International Journal of Biology Research
ISSN: 2455-6548, Impact Factor: (RJIF 5.22)
www.biotechjournals.com
Volume 1; Issue 1; March 2016; Page No. 50-52

Multienzyme producing bacteria isolated from agricultural fields in Thiruvannamalai, Tamil Nadu, India

[*1] Kuzhadhaivel Jayaprakash, [2] Mani Gopu, [3] Shanmugam Anbu, [4] Vadivel Balamurugan

[1, 2] Department of Biotechnology, Shanmuga industries arts and science college, Thiruvanamalai-606 603.

[3, 4] PG& Research Department of Biotechnology, Sri Vinayaga College of Arts and Science Ulundhurpet-606 107

Abstract
Soil Microorganisms as potential source of novel and or improved products of industrial importance have gained highest importance in Microbial technology and bioprocess engineering. The present study was carryout the isolated the multi-enzyme producing bacteria from agricultural field at Arni, Thiruvannamalai District, Tamilnadu, India. Totally 26 morphologically different colonies were isolated namely SCBT01 to SCBT26 and checked different enzyme activities such as Amylase, Protease, Lipase and Cellulase. There are 12 strains it ability to produce the enzymes and particularly SCBT06 it produced Amylase, Protease and SCBT19 it produced the Amylase, Protease, Lipase and Cellulase in this strain Concerted as potential strain. In the strain to suggest to industry which have the capacity to produce commercially important enzymes on industrial scale; Microbial enzymes are generally cheaper to be produced and are obtained in high yields.

Keywords: Multi-enzyme, Amylase, Protease, Lipase, Cellulase and Soil Microorganisms.

1. Introduction
Biotechnology is application of living organism and their components to industrial products and processes that requires less energy and is based on renewable raw materials **(**Awan, 1993; Rolin, 1993; Ridley, 2001) [1-3]. Microorganism can live in everywhere, in the air, water and soil, and in the body of human beings and other creatures. Society benefits from microorganisms in many ways. They are necessary for the production of bread, cheese, bear, antibiotics, vaccines, vitamins, enzymes, and another important product. Microorganisms are indispensable components of our ecosystem (Han *et al*., 2005) [5]. In favor of industrial applications, enzymes must be established under process conditions. Generally, enzymes are preferred over chemical catalysts. There are habited microorganisms it ability to produce different kinds of enzymes. A few numbers of microorganisms it shown multi-enzyme activity. Microbial soil Enzymes have become big business, with a wide range of industries using commercial enzymes, in addition to the feed industry. Application of biotechnology to industrial operations for enzyme production is no longer an academic or potentially useful alternative proposition for the future (Suneetha *et al.,* 2011) [10]. Amylases have wide spread applications in textile, paper, food and fermentation industries e.g., in manufacturing of bread, glucose and fructose syrups, fruit juices, sweeteners and alcoholic beverages (Haq *et al.,* 2010) [6]. The present study was those agricultural samples were collected and isolated the bacteria and investigate the multi enzyme activity.

2. Materials and Methods
2.1 Isolation of Bactria and Enzyme Activitys
The soil samples were collected from agricultural fields in Arni, Thiruvannamalai, Tamil Nadu, and India. The sample was aseptically subjected to serial dilutions and plated on Nutrient Agar (NA) and incubated at 37 °C for 24 h. After than 26 morphologically different colonies were isolated and sub-cultured, all the 26 strains screened for Amylase, Protease, and Lipase and Cellulase enzymes activities were used for following medias Starch, Skimmed milk, Tween80 and Car boxy methylcellulose (CMC). The enzyme visualized there folding the reagent on the plate such as Grams iodine and 0.3% Congo red, the formation of a clear zone of hydrolysis indicated Starch and protein degradation. The opaque colonies is indicated the Lipase activity.

2.2 Mass Culture and Partial Purification of Enzymes
The potential strains were selected by based on the zone diameter (SCBT01, SCBT21 and SCBT25) and mass cultured after incubation the culture was centrifuged at 10000 RPM for 10 minutes at 4ºc the supernatant was taken and saturated by solid ammonium sulphate and the mixture was left overnight at 40C for precipitation in the precipitates were collected by centrifugation in partially purified enzyme was dialyzed the final product was assayed for enzyme activity and further studies.

3. Results and Discussion
In the present study there are 26 morphologically different colonies (Table 1) were isolated from agricultural fields in Arni, Thiruvannamalai, Tamil Nadu, India

Table 1: Culture characteristic of the isolate in plate.

