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ORIGINAL ARTICLE

Quorum sensing involvement in response surface methodology for optimisation of sclerotiorin production by *Penicillium sclerotiorum* in shaken flasks and bioreactors

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Abstract

Purpose Sclerotiorin, an azaphilone produced by some filamentous fungi including *Penicillium sclerotiorum*, is a pigment with variety of biological activities including lipoxygenase inhibition, reduction of cholesterol levels, and anti-cancer properties. Sclerotiorin has potential use in pharmaceutical as well as food industries. In this context, the purpose of this study was to provide a simple and robust procedure for optimised production of sclerotiorin by *P. sclerotiorum* using a central composite design developed through response surface methodology (RSM) and to identify the molecule(s) involved in the signalling mechanism in *P. sclerotiorum*.

Methods The optimisation of sclerotiorin production was carried out using RSM in shaken flasks and the obtained results were then replicated using a 2-L stirred tank bioreactor. *Penicillium sclerotiorum* ethyl acetate culture extract was analysed using thin layer chromatography (TLC) and potential signalling molecules were identified using Gas chromatography-mass spectrometry (GC-MS).

Results The experimental studies suggested an increase in the sclerotiorin production by 2.1-fold and 2.2-fold in shaken flasks and stirred tank bioreactors respectively. Further analysis of *P. sclerotiorum* ethyl acetate culture extract reported the presence of ricinoleic acid, an oxylipin, belonging to a family of signalling molecules tentatively involved in the enhancement of sclerotiorin production.

Conclusion This paper has highlighted the positive effect of the optimal supplementation of *P. sclerotiorum* culture extracts for enhanced production of sclerotiorin. It has also examined potential molecules involved in the signalling mechanism in *P. sclerotiorum* culture extract for the overproduction of sclerotiorin.

Keywords Oxylipin signalling · Quorum sensing · *Penicillium sclerotiorum* · Response surface methodology · Ricinoleic acid · Sclerotiorin

Introduction

Natural and biologically active compounds have been studied for decades for their potential roles in various industries; including pharmaceutical, food, agricultural, and cosmetic industries. Amongst the filamentous fungi, *Penicillium* is one of the most studied genera, particularly due its ability to produce several valuable products such as statins, polyketides, and penicillins (Keller et al. 2005).

Penicillium sclerotiorum is studied as a source of sclerotiorin, a chlorinated azaphilone, with strong orange colour (Maccurin and Reilly 1940). Azaphilones are pigment molecules, classified under a subset of polyketides, a class of secondary metabolites that is characterised by a highly oxygenated bicyclic core containing a chiral quaternary centre (Turner 1971; Gill and Steglich 1987; Osmanova et al. 2010). The biosynthesis of sclerotiorin occurs via acetate-malonate pathway involving type I polyketide synthases (PKS), a family of multi-domain enzyme complexes (Holker et al. 1964).

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Azaphilones are renowned for a wide range of pharmaceutical applications as a cytotoxic, anti-inflammatory, anti-viral, antioxidant, and antimicrobial agents (Osmanova et al. 2010). In addition to its antibacterial, antifungal (Chidananda et al. 2006; Lucas et al. 2007), and anti-cancer activities (Giridharan et al. 2012), sclerotiorin also acts as an endothelin receptor binding molecule (Pairet et al. 1995), as well as it inhibits Grb-2-Sch interaction (Nam et al. 1998), gp120-CD4 binding (Matsuzaki et al. 1995), cholesteryl ester transfer protein (Barter and Rye 1994), and the activity of lipase (Lucas et al. 2007), aldose reductase (Kador 1988), soybean lipoxygenase (LOX-I) (Chidananda and Sattur 2007), and monamine oxidase (Fujimoto 1990).

Although there is industrial interest for sclerotiorin production, little efforts have been made at developing an optimised procedure for large-scale industrial production. The low natural yield of sclerotiorin acts as a limiting factor for further developments at an industrial level. Therefore, this study focuses on overcoming the yield limitation and aims to set an optimised and scalable fermentation procedure for improved production. Previous studies at our laboratory by Raina et al. (2010) reported a quorum sensing-like behaviour in *P. sclerotiorum* culture and indicated its ability to enhance the production of the secondary metabolite, sclerotiorin. This stimulated an interest to pursue further research to address the objective in detail.

