSII#1 as compared to most studies in the literature. In 10 ml culture volume *Streptomyces* sp. strain BSII#1 produced 1.30±0.04 U ml^{-1} peroxidase in the presence of 0.1 mM veratryl alcohol on the fifth day of incubation (Figure 3). The majority of reports on actinomycete-based peroxidase production indicate enzyme activities that are generally less than 1 U ml^{-1} (Iqbal *et al.* 1994; Bon *et al.* 1999; Tuncer *et al.* 1999; Macedo *et al.* 1999; Zerbini *et al.* 1999; Antonopoulos *et al.* 2001; Tuncer *et al.* 2009). Induction by veratryl alcohol in the production of lignolytic peroxidases has been reported in several white rot

fungi including *Phanerochaete chrysosporium*, *Bjakandera* sp. and *Phlebia radiata* (Niku-Paavola *et al.* 1990; Mester *et al.* 1995; Couto *et al.* 1999), but has not been shown for an actinomycete.

Studies reporting on the effect of lignin model chemical inducers on lignolytic enzyme production have been based mainly on laccase induction. In a report on laccase production by *Streptomyces psammoticus*, 1 mmol l^{-1} pyrogallol and 1 mmol l^{-1} para-anisidine enhanced enzyme production by 50% (Niladevi and Prema, 2007). Dekker *et al.* (2001) reported the induction of laccase activity above constitutive levels in *Botryosphaeria* sp. by veratryl alcohol in basal media containing softwood kraft lignin. In our findings, 0.1 mmol l^{-1} veratryl alcohol effectively induced peroxidase production, but higher concentrations suppressed production due to growth inhibition. Biomass growth was completely arrested at 1 mmol l^{-1} concentration and no peroxidase production occurred. Similar trends where increased concentrations of lignin-model compounds inhibited growth, were observed with veratric acid, pyrogallol, anisaldehyde and guaiacol.

The continuous production and secretion of peroxidase into the medium is a growth-associated process in actinomycetes (Tuncer *et al.*, 1999; Ramachandra *et al.* 1988; Niladevi and Prema, 2007). Factors that affect the growth of the microorganism in culture, such as temperature and initial pH of incubation, oxygenation rate as well as medium components will therefore also have a bearing on peroxidase production. Typically, actinomycetes are terrigenous microorganisms and grow well within the mesophilic temperature range (McCarthy, 1987). *Streptomyces* sp. strain BSII#1 was isolated from a hot spring locality and peroxidase production occurred at 37°C. However, the growth of *Streptomyces* sp. strain BSII#1, and hence peroxidase production, was inhibited at 45°C. The scale-up experiments were carried out at room temperature (approximately 25±2°C) with the objective of designing a simpler system with minimal production costs, without compromising enzyme yields.

Maximum peroxidase production occurred at alkaline initial medium pH (pH 8.0), and this is in agreement with other reports on actinomycete-based peroxidase production (Tuncer *et al.* 1999).

Identifying feasible enzyme production protocols is an essential prerequisite for improving the cost-effectiveness of biotechnological applications. Soil actinomycetes are recognized lignin-degrading microorganisms, and because their primary substrate in soil is inevitably plant biomass (Godden *et al.* 1992), it was thought that lignocellulose-containing residues could stimulate enzyme production by the organism. The use of inexpensive growth substrates, such as agri-industrial residues, enables the simultaneous removal of unwanted materials from the environment and value addition. As such, the potential of a variety of agri-industrial residues such as wheat bran, banana and orange peel, to stimulate peroxidase production by *Streptomyces* sp. strain BSII#1 was investigated. Kapich *et al*. (2004) suggested that some compounds derived from lignocellulosic substrates may function as inducers for the production of ligninolytic peroxidases. The selected lignin model compounds used in this study were selected on the same basis.

Of the natural materials tested, wheat bran appeared to be the most promising candidate for peroxidase induction in *Streptomyces* sp. strain BSII#1, with peroxidase activity reaching 0.94±0.1 U ml^{-1} on day 6 of cultivation (Figure 4). While the addition of 1.5% (w/v) wheat bran increased peroxidase production (78.4% improvement), it also increased the time required for maximum peroxidase production from 4 days (without wheat bran) to 6 days (Figure 4). Wheat bran is an important and readily-available by-product of the cereal industry, which makes it quite attractive as a raw material feedstock for peroxidase production. While wheat bran produced the highest induction (78.4% compared to 55.2% and 51.3% for 0.1 mmol l^{-1} veratryl alcohol and 0.1 mmol l^{-1} pyrogallol, respectively) albeit at the cost of a longer retention time, it was an impractical addition for the scale-up process. This

was due to the fact that, in practice on a larger scale, the wheat bran adhered to the sides of the vessels (above the liquid level) and did not have an effect on peroxidase production. The remaining natural lignocellulosic substrates tested showed no significant induction of peroxidase activity. The finding that that there was no appreciable induction of peroxidase production in the presence of indulin lends further support to previous reports that indulin is not a good growth substrate for actinomycetes (Godden *et al.* 1992; Giroux *et al.* 1988).

