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PRODUCTION OF KERATINASE FROM FEATHER DEGRADING STENOTROPHOMONASMALTO PHYLA AB20

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ABSTRACT :

Keratinolytic bacteria Stenotrophomonasmaltophyla AB20 have feather degrading ability which is isolated from feather waste soil,thus bacterial strains were identified by physical, biochemical characteristics, and 16S rRNA sequences. Keratinase production maximize through medium optimization of the selected keratinase producing Isolate StenotrophomonasmaltophylaAB 20, and production checked through submerged fermentation with various single parametric factors. Maximum keratinase production was obtained with incubation period at 6th day 75 U/ml of final production medium, with each effective factor likeTemperature 30℃ (72 U/ml), pH7(68 U/ml), Agitation rate 150 rpm (63.2U), Carbon source Maltose (65U/ml), Organic nitrogen sourcesYeast extract (63.7U/ml), Inorganic nitrogen source Sodium nitrate (60.8U/ml) and Activators metal ions $MgCl_2$ (58U/ml) that were tested with specific keratinase activity. Keratinases which are produced by these keratinolytic organisms could be used to degrade keratinous waste and further the digested products could be an excellent material for producing animal feed and fertilizers.

KEYWORDS : *Feather waste, Stenotrophomonasmaltophyla, Keratinase production.*

INTRODUCTION

Poultry processing plant produced million tones of feather waste as by product, and it creates environmental pollution. Keratin is an insoluble macromolecular protein which is resistance to extra cellular proteases for biodegradation. Keratins secondary structural protein packed tightly either in α-helix or in β-sheet structures linked by disulphide bonds through responsible for the stability of keratin. Keratins are two types hard keratins (feather, hair, hoof and nail) and soft keratins (skin and callus) (Gradisar, *et al.,* 2005)
The degradation of keratinous material is important medically and agriculturally, a current use of feathers is conversion to feather meal, a digestible dietary protein for animal feed, using physical and chemical treatments. These methods can destroy certain amino acids and decrease biological value of protein quality and digestibility (Brandelli et al 2010). Secretion of keratinolytic enzymes is associated with number of soil microorganisms or which recalcitrant keratin protein. It is degraded a number of feather degrading species belong to bacteria, actinomycetes and fungi have been used for the production of keratinase enzyme in submerged as well as in solid state fermentation. Most of Keratinase enzymes from microbial sources are extracellular and inducible by keratin waste trough submerged fermentation (Gupta and Ramnani, 2006).However, much current research is centered on the potential use of keratinase of bacterial origin for the industrial treatment of keratin-containing compounds, *Pseudomonas* sp.(Linang et al 2011)keratinolytic enzyme by a newly isolated *Stenotrophomonasmaltophilia* R13 that produces plant growth-promoting activity (Jeong et al 2010).These enzyme finds important application in leather industry, a biological

approach could be advantages over the thermal and chemical methods since it is a friendly environment and energy conservation process resulting in its feather products could have a potentially valuable source of protein as animal. The present study describes the production of Keratinase for single factor parametric approaches produced by *Stenotrophomonas* sp.AB 20 isolated from poultry waste.

MATERIALS AND METHODS

Isolation and identification of Feather-degrading bacteria were employing enrichment culture techniquefrom the soil. Primary and secondary screening approach, Individual colonies with clear zones resultingfrom proteolysis were picked and grown basal feather medium containing1% chicken feather as the sole carbon and nitrogen source. During the cultivationat 30°Cand at 120 rpm, keratinolytic activity and feather degradation rate werechecked at 24 hr time intervals. The isolate exhibiting the highest activity waschosen for further investigation. The basal feather medium used in this study comprisedof (g/L) NaCl 0.5, K_2HPO_4 1, KH_2PO_4 0.6, $MgCl_2.6H_2O$ 0.1, Feather 10, pH 7.5. It was incubated at 30°C for 5 days at 120 rpm (Brandelli A, Riffel2005; Agrahari and Wadhva 2010)

