Octa Journal of Biosciences
International peer-reviewed journal
Octa. J. Biosci. Vol. 4(2):53-57

ISSN 2321 – 3663
July-Dec 2016

Octa Journal of Biosciences

journal homepage: www.sciencebeingjournal.com

Study of The Biological Activity Of 1-Octen-3-Ol In *Penicillium Canescens*

Assamoi A. Antoine[1], Soro-Yao Amenan Anastasie[2], Micael E. Bedikou[1], Destain Jacqueline[3], Thonart Philippe[3] and Sébastien Lamine Niamké[1].

1. Laboratoire de Biotechnologies, Université Félix Houphouët-Boigny d'Abidjan-Cocody, Unité de Formation et de Recherches Biosciences, 22 BP 582 Abidjan, (Côte d'Ivoire).
2. Université Nangui Abrogua, UFR STA, Laboratoire de Biotechnologie et Microbiologie Alimentaire, 02 BP 801, Abidjan (Côte d'Ivoire).
3. Unité de Bio-industries, Gembloux Agro-Bio Tech, Université de Liège. 2, Passage des Déportés. 5030, Belgium.

ARTICLE INFO

Received 02 Sept 2016
Revised 10 Oct 2016
Accepted 07 Nov 2016
Available online 01 Dec 2016

Keywords: Fermentation; *Penicillium*; Quorum sensing; Enzymes; Fungi

Email: assantoine@yahoo.fr

ABSTRACT

This work reports for the first time that *Penicillium canescens* synthesises a probable quorum sensing substance (QRS) 1-octen-3-ol. The impact of this molecule on the morphological changes of this fugus and its biosynthesis of industrial metabolites endo-β-(1, 4)-xylanase and β-galactosidase was highlighted. After seven days of culture, 11 1 and 17 1 µg of 1-octen-3-ol/ml were quantified in dextrose-based medium and in submerged fermentation media (SFM), respectively. In solid-state fermentation (SSF), 10^5, $10^6$and 10^7 spores/g as initial inoculum caused the productions of 363 36, 636 39 and 1000 38 µg of 1-octen-3-ol/g of soya oil cake, respectively. However, under growth circumstances, the level of 1-octen-3-ol was too weak (inferior to 84 µg/ml on dextrose-based medium and 5859 µg/g of soya oil cake, respectively) to influence the growth of *P. canescens* and its production of endo-β-(1, 4)-xylanase and β-galactosidase. Also, the inductive conidiation effect of 1-octen-3-ol was not obvious, and so, it can't be considered as a quorum sensing substance in *P. canescens*.

INTRODUCTION

A considerable amount of research reported the synthesis of volatiles compound by fungi which impact their morphogenesis, filamentation, pathogenesis as well as various other functions (Jorge, 2015). Among them, the major compound 1-octen-3-ol is a self-inhibitor of *Penicillium paneum* by the "crowding effect" phenomenon. In addition, 1-octen-3-ol induced the microcyclic conidiation in *P. paneum*, suggesting an additional role as a factor leading the fungal colony to spore production (Raina et al., 2012). Recently, 1-octen-3-ol, 3-octanol and 3-octanone were recognized as signalling molecules which inducing conidiation in *Trichoderma spp.* (Nemcovic et al., 2008). The formation of 1-octen-3-ol (a C_8 alcohol) is simultaneously accompanied by the formation of a C_{10} aldehyde 10-oxo-trans-8-decenoic acid (ODA) (Mau et al., 1992), which was reported to stimulate mycelial growth, stipe lengthening and fruiting initiation in *Agaricus bisporus* (Champavier et al., 2000). The two substances 1-octen-3-ol and ODA may act in concert as a complex of growth regulating substances to induce the fungal morphology during their growth (Chitarra et al., 2004).

P. canescens is an hyperproducer of extracellular hydrolases such endo-β-(1, 4)-xylanase and β-galactosidase (Sinitsyna et al., 2003). In this study, the role of a produced molecule 1-octen-3-ol by this fungus which would be its Grow Regulating Substance or a quorum sensing molecule able to affect its morphologic changes as well as its production of β-galactosidase and endo-β-(1, 4)-xylanase is investigated.

MATERIALS AND METHODS

Carbon sources

The agro-industrial substrates (soya oil cake and wheat straw) were supplied by Ets Brichart S.A. (Sombreffe, Belgium). The materials were chopped in a Gladiator laboratory hammer mill (Bruxelles, Belgium) to a 1 mm particle size before being used as carbon sources for endo-β-(1,4)-xylanase and beta-galactosidase production by P. *canescens*.

