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Studies on Gibberellic Acid Production by Bacillus Licheniformis DS3 Isolated from Banana Field Soils

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ABSTRACT

The current study reports the Gibberellic acid production by Bacillus licheniformis DS3 isolated from banana field soils, Tadepalli, Guntur district of Andhra Pradesh, India. Various physical conditions (incubation period, pH, effect of mevalonic acid, carbon and nitrogen sources) affected by gibberellic acid production. The highest level of gibberellic production (83.7µg/ml) was obtained in the nutrient broth media when the Bacillus licheniformis DS3 was incubated for 48 h of incubation and at pH 7.0. Maximum production of (67.5µg/ml) was recorded in 60 mM concentration of mevalonic acid. Carbon and nitrogen sources also greatly influenced the Gibberellic acid production. Maximum production was recorded at 1% glucose and 0.5% yeast extracts were used as carbon and nitrogen sources. Possession of plant growth promoting traits, like Bacillus licheniformis DS3 a potential strain to be developed as multifunctional biofertilizer.

Keywords: Bacillus licheniformis, Gibberellic acid (GA3), PGPR.

I. INTRODUCTION

Gibberellic acids, also known as gibberellins, are the complex organic molecules acting as plant growth hormones. They are chemically known as di terpenoid acids having molecular formula $C_{19}H_{22}O_6$. Gibberellins produced by plant growth promoting rhizobacteria (PGPR) promote the plant growth and increases yield of many crop plants (Piccoli *et al.,* 1997). Among all, 136 GAs have been identified from higher plants (128 species), 28 GAs from fungi (7 species), and only 4GAs (GA1, GA3, GA4, and GA20) from bacteria (7 species) (MacMillan, 2002). Gibberellic acid (GA3), the main product of gibberellins in fungi and bacteria, is a terpeanoid hormone that is an important phyto hormone regulating plant growth and development.

Plant growth promoting rhizobacteria act as biofertilizers directly when they help to provide nutrients to the host plant, and indirectly by their positive influence to the growth of roots and morphology or in aiding in some other beneficial symbiotic relationships though not all PGPR are biofertilizers. A number of PGPR stimulate the plant growth by controlling pathogenic organisms (Vessey, 2003).

These beneficial effects of PGPR can be involved in either direct or indirect mechanisms and these bacterial PGPR were used as biofertilizers. Direct promotion of growth by PGPR occurs when the rhizobacteria produce metabolites that promote plant growth such as auxins (Asghar *et al.*, 2002), cytokinins (Arkhipova *et al.*, 2005) and gibberellins (Gutierrez-Manero *et al.*, 2001; Joo *et al.*, 2004) as well as through the solubilization of phosphate minerals (Freitas *et al.*, 1997). Indirect growth promotion occurs through the elimination of pathogens by the production of cyanide (Owen and Zlor, 2001). As much as most of the fertilizers are associated with environmental pollution, plant growth hormones like gibberellic acid (GA3) have to be produced cost-effectively in huge amounts

in order to enhance the quantity of agricultural products (Bilkay *et al*., 2010).

Many species shown that the *Bacillus* species isolated from rhizosphere of *Alnus glutinosa*, *B. pumilus* and *B. licheniformis*, had a strong growth-promoting activity (Probanza *et al*., 1996). Although it was soon found that both were auxin producers (Gutie´rrez-Man˜ero *et al*., 1996), the characteristics of the induced growth are also suggestive of GA-like promotion.

Bacillus is the most abundant genus in the rhizosphere and the PGPR activity of some of these strains has been known for many years, resulting in a broad knowledge of the mechanisms involved. There are number of metabolites that are released by these strains (Charest et al., 2005), which strongly affect the environment by increasing nutrient availability of the plants. From the available literature the most important gibberellic acid production by *Bacillus* species were very merge. Hence it was proposed to carry out the optimization of gibberellic acid production by *B. licheniformis* DS3 isolated from banana field soils.