Here, we report optimal conditions for improvement of the overall sclerotiorin yield. Experiments were conducted using culture extracts collected at different time points of *P. sclerotiorum* fermentation. The optimal concentration and time of addition were statistically calculated using RSM. ANOVA analysis was also performed to evaluate the significance of the effects of concentration and time of addition of the culture extracts. The optimisation experiments were performed in shaken flasks to validate the robustness of the experiments' design. The small-scale experiments were then scaled-up to 2-L fermenters to confirm the process feasibility under controlled conditions of bioreactors. Sclerotiorin concentration was quantified using high performance liquid chromatography (HPLC). Thin layer chromatography and gas chromatography-mass spectrometry (GC-MS) were used to determine the constituents of the culture extracts. The results reported in this study were obtained as part of research investigating quorum sensing in *P. sclerotiorum* (Amache 2014).

Materials and methods

Microbial strain maintenance

P. sclerotiorum IMI 104602 was obtained from CABI Biosciences (Surrey, UK). The *P. sclerotiorum* stock cultures were maintained on modified Czapek-Dox agar slopes prepared by adding the following: sucrose (30 g/l), sodium nitrate (2 g/l), potassium chloride (0.5 g/l), magnesium glycerol-phosphate (0.5 g/l), potassium sulphate (0.35 g/L), ferrous sulphate (0.01 g/l), and agar (12 g/l). The slopes were grown at 28°C for 8 days for generation of spores and subsequently stored at 4 °C.

Medium and growth conditions

Penicillium sclerotiorum was grown in 500-ml shaken flasks using 100 ml of Raulin-Thom medium (Gudgeon et al. 1979; Raina et al. 2010) where 1 ml of 10^7 spores/ml was used for the inoculation of *P. sclerotiorum* growth medium prior to incubation at 27 °C at a speed of 150 rpm. For sclerotiorin production in shaken flasks, 1 mL of *P. sclerotiorum* spore suspension was used to inoculate 100 mL of potato dextrose broth (PDB) which was then incubated at 27 °C at a speed of 220 rpm for 8 days.

Preparation of crude extract

An ethyl acetate extract containing putative signalling molecules was prepared from *P. sclerotiorum* culture grown in Raulin-Thom medium. *Penicillium sclerotiorum* culture was filtered using Whatmann no.1 filter paper via a Buchner filter pump, and then extracted three times with an equal volume of ethyl acetate using a separatory funnel until the organic phase was colourless. The aqueous solution was discarded, whereas the organic phase was concentrated in a Rotavapor RE-120 (Büchi). The resulting pellet was re-suspended in 1/50th of the initial volume of ethyl acetate to obtain 50-fold concentrated culture extracts which were then stored at 4 °C for future use.

Response surface methodology

RSM was then used to find out the optimal concentration and time of addition of the chosen ethyl acetate extract. The two variables examined were concentration (% v/v) of the added ethyl acetate extracts (X1, %) and the time of addition (X2, Days), while the two monitored responses were the weight of the concentration of biomass (R1, g/l) and sclerotiorin yield (R2, mg/g cell dry weight (CDW)). A two-factor central composite design (CCD) was constructed for 28 runs which included 4 centre points, 4 replicated axial star points, and 2 replicated factorial points the Design Expert Software as shown in Table 1.

Secondary metabolite production

The optimal conditions calculated using RSM were used for liquid culture analysis: shaken flasks and bioreactor studies. The cell dry weight of the cultures was analysed at the end of the fermentation. The conditions were first applied in shaken

Table 1 Response surface methodology experimental design showing the code and levels of the two tested variables

			Levels		$\alpha = 0.5$	
Factors	Code	− 1	0	1	− α	+α
Concentration/percentage of added extract %(vol/vol)	A	0.0375	0.075	0.1125	0	0.15
Time of addition	B	1.5	3	4.5	0	6

flask experiments, and once positive results were achieved, scaling-up of the conditions to 2-L stirred tank bioreactors was performed to investigate the feasibility of scale-up.

Shaken flask studies

For the shaken flask studies, the *P. sclerotiorum* culture extract dissolved in ethyl acetate to a final volume of 200 μl was added to 100 mL of potato dextrose broth inoculated with *P. sclerotiorum* spore suspension, prior to incubation at 27 °C and 220 rpm. The concentration of extracts and the addition time were calculated using RSM. The sclerotiorin yield was analysed from the 5th to 8th day of fermentation.

