PRODUCTION OF PROTEASES BY *STAPHYLOCOCCUS EPIDERMIDIS EFRL 12* USING COST EFFECTIVE SUBSTRATE (MOLASSES) AS A CARBON SOURCE

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ABSTRACT

Staphylococcus epidermidis EFRL 12 capable of producing extracellular protease isolated from soil. The protease production was investigated in batch wise submerged fermentation. The protease production was studied with varying carbon sources with (0.5 and 1.0%) concentrations, peptone (0.25-1.5%) and different nitrogen sources (1.0%).The effect of sugarcane bagasse, rice husk, molasses and date syrup were checked along with pure carbon sources. The best production was obtained when microorganism was grown on mineral medium containing 1.0% molasses as carbon source and 1.0% ammonium nitrate as nitrogen source after 6 hours of incubation at 37°C and initial pH was adjusted to 7.5.

INTRODUCTION

Agro-industrial waste causes pollution, which can be utilized as raw material (low cost substrate) for the production of bulk valuable goods such as, ethanol, enzymes, single cell protein, amino acids and organic acids. Among various low cost raw materials molasses focused considerable attention as they support microbial growth and enzyme production (Kanekar et al., 2002). Molasses is the sugar industry by-product. The percentage wise compositions of molasses is water, 30.5; sucrose, 29.75; dextrose, 3.25; fructose, 4; other reducing sugars, 1.0; ashes, 15.0; nitrogenous contents, 4.5; non-nitrogenous contents, 8.5, waxes and sterols, 1.5 and other compounds, 2.0 as reported by Shikha et al., (2007). Proteases (E.C. 3.4.) are also known as peptidyl peptide hydrolases, which catalyze the hydrolysis of peptide bond in a protein molecule stepwise into smaller peptides, dipeptide and amino acids. Proteolytic enzymes are important industrial enzymes contributing 65% of worldwide industrial enzyme market (Johnvesly et al., 2001, Mei et al., 2005, Olsson et al., 1992) and 25% of the total global enzyme production (Layman et al., 1986, Joo et al., 2005). In recent years the growing demand of proteases is increasing due to its wide applications in different industries such as detergent additives, in waste treatment process, medical and basic research, silver recovery, leather and pharmaceutical industries (Godfrey et al., 1996, Gupta et al., 2002, Mei et al., 2005).

In the present study, protease production was carried out by low cost agro-industrial waste material (molasses) to best of my knowledge *S. epidermidis* has been not utilized for protease production using molasses as substrate.

MATERIAL AND METHODS

Organism: The *Staphylococcus epidermidis EFRL 12* was isolated from

soil sample collected from lawn of the Institute of Chemistry, University of Sindh, Jamshoro and grown in Enzyme and Fermentation Research Laboratory, Institute of Biotechnology and Genetic Engineering.

Cultivation condition: For maintenance of the culture and as a control culture the organisms was grown on medium containing 0.5% glucose, peptone (0.5%), $MgSO_4.7H_2O$ (0.5%), KH_2PO_4 (0.5%), Fe_2SO_4. $7H_2O$ (0.01%) and incubated at 37°C for 17 hours with initial pH 7.5. The fermentation medium with slight modification is utilized as reported by many workers in the field (Folsade et al., 2005; Kunamneni et al., 2003) The microorganism was grown in 250 ml conical flask 50ml of sterilized medium The sterilized medium was inoculated with 0.5ml of *Staphylococcus epidermidis EFRL 12* a-17 hours old culture and incubated for 12 hours at 37°C. The bacterial biomass was separated from culture broth after 1 hour interval of incubation period through refrigerated centrifuge machine and further analysis was carried out from culture broth.

Effect of Carbon sources on the production of Proteases: The effect of carbon source (0.5 and 1.0%) on growth and enzyme production was determined by replacing glucose with other carbon sources (glucose, fructose, galactose, sucrose, lactose and maltose, citric acid, trisodium citrate) industrial waste (molasses and date syrup) and agri-cultural waste (sugarcane bagasse and rice husk

Effect of nitrogen sources on the Proteases Production: First of all different concentrations of peptone were used to optimize the concentration of peptone (0.25-1.50 %). In next step different nitrogen sources were supplemented in fermentation medium replacing 1.0% peptone with other nitrogen sources (casein soluble, casein hydrolyzed, tryptone, corn steep liquor, yeast extract, urea ($NH_2–CO–NH_2$), sodium nitrate ($NaNO_3$), ammonium nitrate, potassium nitrate (KNO_3), ammonium chloride and ammonium sulphate).