Morphological and cultural properties of the strain AB 20 were studied accordingto methods for general and molecular bacteriology.The sequence of 16S rRNAgene was determined after genomic DNA extraction and polymerase chain reaction, amplification based on a previous work. Databases in GenBank were searchedfor sequences similar to the 16S rRNA gene sequence. The nucleotide sequence determined in this work has been depositedin GenBank database with accession number KF201645 (Birari et al 2013)

Medium improvement for keratinolytic enzyme production to investigate the effect of culture conditions for the keratinolytic enzyme production cultured over a range of Initial pH range 5.0–10 (Fig.1), Temperature range 20–55°C (Fig.2), In order to various carbon sources glucose, maltose, sucrose, fructose, starch, xylose, mannose, arabinose, lactose (Fig.3) then nitrogen sources peptone, yeast extract, meatextract,soya peptone, tryptone, urea (Fig.4)In organic nitrogen source ammonium nitrate, ammonium chloride, ammonium sulphate, sodium nitrate, potassium nitrate (Fig.5),metal ions CaCl2, BaCl2, MgCl2, FeCl3, CoCl2, MgSO4, MnSO4, ZnSO4, CuSO4, AlSO4 (Fig.6) All these mediumwere supplemented individually to the basal feather medium containing 1% chicken featherwith respective final concentration of 0.1% for above organic and inorganic source respectively and effect of different feather concentrations (0.1–2%) on the keratinolytic enzyme production was also investigated (Fig.7). And finally Enzyme production period also investigated (Fig.8).

For the production of keratinolytic enzyme, 50 ml of the medium was dispensedinto each of 250-ml Erlenmeyer flasks followed by inoculation with 1ml of the strainAB 20 culture (1.4×10^4 cells/ml) grown in nutrient broth at 30 °Cfor 8 h. Cultivationswere carried out at 30°Cand 120rpm for 3 day in a rotary shaker.Assay of keratinolytic activity and soluble proteinwas assayed spectrophotometrically by using Azokeratin as substrate the culture supernatants obtainedby centrifugation at 10000 rpm for 15 min. one unit of Enzyme activity is defined as increase in absorbance at 440 nm with 0.01 U/ml the Keratinase assayed was prepared bythe method as described previously (Brandelle et al 2005). Soluble protein concentration was determined by Lowery method(Lowery 1951)with bovine serum albumin as the standard.All experiments were repeated three times.

RESULTS

The keratinolyticactivity of the strain *Stenotrophomonas*maltophilia AB 20was assayed in the basal feathermedium containing 1% chicken feather as sole carbon and nitrogensource. The level of keratinolytic activity in the medium showed maximum after48 h of incubation.

The influence of initial pH on the of *Stenotrophomonas*maltophilia AB 20keratinolytic enzyme production was investigated in optimum at pH 7.0 (68 U/ml) Fig.1 The influence of temperature on the keratinolytic enzyme production observed optimum level of production was achieved at 30 °C. (72 U/ml) Fig.2. Optimum enzyme activity also observed at 150 rpm Agitation rate (63.2 U/ml). In order to study the influence of co-carbon sources onthe keratinolytic enzyme production, various co-carbon sourceswere

added to the basal feather medium Maltose promoted thegreatest degree of keratinolytic enzyme production (65 U/ml) Fig. 4. To investigate the influence of co-nitrogen sources on the keratinolyticenzyme productionthe highest keratinolyticenzyme production was obtained with Yeast Extract (63.7 U/ml) Fig.5 and In organic nitrogen source Sodium nitrate (60.8U/ml) As shown in Fig.6 The influence of different Metal ions on the keratinolyicenzyme production was investigated. Maximal keratinolyticenzyme production was found in $MgCl_2$ (58U/ml)Fig. 7.And finally enzyme production of optimized medium is 75 U/ml at 4th day Fig. 8 as compared to un optimized medium 52.82U/ml increase 1.41 fold keratinase production