Bioreactor analysis

Bioreactor studies were performed using two of 2-L stirred tank reactors (STR-Electrolab Ltd, UK) containing 1.5 L PDB medium inoculated with 1% of *P. sclerotiorum* spore suspension. A repeat of the optimal conditions used for shaken flask analysis was applied for the bioreactor studies. The internal diameter of the bioreactor was 10 cm, the shaft length was 20 cm, and two Rushton turbine impellers were placed h/2 distance apart inside the bioreactor, where height of the liquid is represented as *h*. The stirring speed, air flow rate, and temperature were programmed at 220 rpm, 1.0 vvm, and 27 °C respectively. The dissolved oxygen tension (% DOT), pH, and temperature were monitored throughout the course of fermentation. The sclerotiorin yield was also assayed from the 5th to 8th day of fermentation.

Detection and quantification of sclerotiorin

Sclerotiorin production was quantified as per Raina et al. (2010), where 0.4 g freeze-dried *P. sclerotiorum* mycelia were extracted using 60-fold (v/w) HPLC-grade methanol. The resultant filtrate was analysed using the HPLC system (Dionex). The mobile phase consisted of acetonitrile:water at a ratio of 65:35 (v/v), the flow rate was 1 mL/min, injection volume was 20 μL, and peak detection was performed at 370 nm. Sclerotiorin standards ranging from 0–100 μg/mL concentration was prepared to generate a standard curve.

Thin layer chromatography

Thin layer chromatography (TLC) was performed to find out the components of the ethyl acetate extract and to understand the variation in ethyl acetate extract's constituents collected at different time points throughout fermentation (Fig. 1). Analytical and preparative TLC were performed using 0.2-mm silica gel plates (60 Å) and fluorescent indicator (200 × 200 mm, Merck Kieselgel).

For analytical TLC, samples of the ethyl acetate extract were spotted on the TLC plates, then placed in the development chamber for optimal separation of the molecules using hexane:ethyl acetate at a ratio of 6:4 as the mobile phase. Upon development; the separated compounds on the silica gel plates were visualised as dark spots under ultraviolet light (254 nm) in a fluorescent green background. For preparative TLC, the separated compounds were scraped off their corresponding silica and transferred to clean glass beakers. The compounds were extracted from the silica gel with 5 mL of the developing solvent (hexane:ethyl acetate 6:4). The silica was removed by filtration using glass wool, and the collected solvent was evaporated under nitrogen gas (N_2). The pellets were stored at 4 °C for later analysis using gas chromatography-mass spectrometry (GC-MS).

Gas chromatography coupled mass spectrometry

The 200 μl resultant solution acquired from preparative TLC was mixed with 20 μl of 0.5 mg/ml heptadecanoic acid, and was dried by evaporating under nitrogen, prior to treatment with 50 μl each of acetonitrile and BSTFA (N,O-bis-(trimethylsilyl)-trifluoroacetamide) containing 1% trimethyl chlorosilane (v/v). The prepared solution was heated for 30 min at 80 °C. GC-MS equipped with bench-top HP-5973B MSD quadrupole (Hewlett Packard) connected to HP-6890 series GC system (Hewlett Packard) was used for analysis. HP-7683 series auto-sampler (Hewlett Packard) was used for injecting 1.0 μl samples. Helium was used as the carrier gas and the flow rate was set to 1 ml/min. The injection port was operated either in split less or split mode (10%). DB-5MS + DG 30 m × 0.25 mm I.D. × 0.25 μm column (J&W Scientific) with 10-m Duro-Guard inserted into the ion source was

used. The oven temperature and rate were programmed from 50 to 290 °C at 10 °C per minute respectively and the final temperature was held for 6 min. The ionisation energy for operating the mass spectrometer was set at 70 eV, scanning over a mass range (m/z) of 10-600. G1701BA Enhanced ChemStation software version B.01.00 (Hewlett Packard) was used for data collection.

