Biospectra : ISSN : 0973-7057, Vol. 13(1), March, 2018, pp. 91-96

An International Biannual Refereed Journal of Life Sciences

Plant Science

ISSN : 0973-7057

Indexed in Thomson Reuters (Clarivate Analytics) as Zoological Records Sl. No.663 (www.mjl.clarivate.com)

Production of Tannase from *Bacillus licheniformis KBR6* through solid state fermentation.

Arpita Chakraborty[a]*, Bikash Ranjan Pati[b] & Jyoti Kumar[c]

[a]Department of Botany, Narajole Raj College, Narajole, Paschim Medinipur ,West Bengal, India

[b]Department of Botany, Vidyasagar University, Midnapur, Paschim Medinipur, West Bengal, India

[c]University Department of Botany, Ranchi University, Ranchi, Jharkhand, India

Received : 17th December, 2017 ; Revised : 3th February, 2018

Abstract : Production of Tannase by *Bacillus licheniformis* KBR6 has been done through solid state fermentation. Different materials like rice bran, saw dust, rice straw and sugar cane baggage were used as substrates for solid state fermentation. Rice bran with moisture ratio (1:4) at p^H 5.0, incubation period of 168hr. and temperature 35°C was found most suitable for enzyme production.

Keywords : *Bacillus licheniformis KBR6*, solid state fermentation, rice bran, saw dust, sugarcane baggage.

INTRODUCTION

Tannase (Tannin acyl hydrolase E.C.3.1.1.20), discovered by Scheele (1886), has been known to hydrolyze the ester linkage of tannic acid into gallic acid and glucose. It is an industrially important enzyme and is reported to fine use in the manufacture of instant tea (Cogoon and Sanderson, 1972), mediarization of wine (Yamada and Tanaka, 1972; Chae and Yu, 1983) and clarification of coffee flavoured soft drinks (Vermeire and Vandamme, 1988; Bajpai and Patil, 1996) and clarification of beer (Lekha and Lansane, 1997). The enzyme is mainly used for the preparation of gallic acid (Misra *et al.*, 1997; Bajpai and Patil, 1997), high grade leather tannin etc. The product, gallic acid, has also different uses like preparation of propylgallate, widely used as food antioxidant (Gathon *et al.*, 1989), trimethoprim, a pharamaceutical antibacterial agent (Hadi *et al.*, 1994), Pyrogallol, dye, fur (Hadi *et al.*, 1994) and also used as photosensitive resin in semiconductor production (Yamada *et al.*, 1989). Most of the reported tannase producting organisms are fungi (Aoki *et al.*, 1976; Bhat *et al.*, 1998; Mondal *et al.*, 2001a) and a few are bacteria (Deschamps *et al.*, 1983; Mondal and Pati, 2000; Mondal *et al.*, 2001b). Most of the authors studied tannase production from different microorganism in the fermented media containing pure tannic acid. Tannic acid in the medium is used as inducer as well as available carbon source.

Modern society is critically dependent on wide variety of products derived from the processing of raw materials from nature with the recent progress in Biotechnology and Biochemical engineering, the production of chemicals and bioactive compounds (e.g. antibiotics) using biological agents such as microorganism cells or enzyme is being considered as a superior alternative to the traditional chemical methods.

Most industrial fermentation use the submerged fermentation process (SMF) where the organism are

*Corresponding author :
Phone : 9434810271/8372878541
E-mail :arpita_scm@rediffmail.com

grown in liquid media that are stirred or aerated within large vessel.

However another fermentation process called solid state fermentation (SSF) where the growth of the micro organism occurred on solid materials without the presence of free water has been shown to be a promising alternative as biotransformation process.

This technique offer distinct advantages over submerged fermentation (SMF) including economy of the space needed for fermentation media, greater campactness of the fermentation vessel owing to a lower water volume; greater product yields, lower capital and recurring expenditure in industry: absence of foam build-up the low moisture level. Additionally such a system find greater application in the areas of solid waste management biomass energy conversion and in the production of secondary metabolites.

Now-a-days a number of microbial enzymes have been produced by using solid state fermentation.

In this project, tannase production has been studied by the bacterial strain *Bacilus licheniformis* KBR6 through solid state fermentation.

MATERIALS AND METHODS

Microorganism : Previously isolated soil bacterium *Bacillus licheniformis* KBR6 has been used in present study.

Chemicals : Tannic Acid (Analytical grade) was obtained from Qualigens Fine chemicals, Mumbai, India. Bovine serum Albumin was purchased from Sigma chemicals, U.S.A.

All other chemicals used for the study were analytical grade.

Growth and media : Organism was grown in tannic acid broth of the following composition as –

Tannic acid, 1%; $MgSO_4$, 0.05%, KH_2PO_4, 0.05%; K_2HPO_4, 0.05%; NH_4Cl, 0.3%. The broth was then autoclaved at 15 1b pressure for 20 minute. Media pH was adjusted to 5.0.