Assay of protease activity: Protease activity was determined by (Penner and Ashton, 1967).

One unit of protease activity was defined as the amount of protease required to catalyze the liberation of 1 µg of tyrosine under the assay conditions. Protein content was determined by Lowry et at., method [1951].

RESULTS AND DISCUSSION

Number of microorganism including mould, yeasts and bacteria produces proteases but bacteria are preferred because of their short span of life cycle. The *Staphylococcus epidermidis EFRL 12* was isolated from soil and screened by zone hydrolysis method. Chi et al., (2007) and Alagarsamy et al., (2005) have isolated the fungal culture through screening for protease production using casein agar medium and identified *Aspergillus oryzae* NRRL 1989 and 2217, (Folasade et al., 2005) have checked protease secretion using skim milk agar by *Bacillus sp.* The isolate hydrolyzed the soluble casein was identified as *Staphylococcus epidermidis EFRL 12* according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). The effect of various pure carbon sources such as (glucose, fructose, galactose, sucrose, lactose, maltose, citric acid, trisodium citrate),

industrial waste (molasses and date syrup) and agricultural waste (sugarcane bagasse and rice husk) was checked on the growth and enzyme yield. It is clearly observed from Figures-1-2 that *S.epidermidis* grow better and secreted maximum protease production when 1% molasses was supplemented in the fermentation medium as a substrate at 37 ± 2°C after 6 hours of incubation in comparison to other sugars. Figure 3 shows the effect of peptone concentration on biosynthesis of protease by *S.epidermidis* using mineral medium supplemented with 1.0% molasses. Protease synthesis increased with increasing peptone concentration up to 1.0% and then it falls because the higher peptone concentration acts as repressor. Figure-4 shows the protease production by *S.epidermidis* when grown on various nitrogen sources (1.0%) added in mineral medium containing 1.0% molasses. In this study various organic and inorganic nitrogen sources such as ammonium chloride, potassium nitrate, sodium nitrate, ammonium nitrate, urea, yeast extract, corn steep liquor, casein hydrolyzed, casein soluble and tryptone were used in place of peptone in culture medium. It is noted that the *S. epidermidis* produced maximum protease when it was grown in 1.0% ammonium nitrate containing molasses mineral medium in comparison to other nitrogen sources and these results are in agreement with the results of Wellingta et al., (2004) in the case of protease production by *Bacillus sp*.

CONCLUSION

In the present work *Staphylococcus epidermidis EFRL 12* was exploited for protease production using molasses. The organism is pathogenic in nature but can be used for commercial production either inactivating the pathogenic activity or by isolating the gene and insert in some suitable organism. The maximum proteases production was noted when *S. epidermidis* was grown on mineral medium containing 1.0% molasses and 1.0% ammonium nitrate at 37°C for 6 hours with initial pH 7.5.

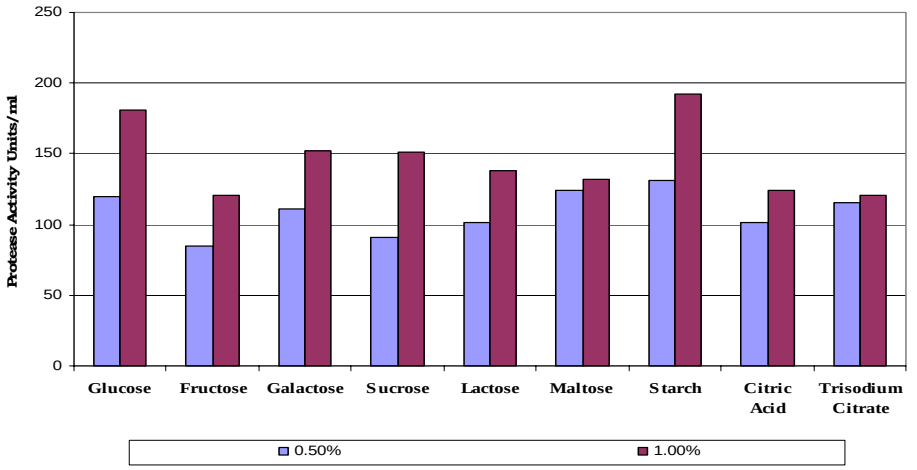
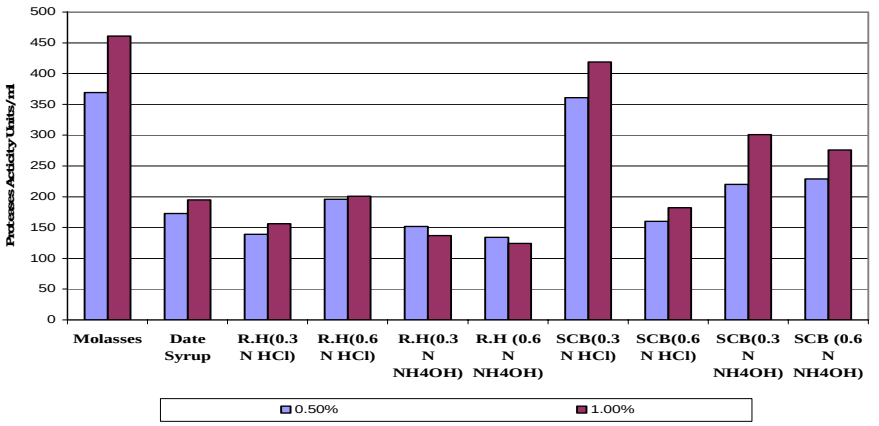


