# EFFECT OF FARNESOL ON *PENICILLIUM DECUMBENS*'S MORPHOLOGY AND CELLULASE PRODUCTION

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It is possible to improve cellulase production by controlling fungal morphology. Farnesol, the first quorum-sensing molecule found in eukaryotic organisms, is reported to influence the morphology of fungi. In this work, farnesol was investigated for its effect on morphology and cellulase production of *Penicillium decumbens*. Scanning electron microscopy (SEM) revealed that farnesol promoted the growth of hyphae, making possible and facilitating a higher yield of cellulase secretion. Enhanced interaction with the substrate in fermentation led to greater cellulase production. These findings are associated with the subsequent cellulase production of the fungus. Compared with a control medium, exogenously added 1 mM farnesol resulted in 1.32-fold increase in maximal filter paper activity with no significant change in the activity per unit of protein. These results provide a novel way to improve the cellulase production, promoting the commercial application of cellulase.

*Keywords: Quorum sensing molecule; Farnesol; Cellulase; Morphology; Penicillium decumbens*

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## INTRODUCTION

The global energy crisis, caused by decreasing production of fossil fuels, has led to the consideration of biofuels as important substitutes. As an important biofuel, bioethanol can be produced from fermentation of biomass in a sustainable way (Hahn-Hagerdal et al. 2006; Rass-Hansen et al. 2007). However, the shortage of sugar or grain, the raw material base used for the production of most of bioethanol today, has limited the production of bioethanol on a large scale (Hahn-Hagerdal et al. 2006). An attractive alternative for the generation of sugar is enzymically hydrolyzing cellulose by cellulase (Hui et al. 2010). However, the cost for cellulase production is a bottleneck for its application as biocatalysts in commercial processing, which accounts for approximately 27 to 40% of the cost of ethanol production from lignocellulosic materials (Ferreira et al. 2009; Ma et al. 2008). Thus, decreasing production cost by enhancing cellulase production through alternative strategies is needed to advance the commercial application.

It has been reported that cellulase production can be strongly impacted by the morphology of the fungus. Several studies have described how changing fermentation

parameters, such as buffer conditions (Ferreira et al. 2009), culture medium composition (Ahamed and Vermette 2009), and agitation conditions (Ahamed and Vermette 2010), can obtain a desired morphology that could enhance cellulase production. Farnesol is the first quorum-sensing molecule found in eukaryotic organisms, which has been reported to have an effect on the morphology of several fungi. In *Candida albicans*, it prevents the fungal transition from yeast to mycelium, disrupts biofilm formation, and mediates cell death (Derengowski et al. 2009; Langford et al. 2009). Farnesol can block the growth of *Saccharomyces cerevisiae* by raising the concentration of mitochondrial reactive oxygen species. Interestingly, externally added farnesol triggers morphological features characteristic of apoptosis in the filamentous fungus *Aspergillus nidulans* (Savoldi et al. 2008; Semighini et al. 2006). Also, farnesol acts as an inhibitor of conidiation, exhibiting a colony morphology resembling the "fluffy" phenotype of *Aspergillus niger* (Lorek et al. 2008). In this work, the influence of farnesol on the morphology and cellulase production of filamentous fungus *Penicillium decumbens* was investigated.

## EXPERIMENTAL

### Fungal Strain and Culture Conditions

The *P. decumbens* strain used in this study was a mutant obtained from the wild strain 114-2. It is a catabolic-repression resistant mutant screened by Qu et al. and has been used to prepare cellulase production industrially (Qu et al. 1991). The fungus was cultivated in 50 mL of modified Mandel's sodium solution that contained wheat bran and microcrystalline cellulose as carbon source. The medium contained 20 g/L of wheat bran; 15 g/L of microcrystalline cellulose; 3.0 g/L of $KH_2PO_4$; 2.0 g/L of $(NH4)_2SO_4$; 0.5 g/L of $MgSO_4 \cdot H_2O$; 0.5 g/L of $CaCl_2$; 0.0075 g/L of $FeSO_4 \cdot 7H_2O$; 0.0025 g/L of $MnSO_4 \cdot H_2O$; 0.002 g/L of $ZnSO_4$; 0.003 g/L of $CoCl_2$; and 0.5 g/L of urea.

*P. decumbens* grew at 30°C with shaking at 180 rpm. After 48 h of cultivation, 5 mL of the broth containing mycelia was transferred to 50 mL of the same medium. 1 mM farnesol (Sigma) was added to the medium at the time of 48 h incubation. The control was treated with an equal volume of ethanol as that of added farnesol. The strains were further cultivated for three days.