II. MATERIALS AND METHODS

1. Isolation of Bacteria

The Amylolytic bacteria (*Bacillus licheniformis* DS3) was isolated from banana fields and screened for α-amylase production. The starch medium used for the isolation of bacteria contained (g/L): Starch , 10.0; yeast extract, 5.0; peptone, 2.0; $MgSO_4.7H_2O$, 0.5; KH_2PO_4, 0.5; NaCl, 1.5; $CaCl_2$, 0.1; Agar, 20.0. Initial pH was adjusted to 7.0. One gram of each soil sample was suspended in 9.0 ml of sterile water and 0.1 ml of suitably diluted suspension was spread on the agar plates. The plates were incubated at 35^0C, for 24 to 48 hours. The isolated colonies were flooded with iodine solution. Pure Colonies with best colourless halos around them were picked and maintained on starch agar slants at 4^0C and further assessed for enzyme production in liquid medium. The characterization and identification of the isolate was made following Bergey's Manual of Systemic Bacteriology (Sneath, 1986).

2. Gibberellic acid extraction and determination

Culture media were filtered, and then samples were acidified to pH 2.5 with HCl and extracted using liquid-liquid (Ethyl acetate/NaHCO3) extraction (Cho *et al*., 1979). Gibberellic acid in the ethyl acetate phase was measured by UV spectrophotometer (Jenway 6105 UV/VIS) at 254 nm (Bruckner B, Blechschmidt, 1991). The amount of gibberellic acid was calculated from the standard curve.

3. Optimization of Physico-Chemical parameters for Gibberellic Acid Production

i). Effect of incubation time on Gibberellic acid production

To determine the optimal incubation time for GA3 synthesis, *Bacillus licheniformis* DS3 was inoculated into nutrient broth incubated for 12, 24, 36, 48, and 72 hours at 30^0 C on a rotary shaker (200 rpm). After incubation, bacterial growth and the GA3 amount were estimated by using spectrophotometer.

ii). Effect of mevalonic acid (MVA) on Gibberellic acid Production

Stimulation of gibberellin production in submerged culture using a commonly known precursor, mevalonic acid (MVA), was investigated in terms of growth and gibberellic acid production. Different 20-100 mM concentrations of mevalonic acid were introduced in to the nutrient broth *Bacillus licheniformis* DS3 was inoculated for 48 hours at room temperature (30^0 C) on a rotary shaker (200 rpm). After incubation, bacterial growth and the amount of Gibberellic acid were estimated by using spectrophotometer.

iii). Effect of pH on Gibberellic acid production

Different pH (5.0, 6.0, 7.0, 8.0 and 9.0) were introduced in to the nutrient broth *Bacillus*

licheniformis DS3 was inoculated for 48 hours at room temperature on a rotary shaker (200 rpm). After incubation, bacterial growth and the amount of Gibberellic acid were estimated by using spectrophotometer.

iv). Effect of sugars on Gibberellic acid Production

To study the gibberellic acid production various (1%) sugars (Arabinose, Starch, Maltose, Glucose, lactose and fructose) were supplemented into the nutrient broth medium. After inoculation of *B. licheniformis* DS3 for 48 hours of incubation, bacterial growth and the amount of Gibberellic acid were estimated by using spectrophotometer.

v). Effect of nitrogen sources on Gibberellic acid Production

To study the effect of gibberellic acid production in various (0.5%) nitrogen sources (Peptone, Ammonium sulphate, Beef extract, yeast extract, potassium chloride and L-Aspargine) were supplemented into the nutrient broth medium. After inoculation of *B. licheniformis* DS3 for 48 hours of incubation, bacterial growth and the amount of Gibberellic acid were estimated by using spectrophotometer.

vi). Statistical analyses

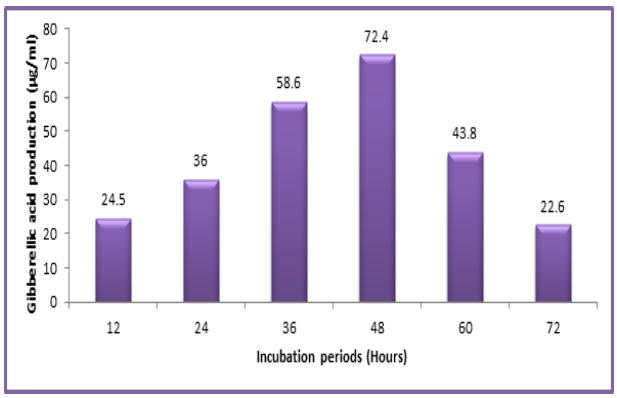
Results are mean of three replicates. Means of growth and production parameters were compared using analyses of variance and significant differences were identified with the Duncan multiple range test (P<0.05).

