Journal of Food Technology 1 (4): 173-177, 2003

Biosynthesis of Alpha Amylase by *Bacillus licheniformis* using Extracts of different Brans

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Abstract: The present study is concerned with the production of extracellular alpha amylase by *Bacillus licheniformis* GCUCM-30. The extracts of different brans such as soybean meal, rapeseed meal, canola meal, wheat bran, cottonseed meal, guar meal, sunflower meal and rice bran were supplemented to the fermentation medium and tested for alpha amylase production in 250 ml Erlenmeyer flask. Of all the extractants examined, 30% wheat bran and rice bran extract at the ratio of 7.5:2.5 was found to be the best for the synthesis of alpha amylase (574 U ml^{-1} min^{-1}). The production of enzyme was reached maximum, 48h after inoculation.

Key words: Production, alpha amylase, *Bacillus*, wheat bran and agricultural by-products

Introduction

Alpha amylase, an extracellular enzyme, degrades ∝, 1-4 glucosidic linkages of starch and related substrates in an endo-fashion producing oligosaccharides including maltose, glucose and alpha limit dextrin (Leach and Schoch, 1961; Calik and Ozdamar, 2001 and Haq *et al.*, 2002). This enzyme is extensively used in many industries including starch liquefaction, brewing, food, paper, textile and pharmaceuticals (Nigam and Singh, 1995). The selection of suitable fermentation media in submerged fermentation also plays a very important role in the production of alpha amylase. The production of alpha amylase in shake flask using agricultural by-products have been reported by many workers (Gosh and Chandra 1984; Bajpai and Sharma, 1989). Remarkable increase in alpha amylase production was obtained when various oil seed cakes and rice husks were used by replacing peptone. An economical medium is usually preferable than the costly medium for commercial production of alpha amylase by *Bacillus* species. Bajpi and Sharma (1989) have developed a low cost medium for the biosynthesis of alpha amylase by *Bacillus licheniformis* TCRDC-B13. The low cost medium produced 5 times more enzyme than the high cost synthetic medium using yeast extract and peptone as nitrogen source were replaced with defatted cotton seed and defatted soybean.

Pakistan being an agricultural country has many agricultural by-products such as wheat bran, rice bran, cottonseed meal, soybean meal etc. These agricultural by-products are cheaply available in the market for their utilization in fermentation process. The present study is concerned with the exploitation of agricultural by-products for the production of extracellular alpha amylase by *Bacillus licheniformis*.

Materials and Methods

Organism: The *Bacillus licheniformis* GCUCM-30 (UV and NTG treated strain) was obtained from Biotechnology Research Laboratory, Department of Botany Government College Lahore. The strains were maintained at nutrient starch agar slopes. The strains were stored in paraffin oil.

Inoculum Preparation: Vegetative inoculum was used in present studies. Fifty ml of inoculum medium containing (%w/v) nutrient broth 1.0, soluble starch 1.0, lactose 0.5, NaCl 0.5, $CaCl_2$ 0.2 in 100 ml of phosphate buffer was transferred to each of 250 ml cotton plugged Erlenmeyer flask and sterilized at 15lb pressure (121°C) for 15 minutes. After cooling at room temperature, a loopful of bacteria was aseptically transferred to each flask. The flasks were then rotated in the rotary shaking incubator (200 rpm) at 40 °C for 24 hours.

Fermentation Technique

Shake Flask: The fermentation was carried out in 250 ml Erlenmeyer flask. Fifty ml of the fermentation medium containing (g/l) soluble starch 20.0, lactose 10.0, nutrient broth 15.0, $(NH_4)_2SO_4$ 5.0, $CaCl_2$ 2.0, NaCl 2.0 in 1000 ml of phosphate buffer (pH 7.5), was transferred to 250 ml cotton plugged Erlenmeyer flask. The flasks were sterilized in the autoclave and cooled at room temperature. One ml of vegetative bacterial inoculum (24h old) was transferred to each flask. The flasks were then placed in the rotary incubator shaker (200 rpm) at 40°C for 48h. After 48 h, the fermented broth was centrifuged at 7000 rpm for 15 min. The solids free supernatant was used for the estimation of alpha amylase and biomass. All the experiments were run parallel in triplicates.

