Production of indole-3-acetic-acid (IAA) by the white rot fungus *Pleurotus ostreatus* under submerged condition of Jatropha seedcake

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The synthesis of phytohormone, indole-3-acetic acid (IAA), is not only confined to flowering plants but bacteria (especially plant growth-promoting rhizobacteria and few pathogens), yeasts, and other fungi are also known to produce this hormone and in many cases even at higher levels than plants. Three white rot fungi, *Trametes versicolor*, *Pleurotus ostreatus*, and *Phanerochaete chrysosporium*, were examined for their ability to produce IAA when incubated with L-tryptophan. The maximum IAA production (473.55 ± 3.32 µg ml\(^{-1}\)) was observed upon 18 d of incubation at 37 °C using a medium containing 2\%(w/v) Jatropha seedcake as substrate, with pH adjusted to 7.0. The IAA produced by *P. ostreatus* was further confirmed and characterized by thin layer chromatography and Gas Chromatograph-Turbomass Mass Spectrometer. The biological activity of IAA obtained from the culture supernatant of *P. ostreatus* was determined using wheat coleoptile bioassay.

Keywords: *Pleurotus ostreatus*; indole-3-acetic acid; Jatropha seedcake; wheat coleoptile bioassay

Introduction

Phytohormones are the signal molecules, acting as chemical messengers that control plant growth and development. Apart from their role in plant development (Davies 2010), hormones are also the principle agents that regulate plant response to changes in environmental conditions (Morgan & Drew 2006; Tuteja 2007; Malhotra & Srivastava 2009). Nowadays, their use in a variety of agronomic applications for controlled plant development has gained momentum. One of such commercially important phytohormone is indole-3-acetic acid (IAA), a principle auxin. Apart from its synthesis by plants, they are also produced by bacteria (Apine & Jadhav 2011), yeast, (Xin et al. 2009) and fungi (Ünyayar et al. 2000; Yürekli et al. 2003; Maor et al. 2004). Although the discovery of IAA in yeast extracts goes back to more than half a century, its production by filamentous fungi is relatively new. However, such reports on the production of phytohormones using fermentation are sparse and only a little data on the actual quantity of IAA synthesized by fungi is known (Yürekli et al. 1999; Chung et al. 2003). Researches in biotechnology have led to an increased awareness of the importance of industrial fungi for the production of plant growth hormones (Berry 1988).

Several fungi including white rot species are widely used in biotechnological and biochemical applications such as bioremediation and delignification. However, there are limited studies on white rot fungi for production of plant growth factors. Crocoll et al. (1991) reported the abscisic acid production by white rot fungi. Later on, Yurekli et al. (1999) demonstrated *Funalia trogii* ATCC 200800 and *Trametes versicolor* to produce gibberellic acid, abscisic acid, indole acetic acid, and cytokinin employing olive oil mill and alcohol factory wastewaters as raw materials for fermentation. Ünyayar et al. (2000), Ünyayar (2002), and Yurekli et al. (2003) reported exogenous IAA production by *Phanerochaete chrysosporium* strain ME446, *Funalia trogii*, and *Lentinus sajor-caju*, respectively.

The physiological role of auxins in fungi is not well understood. One of the roles suggested for production of IAA by fungus is to mediate fungal–plant interaction. High concentrations of IAA can inhibit the hypersensitive response (Robinette & Matthysse 1990; Jouanneau et al. 1991) and may suppress expression of plant defense genes (Yamada et al. 1985; Shinshi et al. 1987). However, direct evidence for the involvement of IAA in plant diseases was available only for plant pathogenic bacteria (Patten & Glick 1996; Manulis et al. 1998) until Maor et al. (2004) showed in-plantae production of IAA by a plant pathogenic fungus, *Colletotrichum gloeosporioides* f. sp. *aeschynomene*, thus suggesting involvement of IAA in fungal phyto-pathogenesis as well.

The aim of the present study was to determine the effect of culture conditions for production of IAA by an
industrially important white rot fungus, *Pleurotus ostreatus*. Although few agricultural residues have been shown as suitable substrates for microbial IAA fermentation (Yurekli et al. 2003; Swain & Ray 2008), there is no report on synthesis of IAA by *P. ostreatus* employing Jatropha seedcake (JSC) as raw material so far to the best of our knowledge.