Preparation of preinduced inoculum :

Tannase is an inducible enzyme. So preparation of preinduced inoculum is very much necessary. The tannic acid medium was inoculated with 24h. Culture broth of *Bacillus licheniformis* KBR6 and incubated in shaker at 35°C for 20h. Preinduced inoculum thus prepared was preserved at 4°C for further work.

Enzyme Production :

5 gms of solid substrate was taken in 100 ml Erlenmeyer flask and autoclaved. 20 ml of tannic acid broth medium (pH 5.0) was added to the solid substrate and inoculated with the organism. After that the flask was incubated at 30°C for enzyme production.

Parameter concerned in production of tannase : Following parameters have been studied in our work for the production of tannase through SSF.

1. Effect of Incubation period :

To study the effect of incubation period on the production of tannase the medium was inculated for different time period (24-192 hr.)

2. Effect of Temperature :

Tannase production was carried out at various temperature (25°C, 30°C, 35°C, 40°C) to determine the optimum temperature for the maximum production of enzyme.

3. Effect of Tannic acid concentration :

Different concentration grades of tannic acid (from 1 – 5.5%) were taken at different conical flasks to achieve the optimum tannase production.

4. Effect of moisture level :

Tannase production was carried out at various moisture level (5ml, 15 ml, 25 ml & 30 ml media) in different percentage and inculated at 30°C.

5. Effect of Innoculum percentage :

Rice bran (5 gm) moistured with 20 ml nutrient solution were taken and inoculated with 10%, 20%, 30%, 40% and 60% inoculum. The enzyme was extracted and assayed from each set following an incubation of 24 hrs at 30°C.

Enzyme Extraction :

From the fermented solid substrate enzyme was extracted by using 25ml NaCl solution (1%) as solvent. First the fermented material were properly mixed with this solvent and flasks were kept on rotary shaker at 150 rpm for 1 hrs. Then the enzyme was separated from that solid substrate, filtering using filter paper. The extract was centrifuged at 5000 rpm for 5 mins. The clear supernatant was used for enzyme assay.

Assay of tannase :

Tannase activity was determined by the newly developed colorimetric method of Mondal *et al.* (2001). For assay 0.1 ml of substrate tannic acid (1.0% w/v in 0.2 M acetate buffer, pH-5.0) was taken in a test tube and

0.05 ml of enzyme solution added and incubated at 50°C for 30 min. The reaction was terminated by the addition of 3 ml BSA solution (1 mg / ml) which also precipitates the residual tannic acid. A control reaction was also done side by side with heat denaturated enzyme. The tubes were then centrifuges (5000g, 10 min) and precipitate was dissolved in 2 ml of SDS-triethanolamine (1% W/V SDS in 5% W/V, triethanolamine). The absornancy was measured at 530 nm after addition of 1 ml of $FeCl_3$ (0.13 M) solution. One unit of tannase activity was defined as the amount of enzyme which is able to hydrolyse 1 mmole of ester linkeage of tannic acid in 1 min at specific condition.

RESULTS AND DISCUSSION

i) Effect of different inert materials :

For production of maximum tannase under solid state fermentation by *Bacillus licheniformis* KBR6, different inert materials like, Rice bran, saw dust, Rice straw and sugar cane pith bagges were tested. Among the materials tested the rice bran showed maximum production of tannase (Fig.1).

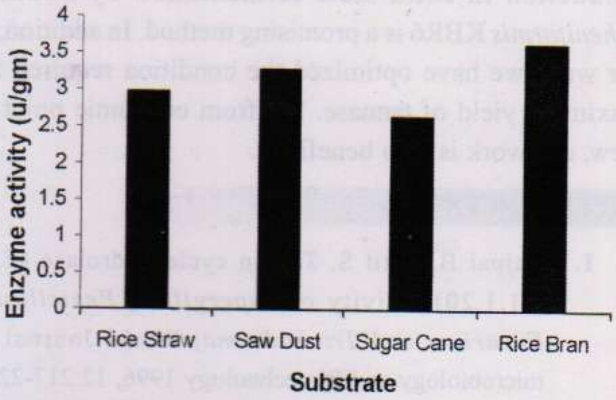

Fig. 1 : Effect of different solid substrates (carrier) on tannase production by *B. licheniformis* KBR6 using solid state fermentation

ii) Effect of Incubation period :

A low level of tannase appeared in the early stages of incubation and the enzyme level steadily reached a maximum at 168 hrs. (Fig. 2) and beyond this period no further increase in enzyme production takes place. Earlier maximum extraceullar tannase production in *Aspergillus niger* was obtained after 4 days through solid state fermentation (Lekha and Lonsane, 1994) whereas maximum enzyme production was obtained after 5 days in *Rhizopus oryzae* (Chatterjee *et al.*, 1996).