Enzyme Assay: Alpha amylase was estimated according to the method of Rick and Stegbauer (1974). The enzyme solution at pH 7.5 was incubated at 60°C

using 1% soluble starch solution. The reducing sugars were measured by adding 3, 5-dinitro salicylic acid reagent, boiling for 5 min, cooling and measuring the O.D at 540 nm in the spectrophotometer (Model CECIL CE7200) against maltose as standard. One unit of activity is equivalent to that amount of enzymes, which in 10 minutes librates reducing group from 1% Lintner's soluble starch corresponding to 1 mg maltose hydrate.

Biomass: The biomass was determined turbidimetrically at 650 nm in spectrophotometer and read against graph plotted for dry weight Vs. O.D. at the same wavelength. Biomass was converted into g/l according to the method of Hariuchi *et al.*, (1993).

Statistical Analysis: Post Hoc Multiple Comparisons were applied for different tests (ANOVA I design). Significance has been presented in the form of probability (p) values (Snedecor and Cochrane, 1998).

Kinetic Study: Kinetic parameters for batch fermentation were determined according to the method describe by Pirt (1975) and Lawford and Rouseau (1993).

Results and Discussion

The production of alpha amylase using an economical medium has been a worth prasing achievement in the field of industrial biotechnology. Pakistan being agricultural country has many cheaply available agricultural by-products. These agricultural by-products can be used for their exploitation as substrate for enzyme formation (Bajpi and Sharma, 1989). In present study the extract of different agricultural by-product such as soybean meal, rapeseed meal, canola meal, wheat bran, cottonseed meal, guar meal, sunflower meal and rice bran were tested for alpha amylase production (Fig.1). The production of enzyme following growth of organism was found to be maximum by wheat bran or rice bran extract and varied significantly ($P < 0.05$) then the other bran extracts. It might be due to these brans provided essential nutrients (proteins 1.32 % carbohydrates 69.0 % fats 1.9 %, fibers 2.6 % ash 1.8 %, Ca 0.05 %, Mg 0.11 %, K 0.45 %, S 0.12 %) for the growth of microorganism as well as for the production of alpha amylase. The production of enzyme was greatly inhibited as the guar meal or cottonseed meal extract were used in fermentation medium. It might be due to these by-products contain very low concentration of carbohydrates which were essential for the production of alpha amylase. As the amount of carbohydrate was reduced, the production of alpha amylase was also reduced (Malhotra *et al.*, 2000).

As the wheat bran and rice bran extract gave maximum production of alpha amylase therefore, these brans were partially replaced at the ratio of 10:00, 7.5:2.5, 5.0:5.0, 2.5:7.5 and 0:10 (Fig. 2). Of all the ratios tested, the maximum production of enzyme was produces as the wheat bran extract was partially replaced with rice bran extract at the ratio of 7.5:2.5.