III. RESULTS AND DISCUSSION

Plant growth promoting *B. licheniformis* DS3 isolated from banana field soils and tentatively identified on the basis of biochemical tests and sugar fermentation tests as described in Bergey's Manuel of Determinative Bacteriology. Further this strain was tested by plant growth promoting ability was showed multiple plant growth promoting characterstics. This paper particularly describes the Gibberellic acid production by *B. licheniformis* DS3. Maximum gibberellic acid production (83.7µg/ml) was recorded at 48 h of incubation at pH 7.0. Sivasakthi, (2013) reported that the maximum gibberellic acid production (4.8 µg/ml) by plant growth promoting *Bacillus subtilis* strain isolated from paddy rhizosphere. Similarly Apastambh *et al.*, (2016) studied the isolates of *Bacillus* species Yb1 were positive for Gibberellic acid (GA) production was the highest producer of GA (52 µg/ml) the strain isolated from banana rhizosphere.

1. Effect of incubation period on Gibberellic acid production

Gibberellic acid production was measured at every 12 h of intervals i.e. 12 to 72 hours. Maximum gibberellic acid production (72.4 µg/ml) was recorded at 48 h of incubation period (Figure -1). Therefore the incubation period at 48 hours was considered as maximum for gibberellic acid production. Further increase in incubation period the gibberellic acid production was decreased. A previous report in *Pseudomonas* species showed the gibberellic acid (GA3) production started at 12 h of incubation and reached a maximum level (279.6 mg/l) at 72 hours was reported by Karakoc S. and Aksoz N. (2006).



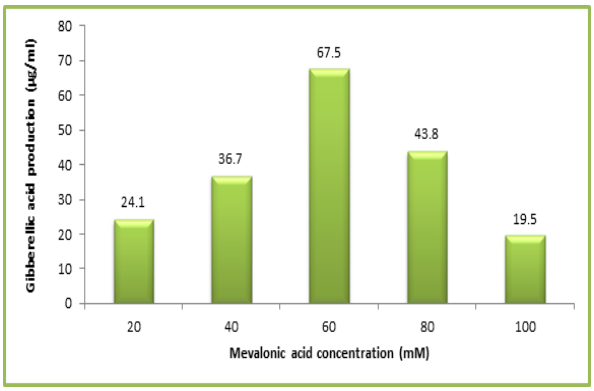
*Significant at p<0.001

Figure 1. Effect of incubation period on Gibberellic acid production

2. Effect of mevalonic acid

Gibberellic acid is obtained in an improved metabolic process which comprises cultivating an active strain of

Bacillus licheniformis DS3 in a nutrient medium, checking active growth to promote gibberellic acid production and adding mevalonic acid as a precursor to the nutrient medium thereby increasing gibberellic acid production. Different concentrations 20 to 100 mM of mevalonic acid were added to the nutrient medium. Maximum production (67.5µg/ml) was recorded in 60 mM concentration of mevalonic acid. Production was increased with increasing mevalonic acid up to 80 mM concentration. Further increase in mevalonic acid the production was decreased (Figure - 2).

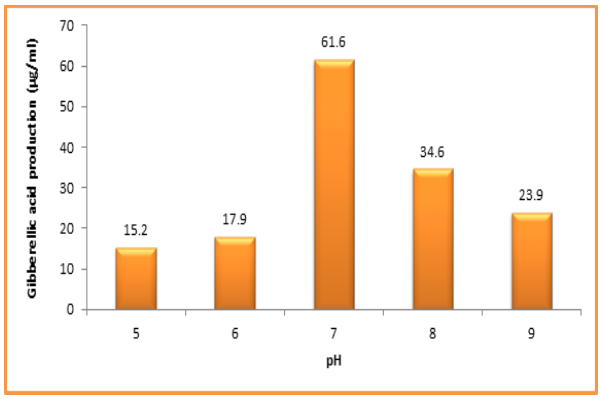


*Significant at p<0.001.

Figure 2. Effect of mevalonic acid on Gibberellic acid production

3. Effect of pH

Generally the pH of the medium enormously effected the growth and gibberellic acid production. Maximum production was observed at pH 7.0. with 61.6 µg/ml. Less amount of gibberellic acid was recorded in above and below neutral pH 7.0. (Figure -3). Sagar Desai (2017) reported that the Maximum gibberellic acid production of 40.8 µg/ml was observed at pH 8.0 with the strain *Pseudomonas Spp.* K8. isolated from rhizospheric soils from sugarcane farms in the vicinity of Surat. Similarly the maximum yield was obtained at pH 7.0, while lower GA3 production and bacterial (*Pseudomonas* sp.) growth were obtained between pH 5.5 and 6.0 reported by Karakoc S. and Aksoz N. (2006).