Inoculum

Pure culture of Penicillium canescens 10-10c was supplied by G.I Kvesidatse, Institute of Plant Biochemistry, Academy of Sciences (Tbilisi, Georgia). Spores or conidia suspension from P. canescens used for inoculum preparation were prepared by adding 2 × 9 ml of sterile peptone saline solution (0.1% (W/V) of casein peptone, 0.5% (W/V) of NaCl and 0.1% Tween 80) to the stock culture (slants inoculated with mycelia and incubated at 30°C for one month) whose surface was gently scraped with a sterile wire loop. The spores were counted in a DHC-B02 hemocytometer (Bürker Türk, INCYTO, Korea). The spore suspensions were adjusted in order to obtain a spore count within the desired range.

Cultural conditions on soluble substrates

The inoculum of P. canescens was cultivated at 30°C and 150 RPM in 250 ml Erlenmeyer flasks containing 100 ml of sterile dextrose-based medium (20 g/l of dextrose, 10 g/l of casein peptone and 10 g/l of yeast extract) or sterile liquid conidiation medium (2 g/l of dextrose and 2 g/l of yeast extract). When cultivated in solid conidiation medium, 12 g of agar was added to the liquid conidiation medium before sterilization (at 121°C for 20 min).

Cultural conditions on insoluble substrates

Solid state fermentation: it was performed in 250 ml Erlenmeyer flaks containing 5 g of 1 mm soya soil cake and casein peptone at 0.75% (W/V) in 20 ml of distilled water. The medium was autoclaved (121°C for 20 min) and inoculated after cooling, by 15, 150 and 1500 µl suspension equivalent to 105, 106, 107 spores/g of soya oil cake, respectively. The fermentation was then performed at 30°C under static conditions for three, seven and twelve days.

Submerged fermentation: it was performed in 250 ml Erlenmeyer flaks containing 3 g of 1 mm wheat bran, 100 ml of a nutritive solution composed by yeast extract at 0.5% (W/V), (NH4)2SO4 at 0.2% (W/V), Na2HPO4.2H2O at 1% (W/V), KCl at 0.05% (W/V) and MgSO4.7H2O at 0.015% (W/V). The medium was autoclaved (121°C for 20 min) and inoculated after cooling at 105spores/ml by 0.15 ml spore suspension. The fermentation was then performed at 30°C and 150 rpm for three, seven and twelve days with constant agitation.

2.5. Enzyme extraction

After fermentation, the fermented solid soya oil cake was complemented with distilled water at 75% (V/W) to obtain a liquid mixture. This mixture and the submerged fermented medium were separately centrifuged at 10000 RPM for 15 minutes at 4°C using a Avanti TM J-25 I centrifuge (Beckman, Palo Alto, USA) to remove the residues. These extract solutions were filtrated through a folded filter (ø = 150 mm, Ref. No. 10311645) (Schleilcher and Schuell, Dassel, Germany) and the resulting clear supernatant filtrate was used as the enzyme source.

Enzymes assays

The Endo-β-(1, 4)-xylanase activity was measured using 1% birchwood xylan (X0502, EC 232-760-6) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) (Bailey et al., 1992).

The β-galactosidase activity was measured by the extent of hydrolysis of o-nitrophenyl-beta-galactopyranoside (ONPG) (Goulas et al., 2007).

Assays of fresh and dried matter of P. canescens

The fresh matter of P. *canescens* was obtained after filtration through a Supor 450 membrane (Pall Corporation, USA) with 0.20 µm pore

size of the biomass issued from two days culture of P. *canescens* on dextrose-based medium or liquid conidiation medium (supplemented or not by pure 1-octen-3-ol). The dried matter was obtained after drying the fresh matter for 48 hours at 105°C.

Isolation of volatiles compounds by microdistillation-extraction procedure

The volatile compounds from culture filtrates of P. *canescens* were isolated by microdistillation extraction procedure according to Renata (2004) with ether-pentane (1:1, V/V) as the extraction reagent (8). Isolation of volatile compounds was performed with a Likens-Nickerson apparatus (extraction time: 90 min) for 10 ml of P. *canescens* culture filtrates and 5 ml of the extraction solvent was injected in an Agilent 6890 N gas chromatograph (Agilent Technologies, Eindhoven, Netherlands) recorded to an Agilent 5973 mass spectrometer for the identification and measurement of 1-octen-3-ol. The recovery of added 1-octene-3-ol was 75 4%. The quantification of 1-octen-3-ol was calculated on the basis of a calibration curve in the range of 0.4185 µg-0.004185 µg. A standard solution of 1-octen-3-ol (Acros Organics, Geel, Belgium) in ether-pentane (1:1, V/V) was used for quantifications purposes. The linear correlation coefficient of the standard curve of 1-octen-3-ol was confirmed within the range of 0.990-0.998.

