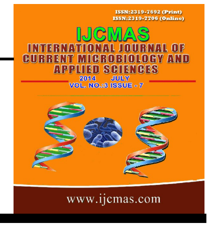
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 7 (2014) pp. 691-697**
http://www.ijcmas.com



Original Research Article

Application of partially purified α-amylase produced by *Brevibacillus borostelensis* R1 on sewage and effluents of Industries

K.Suribabu[1*], T.Lalitha Govardhan[1] and K.P.J Hemalatha[2]

[1]PG Department of Microbiology and Research Centre, Dr.Lankapalli Bullayya Post-graduate College, Visakhapatnam-530 013, A.P, India
[2]Department Microbiology, Andhra University, Visakhapatnam-530 003, A.P, India
**Corresponding author*

Keywords

Brevibacillus borstelensis R1; α-amylase; Sago and Rice effluent; Sewage water; fodder

A B S T R A C T

To test the potency of partially purified α-amylase produced by *Brevibacillus borstelensis* R1 in applications such as sago and rice industrial effluent treatment, sewage water treatment and fodder production. In test (5% *Brevibacillus borstelensis* R1) the maximum production of maltose (1167±58 μg/ml) was observed on the 18th day in sago effluent and in rice effluent the maximum production of maltose (1283±12μg/ml) was observed on the 21st day. The biomass (0.96±0.002gm/ml) and maltose (1057±29μg/ml) production have increased throughout the study on sewage water and reached maximum on the 30th day. The α-amylase was useful in solving the problem of water pollution of industrial effluents and sewage water by hydrolyzing the substrates. The α-amylase was also found to be useful in bakery, food, fodder for poultry, automation dishwashing and in laundry industries.

Introduction

Alpha-amylase obtained from the *Bacillus spp.: B. subtilis, B. stearothermophilus, B. licheniformis* and *B. amyloliquefaciens* is widely used for various applications. Today in the global enzyme market, α-amylases account for about 30% (Sivaramakrishnan *et al.,* 2006). These enzymes have applications in starch processing, desizing of textiles, paper sizing, detergent additive, bread improvement, ethanol production, sewage treatment, effluent treatment and other fermentation processes (Pandey *et al.,* 2000; Maarel *et al.,* 2002; Haki & Rakshit, 2003; Gomes *et al.,* 2005).

The feed of poultry usually constitutes many starches or barley material; The addition or pre-treatment of the feed with α-amylases improves the nutritional value and the digestibility of fiber in the feed (Ismail *et al.,* 1992; Uhlig, 1998; Van der Maarel *et al.,* 2002; Gavrilescu & Chisti, 2005; Ghorai, *et al.,* 2009)

In any treatment system, food processing wastewater offers a unique challenge; often containing multiple types of contaminants that pose serious threat to the ability of standard sewage treatment. By biotechnological effluent treatment of food processing starch waste water can produce valuable products such as microbial biomass protein and purified effluent (Aiyer, 2005).

Materials and Methods

Treatment of Sago effluent

The sago effluent was collected in sterile BOD bottles at Sri Ravi Sago Factory, Hussenpuram Samalkot, East Godavari District, Andhra Pradesh, India and brought to the laboratory for testing. Hundred ml of the effluent was taken in two Erlenmeyer flasks. One was inoculated with 5% of *Brevibacillus borstelensis* strain R1 as test and another served as control without inoculation of the organism. The Erlenmeyer flasks were incubated for 30days at room temperature (25^0C). The α-amylase activity (U/ml) was determined by collecting one ml of the sample at regular intervals of 3days for 30days.

Treatment of Rice effluent

The rice effluent was collected from Sirius Overseas Pvt. Ltd., Peddapurum, East Godavari District, Andhra Pradesh, India in sterile BOD bottles and brought to the laboratory. Effluent sample (100ml) was taken in two Erlenmeyer flasks labeled as test (inoculated with 5% of *Brevibacillus borstelensis strain* R1) and control without inoculation. Then the Erlenmeyer flasks were incubated for 30 days at room temperature (25^0C). The α-amylase activity was estimated by collecting one ml of the sample from the incubated flasks at regular intervals of 3days for 30days.

Sewage water treatment

The sewage water with major content of rice porridge was collected from house hold kitchen with sterile BOD bottles and brought to the laboratory for further work. In each Erlenmeyer flask 100ml of sample was dispensed. Flask inoculated with 5% of *Brevibacillus borstelensis* strain R1 was labeled as test and flask without inoculation was used as control. All the samples were incubated for 30 days at room temperature (25^0C). The α-amylase activity (U/ml) was estimated by DNS method.