In aerobic fermentation processes, oxygen transfer is commonly a rate-determining step for cell growth or product formation because of the low solubility of oxygen in the medium (Garcia-Ochoa and Gomez, 2009). Scale up to 100 ml culture volume in flat-bottomed Erlenmeyer flasks resulted in significant loss of enzyme production (over 90%). This was attributed to depressed aeration rates in the Erlenmeyer flasks as the use of baffled flasks, which improve aeration efficiency, restored activity to a large extent (Figure 5). In addition, comparison of the 100 ml and 400 ml culture volumes in baffled flasks shaking at the same speed (culture volume was 20% of flask volume) indicated better enzyme production in the smaller vessel where aeration was greater. The extent of growth observed in the 400 ml culture volumes was reduced compared to that typically achieved with 10 ml and 100 ml culture volumes. The poorer growth in the larger culture volume was attributed to oxygen transfer limitations, resulting in reduced peroxidase production.

The bubble column bioreactor was used to improve the aeration efficiency and resulted in relatively high production of peroxidase (2.10±0.56 U ml^{-1}; Figure 6) as compared to the highest activity recorded in literature (0.9 U ml^{-1}; Iqbal *et al* 1994). Oxygen Transfer Rate (OTR) is a frequently employed parameter for quantifying the physiological state of an aerobic culture (Suresh *et al*. 2011). Shake flask culture systems depend on agitation of the vessel for oxygen transfer into the growth medium. Bubble column bioreactors, on the other hand, introduce oxygen directly into the growth medium. Improved aeration facilitated by

direct introduction of air into the medium in a bubble column bioreactor resulted in improved growth of the aerobic *Streptomyces* sp. strain BSII#1 and hence peroxidase production increased.

Enzyme production was highest in the airlift bioreactor (4.76±0.46 U ml^{-1}; Figure 7). The design of the airlift bioreactor, which incorporates a central draft tube, enabled more efficient mixing and oxygenation of the culture in the medium. As the airlift bioreactor system does not rely on shaking or agitation effected by baffles or impellers, minimal shearing occurs. The gentler mixing and improved aeration enabled faster growth of *Streptomyces* sp. strain BSII#1, and greater enzyme production over a shorter time period.

The enhanced biomass growth associated with increased peroxidase production may be problematic on larger scales in batch culture, as increased biomass growth reduces the volume of crude extracellular extract that is recovered. However, a continuous process may be useful to minimise this problem and should be investigated.

As with most bioprocesses, it was found that a linear scale-up of peroxidase production could not be achieved. An incremental scale-up was successful in identifying the key factor for restoring biomass growth, thereby enhancing peroxidase production by *Streptomyces* sp. strain BSII#1 on a larger scale without the use of inducers: aeration. This study shows, to the authors' knowledge, the highest peroxidase production by an actinomycete in an airlift bioreactor.

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Conflict of Interest

No conflict of interest declared.

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Figure 7: Peroxidase production by *Streptomyces* sp. strain BSII#1 in 3 l culture volume in an airlift bioreactor, pH 8.0, room temperature (mean±SEM; n=2).

