

Original Research Article

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Endophytic Fungi from *Oryza sativa* L.: Isolation, Characterization, and Production of GA₃ in Submerged Fermentation

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ABSTRACT

In the present investigation, an attempt was made to isolate endophytic fungi from rice plant (*Oryza sativa* L.). A total of 48 fungi were isolated from 288 segments, from 4 varieties (Gitanjali, Hiranyamayee, Khandagiri and Lalat) of rice plant (*Oryza sativa* L.). The colonization frequency of the endophytic fungi were observed to be 16.87% and 16.40% in leaf and stem respectively. All the isolates were screened for GA₃ production both qualitatively and quantitatively under submerged fermentation condition. Two isolates OSLST-4 and OSLL-4 exhibited maximum GA₃ yield were preferred to study the effect of various physical (pH, temperature, incubation period) and nutritional (Different media, salts, carbon sources, nitrogen sources) parameters on GA₃ production. It was observed that isolate OSLST-4 produced maximum amount of GA₃ (96.821 µg/ml) at pH-8, temperature-30°C on 192hrs (8 days) of incubation under submerged fermentation in a medium containing (5%) of NaCl, (0.5%) of sucrose and (0.5%) of sodium nitrate. Whereas, the isolate OSLL-04 produced maximum amount of GA₃ (78.656 µg/ml) under submerged fermentation in a medium containing (3%) of NaCl, (1%) of starch and (0.3%) of ammonium chloride at pH-6, on 240 hrs (10th days) of incubation period at 25°C. Extracellular production of GA₃ into the medium by the isolates was confirmed by TLC & FTIR analysis. Efforts are on, in our laboratory for further characterization of the isolates to exploit their potential for PGP activities for sustainable agriculture.

Keywords

Oryza sativa,
Endophytic fungi,
Submerged
fermentation, GA₃,
Condition
Optimization, TLC,
FTIR

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Introduction

In recent times it has been proclaimed that endophytic fungi can produce phytohormones chiefly gibberellic acids (GAs) that enhance crop growth and mitigate the pernicious effect of abiotic stress (Khan *et al.*, 2011).

Gibberellins are tetracyclic diterpenoid acids that regulate various plant developmental and physiological processes including seed germination, seedling development stem and leaf growth, floral initiation and flower and fruit setting (Crozier, 2000; Davies, 2010). Gibberellins also enhance other physiological

process in plants such as root growth and root hair development and inhibit floral bud differentiation in woody angiosperms. Though, 136 known gibberellins are reported from bacteria, plant and even by fungi, GA₁, GA₃, GA₄ and GA₇ are prominently bioactive. Rice is considered as an cardinal food crop universally, as rice offers food to almost half of the world's population and its consumption has been gaining importance with an escalation in the world's population (Lee *et al.*, 2001; Gyaneswar *et al.*, 2001). Employment of plant micro-biota has aptitudes to produce phyto-hormone like constituents could be an substitute to not only upsurges crop production but also to lessen the plant disease phenomenon, diminish chemical inputs and decrease emissions of greenhouse gasses, for sustainable agricultural practices. Several reports in literature stresses colonization and isolation of endophytic fungi from different parts of rice plant. Endophytic fungi have mostly been reported for their behaviour to enhance plant growth as it plays pivotal role in plant physiology and host's protection against biotic and abiotic stresses by producing various kinds of secondary metabolites similar to phytohormones.

Phytohormone production by microbes totally rely upon the processed parameters like pH, temperature, incubation period, growth potential and various nutritional conditions (Khan *et al.*, 2012; Wei *et al.*, 2013). Selection of optimal growth condition is necessary to outline the strategies for industrial production of gibberellic acids (GAs).

Computation of such potential would satisfy dual benefits in the enhancement of crop growth and sustainable agricultural yield. The current study was therefore, carried out to evaluate the potency of a novel GA₃ producing endophytic fungi isolated from four varieties of *Oryza sativa L.* (Family-Gramineae or Poaceae, Asian rice), and optimize the effect of various

physico-chemical parameters on the maximum gibberellic acid production under submerged fermentation.

Materials and Methods

Sample collection

Healthy (showing no visual symptoms) & matured seedlings of four varieties (Khandagiri, Lalat, Gitanjali & Hiranyamayee) of rice plants (*Oryza sativa L.*) were collected from OUAT (Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India) field (Lat 20°16'N, Long 85°47'E). Seedlings were transported to the laboratory aseptically in sterile polythene bags and processed within 24 hours of collection.