Fodder production

Fodder production of *Brevibacillus borstelensis* strain R1 was analyzed from the test by dry weight method. From the incubated sample, 5.0ml was taken in a centrifuge tube and subjected to 5000rpm for 15minutes. The supernatant was discarded and dried in an oven for 30minutes and the weight of the centrifuge tube was recorded. The dry weight was calculated as the weight of the dried centrifuge tube minus weight of the empty centrifuge tube. The dry weight was estimated after every three consecutive days for 30days.

Estimation of Maltose by Dinitro salicylic acid (DNS method)

The enzyme extract (0.5 ml) was transferred to a test tube containing 0.5 ml of 1.0% soluble starch solution. The mixture was incubated at 37ºC for 10 min. Then 1.0 ml of dinitrosalicylic acid reagent (DNS) was added to each test tube. The tubes were placed in boiling water for 5 min and cooled at room temperature. The contents of tubes were diluted up to 10 ml with distilled water. The absorbance was read at 546 nm using a spectrophotometer and converted to mg of maltose from the standard (Miller, 1959). One unit of enzyme activity was defined as the amount of enzyme that releases 1.0 mmol of reducing sugar (maltose) per minute under the assay conditions.

Statistical analysis

All the experiments were conducted in triplicate. The results were given as mean value ± standard deviation. The conditions were analyzed to determine the significant difference between the variables by one way ANOVA, two way ANOVA and correlation analysis by using the scientific graph pad (Prism 6.1version software).

Results and Discussion

Partially purified α-amylase from *Brevibacillus borstelensis* R1 was purified to 3.9 fold purification, when the crude enzyme was subjected to $(NH_4)_2S0_4$ precipitation and gel filtration. Alpha amylase proved it's potency in many fields: in treatment of effluents from sago and rice industries, sewage water treatment and in fodder production.

Treatment of sago effluent industry

Sago industry effluent was harmful to the water bodies and soil due to the presence of sago starch. The samples were collected from local sago industry (Figure 1A). Two erlenmeyer flasks each with 100ml of sample were taken as control and test (inoculated with 5% *Brevibacillus borstelensis* R1) (Figure 1B). The maltose (µg/ml) was estimated at regular intervals during 30 days of study. In the test the maximum maltose concentration was observed on 18th day (1167±58µg/ml) whereas no increase in maltose concentration was observed in control (Figure 1C).

Treatment of rice effluent industry

The rice industry effluent was collected in BOD bottles from the preparation area of the parboiled rice and rice flakes (Figure 2A).The Erlenmeyer flasks were labeled as control and test (treatment with 5% *Brevibacillus borstelensis* R1 inoculum) (Figure 2B). The maltose (µg/ml) was determined for 30 days (Figure 2C). In test the maltose concentration reached maximum (1283±12µg/ml) on 21st day and later decreased.