Figures

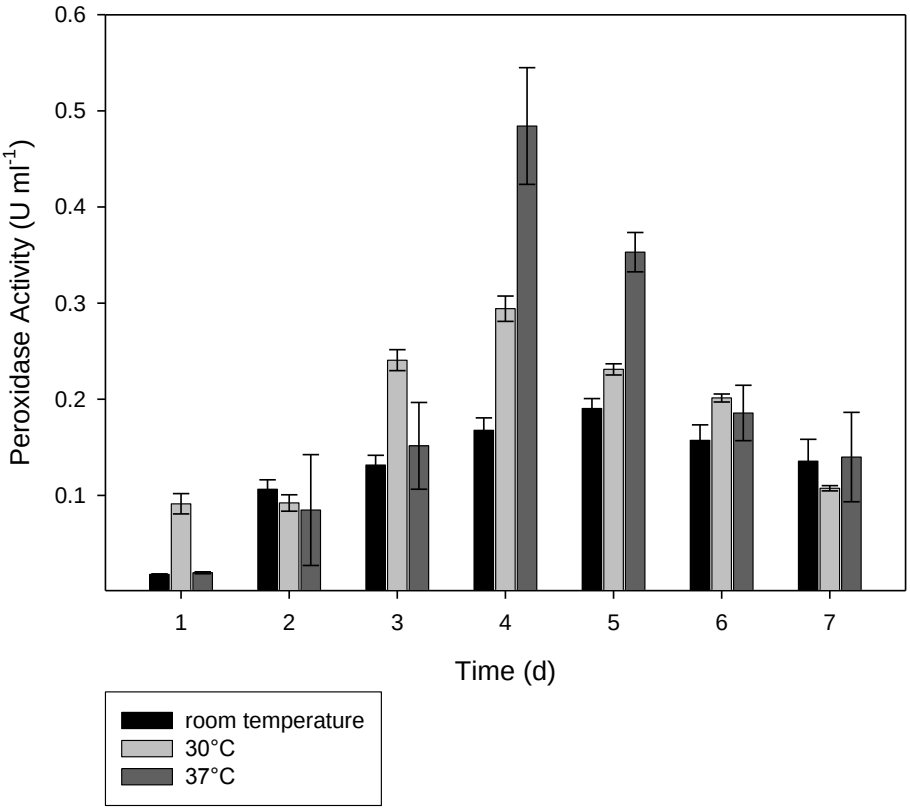

Figure 1

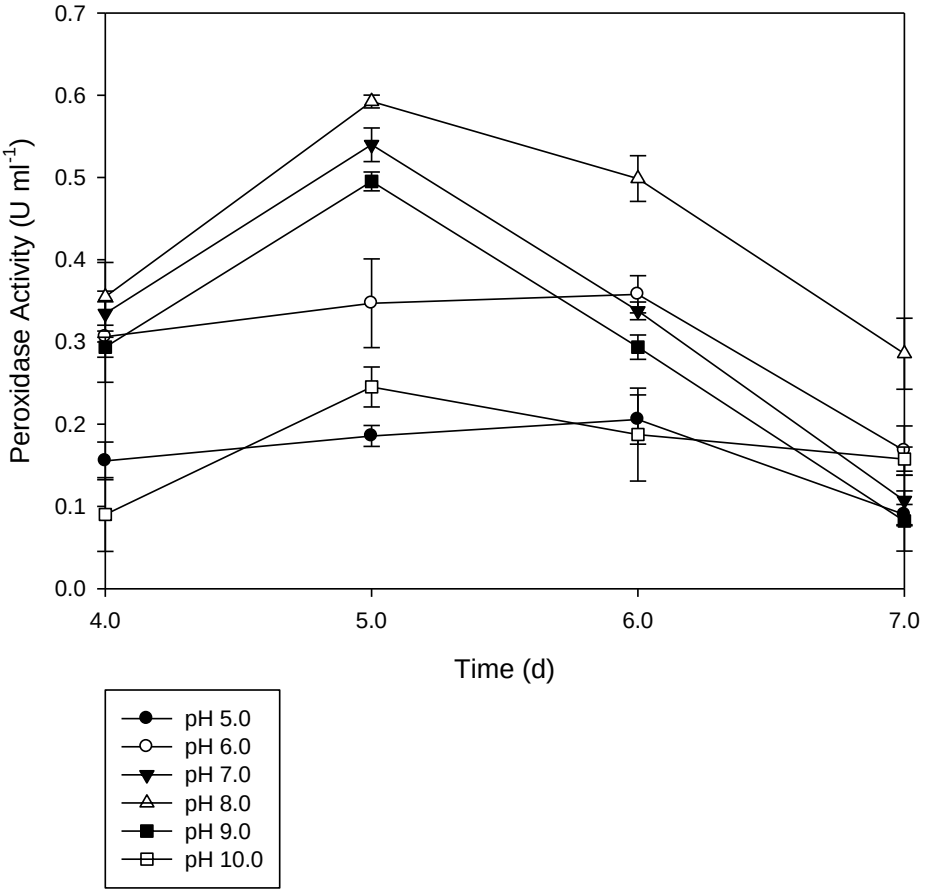
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Peroxidase Activity (U ml-1)
4.0
5.0
6.0
7.0
Time (d)
pH 5.0
pH 6.0
pH 7.0
pH 8.0
pH 9.0
pH 10.0