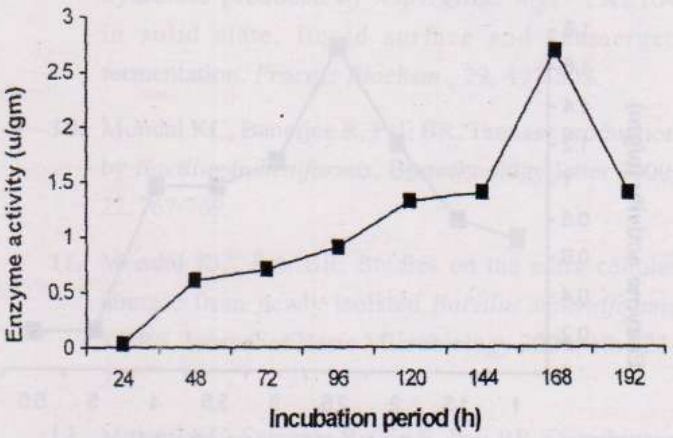

Fig. 2 : Effect of Incubation period (h) on tannase production by *B. licheniformis* KBR6 using solid state fermentation

iii) Effect of temperature :

Production of tannase was studied by incubating the flasks at 25°C, 30°C, 35°C and 40°C and highest production was noticed at 35°C (Fig. 3). Maximum tannase production takes place at 30 – 35°C in liquid submerged fermentation (Lekha and Lonsane, 1997).

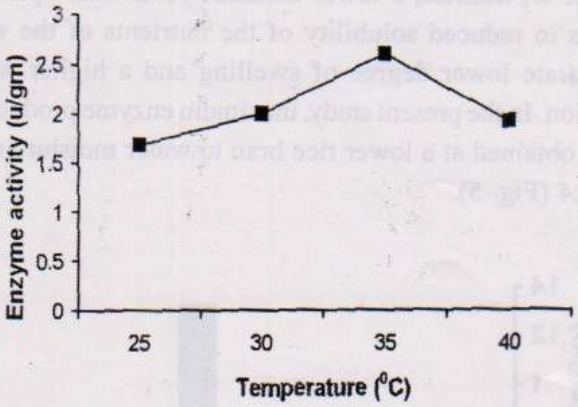

Fig. 3 :Effect of Temperature (°C) on tannase production by *B. licheniformis* KBR6 using solid state fermentation.

iv) Effect of tannic acid concentration :

Effect of substrate concentration on tannase production was studied with different concentrations of tannic acid and the result of the same has been shown in the Fig. 4. It has been found that 2.5% tannic acid concentration is optimum for tannase production. Earlier, similar result was obtained in *Rhizopus oryzae* (Mukherjee *et al.*, 1996).

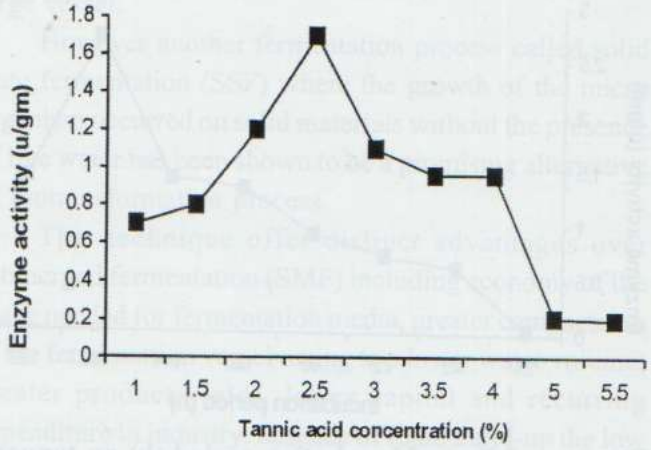

Fig. 4 : Effect of Tannic acid concentration (%) on tannase production by *B. licheniformis* KBR6 using solid state fermentation.

v) Effect of moisture level :

The importance of moisture level in SSF media and its influence on microbial growth and production of biosynthesis may be physical properties of the solid substrate, a higher than optimum moisture level cause decreased porosity, alteration in rice bran particle structure, lower O_2 transfer, a lower moisture level than optimum leads to reduced solubility of the nutrients of the solid substrate lower degree of swelling and a higher water tension. In the present study, maximum enzyme production was obtained at a lower rice bran to water moisture ratio of 1:4 (Fig. 5).