### SEM Observations

The samples after 96 h of cultivation were centrifuged to remove the supernatant. The pellets were fixed in 2.5% glutaraldehyde solution overnight, then 1% osmium tetroxyde for another 1 h. The fixed samples were dehydrated in several stages with a serial dilution of ethanol (40, 70, 90, and 100% v/v) for 15 min, followed by isoamylacetate for more than 2 h. After removing isoamylacetate, the samples were critical-point-dried in $CO_2$. Scanning electron microscopy (SEM) observations were carried out at 10 kV using a Hitachi S3000N (Tokyo, Japan) according to the manufacturer's instructions.

The samples prepared from solid medium were also observed using SEM. Briefly, *P. decumbens* was grown on the wheat bran solid medium at 30°C. The medium contained the dipping sauce of 100g/L wheat bran and 20g/L agar. On the third day, a

sterile filter paper containing 200 μM of farnesol was put on the clones. A sterile filter paper with an equal volume of ethanol instead of farnesol was also put on the clones as a control. After 2 h cultivation, the filter papers were removed and the fungus was cultivated for another three days. A piece of medium (1×1cm) was removed as the sample for SEM studies.

### Determination of Enzyme Activities and Protein Concentration

Certain cultures were centrifuged, and the supernatants were properly diluted for measurement of enzyme activity. One unit of enzyme activity (IU) is defined as the amount of enzyme that liberates l μmol product per minute under the measuring conditions. FPase activity (filter paper activity), endoglucanase activity, cellobiohydrolase activity, and β-glucosidase activity were determined according to the procedure proposed in previous studies (Chahal 1985; Chandra et al. 2009; Ma et al. 2008). The concentration of extracellular protein was determined according to the Bradford method (Bradford 1976), with bovine serum albumin (BSA) as the standard.

## RESULTS AND DISCUSSION

In order to study the effect of farnesol on cellulase production, *P. decumbens* was fermented in Mandel's modified medium with or without farnesol. All of the experiments were carried out in triplicate. Farnesol was added after 48 h cultivation to ensure that *P. decumbens* grew well and produced cellulase normally, since adding farnesol in the first day can inhibit the growth of the fungus (Fig.1).

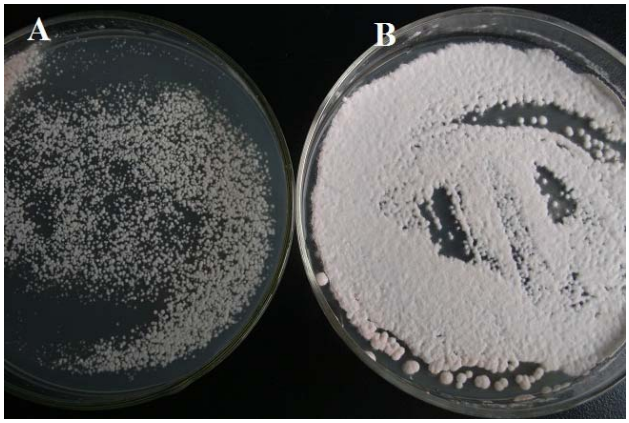


**Fig. 1.** Effect of farnesol on the growth of *P. decumbens*. A: medium containing farnesol; B: control medium without farnesol. The spores were inoculated on agar plates with 200 μM farnesol or not and cultivated for 6 days.

The effect of farnesol on morphology of *P. decumbens* was investigated by SEM analyses. The SEM results showed that no pellet had been formed in either farnesol-added or control medium. The fungus grew as mycelia, and hyphae adhered to the surface of wheat bran and microcrystalline cellulose (Fig.2 A-B). Due to the undissolved solid substrates (wheat bran and microcrystalline cellulose) used in this study interfering the analysis of the morphology of filamentous fungi, a difference was hardly observed

between the strains from farnesol-added medium and the control. This observation is consistent with the SEM analysis of *P. simplicissimum* and *A. niger*, which also form disperse mycelia without pellet formation in the presence of wheat bran and babassu cake, respectively (Gutarra et al. 2009; Papagianni et al. 1999). Then, the effect of farnesol on the morphology of the fungus was determined in the solid medium. The number of spores and the hyphal diameter was measured as shown in Table 1. Each value was the mean of ten projected areas. It can be observed that there were fewer spores, more hypha, and the hypha were thinner in the farnesol-added medium (Table 1; Fig.2 C-D). This observation implies that farnesol hindered the production of spores, but enhanced the growth of hyphae. It is well known that the growth of hyphae is mainly restricted to the tips of them, and protein secretion in filamentous fungi mainly occurs on the tips of growing hyphae (Wosten et al. 1991). Compared to the control medium, more hyphae in the medium with farnesol might due to more active tips during their growth. More active tips may facilitate a higher yield of secretory protein. Meanwhile, thinner and longer hypha in the farnesol-added medium would increase the surface area of the fungus, possibly enhancing the interaction with the substrate in fermentation, and increasing cellulase productivity.