Results

Sclerotiorin production

Raina et al. (2010) reported that the exogenous addition of an ethyl acetate extract from *P. sclerotiorum* at 48-h growth resulted in enhanced sclerotiorin yield of 8.5 mg/g at 168-h post inoculation; this is a 1.8-fold increase compared with control (with no addition of crude ethyl acetate extract). In this study, RSM was used as a method to find out the optimal concentration and time of addition of the chosen ethyl acetate extract as a method to further enhance the overall production of sclerotiorin.

Response surface methodology

A two-factor central composite design (CCD) was constructed consisting of 28 runs, including 4 centre points, 4 replicates of axial star points, and 2 replicates of factorial points. The design variables were the percentage of culture extract concentration added (X1, %) and the addition time (X2, days), whereas the two monitored responses were the cell dry weight (R1, g/l) and sclerotiorin yield (R2, mg/g cell dry weight). The sclerotiorin yield, quantified via HPLC, and the cell dry weight were measured on the 8th day of the course of fermentation.

The 3D response surface and the 2D contour plots for the two responses (sclerotiorin production and the cell dry weight) are the graphical representations of the regression equation. Both plots are represented in Fig. 2.

The main goal of response surface is to detect the optimum values of the two tested variables such that the response is maximised. Each plot depicts the interaction of the two variables and represents an infinitive number of their combinations. Based on the obtained results, it was found that the addition of high cell density extracts has a limited or no impact on the cell dry weight. However, sclerotiorin production increases with the increasing concentrations of the added culture extract. Point prediction of the design expert software was used to determine the optimum values of the factors for maximum sclerotiorin production as it is not possible to analyse the response surface plots simultaneously. The optimum values (with a desirability of 1.00) for maximum production was a concentration of 0.15% v/v crude extract to be added after 2.06 days. The predicted optimum values for the extract concentration and its addition time were validated in shaken flask studies and taken to scale-up in a 2-L stirred tank reactor.

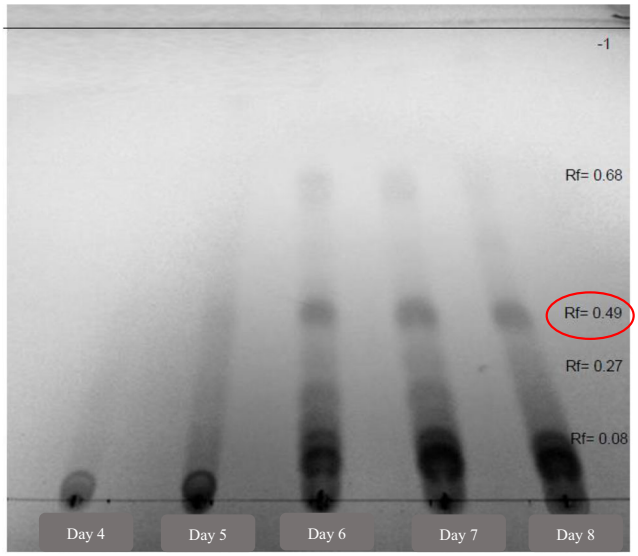

Fig. 1 Thin layer chromatography analysis of *P. sclerotiorum* crude ethyl acetate extracts using hexane:ethyl acetate (6:4). Analysis of ethyl acetate extracts collected at different times of the fermentation; days 4 to 8

Statistical analysis

The optimal concentration and time of addition were statistically calculated using RSM. ANOVA analysis was also performed to evaluate the significance of the effects of concentration and time of addition of the culture extracts. The results obtained from RSM methodology clearly pointed out the significance of the chosen variables with respect to the secondary metabolite formation. The calculations from the quadratic regression model of sclerotiorin production were highly significant with low probability values from Frischer's F test with P model > F = 0.0001. The percentage of coefficient of variation (CV %) values were considerably low measuring 10.77 for sclerotiorin production, indicating a precise and reliable experiment.

The ANOVA of quadratic regression model (Table 2) demonstrates that the model is not significant, also evident from the Fisher's F test with a probability value [($P_{model} > F$) = 0.1176)]. Furthermore, the low value of the coefficient of variation (CV % = 6.33) indicates precision and reliability of the experiment.

Furthermore, the ANOVA of quadratic regression model (Table 2) demonstrates that the model is highly significant, as evident from the Fisher's F test with a very low probability value [($P_{model} > F$) = 0.0001)]. The low value of the coefficient of variation (CV % = 10.77) indicates precision and reliability of the experiment.