Solid phase microextraction

The extraction of headspace volatile compounds was done with a Solid phase microextraction device (Supelco, Inc. Bellefonte, PA) using a 85 µm carboxen/polydimethylsiloxane fibre (stableflex/SS-Lt Blue/plain-57295-U). Before the analysis, the fibre was preconditioned in the injection port of the gas chromatography at 300°C for 1 h. The culture filtrates of P. canescens (2 ml) were inserted into 10 ml headspace vial and sealed with a PTFE-faced silicone septum (Supelco, Inc. Bellefonte, PA). The Solid phase microextraction fibre was exposed to the headspace of the vial while maintaining the sample at 30°C with constant stirring during 30 min. The compounds absorbed by the fibre were identified and quantified by gas chromatography analysis with mass selective detector (Agilent 5973). The compounds sorbed by the fibre were desorbed from the injection port of the gas chromatography (Agilent 6890 N) for 6 min at 220°C with the purge valve off (splitless mode). After, the fibre was heated to 220°C for an additional 25 min to avoid an analyte carry-over between samples. So, no compounds were present when the fibre was reinjected.

Gas chromatography analysis

A GC system 6890N gas chromatograph (Agilent Technologies, Eindhoven, Netherlands) coupled to an Agilent 5973 mass selective detector was used for the analyses. The compounds were separated on a DB-264 capillary column (30 m, 0.25 mm i.d., film thickness 1.4 µm) (JW Scientific, California, USA). Helium was used as carrier gas with a linear velocity of 27.3 cm/s. The gas chromatography oven temperature programme began when the fibre was inserted and held at 38°C for 13 min, ramped to 110°C at 3°C per min, then to 150 at 4°C per min and to 210°C at 10°C per min and finally, held at 210°C for 5 min. The total run time was 58 min and the gas chromatograph-mass spectrometer interface was maintained at 240°C. Mass spectra were obtained by electron impact at 70 eV, and data were acquired across the range of 29-400 uma. The compounds were identified by comparison with their mass spectra from a Wiley 275.L database (Wiley Online Library, Oxford, UK) Kovats retention index and by comparison with authentic standard. Quantification was based on either a total or single ion chromatogram on an arbitrary scale.

Effect of 1-octen-3-ol on isotropic growth of P. canescens and on the beta-galactosidase and endo-beta-(1, 4)-xylanase production

The diluted 1-octen-3-ol (in distilled water) was aseptically filtered (sterile syringue filter, 0.45 µm cellulose acetate) (VWR, Leuven, Belgium) and then added to the sterile culture of P.canescens. After the action of 1-octen-3-ol, the enzymatic activities (endo-β-(1, 4)-xylanase and β-galactosidase) of the fresh and dried matter, the P. canescens pellets size (diameter) were determined and compared to the controls without addition of 1-octen-3-ol.

Data Analysis

Each test was done in triplicate. The average value was validated when the variation between tests values were less than 8.0%. The results of gas chromatography-mass spectrometry analysis were expressed as means of three replicates for each experiment point.

RESULTS AND DISCUSSION

In all the *P. canescens* culture filtrates, 1-octen-3-ol represented more than 90% of the total area of the volatiles compounds extracted by the solid phase micro-extraction analyses (Figure I). The production was influenced by the culture media and the initial inoculum level (Figure II). During the 7th and 12th day, the growth of *P. canescens* was finished and the culture media were poor, causing the volatilization of the 1-octen-3-ol maybe due to the agitation of the flasks, excepted for the cultures in the SSF where with 10^5 spores/g of soya oil cake as initial inoculum size, the *Penicillium* strain grew until the 12th day in parallel to the increase of the level of 1-octen-3-ol.