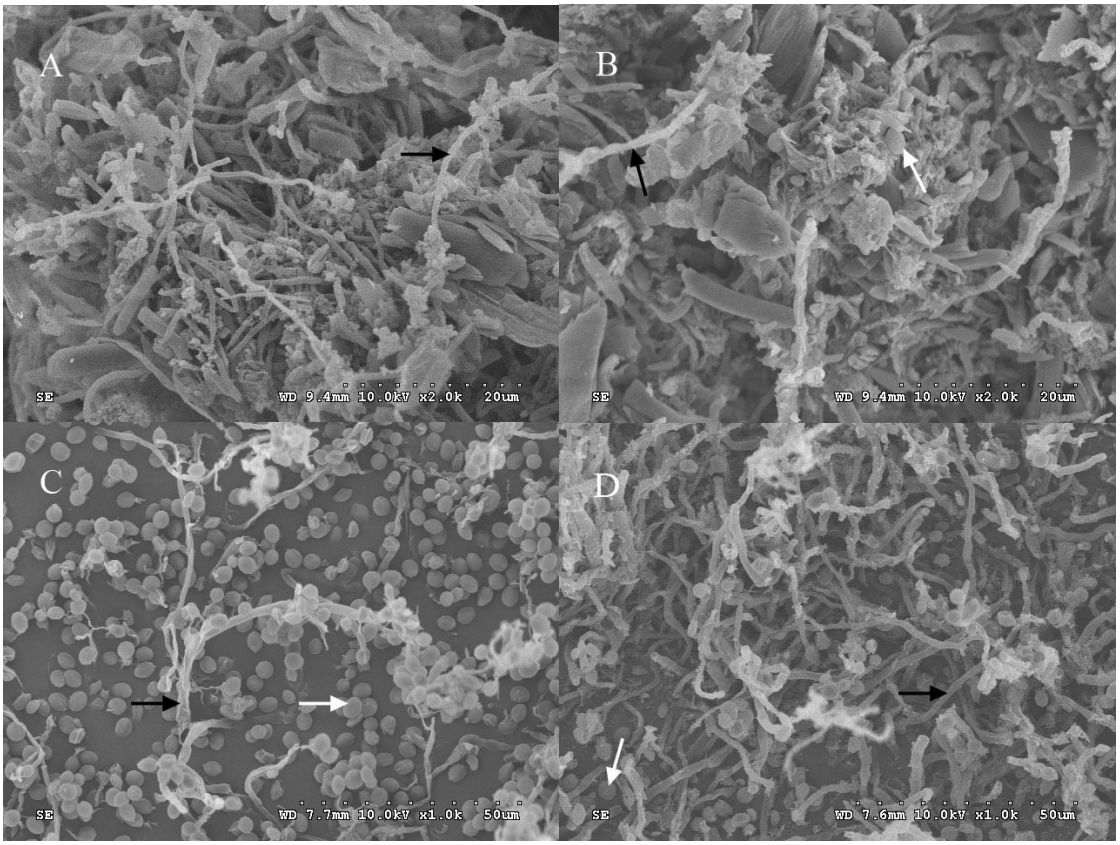


**Fig. 2.** SEM pictures revealing morphological characteristics of *P. decumbens* grown in fermentation medium containing farnesol (B) or not (A) and solid medium containing farnesol (D) or not (C). Hyphae (black arrow); spores (white arrow)

**Table 1.** Morphological Features of *P. decumbens* Grown in Solid Medium containing Farnesol or Not (control)

| | Control | Farnesol |
|---|---|---|
| Amount of extracellular secretory protein (mg/mL) | 1.12±0.09 | 1.34±0.05 |
| Number of spores/projected area | 109.80±23.30 | 52.80±10.73 |
| Hyphal diameter (μm) | 2.11±0.36 | 1.65±0.41 |