Figure 2

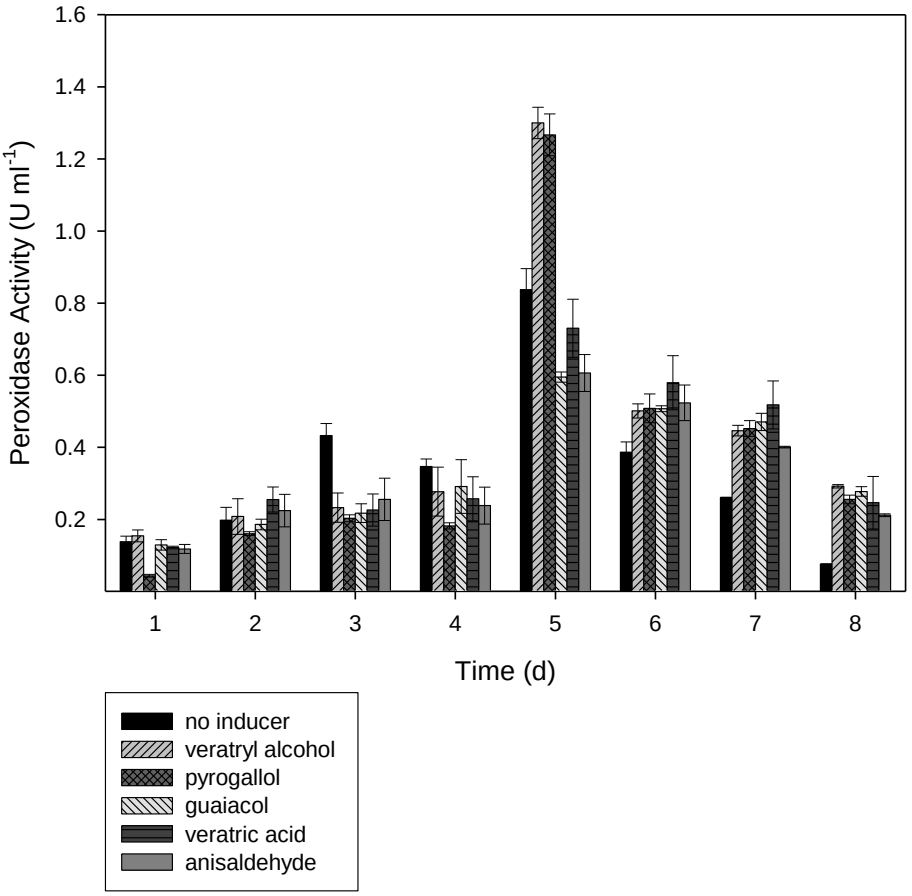
Peroxidase Activity (U ml-1)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
1
2
3
4
5
6
7
8
Time (d)
no inducer
veratryl alcohol
pyrogallol
guaiacol
veratric acid
anisaldehyde

Figure 3

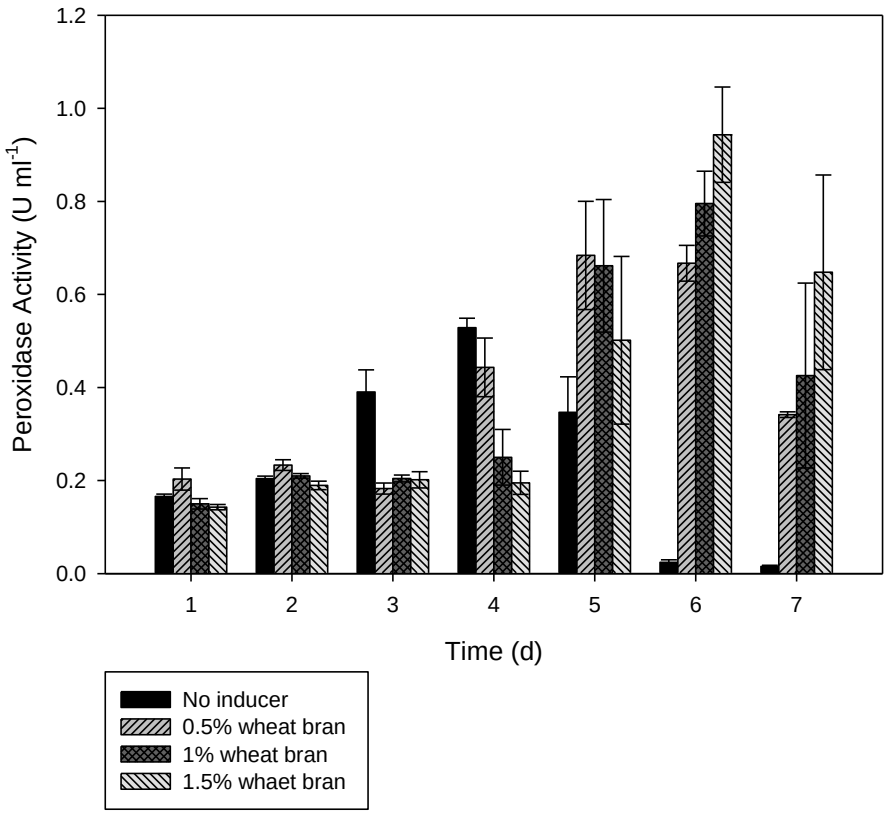
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Peroxidase Activity (U ml-1)
1
2
3
4
5
6
7
Time (d)
No inducer
0.5% wheat bran
1% wheat bran
1.5% whaet bran