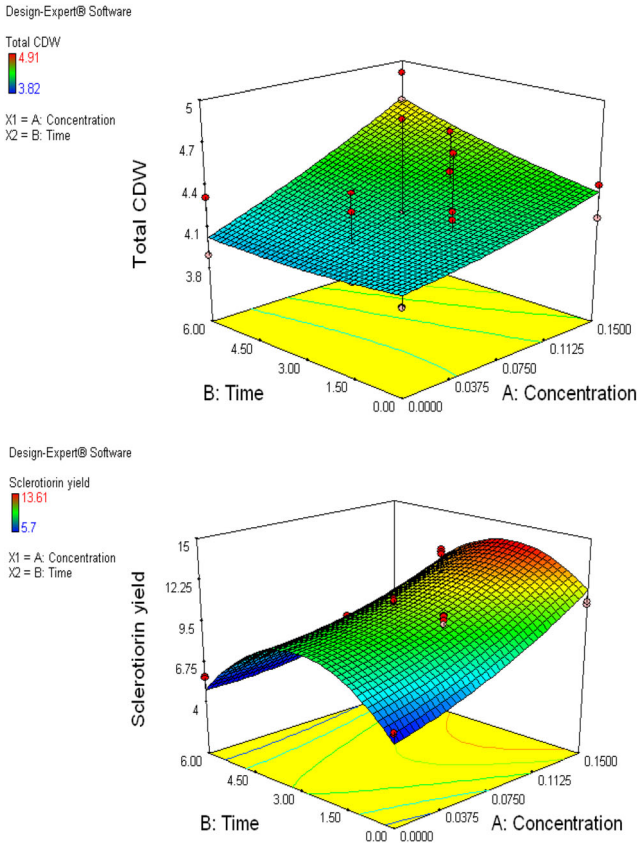

Fig. 2 Response surface plot (3D) depicting the regression equations calculated using RSM showing the effect of the high cell density culture extract on *P. sclerotiorum*. The effect of two factors concentration of culture extract and its time of addition were investigated on cell dry weight (CDW g/L) and sclerotiorin yield (mg/g of cell CDW) in shaken flasks

Sclerotiorin production in shaken flasks

The validation of calculated optimal values (concentration 0.15% (v/v) and 2.06 days) as per RSM was initially done using shaken flask experiments. The secondary metabolite production was examined throughout the incubation period, to analyse the impact of the calculated optimal values on *P. sclerotiorum*. A 2.1-fold increase in sclerotiorin production was noticed in the shaken flask studies with a measured value of 13.89 mg/g CDW (Fig. 3). A proportional increment was observed between the concentration of added extracts and sclerotiorin production.

Sclerotiorin production in bioreactors

Bioreactor studies were then conducted in order to examine whether the results obtained from the shaken flasks are scalable. The bioreactor studies used the RSM optimal conditions previously tested in shaken flasks. The upscaling experiment was performed to validate the results obtained from the shaken flask studies where a 2.2-fold increment in the secondary metabolite production (sclerotiorin) was observed (Fig. 4). The results provide an opportunity for large-scale production of sclerotiorin, which is crucial for various industrial applications.

Thin layer chromatography and GC-MS

The TLC analysis of the culture extracts resulted in the separation of the different components as indicated with the several RF spots on the TLC plate. Components with Rf of 0.49 were only available at a high cell density (corresponding to days 5, 6, 7, and 8 of *P. sclerotiorum* culture). Analysis of whole extracts, as well as compounds with Rf of 0.49, indicated the presence of several compounds which are represented in Table 3. Some of the identified compounds were found to be ricinoleic acid, which is an oxylipin catalysed by the enzymes, dioxygenase and linoleate-diol synthase, mostly known for oxylipin production. The acquired chromatogram is presented in the Fig. 5. Based on the obtained results, it is suggested that sclerotiorin production in *P. sclerotiorum* can be influenced by the presence of oxylipins in addition to the compounds previously reported by Raina et al. (2010).