Strain Number	Form	Margin	Elevation	Size
SCBT01	Circular	Entire	Raised	Small
SCBT02	Circular	Entire	Flat	Small
SCBT03	Circular	Entire	Convex	Small
SCBT04	Circular	Entire	Flat	Big
SCBT05	Irregular	Undulate	Flat	Small
SCBT06	Circular	Entire	Convex	Big
SCBT07	Irregular	Undulate	Flat	Small
SCBT08	Circular	Entire	Raised	Big
SCBT10	Irregular	Undulate	Umbonate	Small
SCBT11	Irregular	Undulate	Convex	Small
SCBT12	Irregular	Undulate	Lowe convex	Small
SCBT13	Circular	Entire	Convex	Small
SCBT14	Irregular	Undulate	Flat	Big
SCBT15	Circular	Entire	Flat	Big
SCBT16	Irregular	Undulate	Umbonate	Small
SCBT17	Circular	Entire	Convex	Big
SCBT18	Circular	Entire	Convex	Small
SCBT19	Circular	Entire	Flat	Small
SCBT20	Irregular	Undulate	Umbonate	Small
SCBT21	Irregular	Undulate	Umbonate	Small
SCBT22	Circular	Entire	Convex	Small
SCBT23	Irregular	Undulate	Flat	Big
SCBT24	Irregular	Undulate	Umbonate	Big
SCBT25	Irregular	Undulate	Convex	Big
SCBT26	Circular	Entire	Flat	Small

The 12 strains have shown extracellular enzyme activity. However, majority of Strains were produced Cellulase enzyme, SCBT19 strain shown the clear zone on the three (Amylase, Protease and lipase) enzyme activity, SCBT01 strain shown positive result on the Amylase and protease, SCBT05 valuable result on Amylase and Lipase activity and SCBT21 strain shown good result on Protease and Lipase activities on the result was clearly demonstrated on (Figure 1 to Figure 4)