Figure 4

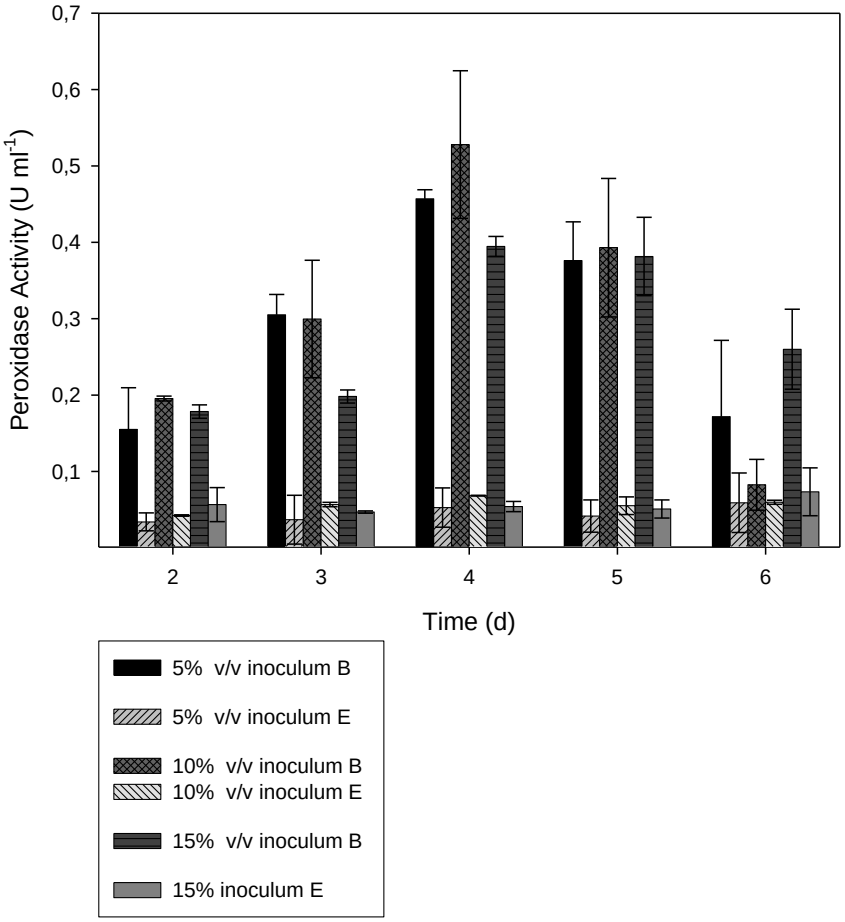
0,7
0,6
0,5
0,4
0,3
0,2
0,1
Peroxidase Activity (U ml-1)
2
3
4
5
6
Time (d)
5% v/v inoculum B
5% v/v inoculum E
10% v/v inoculum B
10% v/v inoculum E
15% v/v inoculum B
15% inoculum E