Sewage water treatment

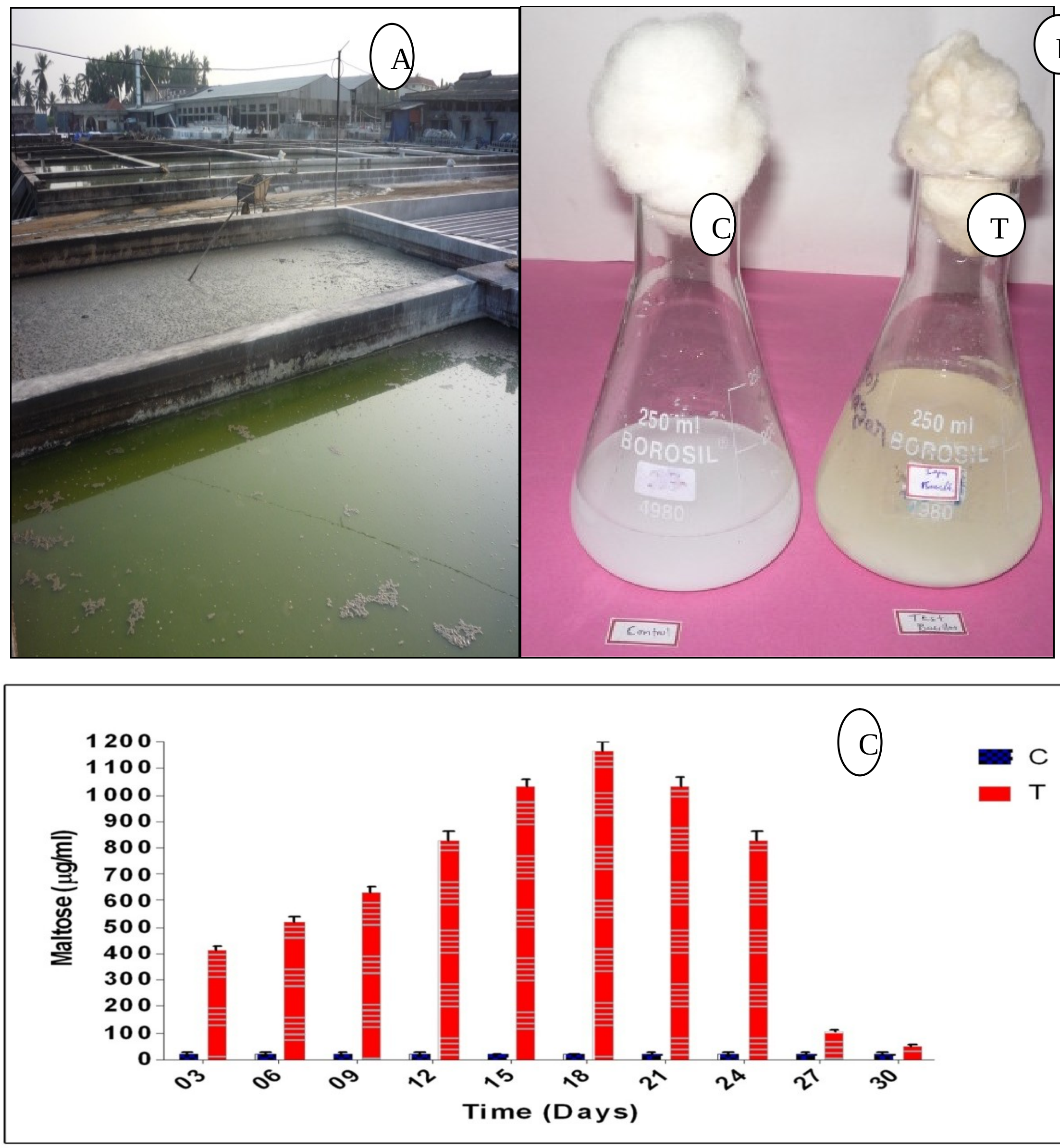
The modern technology for removing the starch from the kitchen sewage was adopted in the present study. The sewage water: control and test (treatment with 5% *Brevibacillus borstelensis* R1) are shown in the figure 3A.

Fodder production

The biomass and maltose concentration during starch degradation were calculated for all samples during 30 days. The biomass (0.96±0.002gm/ml) and maltose concentration (1057±29µg/ml) have increased throughout the study and reached maximum on 30th day (Figures 3b & c).

In the present study, the application of α-amylase produced from *Brevibacillus borstelensis* R1was used in treatment of sago, rice industries effluent and kitchen sewage water. The test sample treated with 5% *Brevibacillus borstelensis* R1 was proved to be the best in degrading the starch and also increased the biomass production subsequently. Aiyer (2005) reported about the treatment of food processing of starch in waste water and standard sewage treatment with amylases and amylase producing microorganisms which produced valuable microbial biomass protein (used as fodder), solving pollution problem and also used in effluent purification.

