JOURNAL OF PURE AND APPLIED MICROBIOLOGY, December 2013. Vol. 7(4), p. 3239- 3242

Gibberellic Acid Production by *Bacillus cereus* Isolated from the Rhizosphere of Sugarcane

N.D. Pandya[1] and P.V. Desai[2]

[1]Department of Microbiology, Arts, Science and Commerce College, Kamrej Cross Road, Surat - 394 185, India.
[2]Department of Biosciences, Veer Narmad South Gujarat University, Surat - 395 007, India.

(Received: 07 February 2013; accepted: 02 April 2013)

Gibberellic acid (GA_3) producing efficient strain was isolated from rhizospheric soil of sugarcane in the vicinity of Surat city, India and characterized as *Bacillus cereus* according to standard methods. This isolate was tested for the gibberellic acid production in a Nutrient medium. Optimization of various physiological conditions (inoculum size, incubation temperature, pH of the growth media, incubation period, and incubation condition) were carried out to achieve maximum production of gibberellic acid. The highest 394.18μg/ml of gibberellic acid production was obtained in Nutrient broth after 9 days of incubation at 6.0 pH and 30°C temperature on a rotary shaker with the10^7 cells/ml of inoculum size.

Key words: Gibberellic acid, Sugarcane, *Bacillus cereus.*

The gibberellins (GAs) are an important group of phytohormones occurring in higher plants. Gibberellins are a typical example of the production of plant growth regulators by microorganisms, and are important biotechnological products which are increasingly used in agriculture and horticulture (Gutierrez-Manero *et al*. 2001; MacMillan 2002). In fungi and bacteria there is no known role for gibberellins, rather they seem to be secondary metabolites that may play a role as signaling factors towards the host plant fixation (Bashan & Levanony 1990) The main product of gibberellin biosynthesis by microorganisms is gibberellic acid (GA_3), which is formed from GA_4 via GA_7. GA_3 is used extensively in agriculture, nurseries, greenhouses, tea gardens, viticulture etc. It has been reported that annual world production of gibberellic acid exceeds about 25 tons with a market value of 100 million USD (Tudzynski, 1999). In this study we used *Bacillus cereus* for GA_3 biosynthesis. In the present study, optimization of production of GA_3 by submerged fermentation, using Bacillus cereus has been investigated.

* To whom all correspondence should be addressed.
E-mail: nile_139@yahoo.com

MATERIALS AND METHODS

Organism

Potent isolate *Bacillus cereus* was isolated from the rhizosphere of sugarcane in the vicinity of Surat, India. Bacterial culture was maintained on Nutrient agar at 4°C in a refrigerator with monthly transfer.

Growth media, Inoculation & Incubation

Culture flasks (250ml) containing 100 ml Nutrient broth medium were sterilized and inoculated with bacterial isolate. The inoculated flasks were incubated at 30 ± 1°C for 48 hours.

Production of gibberellic acid

Production of gibberellic acid was detected by spectrophotometric method. 48 hours old growth of bacterial culture was centrifuged at 10,000 rpm for 15 - 20 min. The pH value of

supernatant was adjusted to 2.5 using stock 3.75 N HCl. Supernatant was extracted using liquid-liquid (ethyl acetate/$NaHCO_3$) extraction method. The amount of gibberellic acid in the ethyl acetate phase was measured by the UV spectrophotometer at 254 nm (Berryos *et al*., 2004).

Influence of Various Environmental Conditions Gibberellic acid Production

The effect of various inoculum sizes (10^4 - 10^9 cells /ml), temperatures (20° - 40°C), pH4 - 8 and mode of cultivation (static and shaking) on gibberellic acid production had been examined 12 hours onwards up to 84 hours. The cell free culture supernatants were examined for gibberellin production.

RESULTS AND DISCUSSION

A total of 59 rhizobacteria obtained were screened for their ability to produce gibberellin in Nutrient broth medium. 48 hours old growth of bacterial culture was centrifuged at 10,000 rpm for 15 - 20 min. The culture supernatants were used to detect GA_3 production by spectrophotometric method. Out of 59, many of rhizobacteria reported as GA_3 producer by spectrophotometric method. Amongst them NPF 13 identified as *Bacillus cereus*

Table 1. Effect of mode of cultivation (static - shaking) on growth (OD at 600 nm) and GA_3 production (µg/ml) by bacteria

Isolates	Mode of cultivation	Growth	GA_3 Production
Bacillus cereus	Static	0.76 ± 0.010	284.00 ± 0.18
	Shaking	0.92 ± 0.025	369.88 ± 0.21

produced 88.04 µg/ml of GA_3, which was significantly more than the other isolates.