In parallel to the production of 1-octen-3-ol, *P. canescens* growing on insoluble substrates in mycelial filament, produced important level of extracellular endo-β-(1, 4)-xylanase and β-galactosidase (Figure III) in relation to the initial inoculum level whereas these levels were too weak when the strain adopted a visual pellet form on soluble substrates (glucose, xylose, sucrose...). Many studies described a relationship between the production of hemicellulolytic enzymes and beta-galactosidase and the fungal morphologic changes such conidiation or filamentation (Grimm et al., 2005). Traditionally, these changes are induced by the physicochemical parameters (pH, temperature, oxygen transfer, mixing conditions) and the composition (carbon, nitrogen…) of the culture (Gaspar et al., 1997). Pheromones which are endogenous factors, inducing intercolony communication are also responsible of these changes (Roncal et al., 2003). A conidiogenone, induced the conidiation activity in *Penicillium cyclopium* (Roncal et al., 2002). Quorums sensing molecules Multicolic acid and related derivatives Butyrolactone induced the morphological changes in *Penicillium sclerotiorum* and *Aspergillus terreus* which control their synthesis and production level of secondary metabolites (sclerotiorin, lovastarin) (Raina et al., 2012). Quorum sensing is a mechanism in which low molecules weight endogenously synthesised by micro-organisms as signals are accumulated in the extracellular medium either at intraspecies or interspecies level in response from the microbial population (Mai *et al*., 2015). This mechanism induced the synthesis of secondary metabolites carbapenem by *Erwinia carotovora*, violacein by *C. violaceum* (Zhu et al., 2011), streptomycin by *Streptomyces griseus*, morphological differenciation *in Streptomyces griseus* and pathogenesis-biofilm formation in *Pseudomonas aeruginosa* (Raina et al., 2012). In Gram-negative bacteria, acyl-homoserine lactones have been identified as signalling molecules whereas in Gram-positive bacteria, these signals are constituted by modified or unmodified oligopeptides. In actinomycetes, γ-butyrolactones such as the A-factor and virginae butanolides have been described for the control of the production of streptomycin and virginiamycin, respectively. In fungi, signalling molecules farnesol and tyrosol are the well described for the regulation of morphological switch between the yeast and the mycelia form in *Candida albicans* (GreguŠ et al., 2010). Oxylipins which are identified as quorum sensing molecules regulate sporulation in *Aspergillus nidulans* and morphological transitions in *Aspergillus flavus*. Nemcovic ***et al. (2008) and*** **Chitarra et al**. **(**2003; 2005) reported also two fungal hormones as growth regulators molecules 1-octen-3-ol and 10-oxo-trans-8-decenoic acid (ODA) which inducing conidiation and mycelial growth in *Trichoderma spp* and in *P. Paneum* by intra- and interspecific colonial, respectively.

So, in subsequent experiments, the supplementation in pure 1-octen-3-ol indicated that the *P. canescens* grew on mycelial pellets form and not in conidial forms on dextrose-based medium and on liquid conidiation medium. The results showed also that 1-octen-3-ol can inhibit the germination of conidia and the growth of *P. canescens*. During growth in the liquid conidiation medium, the minimal inhibitory concentration of 1-octen-3-ol of about 84 µg/ml was able to yield 86 1 mg of dry matter. A supplementation of 837 µg of 1-octen-3-ol/ml induced the death of the inoculated conidia and no growth was possible. Furthermore, the 1-octen-3-ol also reduced the size of *P. canescens* pellets. In the case of liquid conidiation medium, after two days of incubation, the size of the mycelia pellets in the control (3.0 0.2 mm) was two and three fold higher than those observed in cultures supplemented by 209 µg/ml and 419 µg/ml of 1-octen-3-ol (1.5 0.1 and 1.0 0.1 mm, respectively). In the case of supplementation at a level below 84 µg/ml, no (visible) effect on cell growth was detected and the dry matter was similar to that of the control (135 2 mg). None inductive conidiation effect induced by the 1-octen-3-ol supplementation was observed. Our results partially agree with the findings **of Chitarra et al**. **(**2003; 2005). However, the evidence of action of 1-octen-3-ol at weak concentration and inducting conidiation in *P. canescens* has not been established

Also, in SSF before and after germination (three days after inoculation), there was no significant in enzyme production levels (endo-β-(1, 4)-xylanase, β-galactosidase) between samples and the control with the supplementation of 21 mg of 1-octen-3-ol (= 4.19 mg/g of soya oil cake). In the case of addition of 30 mg of 1-octen-3-ol (= 5.9 mg/g of soya oil cake) immediately after inoculation, the relative xylanase activity (% compared to that of the control) was about 29% and 90% after the 7th and 12th day of incubation, respectively. When the addition of 5.9 mg of 1-octen-3-ol/g of soya oil cake was done three days after inoculation, the relative xylanase activity was about 78% and 100% for the same times of incubation, repectively. Similar results were obtained for the β-galactosidase activity. Further decrease in the production levels of β-(1, 4)-xylanase and β-galactosidase was obtained with supplementation of 41.9 mg (= 8.4 mg/g) of 1-octen-3-ol. A 1-octen-3-ol concentration of about 5.9 mg/g was able to induce a decrease in the production of the extracellular β-(1, 4)-xylanase and β-galactosidase. This inhibitory effect affected more the production of extracellular enzymes by conidia than by mycelia of P. canescens.