DISCUSSION

Similar report of keratinase production strain *Pseudomonas* sp. MS 21 was reported to produce optimum keratinase production 43 U/ml Optimum temperature is 37 ℃ and optimum pH 8, Mannitol and $CaCl_2$, $ZnCl_2$ decrease enzyme activity (Tork et al 2010).Similarly approach *Stenotrophomonasmaltophila* R13 use feather as sole carbon and nitrogen source for minimal medium and 0.1% Glucose, 0.12% polypeptone increase enzyme production, optimum pH and Temperature is 7 and 30℃ respectively(Jeong et al 2010)Maximum Keratinase production recorded at 40℃and initial optimum pH is 7.5-8, metal activator $Zn2^+$ activate most of keratinase activity and inhibited by heavy metal ion like $Pb2^+$, Cd 2^+, $Hg2^+$ (Cao et al 2009). Similarly $Ba2^+$, $Ca2^+$activate keratinase production (Lin et al 2009) With regard to optimum temperature *Pseudomonas aeruginosa* at 40℃ (Raju et al 2013) *Bacillus thuringiensis* TS2 with 50℃ (Sivakumar et al 2012). *Bacillus weihenstephanensis* PKD 5 required 120 rpm (Sahoo et al 2012) the optimum amount of inoculums was found to be *Pseudomonas aeruginosa* produced keratinase with 5% (Raju et al 2013). The carbon source, glucose was found to be good co substrate maximum keratinase activity. Among nitrogen sources maximum keratinase was produced*Bacillus pumilus* A1 yeast extract 47.68 U/ml (Zouari et al 2010).

CONCLUSION

The isolate *Stenotrophomonasmaltophila* AB 20 produced a significant amount of Keratinase under submerged fermentation. Organism grows on simple media with feathers as its sole carbon, nitrogen, and energy source. The above report stated the evidence for the production of keratinase with substrate interactions of bacterial strains with maximum keratinase production75 U/ml at 4th day with 1.41 fold keratinase production simple and effective manner. At the same time, it transforms industrial waste (chicken feather) into nutritional feed additives and protects the environment. Thus, utilizing poultry feathers as a fermentation substrate in conjunction with keratin-degrading microorganism or enzymatic biodegradation may be a better alternative toimprove nutritional value of poultry feathers and reduce environmental waste.