Discussion

Over the years, substantial evidence emphasising the role of lactones and oxylipins as signalling molecules has been reported. The role of lactone-containing compounds including acyl-homoserine lactone, gamma-heptalactone, and butyrolactone I in bacteria was reported in a review by

Table 2 ANOVA for response surface quadratic model

(i) Sclerotiorin Production					
Cell dry weight					
Source	SS	DF	MS	*F* value	Prob (*P*) > *F*
Model	0.73	5	0.15	2.01	0.1176 (not significant)
Residual (error)	1.6	22	0.073		
Lack of fit	0.27	3	0.09	1.28	0.3095 (not significant)
Pure error	1.33	19	0.07		
Total	2.34	27			
(ii) Sclerotiorin Production					
Source	SS	DF	MS	*F* value	Prob (*P*) > *F*
Model	126.22	5	25.24	23.72	< 0.0001 (significant)
Residual (error)	23.41	22	1.06		
Lack of fit	22.88	3	7.63	271.00	< 0.0001 (significant)
Pure error	0.53	19	0.028		
Total	149.63	27			

Cell dry weight: R^2 = 0.3131; CV = 6.33%; *SS* sum of squares, *DF* degrees of freedom, *MS* mean square; Adj R^2 = 0.1570

Sclerotiorin production: R^2 = 0.844; CV = 10.77%; *SS* sum of squares, *DF* degrees of freedom, *MS* mean square; Adj R^2 = 0.808

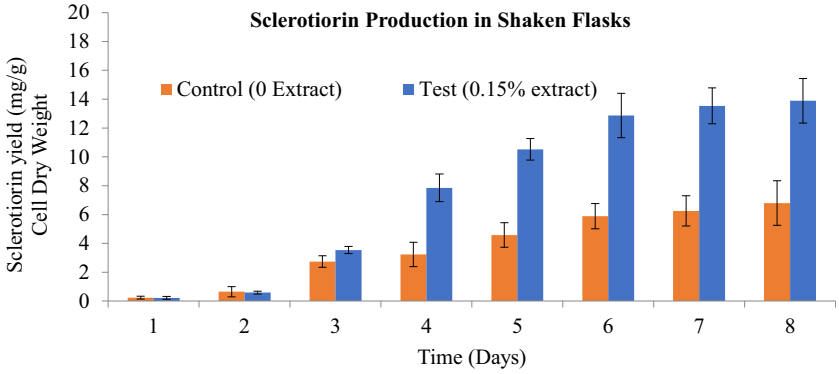

Fig. 3 Graphical representation of sclerotiorin production in shaken-flask analysis using optimised conditions calculated via RSM (0.15% v/v concentration at 2.06 days)

Safari et al. (2014). Secondary metabolism has been also found to be regulated by quorum sensing in filamentous fungi. For instance, the exogenous addition of butyrolactone I enhanced production of the secondary metabolite lovastatin by threefolds and that of sulochrin by twofolds (Schimmel et al. 1998). Similarly, Raina et al. (2012) reported the role of butyrolactone I as a tentative quorum sensing molecule in the filamentous fungus, *Aspergillus terreus*, implicated in an increase in lovastatin yield by 2.5-folds.

Sclerotiorin production increased proportionally with the concentration of the added extract; however, the optimum addition time, as determined by RSM, was around day 2 of the fermentation. In fact, sclerotiorin production increased by 2.1-folds when the extract was added at 0.113% (v/v) on day 3 as compared with the control culture; whereas it had no impact on sclerotiorin production when added at a later stage of the fermentation (day 6). The results can be observed from the 3D plot in Fig. 2. Therefore, it is speculated that the addition of extract containing putative quorum sensing molecules, at early stage of fermentation, trigger changes to the secondary metabolite production pathways, including the induction of sclerotiorin production.

The obtained results are also comparable with those examples where the established quorum sensing molecules were exogenously added to filamentous bacterium, *Streptomyces* spp. For instance, the exogenous addition of the autoinducer γ-butyrolactone A-factor to *S. griseus* cultures resulted in earlier biosynthesis and increased production of streptomycin (Horinouchi and Beppu 1994; Takano et al. 2000). Similarly, the addition of the autoregulator virginiae butanolide C to *S. virginiae* resulted in 2.5-fold increase in the production levels of the secondary metabolites viginamycin M and S compared with the control that had no virginiae butanolide C added (Yang et al. 1996). Moreover, it was also found that the secondary metabolite pristinamycin is regulated by the A-factor in the filamentous bacteria *S. pristinaespiralis* (Paquet et al. 1992).