After the 7th day of incubation, 10^5, 10^6 and 10^7 spores/g on SSF, yielded the productions of 363 36, 636 39 and 1000 38 µg of 1-octen-3-ol/g of soya oil cake, respectively. However, these 1-octen-3-ol levels were low compared to the minimal inhibitory quantity (5859 µg of 1-octen-3-ol/g of soya oil cake) which reduced the productions of endo-β-(1, 4)-xylanase and β-(1, 4)-galactosidase during the SSF. In the case of liquid media, the minimal inhibitory concentration of 1-octen-3-ol was about 84 µg/m

Table 1. Diameter measured from 48 hours of the growth zone at 30°C of the fungi after 16 hours of action of 1-octen-3-ol

	Mycelial growth diameter (cm)	
Fungi at 10^6 spores/ml of LSM	Control	Control + 4.2 mg/ml of 1-octen-3-ol
Penicillium canescens	1.5 ± 0.1	1 ± 0.1
Fusarium oxysporium	2.4 ± 0.2	1.6 ± 0.2
Penicillium restrictum	1 ± 0.1	0.8 ± 0.1
Aspergillus niger	2 ± 0.2	1.8 ± 0.1
Cladosporium cucuncrinum	0.9 ± 0.1	0.6 ± 0.5
Aspergillus awamori	2.2 ± 0.2	1.5 ± 0.1
Botytris cinerea	1 ± 0.1	0.9 ± 0.1

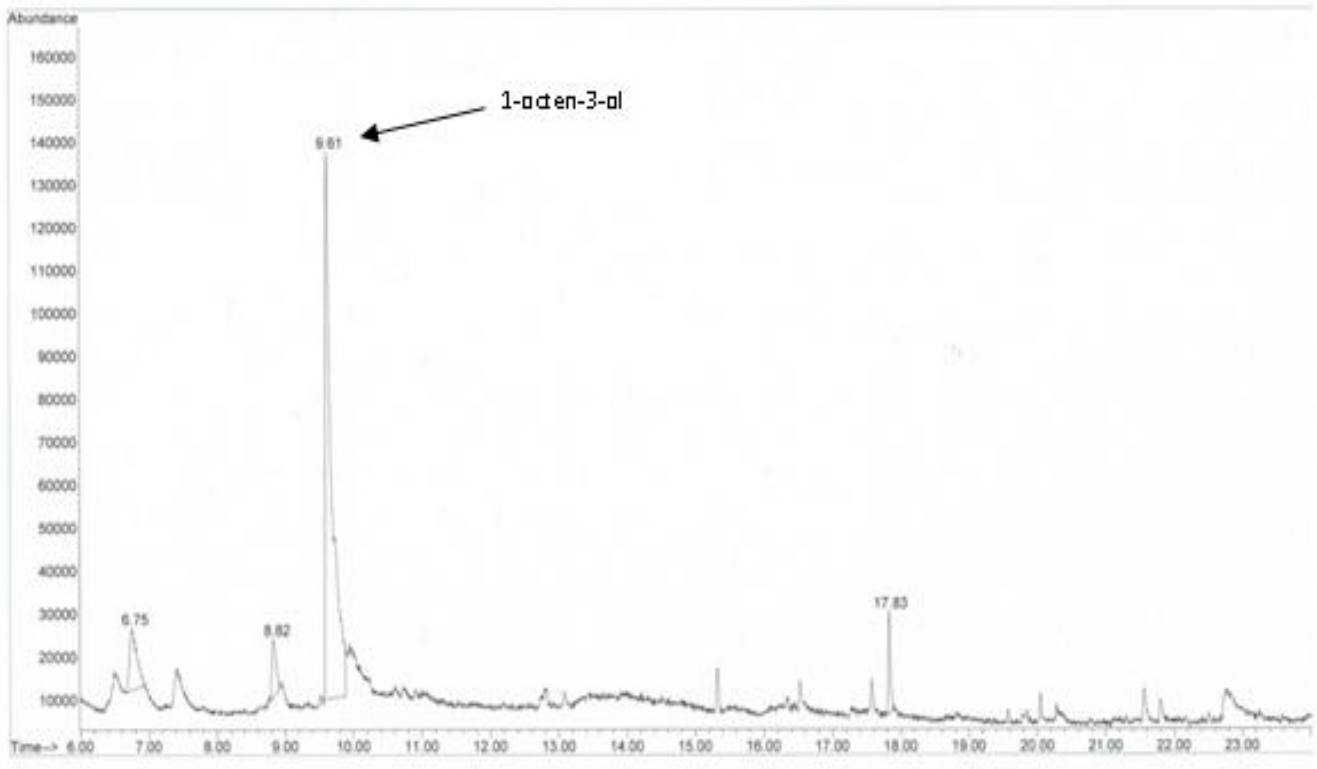

Figure 1. Profile of volatile compounds issued from P. *canescens* and extracted by microdistillation-extraction procedure. The culture conditions are 105 spores/g of soya oil cake incubated in solid-state fermentation at 30°C during 7 days.

whereas 10^5spores/ml as initial inoculums, which induced the productions of 11 1 and 17 1 µg of 1-octen-3-ol/ml on dextrose-based medium and submerged fermentation media, respectively. In spite of its antifungal activity, a wide selection of fungi such *Penicillium*, *Aspergillus*, *Alternaria* and *Fusarium* species have been shown to produce this secondary metabolite (Chitarra et al., 2004) which caused the restriction of the mycelial growth of *P.canescens*, *Fusarium oxysporium*, *Botytris cinerea*, *Cladosporium cucuncrinum*, *Aspergillus awamori*, *Penicillium restrictum*, and *Aspergillus niger*, respectively (Table I).