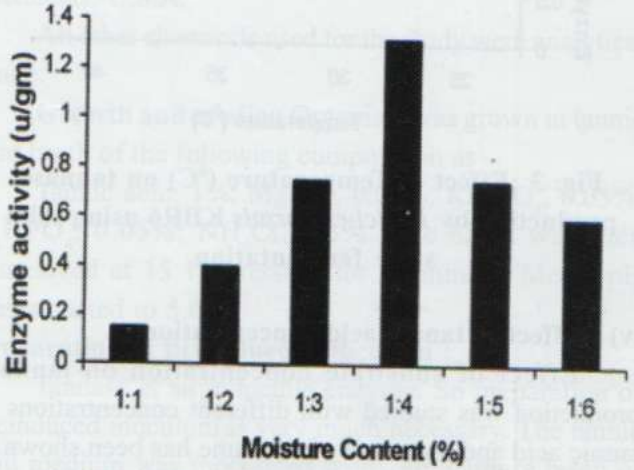

Fig. 5 : Effect of moisture level on tannase production by *B. licheniformis* KBR6 using solid state fermentation.

vi) Effect of inoculum percentage :

A 20% inoculum (based on the initial weight of rice bran) was found to be most suitable to attain a high production of tannase by *Bacillus licheniformis* KBR6 (Fig. 6).

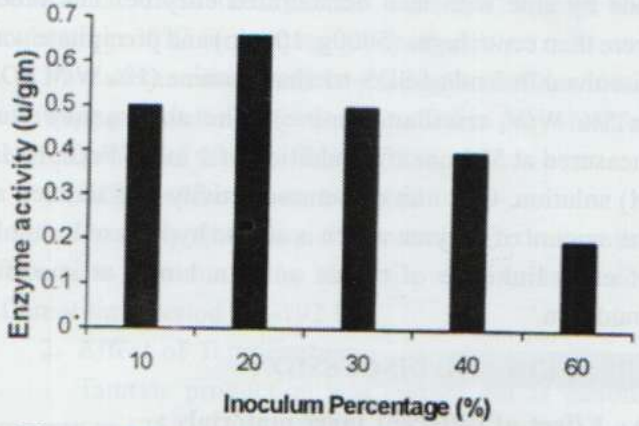

Fig. 6 : Effect of Inoculum Percentage (%) on tannase production by *B. licheniformis* KBR6 using solid state fermentation.

CONCLUSION

From our work it can be concluded that tannase production in solid state fermentation by *Bacillus licheniformis* KBR6 is a promising method. In addition, in our work we have optimized the condition required for maximum yield of tannase. So, from economic point of view, our work is also beneficial.

REFERENCES

1. Bajpai B. Patil S. Tannin cycle hydrolase (E.C. 3.1.1.20) activity of *Aspergillus*, *Penicillium*, *Fusarium* and *Trichoderma*, World Journal of microbiology and Biotechnology 1996, 12:217-220.
2. Banerjee D., Modal KC, Pati BR. Production and Characterization of extracellular and intracellular tannase from newly isolated *Aspergillus aculeatus* DBF9. Journal of Basic Microbiology, 2001.
3. Bhat TK, Singh B., Sharma OP. Microbial degradation of tannis – a current perspective. Biodegradation 1998; 25 : 3434-357.
4. Bradoo S., Gupta R., Saxena RK. Parametric optimization and biochemical regulation of extra cellular tannase from *Aspergillus japonicus*. Process Biochemistry 1997; 32 : 135-139.

5. Chatterjee R, Dutta A, Banerjee R, Bhattacharyya BC, Production of Tannase by Solid State Fermentation, Bioprocess Engineering 14 : 159-162.

6. Coggon P, Sanderson GW. Manufacture of instant tea. Patent Ger offen 2.304079 (cl. A.241) 16 Aug 1973.

7. Hadi TA, Banerjee R, Bhattacharya BC. Optimization of tannase biosynthesis by a newly isolated *Rhizopus oryzae*, Bioprocess Enginerong 1994; 11; 239-243.

8. Lekha, P.K., and Lonsane, B.K. (1997) Production and application of tannin acyl hydrolase : State of the art. *Adv. Appl. Microbiol.*, 44, 215-260.

9. Lekha, P.K., and Lonsane, B.K. (1994) Comparative titers, location and properties of tannin acyl hydrolase produced by *Aspergillus niger* PKL104 in solid state, liquid surface and submerged fermentation. *Process Biochem.*, 29, 497-503.

10. Mondal KC, Banerjee R, Pati BR. Tannase production by *Bacillus licheniformis*, Biotechnology letter 2000; 22: 767-769.

11. Mondal KC, Pati BR. Studies on the extra cellular tannase from newly isolated *Bacillus licheniformis* KBR6. Journal of Basic Microbiology 2000; 40: 223-232.

12. Mondal KC, Samanta S, Giri S, Pati BR, Distribution of tannic acid degrading microorganisms in the soil comparatives study of tannase from two fungal strains. Acta Microbiologica Polonica 2001; 50 (1).

***