Isolation of indigenous endophytic fungi

The four variability of rice seedlings were rinsed gently under running tap water for five minutes for removing dust and debris and were then allowed to air dry. Before surface sterilization the cleaned stems and leaves were cut into 0.5×0.5 cm²size. Isolation and Surface sterilization of endophytic fungi were carried out according to the modified immersion procedure described by (Bills and Polishook, 1993; Strobel, 2002). Every set of plant material was immersed consecutively in 70% ethanol for two minutes, followed by immersion in 4% sodium hypochlorite for four minutes and in 70% ethanol for 45 seconds, then dipping systematically thrice with sterile distilled water. The surface sterilized stems and leaves were then air dried under laminar air flow chamber. The sterilized plant segments were applied over the surface of PDA (Potato dextrose Agar), WA (water agar), RBA (Rose Bengal agar), SDA (Sabour's Dextrose Agar), CDA (Czapeck's dox agar) and MEA (Malt extract agar) plates supplemented with streptomycin

(100mg/L) to prevent the growth of bacteria. The petridishes were incubated at $28 \pm 2^\circ\text{C}$ in BOD incubator. Plates were supervised repeatedly to check the growth of endophytic fungi. Hyphal tips growing out from the edge of the inoculated fragments were instantly transferred into PDA slants, purified, and preserved at 4°C . Non-appearance of any microbial growth form on the media plates drenched with 60 μl aliquots of final wash of water displayed the effectiveness of surface disinfection method (Schulz *et al.*, 1993).

Identification of endophytic Fungi

The fungal isolates were identified based on their morphological and reproductive characters using the standard identification manuals (Gillman, 1971; and Barnett & Hunter 1998) by LCB (Lacto Phenol Cotton Blue) staining technique of sticky tape method.

Calculation of colonizing frequency

The colonization frequency of endophytic fungi was calculated by using the formula given by Fisher and Petrini (1987) as follows.

Colonization frequency was expressed as $(\text{CF}\%) = (\text{N}_\text{C}/\text{N}_\text{T}) \times 100$

(Where N_C = Total number of plated segments colonized by endophytic fungi, N_T = Total number of segments plated)

Preliminary & Secondary screening of fungal isolates for gibberellic acid production beneath submerged fermentation

All the endophytic fungal isolates were screened for the production of gibberellic acid by plate assay as well as spectrophotometric method by using Follin-wu method (Graham and Henderson, 1961, Patil and Patil, 2014). All the isolates were aseptically inoculated on

Czapeck'dox modified agar plate and were allowed to incubate for proper growth. After 4-5 days of incubation the preliminary screening of isolates for gibberellic acid were done by spraying the phosphomolybdic reagent using the method of Graham and Henderson (1961).

Endophytic fungi that unveiled gibberellic acid production activity in the preliminary screening were subjected to spectrophotometric quantification following the above said methodology using Czapeck'dox media (CD Broth). After ten days of incubation the concentration of gibberellic acid in the culture broth was determined spectrophotometrically using phosphor-molybdic acid reagent. Briefly, 1 ml of culture supernatant was taken in a volumetric flask of 25 ml, mixed with 15 ml of phospho molybdic acid reagent and kept in a boiling water bath for one hr.

After one hr. the temperature of the flasks was reduced to room temperature and then the final volume was made up to 25ml with distilled water. The absorbance was measured at 780 nm using UV-Visible spectrophotometer (Systornics, 118). The two isolates OSLST-4 and OSLL-4 that showed maximum amount of GA_3 production were characterized further.

Condition optimization for maximum production of GA_3 by the isolates

Different physical and chemical parameters were optimized for maximum production of GA_3 by the isolates (OSLST-4 & OSLL-4).

Optimization of physical parameters

Effect of incubation period on GA_3 production

To determine the optimal incubation period for GA_3 synthesis, the two fungal isolates

(OSLST-4 & OSLL-4) were inoculated into culture flasks (250ml) containing 100ml Czapeck'dox broth (CDB) medium incubated for 96hrs, 120hrs, 144hrs, 168hrs, 192hrs, 216hrs, 240hrs, 264hrs, 288hrs at $28 \pm 1^\circ\text{C}$.

After 4th day of incubation, GA₃ amount was estimated up to 12th day at 24hrs increment using spectrophotometric method as described earlier.

Effect of temperature on GA₃ production

Culture flasks containing 100 ml of CDB inoculated with the isolates separately were incubated at different temperatures (25, 30, 35, 40 and 45°C) upto 8th day and 10th day. GA₃ production was measured spectrophotometrically as described previously.

Effect of pH on GA₃ production

For pH optimization, both the isolates OSLST-4 & OSLL-4 were cultured separately in 100ml of CDB at different pH (pH 4-12) and the flasks were incubated at 30°C and 25°C and for 8th (192hrs) & 10th (240hrs) day respectively. Production of GA₃ by the isolates were quantified spectrophotometrically as described above.

Optimization of chemical parameters

Effect of media on GA₃ production

To determine the suitable media for GA₃ synthesis, the two fungal isolates OSLST-4 & OSLL-4 were inoculated into culture flasks (250ml) containing 100ml of Different media viz. Potato dextrose broth (PDB), Czapeck'dox broth (CDB), Sabouraud's dextrose broth (SDB), Malt extract broth (MEB), incubated at 30°C and 25°C for 8th (192hrs) & 10th (240hrs) days respectively. GA₃ was estimated using the method of (Patil and Patil, 2014) as described earlier.