**Materials and methods**

**Materials**

Jatropha seedcake (JSC) was obtained from Anand Agriculture University, Anand, Gujarat, India. IAA and L-tryptophan were purchased from HiMedia, Mumbai, India. Malt extract was procured from SRL, Mumbai, India. All other solvents and chemicals used during the experiment were of analytical grade.

**Culture and inoculums**

Three fungal strains, *Trametes versicolor* (TV), *Pleurotus ostreatus* (PO), and *Phanerochaete chrysosporium* (PC), used in this study were gifts from Institute of Forestbotanisches, Gottingen, Germany. They were grown on 2% malt extract agar plates at 27°C, preserved at 4°C, and maintained by subculturing once in 2 months. For inoculation, two agar blocks (1 cm × 1 cm) were removed from actively growing fungi on 2% malt extract agar plates and aseptically inoculated into the flasks containing liquid medium.

**Screening of growth medium and fungal strain for IAA production**

Three different liquid media, viz., (i) 2% malt extract broth (pH 5.5); (ii) 2% (w/v) JSC in basal salt medium (BSM) (5 g l⁻¹ of glucose, 1 g l⁻¹ of KH₂PO₄, 0.5 g l⁻¹ of MgSO₄, 0.5 g l⁻¹ of KCl; pH 5.5); and (iii) 2% (w/v) JSC in distilled water (pH 5.5), all supplemented with 0.1% (w/v) L-tryptophan, were used for IAA production in submerged condition. 100 ml sterilized media in 250 ml Erlenmeyer flasks were inoculated with two blocks (1 cm × 1 cm) of TV, PO, or PC mycelial growth cut from malt extract agar plates. The flasks were incubated on a rotary shaker at 30°C and 150 rpm for 22 d. The uninoculated media served as controls. The 2 ml aliquots were withdrawn regularly after every 2 days during the course of fermentation and analyzed for IAA content.

**Effect of physico-chemical parameters on IAA production by *P. ostreatus***

To study the effects of various physico-chemical parameters for maximum IAA production by white rot basidiomycetes *P. ostreatus*, 250 ml Erlenmeyer flasks with 100 ml media were inoculated with two blocks (1 cm × 1 cm) of uniform growth of fungus. Uninoculated media served as controls. The production media with varying JSC (0.5, 1, 2, 3, 4, 5, and 6%; w/v) and L-tryptophan concentrations of (0.05, 0.1, 0.5, and 1%; w/v) were used to study their effect on IAA production by *P. ostreatus*. Studies were also performed to investigate the effect pH over a range of 4.0–9.0 (adjusted using appropriate buffers) at 30°C on IAA production by *P. ostreatus*. Sodium acetate buffer (100 mM) for pH in the range of 4.0–5.0, sodium phosphate buffer (100 mM) for pH in the range of 6–7, Tris-Cl buffer (100 mM) for pH 8, and Glycine-NaOH buffer (100 mM) for pH 9 were used. The optimum production temperature was determined by monitoring IAA production by *P. ostreatus* at 20, 25, 30, 37, 42, and 50°C. Unless otherwise mentioned, experiments were carried out by incubating the inoculated media and uninoculated controls on rotary shaker at 30°C and 150 rpm for 18 d. Upon incubation, the fermentation broth was centrifuged at 6797 × g for 15 min and supernatant was assayed for IAA. All experiments were done in triplicates.

**Extraction of IAA from fermentation broth**

The culture supernatant containing IAA obtained from a 18-d fermented broth of *P. ostreatus* was acidified to pH 2.0 using (1 N) HCl and extracted twice with double volumes of ethyl acetate each time. The ethyl acetate fractions were pooled and evaporated dry in a rotary evaporator at 40°C. The extract was dissolved in minimum amount of methanol and stored at −20°C till further use.