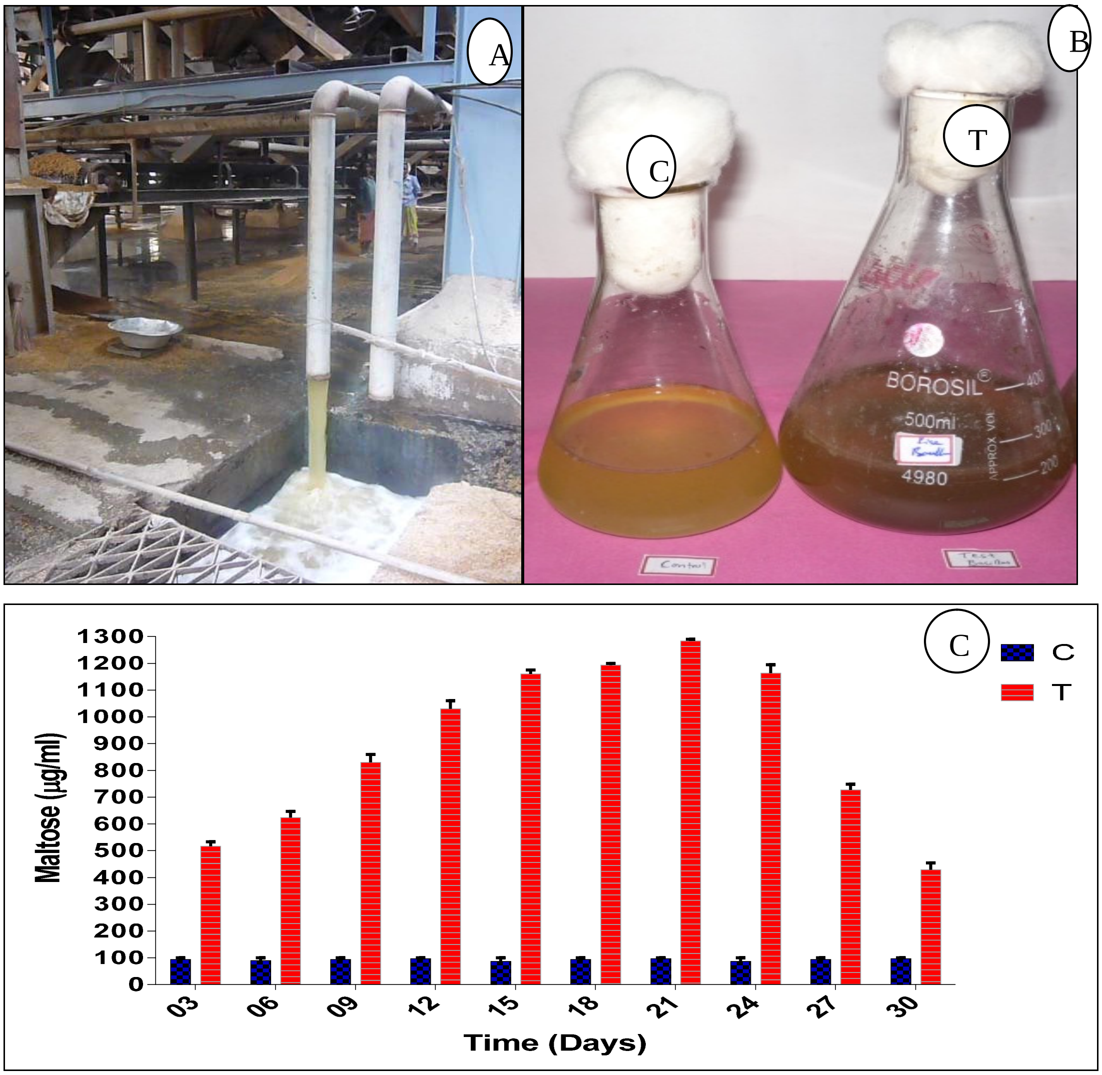
Figure.1 Sago effluent industry (A) sample collecting point (B) control, test (inoculated with 5% *Brevibacillus borstelensis* R1) (C) Figure showing the concentration of maltose in test.



Y bars indicate the standard deviation of mean value.

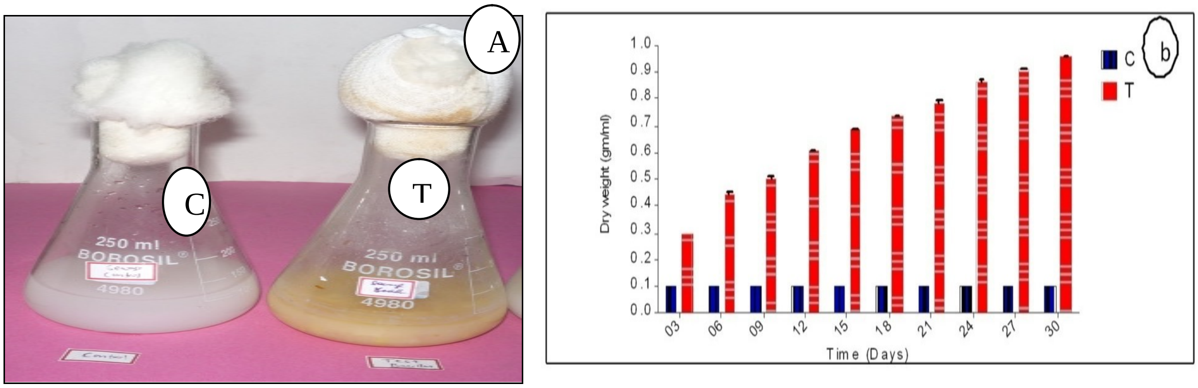
**** P < 0.0001 Values differ significantly at p<0.05.