Effect of carbon sources on GA₃ production

Both the isolates were grown separately in 250ml conical flasks containing 100 ml of CDB supplemented with different carbon sources (sucrose, fructose, maltose, lactose, and soluble starch) at varied concentration (0.5%, 1%, 1.5%, 2%, 2.5%), and incubated at 30°C and 25°C for 8th (192hrs) & 10th (240 hrs) days respectively. GA₃ was estimated as described previously (Patil and Patil, 2014).

Effect of different nitrogen sources on GA₃ production

This experiment was designed to study the effect of different nitrogen sources on GA₃ production by the isolates. Both the isolates were grown separately in 100ml CDB supplemented with different nitrogen sources (sodium nitrate, potassium nitrate, ammonium chloride, calcium nitrate, and urea) at different concentrations (0.1%, 0.3%, 0.5%, 1%) at 30°C and 25°C for 8th (192 hrs) and 10th (240hrs) days respectively maintaining other parameters (physical and nutritional) optimal. GA₃ in the culture filtrate was estimated as described previously.

Effect of NaCl on GA₃ production

To determine the effect of NaCl on GA₃ synthesis, the two fungal isolates OSLST-4 and OSLL-4 were inoculated into culture flasks (250ml) containing 100ml Czapeck'dox broth (CDB) medium supplemented with various NaCl concentrations (1%, 3%, 5%, 7%, 10%) at 30°C and 25°C for 8th (192hrs) and 10th (240hrs) day respectively keeping other parameters constant.

GA₃ amount was estimated following the method of (Patil and Patil, 2014) as described earlier.

Gibberellic acid extraction and separation

Eight days old fermented broth (200 ml) was taken filtered in Whatman filter paper (no.1) and then centrifuged at 10000 rpm for 10 min., and the supernatant was collected, acidified to pH 2-2.5 using 1N HCl. Equal volume of ethyl acetate was added and shaken vigorously for 10 minutes. The ethyl acetate fraction was separated and re-extracted the aqueous layer with 200 ml of ethyl acetate. The ethyl acetate fractions collected and was evaporated by a rotary evaporator (Heidolf, USA) at 40°C. Residues dissolved in 2ml of methanol for analytical purposes (TLC and FTIR), was stored at 4°C.

TLC analysis

The slurry of silica gel was poured on a TLC plate, air dried, and the matrix was activated by keeping the plates on hot air oven at 80°C for 1 hr. Plates were run using mobile phase containing solvent isopropanol: ammonia: water (10:1:1v/v/v). The organic extract (30µl) was injected into the TLC plate and the standard GA₃ [(10mg/100ml (Himedia, Pvt, Ltd)] dissolved in methanol was used as reference by using the capillary tube and run for two hour. The plates were removed, sprayed with 3% sulphuric acid containing 50mg FeCl₃ and heated in oven at 80°C for ten minutes. Plates were observed under UV to detect the presence of greenish fluorescence spots, confirming the presence of GA₃ in the extract (Cavel *et al.*, 2016).

FTIR analysis

Further, the extracted GA₃ was subjected to FTIR analysis following the method of Silverstein *et al.*, (2014). The organic extract of both the isolates were completely dried and loaded to FTIR (Thermo Nicolet-6700 FTIR unit) at the transmission mode from 400-4000 cm⁻¹. Commercial GA₃ obtained from

Himedia, Pvt, Ltd. Mumbai, India was used as standard.

Statistical procedure for data analysis

One way (ANOVA) with Tukey's multiple comparisons test for preliminary qualitative analysis of fungal isolates, two way ANOVA with Bonferroni multiple comparison post test for optimization of incubation days, pH, temperature, carbon sources, nitrogen sources, media, and salt stress for two isolates (OSLST-4 and OSLL-4) on GA₃ production were carried out using Graph Pad Prism software (version 5.0, San Diego, California USA). All data are expressed as means of triplicates (Mean ± SE) and values of P ≤ 0.05 were considered as significant.

Results and Discussion

During the study, total 48 fungal strains were isolated from 288 fragments (leaves and stems) on six different media (MEA, SDA, WA, PDA, RBA, CDA) of the four varieties of *Oryza sativa* L. (Gitanjali, Hiranyamayee, Khandagiri and Lalat) and were used to examine their efficacy to yield gibberellic acid in *in-vitro* condition. The colonization frequency was highest in leaves (16.87%) followed by stem (16.40%) presented in Table 1.

The present study results are in agreement with the results of (Bhattarani *et al.*, 2014 and Radu *et al.*, 2002) who have stated that colonization frequency is observed higher in leaves than in stem. The isolated strains were distinguished primarily on the basis of morphological characters. In the present exploration, most fungal genera were tentatively identified belonged to the class of ascomycetes (*Penicillium*, *Aspergillus*, *Colletotrichum* and *Fusarium spp.*). All the endophytic fungal isolates were pure cultured on PDA slants and were maintained at 4°C for future use.

Screening of GA₃ production through qualitative and quantitative method

Primarily, all the isolates were screened for GA₃ production by plate assay method, (Fig. 1) of which, 15 were positive and screened for GA₃ production quantitatively (Fig. 2), Two isolates OSLST-4 and OSLL-4 that showed better activities through this method were characterized further.