**Analysis of IAA by thin layer chromatography (TLC)**

The concentrated fungal IAA (1 µl) was applied to TLC (Silica gel G f₂₅₄, thickness 0.25 mm, Merck, Berlin, Germany), using LINOMAT (CAMAG, Darmstadt, Germany), and developed with n-butanol:ammonia:water (10:1:10 v/v/v, upper phase). The developed TLC plate was then sprayed with Salkowskia reagent in order to visualize IAA band. The authentic IAA standard (1 µl) was also simultaneously run on TLC in order to identify the IAA band in sample on the basis of *Rf* value.
The fungal strain, PO, was able to grow and produce IAA in JSC. The concentration of IAA produced by PO was measured as 840.46 μg ml⁻¹ after 15 d of fermentation. In JSC, PO and TV did not produce IAA or the amount was negligible, whereas PC produced a maximum of 20 d of incubation, whereas PC produced a maximum of 563 μg ml⁻¹ of IAA in JSC + BSM by 15 d of incubation. In JSC + D/W, PO and TV produced almost equal amounts of IAA upon 15 d of fermentation.

The culture supernatants of all the three fungi grown in different media showed a clear red spot at the R₁ value 0.33 corresponding to standard IAA on silica gel TLC plates, upon spraying with Salkowski reagent. The TLC analysis revealed that high concentration of IAA estimated by colorimetric assay in fermentation broth of PC using JSC + BSM and malt extract medium was due to some other metabolite produced in the fermentation medium that interfered with the assay. But, when PC was grown in JSC + D/W, only IAA was produced. In malt extract medium, PO and TV did not produce IAA or the amount was negligible, whereas in JSC + D/W medium, both these cultures produced significant amounts of IAA. Thus on the basis of results obtained by colorimetric assay and TLC analysis, PO was selected for further study, as it exhibited least production of interfering metabolites in comparison to the other two white rot fungi and produced significant amount of IAA in JSC.

**Results and discussion**

**Screening for growth medium and fungal strain for IAA production**

The JSC contains approximately 49% of carbohydrate, 22% of protein, and 14% of fiber (Rakshit et al. 2008). This composition is suitable to support good microbial growth. The use of JSC as feedstock for fermentation thus appears logical and worth exploring. In the present study, we investigated the suitability of different media compositions for IAA production under submerged fermentation by three white rot fungi, viz., TV, PO, and PC.

All the three white rot cultures were able to produce IAA in 0.1% L-tryptophan-supplemented test media (Figure 1(a)). All the three fungal cultures produced maximum IAA after 15 d of incubation but the extent of IAA produced varied depending on the fungal culture and production medium. In 2% (w/v) malt extract medium, PO produced maximum IAA (563 μg ml⁻¹) 20 d of incubation, whereas PC produced a maximum of (840.46 μg ml⁻¹) IAA in JSC + BSM by 15 d of incubation. In JSC + D/W, PO and TV produced almost equal amounts of IAA upon 15 d of fermentation.

The culture supernatants of all the three fungi grown in different media showed a clear red spot at the R₁ value 0.33 corresponding to standard IAA on silica gel TLC plates, upon spraying with Salkowski reagent. The TLC analysis (Figure 1(b)) revealed that high concentration of IAA estimated by colorimetric assay in fermentation broth of PC using JSC + BSM and malt extract medium was due to some other metabolite produced in the fermentation medium that interfered with the assay. But, when PC was grown in JSC + D/W, only IAA was produced. In malt extract medium, PO and TV did not produce IAA or the amount was negligible, whereas in JSC + D/W medium, both these cultures produced significant amounts of IAA. Thus on the basis of results obtained by colorimetric assay and TLC analysis, PO was selected for further study, as it exhibited least production of interfering metabolites in comparison to the other two white rot fungi and produced significant amount of IAA in JSC.

**Profile of IAA production by P. ostreatus under submerged fermentation**

The time course of IAA production by PO grown in JSC medium containing 0.1% (w/v) L-tryptophan is shown in Figure 2. The fungal strain, PO, was able to grow and produce IAA from the 3rd day of incubation, and the production continued up to 15 d of fermentation. The production of IAA was determined as described by Gordon and Weber (1951). The fermented broth was centrifuged at 12,310 x g for 15 min to separate mycelial biomass. The apical 2 mm was cut and discarded. The next 4 mm sections were used for the bioassay. These sections were floated on distilled water to remove the endogenous IAA. Five such sections were then placed into test tubes containing phosphate-citrate buffer (1.794 g l⁻¹ K₂HPO₄ + 1.019 g l⁻¹ citric acid monohydrate; pH 5.0) supplemented with 2% (w/v) sucrose, in three sets: (1) control (without IAA), (2) serial dilutions (5, 2, 1, 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ μg ml⁻¹) of extracted fungal IAA, and (3) serial dilutions (5, 2, 1, 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ μg ml⁻¹) of synthetic IAA standard. The assay tubes were incubated for 20 h at 25°C in dark with a roller tube apparatus (10 rpm) to avoid curling of coleoptiles (Nitsch & Nitsch 1956). At termination of the bioassay, coleoptile sections were measured using micrometry, and the length of each coleoptile was recorded. All assays were done in triplicates.