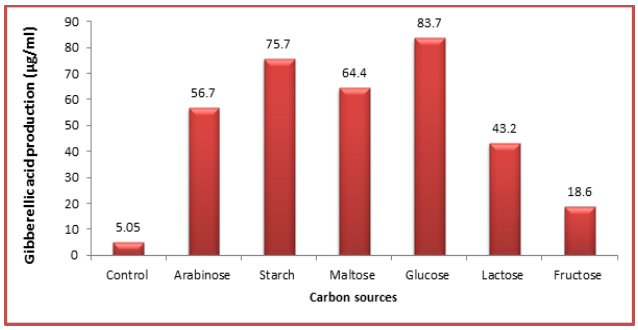


*Significant at p<0.001.

Figure 3. Effect of pH on Gibberellic acid production

4. Effect of carbon sources

From the results maximum gibberellic acid production (83.7 µg/ml) was observed in (1%) glucose was used as the carbon source. For the production of gibberellic acid, glucose was replaced with different carbon sources were tested. Among them starch containing the medium also showed the maximum gibberellic acid (75.7 µg/ml) production. Relatively maximum gibberellic acid production was observed in maltose (64.4 µg/ml) and arabinose (56.7 µg/ml) used as carbon source (Figure- 4). Lowest amount of gibberellic acid production was recorded in the presence of fructose (18.6 µg/ml). Similarly, maximum gibberellic acid production of 87.6 µg/ml was observed in flask containing 1% glucose inoculated with isolate *Pseudomonas Spp.* K8 reported by Sagar Desai (2017). The highest yield of GA3 productivity was found in growth medium supplemented with glucose (445.52 mg/L) by *Bacillus cereus* isolated from the rhizosphere of sugarcane (Pandya and Desai, 2103).

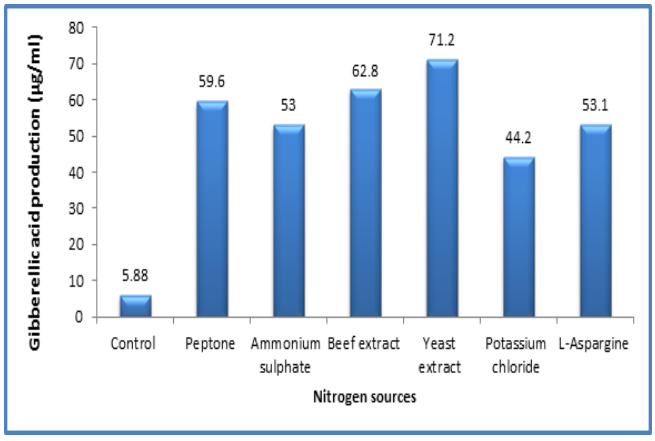


* The overall model is significant with p<0.005

Figure 4. Effect of carbon sources on Gibberellic acid production

5. Effect of nitrogen sources

Various nitrogen sources were tested, yeast extract was identified as the best and suitable nitrogen source for the highest level of gibberellic acid production (71.2 µg/ml). Maximum production (62.8 µg/ml) was also observed in beef extract was used as nitrogen source (Figure -5). Sagar Desai (2017) have reported that the maximum gibberellic acid production of 76.8 µg/ml was observed in flask supplemented with 0.5% ammonium chloride inoculated with isolate *Azotobacter Spp*. K37. Lale and Gadre (2010) also used inorganic nitrogen sources like ammonium chloride for gibberellic acid production.



* The overall model is significant with p<0.005

Figure 4. Effect of Nitrogen sources on Gibberellic acid production

IV. CONCLUSION

From this study it could be concluded bacterial isolate were identified as *B. licheniformis* DS3 had a potential of gibberellic acid production and can be further explored for its utilization for plant growth promoting capacity. The results showed that production of GA3 is highly dependent on the optimization of some cultural parameters. For the first time we are reporting the different mevalonic acid concentrations and also different carbon and nitrogen sources affected the gibberellic acid production by *B. licheniformis* DS3 isolated from banana field soils in the vicinity of Guntur, A.P. India. For the optimization study, GA3 production was maximized (83.7 µg/ml) at 48 hours of incubation at pH 7.0 in the room temperature on a rotary shaker (200 rpm).

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