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Research Article

Optimization of gibberellic acid production from *Pseudomonas* sp. isolated from the soil sample

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Abstract

The present study reports the optimal process conditions for gibberellic acid production by *Pseudomonas* sp. isolated from the soil. A total of 15 bacterial isolates with different colony morphology were tested for the production of gibberellic acid. Among the bacterial isolates, *Pseudomonas* sp. was showed maximum amount of gibberellic acid. Gibberellic acid production was found to be maximum after 8 days of incubation at 30 °C incubation temperature. The organism was grown in culture medium containing varying carbon sources such as sucrose, lactose, glucose, maltose and trehalose. Each of these carbon sources was added to nutrient broth individually at 1% level. Among the carbon sources, glucose significantly enhanced the production of gibberellic acid (49 µg/ml). The maximum gibberellic acid production of was observed in flask supplemented with 1.0% ammonium chloride.

Key words: Plant growth hormones, gibberellic acid, *Pseudomonas* sp.

Intoduction

Soil bacteria known as plant growth promoting rhizobacteria promote plant growth directly or indirectly through many mechanisms. Among the growth promoting mechanisms the release and production of various metabolites such as cytokinin, auxin, cytokinin, gibberellins (GAs) are very important (Kang et al., 2009). GAs influence a wide range of processes including flowering, sex expression, dormancy, seed germination and stem elongation in plant species (Sponsel, 2003). GAs consist of a family of dipertenoid acids related, representing group of phytohormones that promote different effects on development and growth of plants. GAs can significantly influence the expansion, germination of leaves and development of flowers. GAs significantly stimulates the activity of transference, generating good development of phloem and xylem in plants (Haisel et al., 2001). These properties make GAs a valuable tool in agriculture sector (Shukla et al., 2005). Approximately 120 GAs were already isolated and characterized by various researchers across the Globe. Among the 120 GAs, GA3 has attracted much more attention. Presently, commercial application of GA3 is observed in nursery, agriculture and flowers greenhouse (Rodrigues et al., 2012).

GAs are widely produced by plants, fungi, algae and bacteria. However, due to high concentrations of GAs in fungus, industrial production of GAs is mainly performed by using the fungus *G. fujikuroi* in submerged fermentation. Extraction of GAs is not industrially viable from plants because of very low concentrations of GAs (Shi et al., 2016). The mode of action of GAs, biosynthesis

and relationships between activity and structure have been investigated and little information is known about optimization of GAs production (Hollmann et al., 1995). GA3 is mainly produced by submerged fermentation techniques using hyper producing *Gibberella fujikuroi* and *Fusarium moniliforme* (Santos et al., 2003). The bacteria from the genus *Azospirillum* and *Azotobacter* produce GA3 (Rademacher, 1994). Production of GA3 is influenced significantly by various bioprocess conditions. Some of the significant factors in obtaining increase yields of the GA3 include temperature, pH, incubation time, carbon and nitrogen sources (Cihangir and Aks, 1993). In the present study, *Pseudomonas* sp. was isolated from the soil sample and the process parameter were optimized to enhance the production of GA3 in submerged fermentation.

Materials and methods

Chemicals

Most of the chemicals were purchased from Himedia, Mumbai, India. The other chemicals used were analytical grade.

Sample

Soil samples were collected from agricultural land for the isolation of various microorganisms for gibberellic acids production. All samples were collected in plastic bags and stored at 4 °C for further analysis.

Isolation and screening of gibberellic acid producing bacterial isolates

For the isolation of bacterial isolates, 0.1 ml sample was spread on nutrient agar plates and incubated for 24 h at 37 °C. After incubation, nutrient agar plates were analyzed for morphologically different colonies and screened for the production of gibberellic acids. For screening of GA3 producing bacterial strains, 50 ml of nutrient broth was inoculated with a loop full culture of bacteria and incubated at 30 °C for 8 days at 150 rpm. After 8 days of incubation, culture supernatant was used to analyze the amount of GA3 produced by bacterial isolates.

Estimation of GAs

The gibberellins were assayed using a UV-Visible spectroophotometer. To 10 ml of cell free extract, 2 ml of zinc acetate reagent (1 ml of glacial acetic acid + 21.9 g zinc acetate was made upto 100 ml with double distilled water) was added and mixed. After 2 min of incubation, 2 ml of potassium ferrocyanide (10.6% in double distilled water) was added and further centrifuged (2000 rpm for 15 min). To 5 ml of this supernatant 5 ml of HCl (30%) was added and was incubated at 30 °C for 30 min. For reagent blank 5 ml of 5% HCl was added. Finally, the absorbance was measured at 254 nm. The concentration of GAs was calculated by preparing standard curve by GA3 (Hi-media, Mumbai, India) as standard (100-1000 µg/ml).

Optimization of process parameters to enhance the production of gibberellic acid

Effect of fermentation period on gibberellic acid production

To find the optimum incubation period on gibberellic acid production, the culture medium (nutrient broth) was incubated for 8 days at 37 °C. The cell free supernatant was subjected to analyze gibberellic acid production. Samples were removed from the culture medium at regular interval and analyzed for amount of gibberellic acid produced.

Effect of temperature on GA3 production

To optimize the culture temperature, the bacterial suspension was inoculated in nutrient broth and incubated at various temperatures (20 – 40 °C) and kept on a rotary shaker at 150 rpm. The culture medium was analyzed for amount of gibberellic acid produced.