**Analytical methods**

**Indole acetic acid assay**

The production of IAA was determined as described by Gordon and Weber (1951). The fermented broth was centrifuged at 12,310 x g for 15 min to separate mycelial growth. One ml aliquot of appropriately diluted culture supernatant was mixed with 0.2 ml of o-phosphoric acid and 2 ml of Salkowski reagent (50 ml of 35% of HClO₄ and 1 ml of 0.5 M FeCl₃ solution). This assay system was allowed to stand in dark at room temperature for 20 min. The development of pink color indicated IAA production and was quantified by reading the absorbance at 535 nm using Systronics Spectrophotometer 106, Ahmedabad, India.

**Laccase assay**

Laccase activity was determined by monitoring oxidation of o-dianisidine as described by Palmieri et al. (1993). All enzyme reactions were started by adding 200 μl of culture filtrate, and the enzyme activities were calculated from the linear phases of the reaction. One unit of enzyme activity was defined as the amount of the enzyme required to oxidize 1 μmol of o-dianisidine ml⁻¹ min⁻¹ under specified assay conditions.
Figure 1. (a) IAA production by three different white rot fungi on three different media and (b) detection of fungal IAA in ethyl acetate extract of culture supernatant by silica gel TLC (In 1(b): L1-Synthetic IAA (1 mg ml$^{-1}$); L2, L3, L4-PO, TV, PC grown in JSC + BHM, respectively; L5, L6, L7-PO, TV, PC grown in JSC + D/W, respectively; L8, L9, L10-PO, TV, PC grown in malt extract, respectively; L11-JSC control and L12-malt extract control).

Effect of physico-chemical parameters for IAA production by P. ostreatus

Microbial phytohormones are mostly extracellular and are greatly influenced by nutritional and physico-chemical factors, such as pH, L-tryptophan concentration, carbon-nitrogen source, agitation, and dissolved oxygen concentration. PO produced maximum IAA (362.53 ± 3.29 µg ml$^{-1}$) by 18 d of incubation at 30°C under shaking condition, in 0.1% (w/v) L-tryptophan-supplemented JSC broth which was quiet higher than that reported by Ünyayar et al. (2000) for P. chrysosporium ME446 (55.91 µg IAA ml$^{-1}$) in basal mineral medium. Yurekli et al. (1999) reported 111.76 µg ml$^{-1}$ and 75.2 µg ml$^{-1}$ IAA production employing olive oil mill waste as substrate by Funalia trogii ATCC 200800 and Trametes versicolor ATCC 200801, respectively.

The influence of varying concentrations of deoiled Jatropha seedcake on IAA production is shown in Figure 3(a). IAA production increased in media with increasing concentration of deoiled JSC from 0.5% to 2.0% (w/v). The IAA production depends on deoiled seedcake concentration because its protein and carbohydrate content act as feedstock for the growth of white rot fungi and thereby for IAA production. Further increase in seedcake concentration above 2.0% (w/v) JSC did not result in higher yield of IAA. The lower yields at higher concentrations (above 2% (w/v) JSC) may be due to reduced mass transfer as a result of increase in viscosity or may be due to toxicity associated with substrate at that concentration (Schügerl 1981; Saetae & Suntornsuk 2010).

The IAA production was found to increase with an increase in L-tryptophan concentration from 0% to 1% (w/v) in fermentation medium (Figure 3(b)). At higher L-tryptophan concentration in medium, PO exhibited significantly lower yields of IAA.

The culture PO produced significantly higher amount of IAA in the pH range of 7.0–8.0, with maximum IAA in
medium with initial pH 7.0 (Figure 3(c)). Our results are in agreement with the findings of Strzelczyk et al. (1992) who reported that auxin biosynthesis was favored in media over a pH range of 6.0–9.0 in mycorrhizal fungi.