Optimization of culture conditions of optimum GA₃ Production in submerged fermentation

Optimization of physical parameters

Effect of incubation period

It was observed that the isolates OSLST-4 and OSLL-4 produced maximum amount of GA₃ 74.365 µg/ml and 59.490 µg/ml on 8th and 10th days of incubation in CDB medium under submerged fermentation condition (Fig. 3). In corroboration Rangaswamy (2012) reported GA₃ production on 8th day by *Fusarium moniliforme* as observed in this study. In contrast, Kahlon *et al.*, (1986) observed 12 days as optimal incubation period for production of GA₃ by the same species. However, Shukla *et al.*, (2005) and Escamilla *et al.*, (2000) reported optimal production of GA₃ by microorganism varies from 10-18 days. Maximum production of GA₃ with 8-10 days by our isolates is suggestive of that these isolates could be an alternative for GA₃ production industrially. Optimization of other parameters were studied by incubating at 8th and 10th days respectively specific to the isolates.

Effect of temperature

Temperature plays an important role in production of secondary metabolites by microorganisms including growth hormones.

Hence, an experiment was designed to optimize the temperature condition for maximum production of GA₃ by the isolates, keeping other parameters constant. It was reported that isolate OSLST-4 and OSLL-4 produced maximum amount of GA₃ 84.377 µg/ml and 61.207 µg/ml respectively, in the growth medium at 30^o C and 25^o C respectively (Fig. 4). Our above findings in this investigation substantiate with the result of several workers (Kumar and Lonsane, 1990; Pastrana *et al.*, 1995; Cihangir and Aksiiza, 1997; Tomasini *et al.*, 1997; Escamilla *et al.*, 2000; Machado *et al.*, 2002; Corona *et al.*, 2005) who observed maximum production of GA₃ between 25^o-34^o C by microorganisms in different media. Observance of decline in GA₃ production at high temperatures could be due to alteration and denaturation of enzyme action at high temperature. Production of GA₃ at different temperatures by our isolates could find their possibility to be used as PGP candidates under diverse temperature in nature.

Effect of pH

Similarly, the effect of different pH on GA₃ production by the two isolates were studied by culturing the isolates at different pH in CDB. The isolate OSLST-4 produced maximum amount of GA₃ (88.945 µg/ml) on 8th day of incubation at 30^o C, at pH 8, whereas, the isolate OSLL-4 produced maximum amount of GA₃ (66.642 µg/ml) on the 10th day of incubation at 25^o C, at pH 6 (Fig. 5). In agreement to our observations (Patil and Patil, 2014), and (Sagar and Desai, 2017) reported maximum GA₃ production by fungi at pH 8.0. In addition, Pandya and Desai (2013) also reported maximum GA₃ production by the isolate *Bacillus cerus* at pH 6. In contrast to our observations Bilkay *et al.*, (2017) observed highest GA₃ production by *A.niger* and *F.moniliforme* at pH 5.0 and 7.0 respectively. Further characterization of other parameters

was carried out a specific pH for the respective isolates.

Optimization of chemical parameters

Media optimization

Production of GA₃ by the two isolates was studied under submerged condition using (CDB, PDB, SDB, MEB) and culturing under optimal physical conditions. It was observed that both the isolates produced highest amount of GA₃ in CDB(Fig. 6) which could be attributable to the low amount of glucose in the above medium (Barborakova *et al.*,2012). In agreement to our observations Rangaswamy (2012) also reported maximum production of GA₃ in CDB medium.

Effect of different carbon sources

While CDB was supplemented with different carbon sources at varied concentrations and

used to study the GA₃ production by the isolates under fermentation (optimized condition) it was observed that OSLST-4 produced maximum amount of GA₃ (96.821µg/ml) at 0.5% of sucrose and OSL-4 (77.512µg/ml), at 1% soluble starch in the medium (Fig. 7). Soluble starch being poly saccharide demonstrated to be very appropriate for Gibberellic acid production, as this result harmony with the result reported by Kumar (1987). In case of the first OSLST-4 our result reinforced with the result obtained by Rangaswamy *et al.*, (2012) i.e. sucrose was the best carbon source at a final concentration of 15g/l under optimized condition, in contrary with the result reported by Lale and Gadre (2010) i.e. glucose was the supplementary carbon source for the optimum production of Gibberellic acid by microbes. In this case we established that by breaking down of sucrose and soluble starch by the isolates quickly swapped to stationary phase and Gibberellic production was observed.