Effect of carbon supplementation on gibberellic acid production

Effect of supplemented carbon sources, namely, lactose, glucose, maltose, sucrose and trehalose on GA3 production was evaluated. For this, 100 ml of nutrient broth was supplemented with various carbon sources at 1% level. 0.1 ml bacterial suspension was inoculated and Erlenmeyer flasks were incubated at 37 °C at 150 rpm. Samples were centrifuged and checked for amount of gibberellic acid produced.

Effect of nitrogen sources on gibberellic acid production

Effect of nitrogen sources, namely, ammonium chloride, urea, yeast extract and beef extract were tested for gibberellic acid production. For this, 100 ml of nutrient broth was supplemented with various nitrogen sources at 1% level. 0.1 ml of bacterial suspension was inoculated and Erlenmeyer flasks were incubated at 150 rpm for 37 °C. Samples were centrifuged and checked for amount of gibberellic acid produced.

Results and discussion

In the present study, a total of 15 bacterial isolates with different colony morphology were tested for the production of gibberellic acid. In this study, only 3 bacterial isolates showed gibberellic acid production above 10 µg/ml which were 17 µg/ml, 23 µg/ml and 22 µg/ml. Maximum gibberellic acid producing *Pseudomonas* sp. was used for optimization studies. The production of GA3 by submerged fermentation process is mainly controlled by catabolic repression and substrate inhibition. This catabolic repression can be overcome by the addition of slowly consumed carbon sources with the culture medium.

Effect of fermentation period on gibberellic acid production

In the present study, gibberellic acid production was found to be maximum after 7 days of incubation at 30 °C (Fig. 1). The kinetics of biomass and GA3 production by the organism were reported previously by Borrow et al. (1964). During exponential growth phase, the nutrient sources such as, glucose, nitrogen and phosphate are consumed. However, GA3 production starts only after nitrogen is exhausted. The effect of fermentation period on the growth and GAs production was studied in *Fusarium moniliforme* LPB 03. In this case, GA3 production started after two days of incubation and production was found to be high after 6 days of incubation (Rodrigues et al., 2016). Hollmann et al. (1995) reported that GA3 production was optimum at 200 h of incubation and the amount of GA3 produced was decreased sharply after 200 h in *Gibberella fujikuroi*. Escamilla et al. (2000) found that in fungi GA3 production was maximum at the lag phase of growth and decreased latter. The decreased GA in the culture medium after the optimum incubation period is many due to product inhibition, bio-degradation of the product by the culture and chemical decomposition.

Fig.1. Effect of fermentation period on gibberellic acid production

Effect of incubation temperature on GAs production

In the present study, gibberellic acid production was found to be maximum at 30 °C (Fig. 2). In *Fusarium moniliforme*, 30 □ was optimum for the production of GA3 (Rangaswamy, 2012). The effect of incubation temperature on GA3 production is mainly depending on the selected strain. The temperature such as, 25 °C, 27 °C, 28 °C, 29 °C and 30°C were reported as optimum for the production of gibberellic acid (Rodriques et al., 2011). It was previously reported that bacterial growth and GA3 production increased with increasing incubation temperature. The maximum bacterial growth and GA3 production was detected at 30 °C and growth and GAs production started to decrease at higher temperature (35 °C). Zamanian et al.

(1987) stated that maximum GA3 production at 30 ± 2°C in *P. putida*. Rodrigues et al. (2011) reported that temperature is one of the critical factors for the production of GAs.

Fig. 2. Effect of temperature on gibberellic acid production

Effect of carbon sources on GAs production

The organism was grown in culture medium containing varying carbon sources such as sucrose, lactose, glucose, maltose and trehalose. Each of these carbon sources was added to nutrient broth individually at 1% level. Among the carbon sources, glucose significantly enhanced the production of gibberellic acid (49 µg/ml) (Fig. 3). Previously various carbon sources were used for the production of GAs. Sucrose and glucose are often used as carbon sources, but glucose concentrations >20% at the start of fermentation should be avoided, as they cause catabolic repression. The alternative carbon sources such as mannitol, maltose, plant meal or mixture of easily utilized carbon sources are useful to enhance the production of gibberellic acid (Gelmi et al., 2000). Lale and Gadre (2010) applied glucose as potential carbon source and reported increased production of gibberellins. Similar results were obtained by Shukla *et al.* (2005). Gulewicz et al. (1994) reported that rafinose and sucrose were significantly enhanced the production of GA2

Fig. 3. Effect of carbon sources on gibberellic acid production

Effect of nitrogen sources on gibberellic acid production

Effect of nitrogen source, on gibberellic acid production was assayed. The maximum gibberellic acid production of was observed in flask supplemented with 1.0% ammonium chloride inoculated with *Pseudomonas* sp. Supplemented urea also significantly enhanced the production of gibberellic acid (Fig. 4). The quantity and quality of nitrogen in the culture medium is very important for gibberellin production due to the regulation of ammonium. High production of GA3 are reached when the nitrogen concentration in the culture media is very low. The inorganic nitrogen sources like ammonium chloride significantly enhanced gibberellic acid production (Lale and Gadre, 2010).

Fig. 4. Effect of nitrogen sources on gibberellic acid production

Karakoc and Aksoz (2004) optimized carbon-nitrogen ratio to enhance the production of gibberellic acid by *Pseudomonas* sp. In another study, the nitrogen sources such as, KNO_3, NH_4NO_3 and NH_4Cl were tested. GA3 production was found to be maximum by the addition of $NaNO_3$ to the culture medium (Cihangir and Aksoz, 1993).

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