Figure.2 Para boiled Rice & flakes effluent industry (A) Sample collecting point (B) control, and test (treatment with 5% *Brevibacillus borstelensis* R1) (C) Figure showing the maltose concentration in test

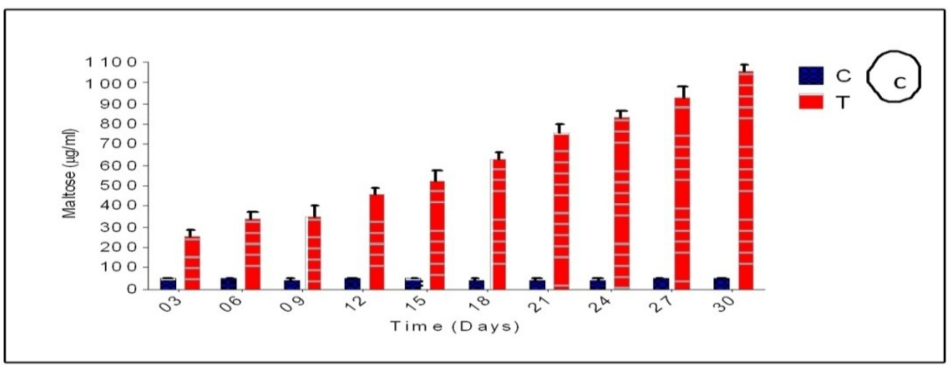


Y bars indicate the standard deviation of mean value.
**** $P < 0.0001$ Values differ significantly at $p<0.05$.

Figure.3 Sewage water (A) control, test (treatment with 5% *Brevibacillus borstelensis* R1) (B) Figure showing production of biomass (C) Figure showing maltose concentration during Submerged Fermentation



Y bars indicate the standard deviation of mean value.
**** P < 0.0001 Values differ significantly at $p<0.05$.



Y bars indicate the standard deviation of mean value.
**** P < 0.0001 Values differ significantly at $p<0.05$.

Partially purified α-amylase from *Brevibacillus borstelensis* R1 was purified of 3.9 fold purification, when the crude enzyme was subjected to $(NH_4)_2S0_4$ precipitation and gel filtration. The α-amylase produced by *Brevibacillus borstelensis* R1 was found to be useful in sago and rice industrial effluent treatment, sewage water treatment, fodder production for poultry. The α-amylase was also useful in solving the problem of water pollution of industrial effluents and sewage water by hydrolyzing the substrates.

Acknowledgements

We thank Management of Dr.Lankapalli Bullayya College, Visakhapatnam for the financial support and facilities provided to make this work possible.

References

Aiyer, P.V. 2005. Amylases and their applications. *African J. Biotech.* 4: 1525-1529.

Gavrilescu, M. and Chisti, Y. 2005. Biotechnology a sustainable alternative for chemical industry. *Biotechno. Adv*. 23:471-499.

Ghorai, S. Banik, S.P., D. Verma, S.Chowdhury, Mukherjee, S. and Khowala, S. 2009. Fungal biotechnology in food and feed processing. *Food Res. Int.* 42: 577–587.

Gomes, E. Sauza, S.R., Grandi, R.P. and Silva, R. 2005. Glucoamylases produced from *Aspergillus flavus* A1.1 and *T. Braz. J. Microbiol* 36: 75-82.

Haki, G.D. and Rakshit, S.K. 2003. Developments in industrially important thermostable enzymes: A review. *Biores. Techno.* 89: 17-34.

Ismail, A.M. S.H. Omar, El-Aassar, S.A. and Gouda, M.K. 1992. Purification of alpha amylase from *Bacillus lentus* cultures. *Appl. Microbiol. Biotechnol.* 38: 312-314.

Maarel, V.M., V.B. Jec Veen, Uitdehaag, J.C. Leemhuis, H. and Dijkhuizen L. 2002. Properties and applications of starch-converting enzymes of the alpha-amylase family. *J. Biotechnol.* 94: 137-155.

Miller, G.L. 1959. Use of Dinitro salicylic acid reagent for determination of reducing sugar. *Analy. Chem.* 31: 426 - 429.

Pandey, A., P. Nigam, C.R.Soccol, V.T. Soccol, Singh, D. and Mohan, R. 2000. Advances in microbial amylases. *Biotechnol. Appl. Biochem.* 31: 135- 152.

Sivaramakrishan, S., D. Gangadharan, K.N.Nampoothiri, Sccol, C.R. and Pandey, A. 2006. α-Amylases from Microbial sources-An Overview on Recent Developments. *Food Techn. Biotechnology*. 44: 173-184.

Uhlig, H. 1998. Industrial Enzymes and their applications. New York: John Wiley and sons Inc. 435.

Van der Maarel, M.J.E.C., B. Van der Veen, J.C.M. Utdehaag, Leenhuis, H. and Dijkhuizen, L. 2002. Properties and applications of starch converting enzymes of the alpha amylase family. *J. Biotechnol.* 94: 137-155.