Table.1 Colonization frequency of the isolates

Characters	Plant parts inoculated vs endophytic fungi occurrence			
	Gitanjali	Hiranyamayee	Khandagiri	Lalat
Plant variety				
Stem	32/9	32/5	32/3	32/4
Leaf	40/7	40/7	40/6	40/7
CF% of stem	28.1%	15.6%	9.3%	12.5%
CF% of leaf	17.5%	17.5%	15%	17.5%

Table.2 Condition optimization for maximum synthesis of GA₃ by the isolates

Condition	OSLST-4 (<i>Aspergillus sp.</i>)	OSLL-4 (<i>Colletotrichum sp.</i>)
Media	Czapeck's dox broth medium	Czapeck's dox broth medium
Incubation period	192hrs(08 th Day)	240hrs (10 th Day)
Temperature	30 ⁰ C	25 ⁰ C
pH	8	6
Carbon source	Sucrose (0.5%)	Starch (1%)
Nitrogen source	Sodium Nitrate (0.5%)	Ammonium chloride (0.3%)
NaCl (%)	5	3

Fig.1 Screening of the isolates for GA₃ production by plate assay method. A:OSLST-4(*Aspergillus sp.*) B: OSLL-4 (*Colletotrichum sp.*)

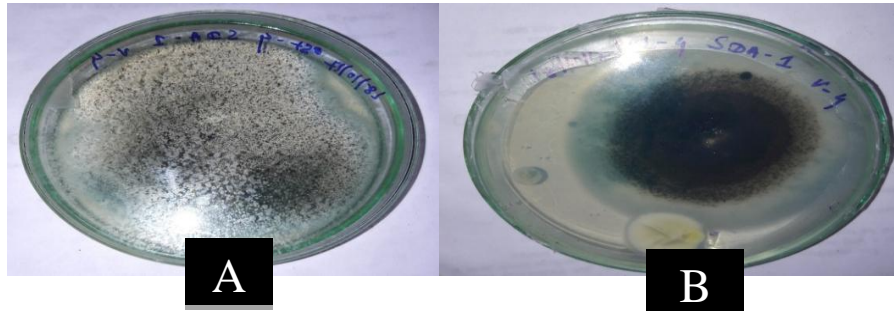


Fig.2 Quantitative Screening for Gibberellic Acid activity by the isolates

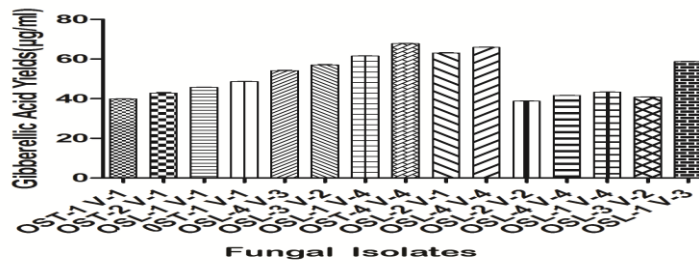


Fig.3 Effect of incubation period on GA₃ production of the isolates [OSLST-4(*Aspergillus sp.*) and OSLL-4(*Colletotrichum sp.*)]

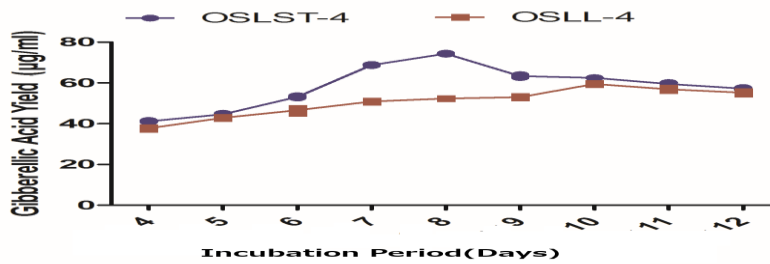


Fig.4 Effect of temperature on GA₃ production by the isolates [OSLST-4 (*Aspergillus sp.*) and OSLL-4 (*Colletotrichum sp.*)]

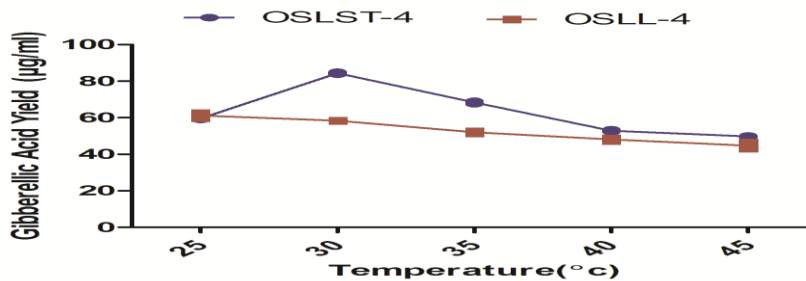


Fig.5 Effect of pH on GA₃ production by the isolates [OSLST-4(*Aspergillus sp.*) and OSLL-4 (*Colletotrichum sp.*)