The IAA production by PO was found to increase with incubation temperature with maximum production at 37°C (Figure 3(d)). Further increase in temperature retarded growth as well as IAA production, which reduced to negligible amount at 50°C when the fungal growth ceased. In contrast to IAA levels, maximum growth was observed at 30°C which gradually decreased with either increase or decrease in incubation temperature.

**Characterization of IAA produced by P. ostreatus**

The silica gel thin layer chromatogram of ethyl acetate extract from culture supernatant of PO exhibited clear red spot of IAA with $R_f$ value 0.33 corresponding to standard IAA when sprayed with Salkowski’s reagent. The TLC findings (Figure 4) are in good agreement with the report of Bayer (1969). In addition to IAA, other compounds were also detected on TLC, which might represent intermediates in biosynthetic or degradation pathways or may be degradation products or structural analogs of IAA that remains to be identified.

The two major peaks with retention time of 12.34 and 19.57 min were detected in ethyl acetate extract of PO cell-free fermentation broth, when analyzed by gas chromatography (Figure 5(a)). The mass spectrometric analysis (Figure 5(b)) of the compound eluted at a retention time of 19.57 min suggested it to be IAA. Upon fragmentation, this compound yielded ions ($m/z$ 128, 129, 130, and 131) corresponding to indole moiety. The mass spectrometry analysis of minor peak with 18.23 min retention time suggested it to
be of indole acetamide. The presence of indole acetamide in culture supernatant of PO indicates the biosynthesis of IAA through indole-3-acetamide pathway in this organism. The indole acetamide pathway was long believed to be restricted to bacteria (Costacurta & Vanderleyden 1995) until Robinson et al. (1998), and Maor et al. (2004), showed indole-3-acetamide as a major pathway utilized by a plant pathogenic fungus Colletotrichum gloeosporioides f. sp. aescynomene to produce IAA.

Etiolated wheat coleoptile bioassay of IAA produced by P. ostreatus
Wheat coleoptile bioassay was performed to determine the potency of fungal auxin in reference to the commercially available synthetic IAA at corresponding concentrations (Table 1). The 0.1 µg ml⁻¹ IAA of synthetic and fungal origin caused 2.85 and 2.89 mm increase in coleoptile length, respectively. The higher IAA concentrations (5 and 2 µg ml⁻¹) were less effective in comparison to the lower concentrations. The yellowing of the coleoptiles and retarded growth observed at higher concentrations might be due to the phytotoxic effect of IAA at supraoptimal concentration (Foster et al. 1952; Badenoch-Jones et al. 1982). A low activity was detected in control where the coleoptile length was 5.13 ± 0.093 mm. This increase in control coleoptile length might be due to the endogenous IAA content. Coleoptile lengths with synthetic and bacterial IAA were very close to each other, and the difference between the coleoptile lengths was not significant. This indicates that the standard synthetic IAA and fungal IAA possessed equivalent biological activities.
Figure 5. (a) Gas chromatogram of crude ethyl acetate fraction obtained from culture supernatant of *P. ostreatus* upon fermentation of 2% (w/v) JSC for 18 d and (b) MS fragmentation pattern of putative IAA with the major fragment ion at *m/z* 130.

Table 1. Comparative data of wheat coleoptile bioassay for IAA.

| Treatments  | IAA concentration (µg ml\(^{-1}\)) | Length before treatment (mm) | Length after treatment (mm)\(^a\) | Growth (mm) |
|-------------|----------------------------------|-------------------------------|-----------------------------------|-------------|
| Control     | 0                                | 4                            | 5.13 ± 0.093                      | 1.13        |
| Synthetic IAA | 5                              | 4                            | 4.69 ± 0.133                      | 0.69        |
|             | 2                                | 4                            | 5.50 ± 0.093                      | 1.50        |
|             | 1                                | 4                            | 6.46 ± 0.074                      | 2.46        |
|             | 10\(^{-1}\)                      | 4                            | **6.85 ± 0.089**                  | **2.85**    |
|             | 10\(^{-2}\)                      | 4                            | 6.13 ± 0.103                      | 2.13        |
|             | 10\(^{-3}\)                      | 4                            | 5.90 ± 0.069                      | 1.90        |
|             | 10\(^{-4}\)                      | 4                            | 5.63 ± 0.067                      | 1.63        |
| Fungal IAA  | 5                                | 4                            | 4.88 ± 0.104                      | 0.88        |
|             | 2                                | 4                            | 5.67 ± 0.077                      | 1.67        |
|             | 1                                | 4                            | 6.32 ± 0.118                      | 2.32        |
|             | 10\(^{-1}\)                      | 4                            | **6.89 ± 0.075**                  | **2.89**    |
|             | 10\(^{-2}\)                      | 4                            | 5.06 ± 0.108                      | 1.06        |
|             | 10\(^{-3}\)                      | 4                            | 6.02 ± 0.09                       | 2.02        |
|             | 10\(^{-4}\)                      | 4                            | 5.52 ± 0.059                      | 1.52        |