Figure 5

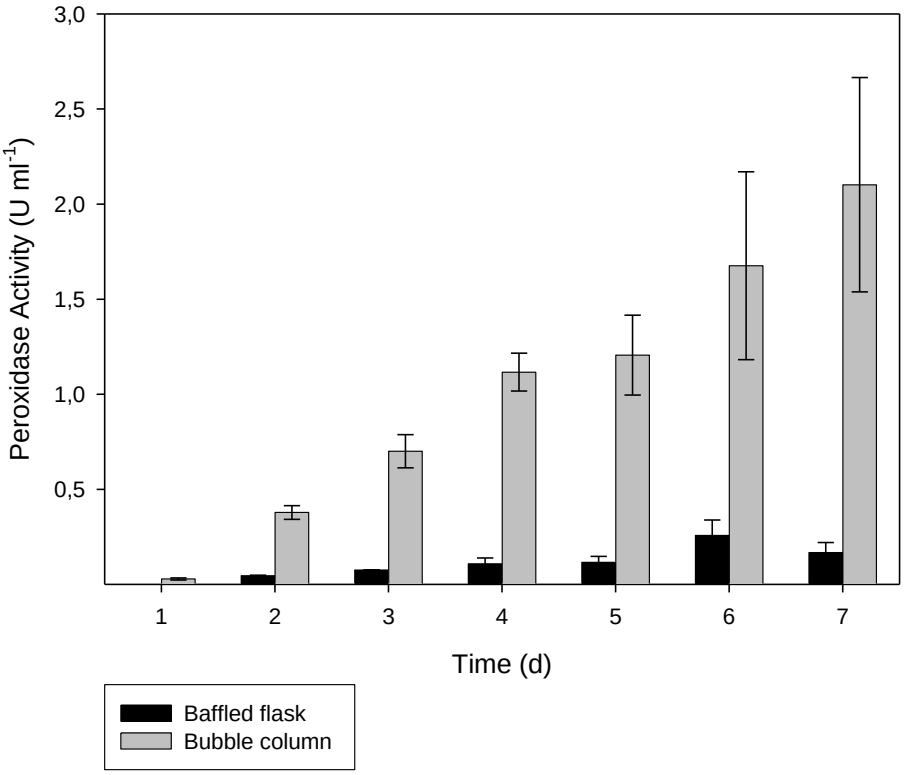
3,0
2,5
2,0
1,5
1,0
0,5
Peroxidase Activity (U ml-1)
1
2
3
4
5
6
7
Time (d)
Baffled flask
Bubble column

Figure 6

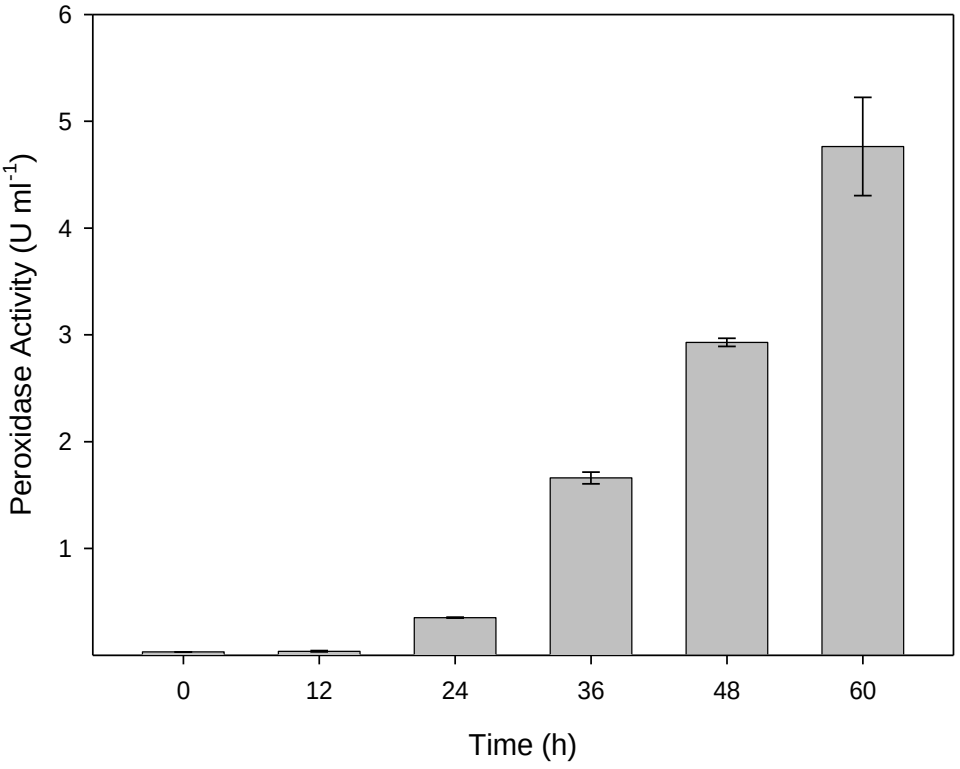

Figure 7