Effect of Inoculum size on Gibberellic acid Production

Bacillus cereus showed maximum GA_3 production (160.06µg/ml) at inoculum size of 10^7 cells/ml after 48 hours of incubation. Inoculum concentration is an important parameter in growth and production of secondary metabolites. Too high or too low inoculum concentration cause low growth and productivity. The observation from Fig.1 is in agreement to the result indicating maximum GA_3 production at 10^7 cells/ml. Both growth and GA_3 production decreased at lower and higher inoculum sizes.

Effect of Temperature on Gibberellic acid Production

The results of effect of various temperatures (20° - 40°C) indicate that all the isolates showed optimum GA_3 production (205.58µg/ml) at 30°C after 48 hours of incubation. Temperature is known to influence the ability of microorganisms to produce secondary metabolites in culture media. The effect of temperature on the GA_3 production is dependent on the strain employed. Values between 25°-34°C are reported as optimum temperature for GA_3 production (Kumar & Lonsane 1990; Pastrana *et al*., 1995; Cihangir & Aksoza 1997; Tomasini *et al*., 1997; Escamilla *et al*., 2000; Machado *et al*., 2002; Corona *et al*., 2005). In present study the optimum temperature for GA_3 production was found to be 30°C, which is probably due to the fact that the enzymes synthesizing GA_3 are comparatively low at higher temperatures (Fig 2).

Effect of pH on Gibberellic acid Production

Effect of initial pH of the medium on GA_3

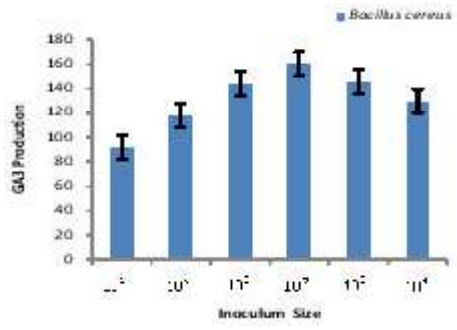

Fig. 1. Effect of Inoculum size on GA_3 production

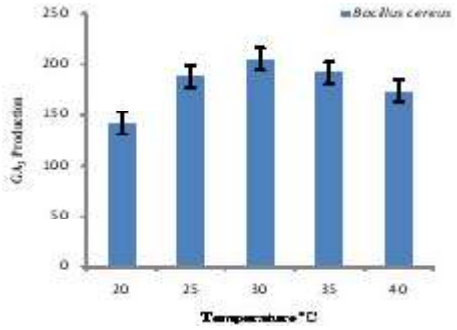

Fig. 2. Effect of Temperature on GA_3 production

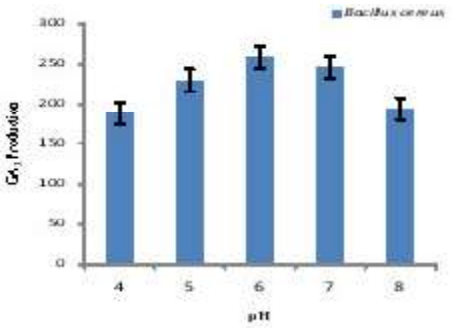

Fig. 3. Effect of pH on GA_3 production

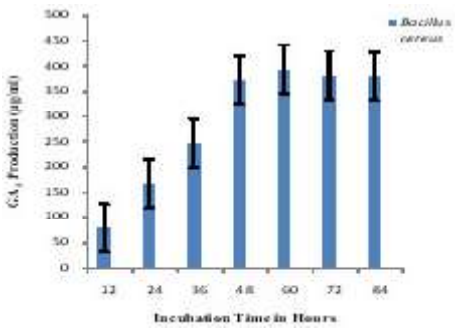

Fig. 4. Effect of incubation time on GA_3 production

production was investigated (Fig. 3). It was noted that initial pH of the medium influence the production of GA_3, highest 258.12 µg/ml of yield was obtained after 48 hours of incubation when the initial pH was adjusted to 6.0. Similar profile was reported by Borrow *et al*, 1965 reported that GA_3 production decreases when the pH was outside the range of 3.0-5.5 in a stirred culture.