Plants infected by fungi emit 1-octen-3-ol, which induced their defensive fungal mechanism. Thus, treated with 1-octen-3-ol, the defensive genes of Arabidopsis plants (turned on by wounding or ethylene/jasmonic acid signalling) were stimulated to respond to the infection. The treatment also enhanced resistance of the plant against *Botrytis cinerea*. It would seem that 1-octen-3-ol may be a signal by which Arabidopis perceives the presence of a fungal pathogen. Some insects synthesized also this molecule during their search for a palatable fungus (Kishimoto et al., 2007). 1-octen-3-ol gets an antibacterial activity and can inhibit the growth of insects (Sawahata et al., 2008). In all cases, the action of the compound is dose-dependant and reversible (Wood et al., 2001). In addition of this extracellular volatile compound, *P. canescens* synthesises extracellular non volatiles compounds such griseofulvin, curvulinic acid, a fungitoxic extrolite and antibacterial substance named Sch 642305 and an antibiotic named canescin (Brian et al., 1953). These all secondary metabolites could aid the strain *P. canescens* in its attack or defensive mechanisms.

CONCLUSIONS

The ubiquist volatile compound 1-octen-3-ol, could be used as an indicator of the growth of *P. canescens*. This compound can inhibit the germination of *P. canescens* conidia. Getting a concentration-dependent effect, the level of 1-octen-3-ol emitted during the cultural conditions is lower to affect the growth of *P. canescens* and the production of extracellular endo-beta-(1, 4)-xylanase and beta-galactosidase. So, this compound can't not be considered as a grow regulating substance or a quorum sensing molecule in *P. canescens*.

cknowledgments

We are very grateful to Vincent Hôte and Danny Trisman (Unité de Chimie Générale et Organique) for their generous assistance in 1-octen-3-ol analysis. We thank also Jean-Marc Aldric (Unité de Bio-Industries) for helpful advices and discussions. Finally, we thank the Government of Côte d'Ivoire for the financial assistance.

Disclosure statement

No potential conflict of interest was reported by the author.