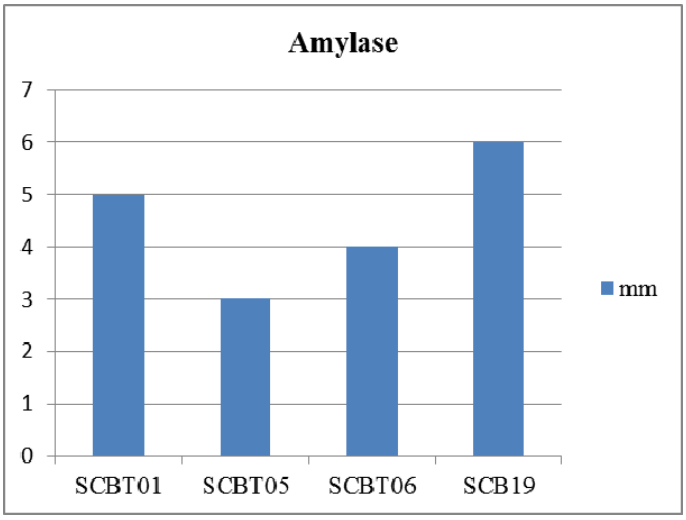

Fig 1: Amylase producing strains

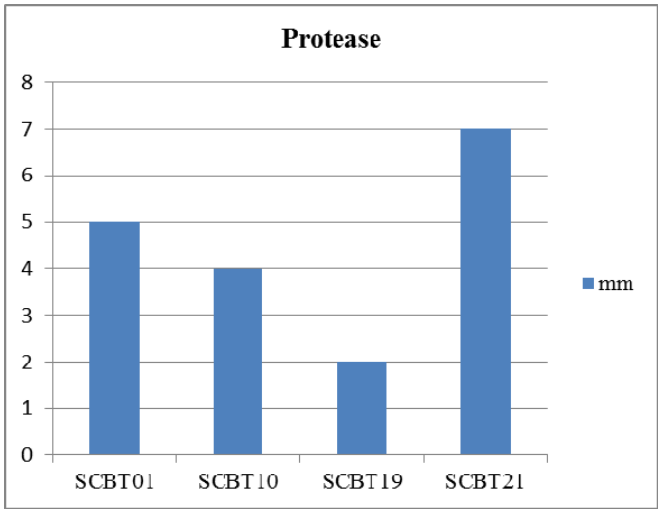

Fig 2: Protease producing strains

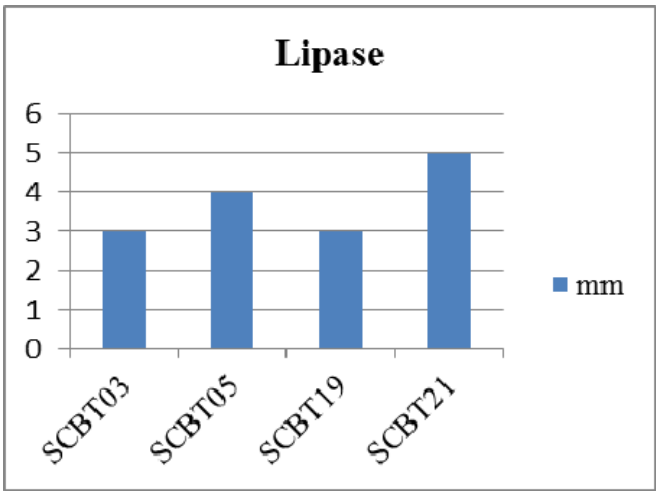

Fig 3: Lipase producing strains

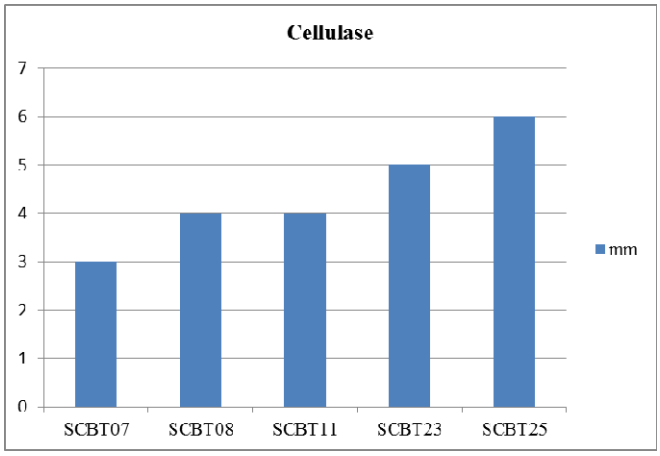

Fig 4: Cellulase producing strains

The purified proteins was good result compared to crud culture activity in this result was clearly demonstrated on (Figure 5) in this similar result already repotted by Shaikh *et al.*, 2013 [8].

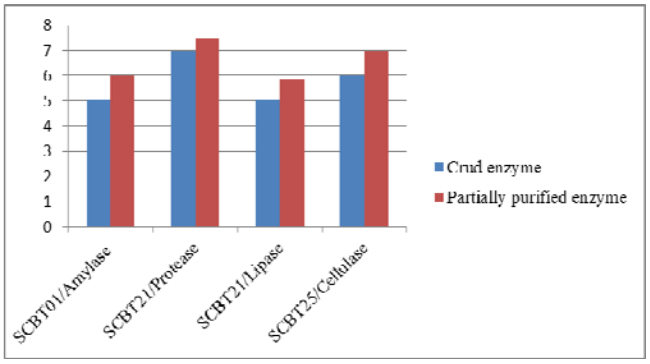

Fig 5: Comparisons of enzyme activity

The production of extracellular lipases and by *Bacillus* sp. was reported by Jung *et al*., (2003) [7]. Commercially useful lipases are usually obtained from microorganisms that produce a wide variety of extracellular lipases (Rohit Sharma *et al.,* 2001) [10]. Most of microorganisms it has the capacity to produce the extracellular and intracellular enzymes, lot of research work has to be done in this aspect. The isolate SCBT19 was taken for further studies.

4. Conclusion

Agricultural Soil Microorganisms as potential source of new and or improved products of industrial importance like enzymes have gained greatest significance in biotechnology. Industrialist focus on enzyme technology is the microorganism good source, which have the capacity to produce the commercially important enzymes on industrial scale, as enzymes occupy the centre stage in all biological processes. In this study suggested to giving a multi enzyme producing strain to the industry.

5. Acknowledgement

The authors would like to thank, the principal and College managements of Shanmuga industries arts and Science College, Thiruvanamalai, Tamilnadu, India. For providing all facilities to this study.

6. References

1. Awan AJ. Elements of food nutrition 1st Ed, 3-press street, Aminpur Bazr Faisalabad, Pakistan, 1993.
2. Rolin C. Pectin in industrial gums: Polysaccharides and their Derivatives, 3rd Ed, 2001, 257-293.
3. Ridley BL, O'Neill MA, Mohnen D. Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry 2001; 57:929-967.
4. Priya V, Sashi V. Pectinase producing microorganisms, International Journal of Scientific and Research Publications. 2014; 4(1). ISSN 2250-3153.
5. Junhong Han, Yu-ichi Tsukada, Eiji Hara, Naomi Kitamura, Toshiaki anaka. Hepatocyte Growth Factor Induces Redistribution of p21CIP1 and p27KIP1through ERK-dependent 16INK4a Up-regulation, Leading to Cell Cycle Arrest at G1 in HepG2 Hepatoma Cells. Journal of Biological Chemistry. 2005; 280:31548-31556.
6. Haq I, Ali S, Javed MM, Hameed U, Saleem A, Adnan F, Qadeer MA. Production of alpha amylase from a randomly induced mutant strain of *Bacillus Amyloliquefaciens* and its application as a desizer in textile industry. *Pak. J Bot*. 2010; 42(1):473-484.
7. Jung YJ, Lee JK, Sung GG, Oh TK, Kim HK. Nonionic detergent induced Activation of an esterase from Bacillus megaterium 20-1. J Molecular Catalysis B: Enzymatic, 2003; 26:223-229.
8. Shaikh NM, Patel AA, Mehta SA, Patel N. DIsolation and screening of cellulolytic bacteria inhabiting different environment and optimization of cellulase production. Universal Journal of Environmental Research and Technology. 2013; 3(1):39-49.
9. Suneetha Vuppu. Bishwambhar Mishra An overview of some reported soil enzyme producing microorganisms, Indian Journal of Fundamental and Applied Life Sciences. 2011; 1(4):180-186. ISSN: 2231-6345
10. Rohit Sharmaa, Yusuf Chistib, Uttam Chand Banerjee. Production, purification, characterization, and applications of lipases, Biotechnology Advances 2001; 19:627-662.