Effect of Environmental condition on Gibberellic acid Production

Tables 1 show the effect of mode of cultivation (shaking and static) on GA_3 production by the rhizosphere isolate. *Bacillus cereus* showed GA_3 production (369.88 µg/ml) under shaking condition after 48 hours of incubation.

Since the biosynthesis of gibberellins involves many oxidative steps, a good aeration of fermentors is critical for an optimal production process. In fact, since the value of oxygen consumption for a growing mycelium in the exponential phase of growth remains constant, the demand of oxygen increases more or less exponentially (Tudzynski, 1999). Hence, shaking condition proved to be conducive to GA_3 production.

Effect of Incubation Time on Gibberellic acid Production

GA_3 production observed from 12 hours and reached a maximum level (394.18µg/ml) at 60 hours of incubation (Fig 4). Thereafter, a gradual decline of GA_3 production was observed up to 84 hours of incubation.

CONCLUSION

The culture conditions were optimized for *Bacillus cereus* in order to increase gibberellic acid production. In the presented study, the increase in gibberellin production was almost 5 times which will be helpful to produce GAs in higher yields at economic costs.

ACKNOWLEDGMENTS

This research was supported by Gujarat State Biotechnology Mission (GSBTM), FAP-2010. The authors wish to thank GSBTM for financial support.

REFERENCES

1. Bashan Y, Levanony H, Current status of *Azospirillum* inoculation technology: *Azopirillum* as a challenge for agriculture. *Can J Microbiol* 1990; **36**: 591–608.
2. Borrow A, Brown S, Jeferrys EG, Kessel RJH, Lloyd EC, Lloyd PB, Rothwell A, Rothwell B & Swait JC, The effect of varied temperature on the kinetics of metabolism of kinetics of Gibberella fujikuroi in stirred culture, *Can J Microbio*, 1965; **10:** 445-466.
3. Cihangir N & Aksiiza N, Evaluation of some food industry wastes for production of gibberellic acid by fungal source, *Environ Technol*, 1997; **18:** 533-537.
4. Corona A, Sanchez D, Agosin E, Effect of water activity on gibberellic acid production by Gibberella fujikuroi under solid-state fermentation conditions, *Proc Biochem*, 2005; **40:** 2655-2658.
5. Escamilla EM, Dendooven L, Magafia IP, Parra R & De La Torre M, Optimization of gibberellic acid production by immobilized Gibberella fujikuroi mycelium in fluidized bioreactors, *J Biotechnol.*, 2000; **76:** 147-155.
6. Gutierrez-Manero F, Ramos-Solano B, Probanza A, Mehouachi J, Tadeo FR, Talon M, The plant-growth-promoting rhizobacteria *Bacillus*

pumilus and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. *Physiol Plant* 2001; **111**: 206–211.

7. Berryos J, Illanes A & Aroca G, Spectrophotometric method for determining gibberellic acid in fermentation broths. *Biotechnol. Lett.* 2004; **26:** 67–70.
8. Kumar PKR & Lonsane BK, Solid-state fermentation: physical and nutritional factors influencing gibberellic acid production, *Appl Microbio Biotechnol.,* 1990; **34:** 145-148.
9. Machado CMM, Soccol CR & Pandey A , Gibberellic acid production by solid sate fermentation in coffee husk, *Appl Biochem Biotechnol.,* 2002; **102:** 179-192.
10. MacMillan J , Occurrence of gibberellins in vascular plants, fungi and bacteria. *J Plant Growth Regul.,* 2002; **20:** 387–442.
11. Pastrana LM, Gonz~ilez MP, Torrado A & Murado M A, A fed-batch culture model for improved production of gibberellic acid from a waste medium, *Biotechnol Lett.,* 1995; **17:** 263-268.
12. Tomasini A, Fajardo C & Barrios-Gonzfiles J , Gibberellic acid production using different solid-state fermentation systems, *World J Microbio Biotechnol.,* 1997; **13:** 203-206.
13. Tudzynski B, Biosynthesis of gibberellins in Gibberella fujikuroi: biomolecular aspects, *Appl Microbio Biotechnol.,* 1999; **52**: 298-310.