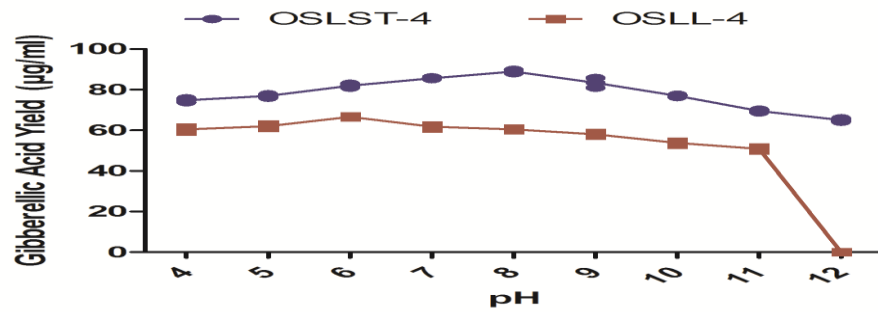


Fig.6 Effect of different media on GA₃ yield by the isolates [OSLST-4 (*Aspergillus sp.*) and OSLL-4 (*Colletotrichum sp.*)

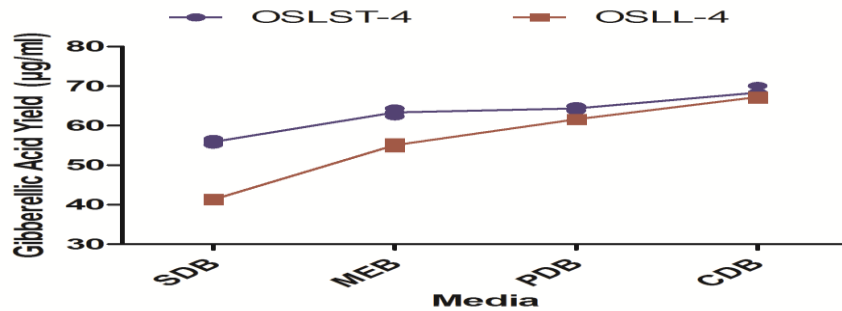
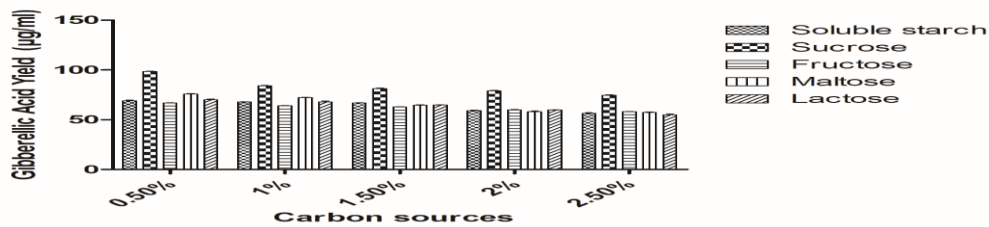
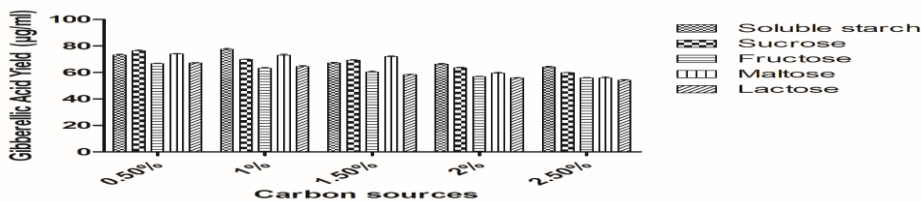


Fig.7 Effect of different carbon sources on GA₃ production by the isolates. A: OSLST-4 (*Aspergillus sp.*) B: OSLL-4 (*Colletotrichum sp.*). Each value is the mean of three replicates (n=3). Error bars showing the ±SE



(A)



(B)

Fig.8 Effect of different nitrogen sources on GA₃ production by the isolates. A: OSLST-4 (*Aspergillus sp.*) B: OSLL-4 (*Colletotrichum sp.*). Each value is the mean of three replicates (n=3). Error bars showing the ±SE

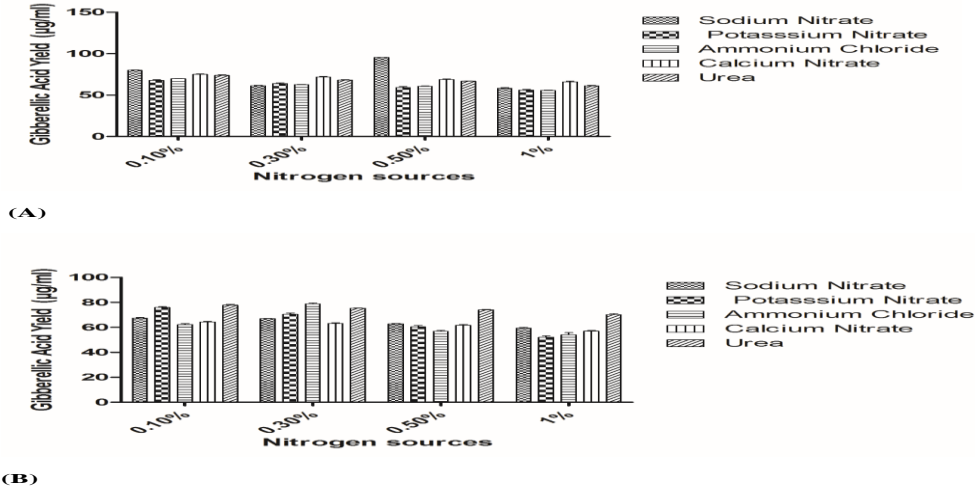


Fig.9 Effect of NaCl on GA₃ production by the isolates [OSLST-4 (*Aspergillus sp.*) and OSLL-4 (*Colletotrichum sp.*)