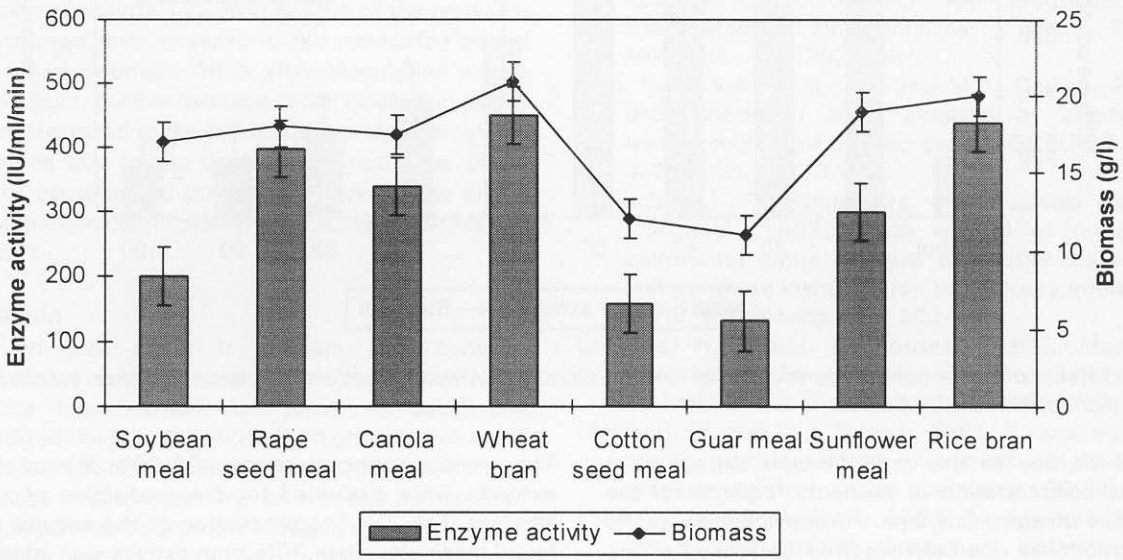
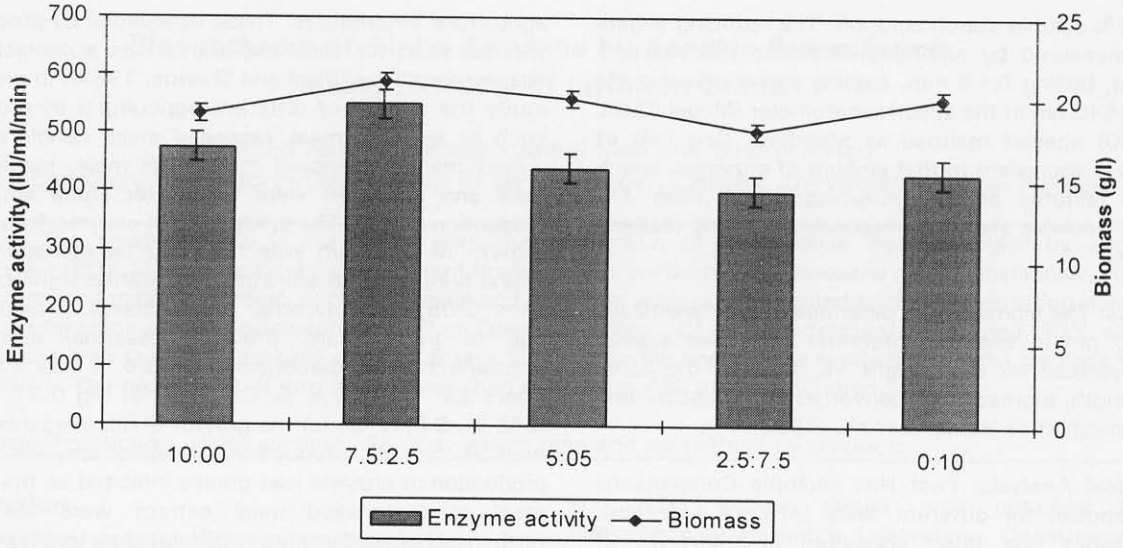


Fig 1: Effect of different agricultural by-product extracts on the production of alpha amylase by *Bacillus licheniformis* GCUCM-30



Each value is an average of three replicates. Y error bars indicate the standard error among the values. The values differ significantly at $P < 0.05$.

Fig 2: Effect of partial replacement of wheat bran extract with rice bran extract on the production of alpha amylase by *Bacillus licheniformis* GCUCM-30