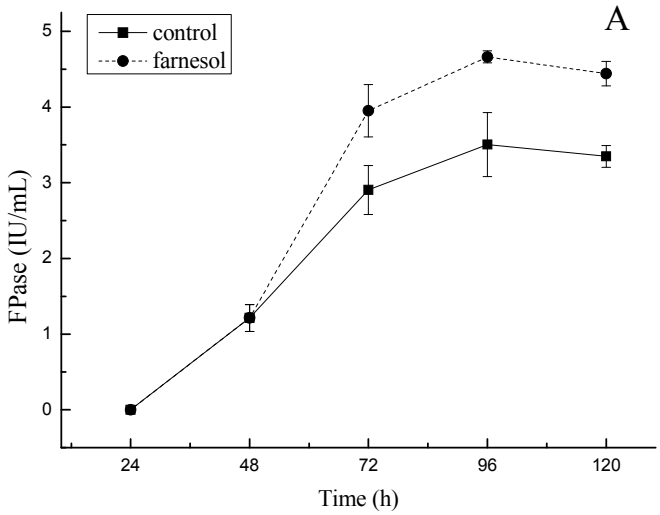


**Fig. 3A.** Effect of farnesol on cellulase activity of *P. decumbens*. The time course of filter paper activities.

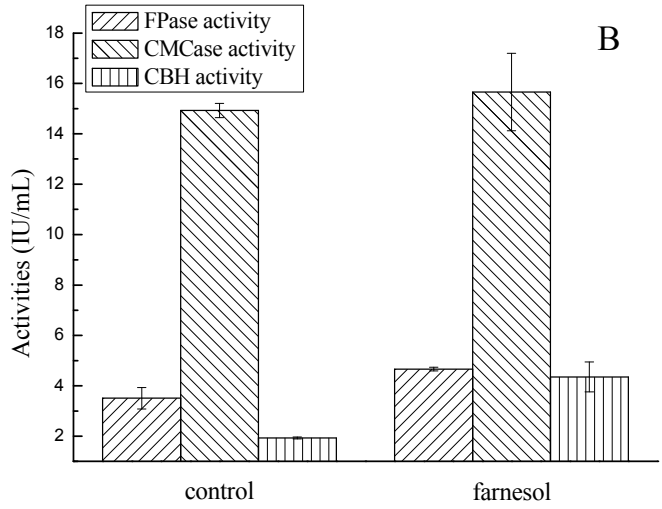


**Fig. 3B.** Effect of farnesol on cellulase activity of *P. decumbens*. FPase activity, endoglucanase (CMCase activity) and cellobiohydrolase (CBHactivity) activities after 96 h of fermentation

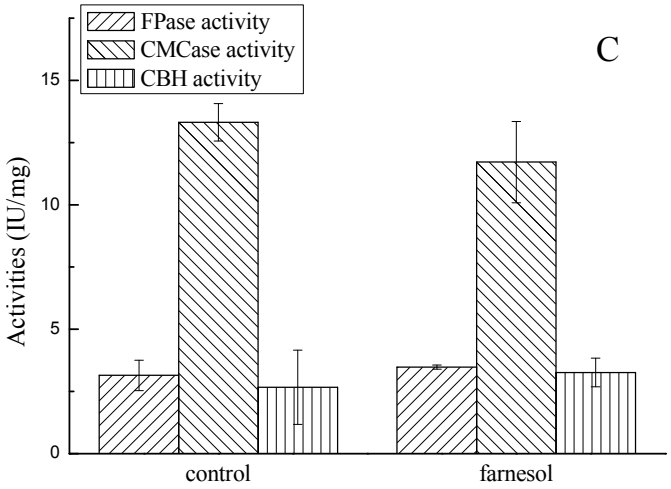


**Fig. 3C.** Effect of farnesol on cellulase activity of *P. decumbens*. Specific activities (IU/mg) after 96 h of fermentation.

Since adding farnesol resulted in changed morphology, which may increase cellulase production, the effect of farnesol on cellulase production of *P. decumbens* was determined. Comparing the effect of farnesol with the control culture, a higher FPase was seen over time after farnesol was added in the medium (Fig. 3A). As shown in Fig. 3B, exogenous farnesol led to a higher filter paper hydrolase activity, endoglucanase activity, and cellobiohydrolases activity at the time of peak cellulase production (96 h), without changing the enzyme component proportion. Consistent with a previous report (Liu et al. 2010), β-glucosidase activity once again was not high in the present study (data not shown). Meanwhile, a higher amount of extracellular secretory protein was also obtained in medium contained farnesol (Tab.1), resulting in no significant changes in the activities per unit protein (IU/mg) (Fig.3-C). Hence, adding farnesol did increase the cellulase activities by enhancing the cellulase production, not by increasing special activity per cellulase molecule.

## CONCLUSIONS

1. Results of this work showed that exogenous addition of farnesol changed the morphology of *P. decumbens*, leading to a higher cellulase production, providing a novel way to improve the cellulase production, promoting the commercial application of cellulase.
2. Since farnesol is a quorum-sensing molecule in eukaryotic organisms and a recent study has reported that exogenous addition of γ-butyrolactones, a quorum-sensing molecule in *P. sclerotiorum*, resulted in 6.4-fold increase in sclerotiorin yield (Raina et al. 2010), the present results raise questions as to whether and how farnesol acted as a quorum-sensing molecule in the cellulase production.

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