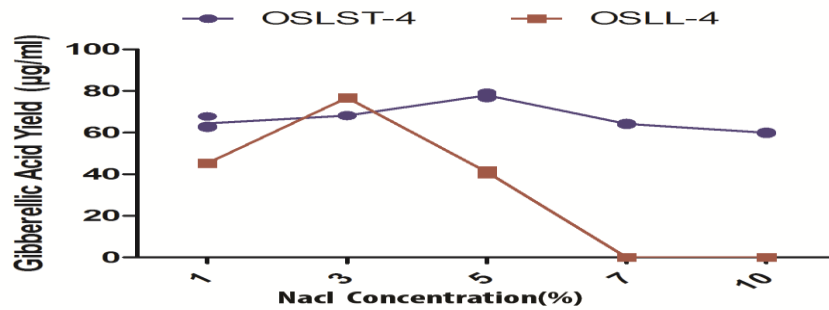


Fig.10 Characterization of GA₃ by TLC. Both standard and extract showed similar R_F value. A: OSLST-4 (*Aspergillus sp.*) B: OSLL-4 (*Colletotrichum sp.*)

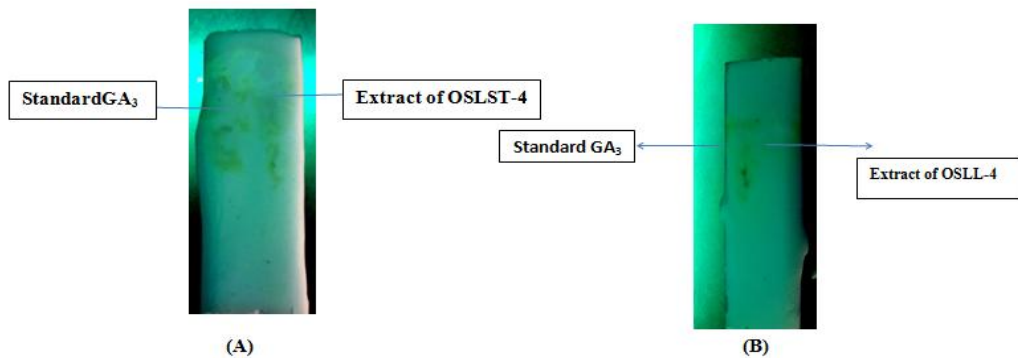
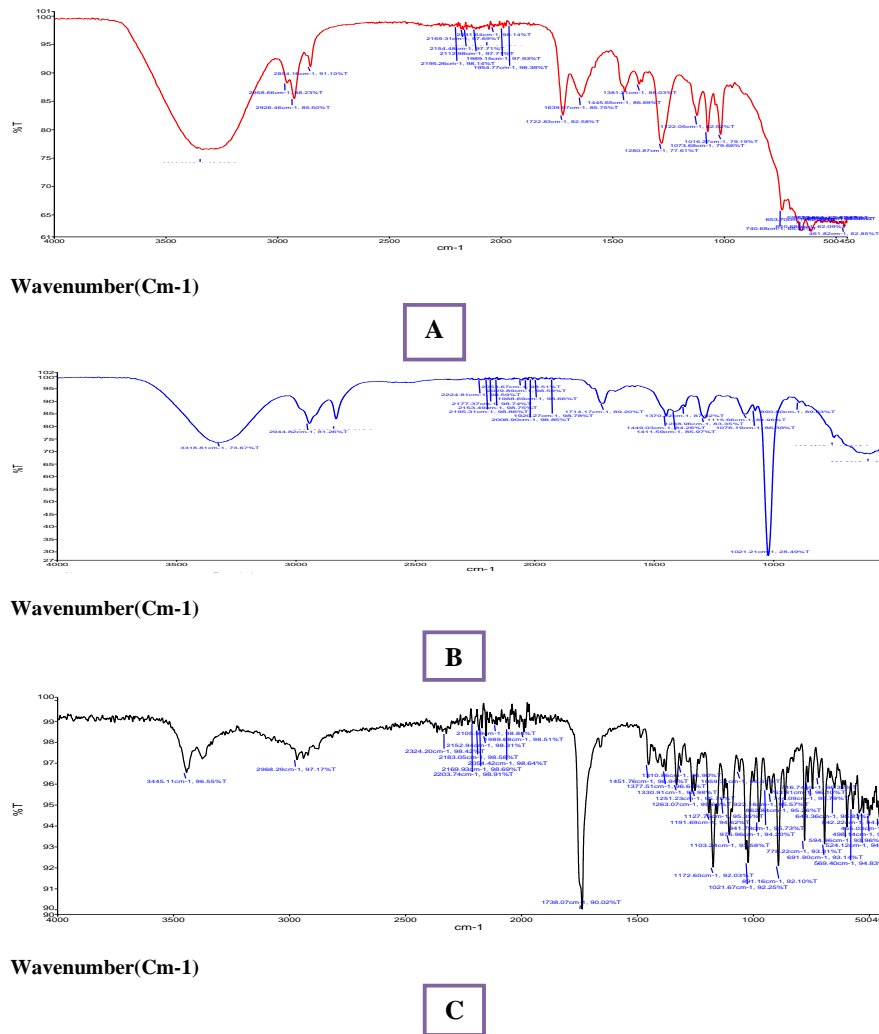