Additionally, the exogenous addition of γ-heptalactone, an endogenously produced signalling molecule in *Aspergillus nidulans*, was extracted from the supernatant of *A. nidulans* at high cell density induced a coordinated response in *A. nidulans* culture including the enhanced production of the secondary metabolite penicillin (Williams et al. 2012).

Furthermore, the increase in sclerotiorin production in the 2-L bioreactor confirms that the results obtained in shaken flasks are scalable; it thus opens an opportunity for utilisation of culture extract as a method for enhanced industrial production of this metabolite. Similarly, Williams (2009) reported that partially purified supernatant extracts from *A. nidulans* enhanced the production of the secondary metabolite penicillin in 2-L and 20-L bioreactors in a similar manner to 500-mL shaken flasks.

In this paper, we have reported biosynthesis of another product, ricinoleic acid by *P. sclerotiorum*. Ricinoleic acid is synthesised by the oxylipins producing enzymes, fungal dioxygenase, and linoleate-diol synthase (Su and Oliw

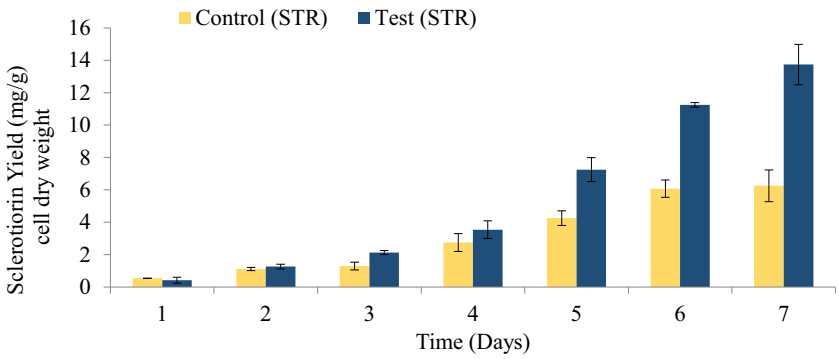

Fig. 4 Impact of RSM-optimised conditions (0.15% concentration after 2.06 days) on sclerotiorin production in 2-L STR

Table 3 Compounds identified by GC-MS in ***P. sclerotiorum*** culture extracts. The identification of the compounds present in the extract was performed by comparison of retention times and mass spectra with reference compounds contained in the NIST (National Institutes of Standards and Technology) Mass Spectral Library

Retention time (min)	Compound	Retention time (min)	Compound
5.7	Benzene	25.8	Monostearin
5.9	3-Amino-N-butyric acid	15.6	Dodecanoic acid (*Lauric acid*)
6.0	Trifluoromethyl ketone	17.2	Azelaic acid
7.3	2-Pyrrolidinone	19.7	Hexadecanoic acid (*Palmitic acid*)
7.6	Propanoic acid	20.7	Internal standard-Heptadecanoic acid
7.8	Hexanoic acid	21.3	11-cis-Octadecanoic acid
9.3	Butanoic acid	21.5	Octadecanoic acid (*stearic acid*)
10.4	Benzoic acid	22.7	Myristic acid
10.6	Glycerol	22.8	9,12-Octadecadienoic acid (*linoleic acid*)
11.9	Nonanoic acid	22.9	Ricinoleic acid
14.6	Octanoic acid	24.3	9,12,15-Octadecatrienoic acid (*α-linolenic acid*)

1996; Tsitsigiannis et al. 2005). Oxylipins were previously reported to act as quorum sensing molecules in fungi (Tsitsigiannis and Keller 2007). Deletion of *Ppo* genes, the oxylipins producing dioxygenase genes, affected the production of two secondary metabolites; the mycotoxin sterigmatocystin; and the antibiotic penicillin. It also effected a shift in spore reproduction from asexual to sexual in *A. nidulans* (Tsitsigiannis et al. 2005). It is therefore speculated that oxylipins play a similar role in *P. sclerotiorum* regulating its secondary metabolism.

