JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2010. Vol. 4(2), p. 823-826

Study on the Amylolytic Activity of Bacteria Isolated from Flour Mill Waste

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(Received: 30 March 2010; accepted: 08 May 2010)

The present investigation deals with the screening and study on the amylolytic activity of bacteria isolated from flour mill waste. Six different bacterial colonies were isolated and tested for starch hydrolysis. Out of them, isolate no A_4 has showed maximum zone of hydrolysis of 3.3 mm in starch agar medium and identified as *Bacillus*. The isolated *Bacillus* was subjected for amylase assay and extra cellular amylase production. The maximum amylase production was achieved with a reducing sugar concentration of 2.25 IU/ml at 37 ^{0}C for 48 hours of incubation.

Key words: *Bacillus,* amylase, starch hydrolysis, extra cellular amylase.

Starchy waste generated during milling processes which often disposed on open dumpsites near the local areas creates a serious problem in the environment and eventually leading to emission of offensive odors. These dumping yards provide rich medium for the growth of amylolytic bacteria. The soil waste around the mills was made use for the isolation of physiologically potential microorganisms which are able to produce starch degrading amylolytic enzymes[1]. Soils around mills, cassava farms after harvesting and treatment of tubers and flour markets represents natural fermented media for isolation of microbial strains producing amylases[2].

Amylases are enzymes which hydrolyse the starch molecules to give diverse products including dextrins and progressively smaller polymers composed of glucose units[3]. These enzymes have great significance with extensive biotechnological applications in bread, baking, food, textile and paper industries[4].

Amylases having approximately 25% of the enzyme market have almost completely replaced chemical hydrolysis of starch in starch processing industry[5-7]. Therefore screening of microorganisms with high amylase activities could therefore facilitate the discovery of novel amylases suitable to new industrial applications[8,9]. The necessity to improve the efficiency of amylase has led researchers to investigate the possibility of using different sources such as flour mill waste as growth medium. The present work was carried out for screening and identification of starch degrading bacteria from flour mill waste and further studies on their amylolytic activity.

MATERIAL AND METHODS

Collection of the soil sample

The soil samples were collected from different sites in Vizianagaram District, Andhra Pradesh, India where generally the flour mill wastes are disposed off. Sampling was done taking all possible aseptic measures and was stored at 4^{0}C. The samples were processed for isolation of bacteria and were screened for amylolytic potential.

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All the chemicals used were of analytical grade and purchased from Merck (India) and Himedia.

Isolation of bacteria

Isolation of bacteria from soil samples was done by standard serial dilution method. Luria agar and nutrient agar media containing nystatin (30 µgm /ml of the medium) were used for the isolation of bacteria. All the plates were incubated at 37^{0}C for 24hrs. Six well isolated colonies were further streaked on starch agar plate to get the starch hydrolysis strains, and were stored on the nutrient media as slants at 4^{0}C for the studies. The selected isolate was identified using morphological characteristics, gram staining, motility test and biochemical tests[10, 11].

Starch hydrolysis assay

The six bacterial isolates (A_1 to A_6) were subjected for starch hydrolysis assay by plating the bacterial isolates on to the starch agar plate and incubating the petri dishes at 37^{0}C for 24hrs. Composition of starch agar medium is 0.5% peptone, 0.2% yeast extract, 1% soluble starch, 0.13%KH_2PO_4, 0.05%,$MgSO_4.7H_2O$, 0.01% $CaCl_2.2H_2O$ and 1.5% agar pH 7.0 supplemented with 30 µgm of nystatin/1ml of medium. Starch hydrolysis assay using 4% lugol solution was performed with all six bacterial isolates to confirm their amylase production capability [12]. The bacterial isolate that exhibited widest zone of hydrolysis indicated their high amylase production.

Extra cellular production of amylase by the selected bacterial isolate

The production medium was prepared by using natural raw material as a carbon source such as 10% cassava flour. Ten grams of cassava flour was weighed and boiled with 200ml of sterile double distilled water and contents were reduced to 100 ml. This extract was added to sterile one hundred ml of nutrient broth (pH 7.0).Then a loopful of isolate from agar plate was inoculated and incubated at 37^{0}C for 24 to 48 hrs in an orbital shaker at 180 rpm . After incubation the supernatant of culture was collected by centrifugation at 4,500 rpm for 15 min at 4^{0}C which was source for the crude enzyme[13].

Amylase activity in the crude was assayed using starch as a substrate. The reaction mixture with 0.5 ml of 1% starch in 0.01 M Phosphate buffer (pH 7.0) and 0.5 ml of enzyme were incubated at 37^{0}C for 30 min, the reaction was stopped by the addition of 1 ml of 3,5 DNS reagent and kept on boiling water bath for 15 min and 10 ml of distilled water was added. Absorbance was measured at 540 nm against blank. The control was rundown simultaneously with the addition of DNS reagent prior to the addition of the enzyme. The enzyme activity which represents the amount of maltose produced in the reaction mixture per ml per unit time was calculated using the standard graph.

RESULTS AND DISCUSSION

Isolation and Identification of the bacterial strain

Six different bacterial colonies were isolated viz A_1, A_2, A_3, A_4, A_5, A_6 from different flour mill waste soil. When each of these were cultured in a starch agar medium, only isolate A_4 has showed maximum zone of hydrolysis of 3.3 mm which indicates its high amylase producing efficiency (Table1). Identification and characterization of the isolated bacterial strain A_4 was done by performing morphological and biochemical tests. The strain A_4 showed positive response towards grams staining, spore staining and motility. The bacteria showed positive response towards starch, glucose and maltose in carbohydrate fermentation. Bacterial strain also shown positive for voges-prauskauer and catalase test. The results of morphological and biochemical tests of isolated bacterial strain A_4 were presented in the Table 2 and Table 3.From these studies it was evident that bacterial isolate (strain A_4) from flour mill waste soil belongs to *Bacillus* and could hydrolyse starch. The characteristics of the microorganism used in this study were compared with Bergey's Mannul of Systematic Bacteriology. However the molecular study and ribotyping will reveal the true identity of the bacteria.

Production of extra cellular amylase

The selected bacterial isolate A_4 was tested for the extra cellular amylase production by using natural source which serves economical and readily available raw material for production of valuable enzymes. In this study, cassava flour was used as a substrate. Cassava is a good

ACKNOWLEDGMENTS

The authors acknowledge the support of the Department of Biochemistry, GITAM University for providing necessary facilities in carrying out the research work.

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