Fig.11 FTIR spectra of GA₃ produced by the isolates. A: A: OSLST-4 (*Aspergillus sp.*); B: OSSL-4 (*Colletotrichum sp.*); C: Standard GA₃



Effect of different nitrogen sources

Effect of additional nitrogen supplementation on gibberellic acid production was premeditated. The maximum gibberellic acid production of 95.390µg/ml was witnessed, while the CDB medium was supplemented with 0.5% sodium nitrate inoculated with the isolate OSLST-4 whereas, the maximum gibberellic acid production of 78.656µg/ml with 0.3% ammonium chloride inoculated with the isolate OSSL-4 (Fig. 8). Our results corroborated with the outcome of Sagar and Desai *et al.*, (2017) i.e who reported NH₄Cl

(0.5%) as the outstanding nitrogen source for GA₃ production for the isolate K-37 in contrast to the report also spotted by the same author i.e. 0.5% urea was also good for another isolate K-8. The results suggested that with magnification in carbon and nitrogen sources concentration the production of GA₃ diminished significantly. This might be endorsed to the fact that the low concentration of carbon and nitrogen source in the medium terminates exponential growth of micro-organism but elicits secondary metabolism (Escmilla *et al.*, 2000).

Effect of NaCl

The isolates OSLST-4 and OSLL-4 produced maximum amount of GA₃ under optimized fermentation condition at 5% and 3% of NaCl supplemented to CDB medium (Fig. 9) with optimal C and N and physical parameters. In agreement to our observations Rasulov *et al.*, (2010) cited that salinity not only affects the growth and development of microorganisms but also regulates the secondary metabolite production in the medium.

The maximum production of GA₃ by the two fungal isolates OSLST-4 and OSLL-4 were observed in the Czapek's dox medium on 8th day (192hrs) and 10th day (240hrs), at 30^oC and 25^oC in pH 8 and 6, by using carbon (sucrose 0.5% and starch 1%) and nitrogen (sodium nitrate 0.5% and ammonium chloride 0.3%) sources, with NaCl concentration (5% and 3%) respectively (Table 2).

Characterization of GA₃ production by the Isolates

TLC Analysis

The crude extracts of the isolates were subjected to preparatory TLC. Standard GA₃ procured from (Himedia, pvt. ltd, Mumbai, India) was served as control. Both the extracts and standard GA₃ showed similar RF value of 0.7 confirming the presence of GA₃ in the extract (Fig. 10).

FTIR analysis

FTIR spectra revealed the broad spectrum absorption band. The major characteristic peaks achieved by FTIR in standard GA₃ (Fig. 11) were at 1000-1260 cm⁻¹ corresponds to (C-O) group (stretching and asymmetrical coupled vibration) i.e. the peak at 1127 cm⁻¹. The band at 1451cm⁻¹ resembles to (C=C) group(stretch consist of asymmetric type).

The peak at 1738cm⁻¹ allied to (C=O) stretching from carboxylic group. Further, the peak perceived at 3445cm⁻¹ corresponds to (OH) group (intra-molecular hydrogen bonding). For extracted GA₃ of OSLST-4 (Fig. 11) the peaks were achieved at 1122cm⁻¹ corresponds to (C-O) group, the peak found at 1722cm⁻¹ attributed to (C=O) group, and the peak endorsed to 3337cm⁻¹ signposted the presence of (OH) group. The peak observed at 1445cm⁻¹ ascribed to (C=C) group (stretching consist of asymmetric). For extracted GAs of OSLL-4 (Fig. 11) the peaks were gained at 1115cm⁻¹ (C-O), 1714cm⁻¹ (C=O), the peak observed at 1449cm⁻¹ (C-C) group (Asymmetric stretching), the peak observed at 3318cm⁻¹ attributed to (OH) group.

The result of the infrared spectroscopy of the extracted GA₃ using FTIR presented that it contained four out of four main characteristic bands existent on the standard GA₃ sample. All the perceived characteristic FTIR peaks communally confirm the presence of GA₃ like substance in the extract.

In conclusion through this scientific investigation we place in record, isolation of endophytic fungi from rice plant with the ability for GA₃ production. The isolates can be exploited industrially for commercial production of GA₃ with further scientific investigations. Though, it is a preliminary endeavour, studies such as this is a prerequisite to exploit the biotechnological potential of microbes of special environment, more specifically the endophytic fungi, for production of growth promoting compounds.

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