Notes: The bold values indicate the optimum values of IAA for coleoptile length and growth.

\(^a\)Each value represents means ± SE of replicates per treatment.
Conclusion

Jatropha oil seedcake can be used as a raw material in the production of IAA by white rot fungi. Amongst the three cultures, *P. ostreatus* was found to synthesize maximum IAA under submerged fermentation using medium containing 2% (w/v) JSC, 0.1% (w/v) L-tryptophan, with initial pH 7.0 at 37°C upon 18 d of incubation. The production of IAA by PO was confirmed by TLC and GC-MS analysis and on the basis of wheat coleoptile bioassay, it was found to be biologically potent as synthetic IAA.

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References

Apine OA, Jadhav JP. 2011. Optimization of medium for indole-3-acetic acid production using *Pantoea agglomerans* strain PVM. J App Microbiol. 110:1235–1244.

Badenoch-Jones J, Summons RE, Djordjevic MA, Shine J, Letham DS, Rolfe BG. 1982. Mass spectrometric quantification of indole-3-acetic acid in rhizobium culture supernatants: relation to root hair curling and nodule initiation. App Environ Microbiol. 44:275–280.

Bayer MH. 1969. Gas chromatographic analysis of acidic indole auxins in *Nicotiana*. Plant Physiol. 44:267–271.

Berry DR. 1988. Physiology of industrial fungi. Oxford: Blackwell Scientific Publications.

Chung K-R, Shilts T, Ertürk Ü, Timmer LW, Ueng PP. 2003. Indole derivatives produced by the fungus *Colletotrichum acutatum* causing lime anthracnose and postbloom fruit drop of citrus. FEBS Microbiol Lett. 226:23–30.

Costacurta A, Vanderleyden J. 1995. Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol. 21:1–18.

Crocoll C, Kettner J, Dörflling K. 1991. Abscisic acid in saprophytic and parasitic species of fungi. Phytochem. 30:1059–1060.

Davies PJ. 2010. Plant hormones: biosynthesis, signal transduction, action! 3rd ed. Netherland: Springer.

Foster RJ, Mcrea DH, Bonner J. 1952. Auxin-induced growth inhibition a natural consequence of two-point attachment. Proc Natl Acad Sci. 38:1014–1022.

Gordon SA, Weber RP. 1951. Colorimetric estimation of indole-acetic acid. Plant Physiol. 26:192–195.

Jouanneau JP, Lapous D, Guern J. 1991. In plant protoplasts, the spontaneous expression of defense reactions and the responsiveness to exogenous elicitors are under auxin control. Plant Physiol. 96:459–466.

Malhotra M, Srivastava S. 2009. Stress-responsive indole-3-acetic acid biosynthesis by *Azospirillum brasilense* SM and its ability to modulate plant growth. Eur J Soil Biol. 45:73–80.

Manulis S, Haviv-Chesner A, Brandl MT, Lindow SE, Barash I. 1998. Differential involvement of indole-3-acetic acid biosynthetic pathways in pathogenicity and epiphytic fitness of *Erwinia herbicola* pv. *gypsophilae*. Mol Plant-Microbe Interact. 11:634–642.

Maor R, Haskin S, Levi-Kedmi H, Sharon A. 2004. In planta production of indole-3-acetic acid by *Colletotrichum gloeosporioides* f. sp. *aschynomene*. App Environ Microbiol. 70:1852–1854.

Morgan PW, Drew MC. 2006. Ethylene and plant responses to stress. Physiol Plantarum. 100:620–630.

Nitsch JP, Nitsch C. 1956. Studies on the growth of coleoptile and first internode sections. A new, sensitive, straight-growth test for auxins. Plant Physiol. 31:94–111.