Figure-1: Effect of 0.5 and 1.0 % pure carbon sources on protease production by *Staphylococcus epidermidis.*



R. H= Rice Husk, SCB= Sugarcane baggasse, N= Normal solution

Figure-2: Effect of 0.5 and 1.0 % agricultural waste and industrial waste on protease production by *Staphylococcus epidermidis.*

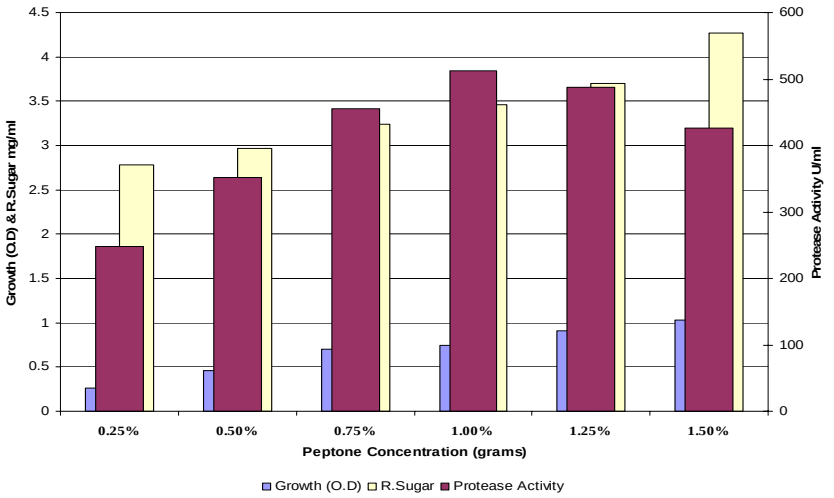


Figure-3: Effect of peptone concentration on protease production by *S.epidermidis* using 1% molasses as a carbon source when initial pH of culture medium was 7.5 and incubated at 37°C for 6 hours.

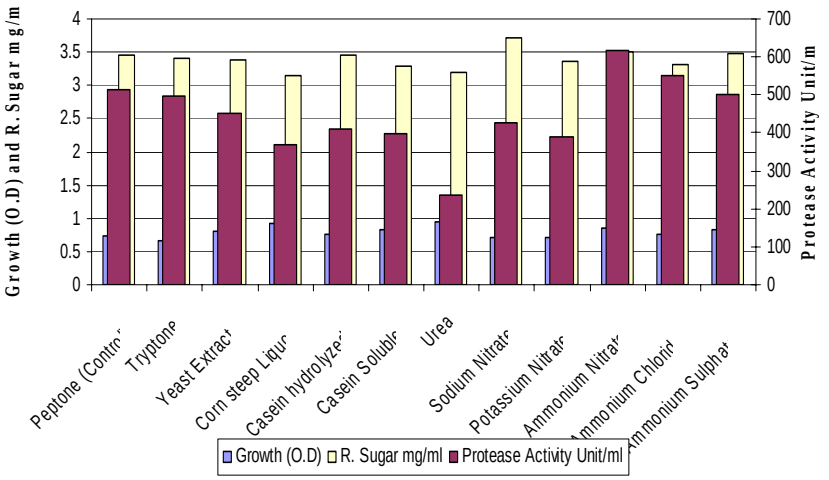


Figure-4: Effect of 1.0% nitrogen sources on protease production by *S.epidermidis* using 1% molasses as a carbon source and 1.0 % different nitrogen sources when initial pH of culture medium was 7.5 and incubated at 37°C for 6 hours.

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