Financial and proprietary interest: Nil
Financial support: Nil

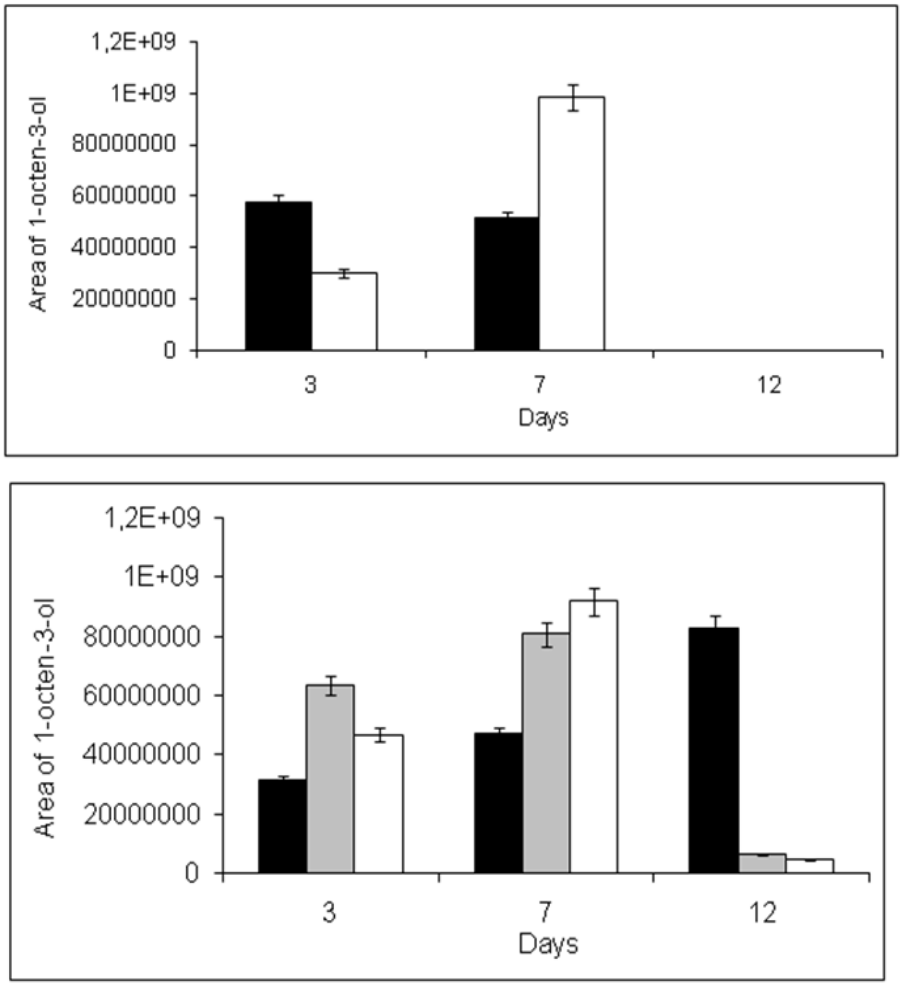

Figure 2. At top) Levels produced of 1-octen-3-ol during the growth of P. canescens on 863 (black) and submerged fermentation media (white) with initial inoculum size of 105 spores/ml and at underneath): .levels produced of 1-octen-3-ol during the growth of P. canescens on SSF with initial inoculum (Spores/g of soya oil cake): 105 (black), 106 (blue) and 107 (white).

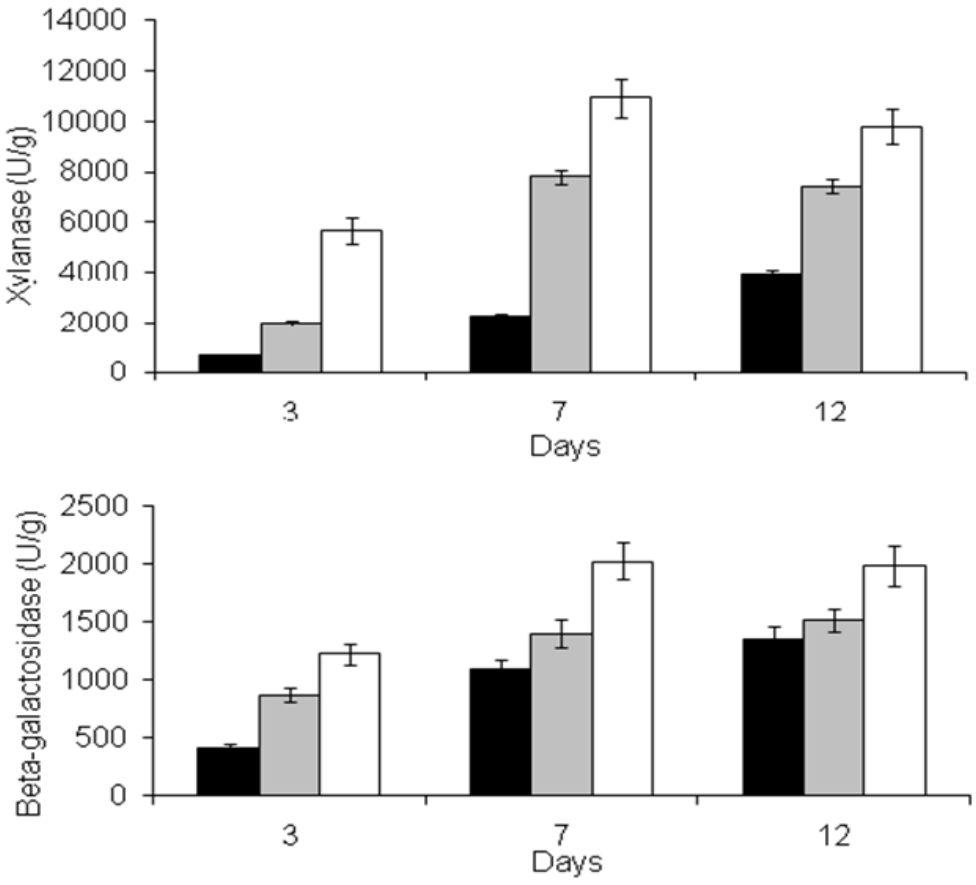

Figure 3. Levels produced of: (At top) endo-beta-(1, 4)-xylanase and (at underneath) beta-galactosidase by P. canescens on SSF with initial inoculum (Spores/g of soya oil cake): 105 (black), 106 (blue) and 107 (white).