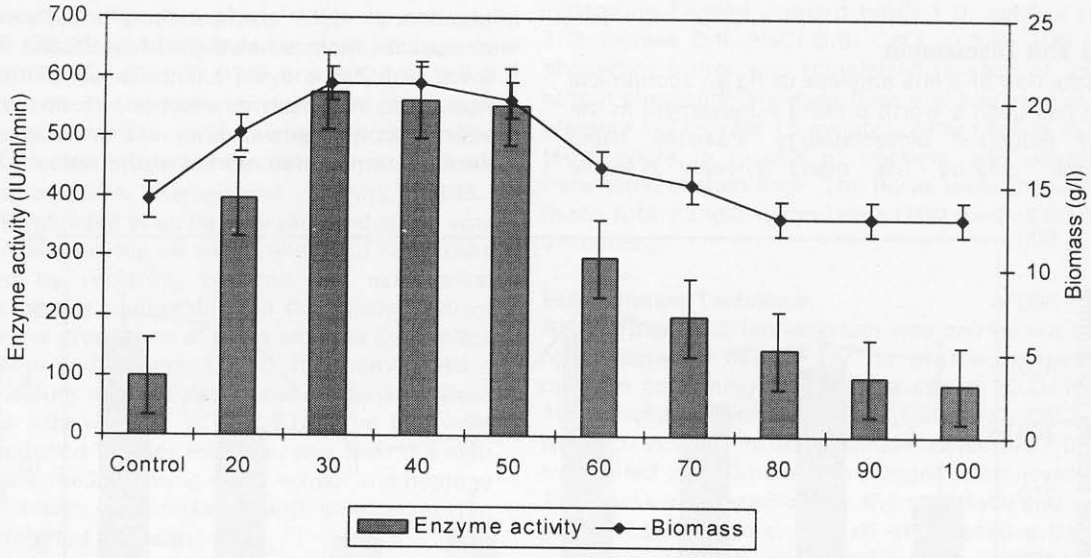
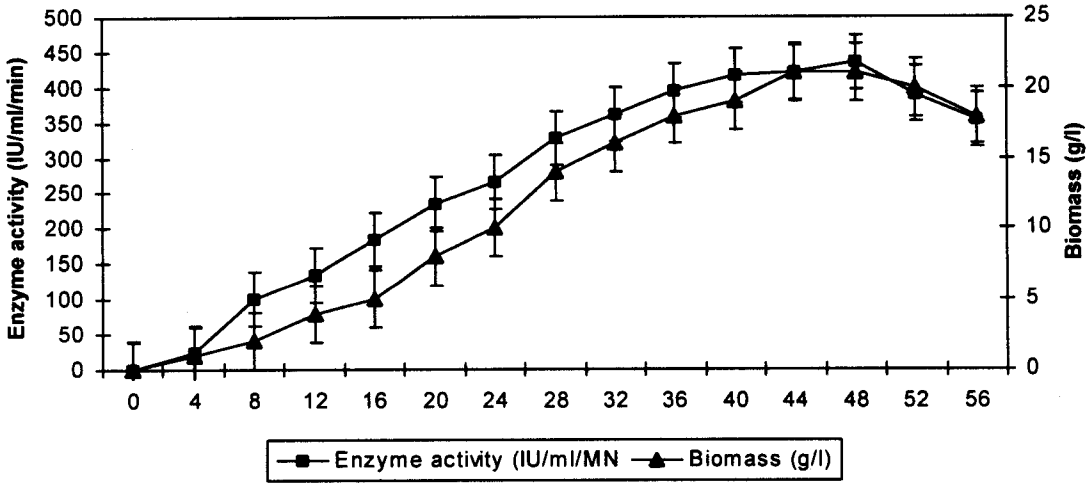


Fig 3: Effect of different concentration of bran extract on the production of alpha amylase by *Bacillus licheniformis* GCUCM-30

It might be due to this ratio of bran extract gave adequate concentration of nutrients required for the production of alpha amylase. Further increase in the concentration of rice bran resulted decreased in the production of alpha amylase by the bacteria strain. Thus, the ratio 7.5:2.5 of wheat bran and rice bran extract was selected.

The various concentrations (20-100 %) of bran extracts were evaluated for the production of alpha amylase (Fig. 3). The production of the enzyme was found maximum when 30% bran extract was added to the fermentation medium. Further increase in the concentration of bran extract resulted decreased in the production of alpha amylase. It might be due to with



Each value is an average of three parallel replicate. Y error bars indicated the stander error from mean value

Fig 4: Rate of alpha amylase fermentation by *Bacillus licheniformis* GCUCM-30

increase in the concentration of nutrients resulted increase in the stationary phase of the bacteria. This increase in the stationary phase of bacteria resulted decreased in the accumulation of alpha amylase by the bacterial culture (Pratima and Umender, 1989; Prescott and Dunn, 1987). Thus, 30 % bran extract (wheat bran 7.5 and rice bran 2.5) was optimized.

The optimization of rate of enzyme production is very essential for maximum production of alpha amylase by the bacterium. In this connection the rate of alpha amylase production was carried out (Fig. 4). The production of enzyme following growth of the organism was increased with increase in the incubation period and reached optimum 48 h after inoculation in the entire medium. Further increase in the incubation period resulted decreased in the production of alpha amylase. It might be due to the depletion of nutrients, death phase of bacteria and production of proteases in the fermentation medium (Lealem and Gashe 1994; Haq *et al.*, 2002).

Conclusion

Based on this study it appears that complex carbohydrate sources such as wheat bran extract along with rice bran extract can serve as basal and standardized medium for obtaining high yields of alpha amylase from *Bacillus licheniformis* GCUCM-30. These substances are inexpensive and could be used to replace expensive carbon and nitrogen sources that are considered to be costly and impractical for the commercial production of enzyme.

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