Palmieri G, Giardina P, Marzullo L, Desiderio B, Nitti G, Cannio R, Sannia G. 1993. Stability and activity of a phenol oxidase from the lignonolytic fungus *Pleurotus ostreatus*. Appl Microbiol Biotechnol. 39:632–636.

Patten CL, Glick BR. 1996. Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol. 42:207–220.

Rakshit KD, Darukeshwara J, Raj KR, Narasimhamurthy K, Saibaba P, Bhagya S. 2008. Toxicity studies of detoxified Jatropha meal (*Jatropha curcas*) in rats. Food Chem Toxicol. 46:3621–3625.

Robinette D, Matthysse AG. 1990. Inhibition by Agrobacterium *tumefaciens* and *Pseudomonas savastanoi* of development of the hypersensitive response elicited by *Pseudomonas syringae* pv. *phaseolicola*. J Bacteriol. 172:5742–5749.

Robinson M, Riov J, Sharon A. 1998. Indole-3-acetic acid biosynthesis in *Colletotrichum gloeosporioides* f. sp. *aschynomene*. App Environ Microbiol. 64:5030–5032.

Saetae D, Suntornsk W. 2010. Antifungal activities of ethanolic extract from *Jatropha curcas* seed cake. J Microbiol Biotechnol. 20:319–324.

Schügerl K. 1981. Oxygen transfer into highly viscous media. Adv Biochem Eng Biotechnol. 19:71–174.

Shinshi H, Mohnen D, Meins F. 1987. Regulation of a plant pathogenesis-related enzyme: inhibition of chitinase and chitinase mRNA accumulation in cultured tobacco tissues by auxin and cytokinin. Proc Natl Acad Sci. 84:89–93.

Strzelecky E, Pokojska A, Kampert M. 1992. The effect of pH on production of plant growth regulators by mycorrhizal fungi. Symbiosis. 14:201–215.

Swain MR, Ray RC. 2008. Optimization of cultural conditions and their statistical interpretation for production of indole-3-acetic acid by *Bacillus subtilis* CM5 using cassava fibrous residue. J Sci Ind Res. 67:622–628.

Tuteja N. 2007. Abscisic acid and abiotic stress signaling. Plant Signal Behav. 2:135–138.

Ünyayar S. 2002. Changes in abscisic acid and indole-3-acetic acid concentrations in *Funalia trogii* (Berk.) Bondartsev & Singer and *Phanerochaete chrysosporium* Burds. ME446 subjected to salt stress. Turk J Bot. 26:1–4.

Ünyayar S, Ünal E, Ünyayar A. 2001. Relationship between production of 3-indoleacetic acid and peroxidase-laccase activities depending on the culture periods in *Funalia trogii* (*Trametes trogii*). Folia Microbiol. 46:123–126.

Ünyayar S, Ünyayar A., Ünal E. 2000. Production of auxin and abscisic acid by *Phanerochaete chrysosporium* ME446 immobilized on polyurethane foam. Turk J Biol. 24:769–774.

Xin G, Glaweb D, Dotyc SL. 2009. Characterization of three endophytic, indole-3-acetic acid producing yeasts occurring in *Populus* trees. Myco Res. 113:973–980.
Yamada T, Palm CJ, Brooks B, Kosuge T. 1985. Nucleotide sequences of the *Pseudomonas savastanoi* indole acetic acid genes show homology with *Agrobacterium tumefaciens* T-DNA. Proc Natl Acad Sci USA. 82:6522–6526.

Yesilada O, Topcuoglu SF, Ünyayar S, Ünyayar A, Fiskin K, Bozcuk S. 1990. Slempe (vinasse) iceren inkübasyon ortamında bazı beyaz çürükcül funguslarda absisik asit (ABA) üretimi. X. Ulusal Biyoloji Kongresi Erzurum. 31–37.

Yürekli F, Geckil H, Topcuoglu F. 2003. The synthesis of indole-3-acetic acid by the industrially important white-rot fungus *Lentinus sajor-caju* under different culture conditions. Mycol Res. 107:305–309.

Yürekli F, Yesilada O, Yürekli M, Topcuoglu SF. 1999. Plant growth hormone production from olive oil mill and alcohol factory wastewaters by white rot fungi. World J Microbiol Biotechnol. 15:503–505.