There are limited studies reporting quorum sensing and signalling mechanisms in filamentous fungi as compared with those in bacteria and unicellular fungi (yeast). For a compound to be classified as a signalling molecule, it must fulfil five criteria as reported by Albuquerque and Casadevall (2012). In this paper, we reported two of the criteria, specifically where the signalling molecule accumulates in the extracellular environment during microbial growth and in a concentration that is proportional to the population cell density with its effects restricted to a specific stage of growth. Additional evidence supporting the fulfilment of the additional criteria are reported in the detailed study by Amache (2014).

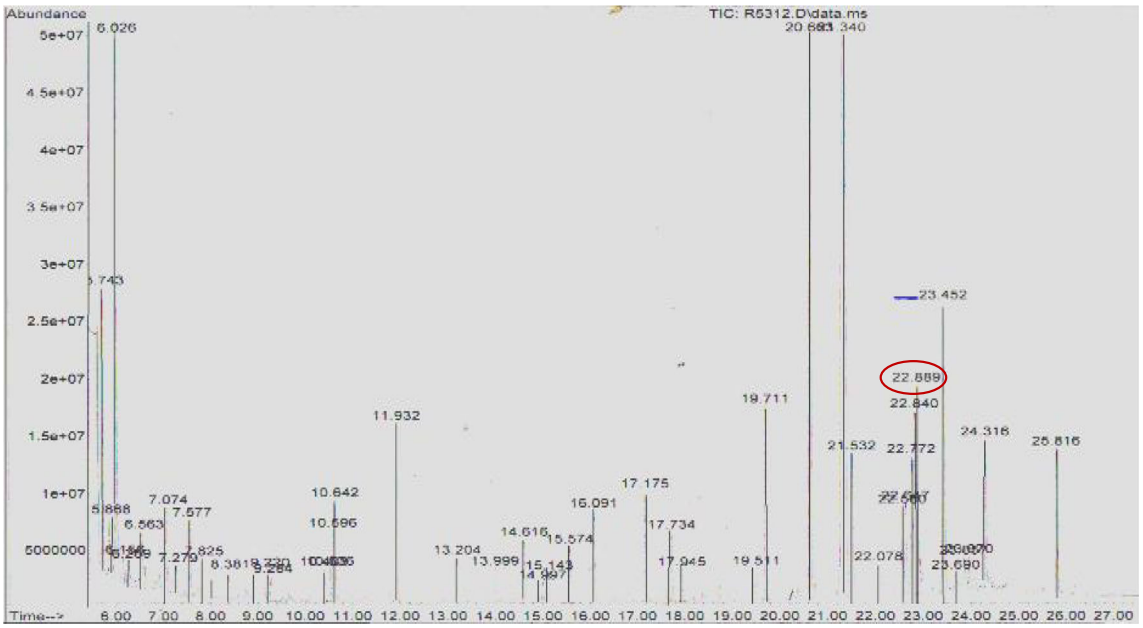

Fig. 5 Chromatographic analysis of *P. sclerotiorum* culture extracts. The identified peaks obtained after the analysis corresponding to the retention time are given in Table 3. Retention peak of 22.9 min indicates the presence of ricinoleic acid

Conclusions and future work

The optimisation of sclerotiorin production was carried out using response surface methodology experimental design. The optimised conditions resulted in 2.1-fold increase in shaken flask within 8 days of fermentation. Furthermore, to determine the efficacy of the obtained results, scale-up experiments were successfully replicated using 2-L stirred tank bioreactor under similar conditions resulting in a 2.2-fold increase. TLC data revealed the presence of compounds with Rf value 0.49. The analysis of the compounds by GC-MS indicated the presence of ricinoleic acid. Therefore, it is suggested that the oxylipin, ricinoleic acid, might be involved in the signalling mechanism in addition to the previously reported quorum sensing molecules. This paper has highlighted the positive effect of the optimal supplementation of *P. sclerotiorum* culture extracts for enhanced production of sclerotiorin. However, to report exact molecule(s) responsible for the observed results, additional studies are necessary to isolate, purify and identify the exact signalling molecules that contribute to the enhancement of sclerotiorin production in *P. sclerotiorum*. Furthermore, gene expression analysis can elucidate the cellular mechanisms involved in the enhancement of secondary metabolites by *P. sclerotiorum.*

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals Not applicable (N/A). This research did not involve human participants and/or animals.

Informed consent Not Applicable (N/A). This research did not involve human participants.

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