REFERENCES

1. Bailey M. J., Biely P. and Poutanen K. (1992); Interlaboratory testing of methods for assay of xylanase activity. *J Biotechnol*; 23: 257-270.
2. Brian P. W., Hemming H. G., Moffatt J. S. and Unwin CH. (1953); Canescin, an antibiotic produced by Penicillium canescens. *Trans Br Mycol Soc*; 36: 243-247.
3. Champavier Y., Pommier M. T., Arpin N., Voiland A. and Pellon G. (2000); 10-Oxo- trans-8-decenoic acid (ODA): production, biological activities, and comparison with other hormone-like substances in Agaricus bisporus. *Enzyme Microb Technol*; 26: 243-251.
4. Chitarra G. S., Tjakko A., Rombouts,M. F., Posthumus M. A. and Dijksterhuis J. (2004); Germination of Penicillium paneum Conidia is Regulated by 1-Octen-3-ol, a Volatile Self-Inhibitor. *Appl. Environ Microbiol*; 70 (5): 2823-2829.
5. Chitarra G. S., Tjakko A., Rombouts M. F., Posthumus M. A. and Dijksterhuis J. (2005); 1-octen-3-ol inhibits conidia germination of Penicillium paneum despite of mild effects on membrane permeability, respiration, intracellular pH, and changes the protein composition. *FEMS Microbiol. Ecol*.; 54: 67-75.
6. Gaspar A., Cosson T., Roques C. and Thonart Ph. (1997); Improvement of oxygen transfer coefficient during Penicillium canescens culture. Appl. Biochem. Biotechnol; 67: 45-58.
7. GreguŠ P, Hana,M., Vladimir B., Jan K., Lucie K. and Lucie N. (2010); Ultra high performance liquid chromatography tandem mass spectrometry analysis of quorum-sensing molecules of Candida albicans. J. Pharmaceutical Biomed. Anal.; 53: 674-681.
8. Grimm L. H., Kelly S., Krull R. and Hempel D. C. (2005); Morphology and productivity of filamentous fungi. *Appl. Microbiol. Biotechnol*.; 69: 375-384.
9. Goulas A., Geoges T. and Glenn R. G. (2007); Development of a process for the production and purification of α- and β-galactooligosaccharides from Bifidobacterium bifidum NCIMB 41171. *Intern. Dairy J*.; 17: 648-656.
10. Jorge B. (2015); Quorum sensing mechanisms in fungi. AIMS Microb.: 1 (1): 37-47.
11. Kishimoto K., Matsui K., Ozawa R. and Takabayashi J. (2007); Volatile 1-octen-3-ol induces a defensive response in Arabidopsis thaliana. *J. Gen. Plant Pathol*.; 73: 35-37.
12. Mai T., Tintillier F., Lucasson A., Moriou C., Bonno E., Petek S., Magré K., Al Mourabit A., Saulnier D. and Debitus C. (2015); Quorum sensing inhibitors from Leucetta chagosensis Dendy. *Lett. Appl. Microbiol*. 61 (4): 311-317.
13. Nemcovic M., Lucia J., Ivan V. and Vladimir F. (2008); Induction of conidiation by endogenous volatile compounds in *Trichoderma* spp. *FEMS Microbiol. Lett*.; 284: 231-236.
14.Raina S., Mark O. and Tajalli K. (2010); Quorum sensing as a method for improving sclerotiorin production in Penicillium sclerotiorum. *J. Biotechnol*.; 148: 91-98.
15. Raina S., Daniela D. V., Elina K. P., Mark O., Annika M. B., Juhani T. S. And Tajalli K. (2012); Is quorum sensing involved in lovastatin production in the filamentous fungus *Aspergillus terreus*? *Process Biochem*.; 47: 843-852.
16. Renata Z. W. (2004); Optical purity of (R)-(−)-1-octen-3-ol in the aroma of various species of edible mushrooms. *Food Chem*.; 86: 113-118.
17. Roncal T., Cordobés S., Sterner O. and Ugalde U. (2002); Conidiation in Penicillium cyclopium is induced by conidiogenone, an endogenous diterpene. Eukaryotic cell.; 1(5): 823-829.
18. Roncal T. and Ugalde U. (2003); Conidiation induction in Penicillium. *Research Microbiol*.;154: 539-546.
19. Sawahata T., Satoshi S. and Masahiro S. (2008); Tricholoma matsutake 1-Ocen-3-ol and methyl cinnamate repel mycophagous Proisotoma minuta (Collembola: Insecta). *Mycorrhiza* ;18: 111-114.
20. Sinitsyna O. A., Bukhtoyarov F. E., Gusakov A. V., Okunev O. N., Bekkarevitch A. O., Vinetsky Y. P. and Sinitsyn, A. P. (2003); Isolation and properties of major components of Penicillium canescens Extracellular Enzyme complex. *Biochem*.; 68 (11): 1200-1209.
21. Wood W. F., Archer C. L. and Largent D. L. (2001); 1-octen-3-ol, a banana slug antifeedant from mushrooms. Biochem. Syst. Ecol.; 29 (5): 531–533.
22.Zhu H, He C.C. and Chu Q.H. (2011); Inhibition of quorum sensing in Chromobacterium violaceum by pigments extracted from Auricularia auricular. *Lett. Appl. Microbiol*. ; 52(3): 269-274.