Figures

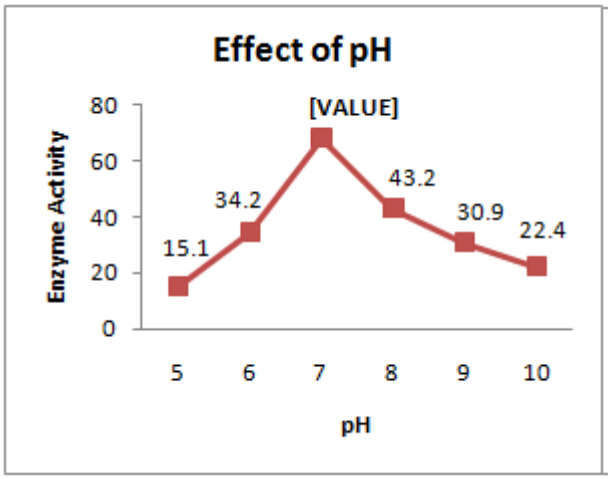


Fig. 1

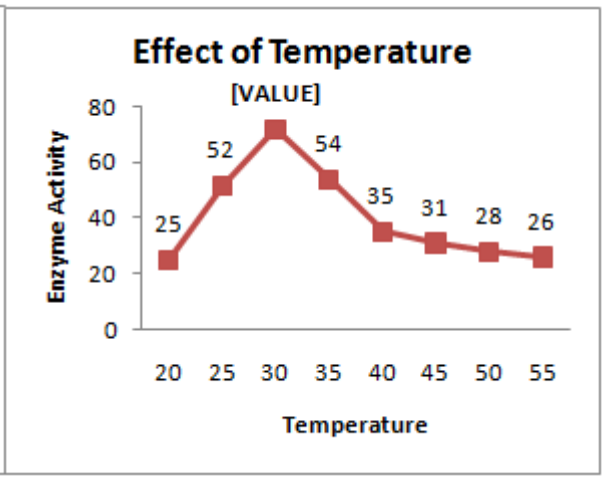


Fig.2

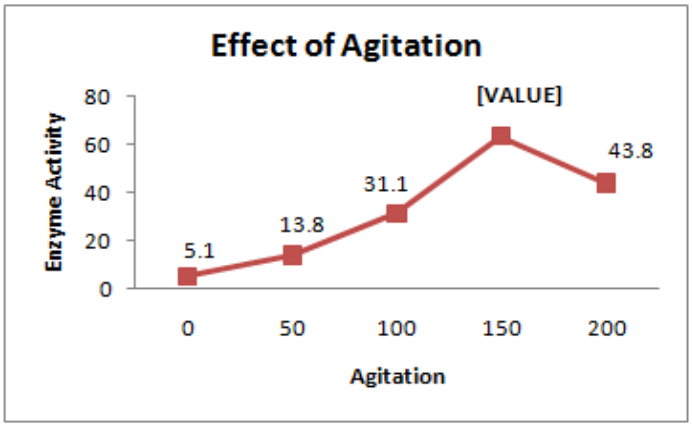


Fig. 3

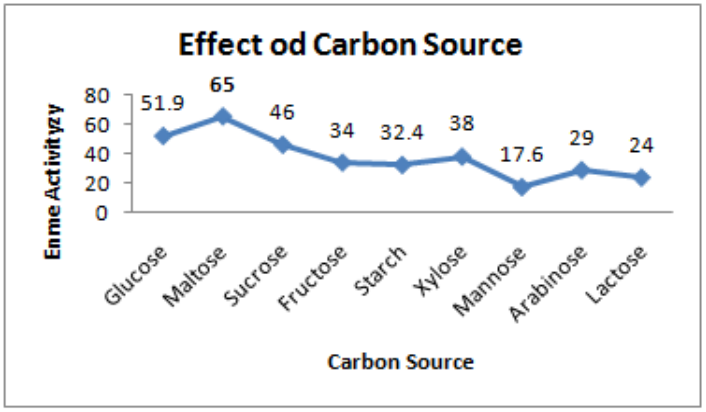


Fig. 4

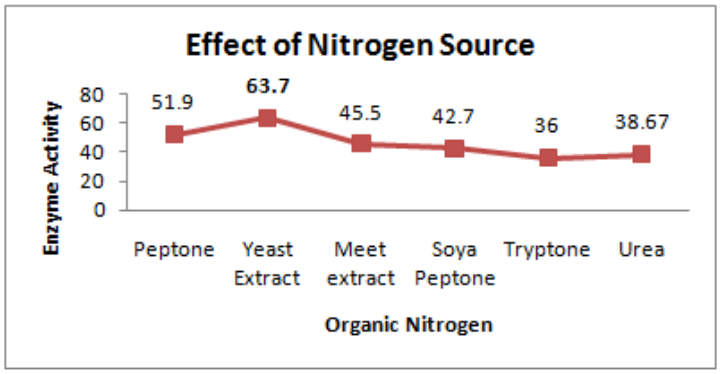


Fig. 5

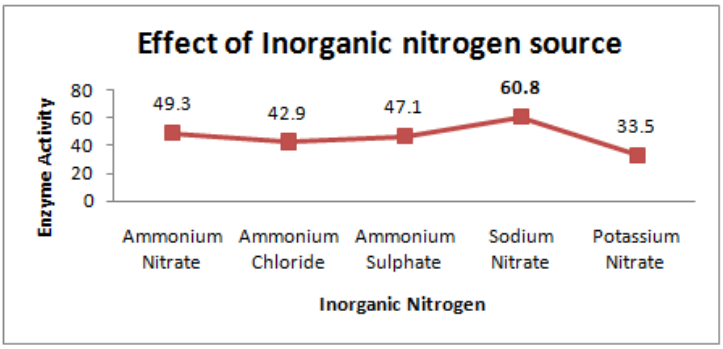


Fig. 6

Fig. 7

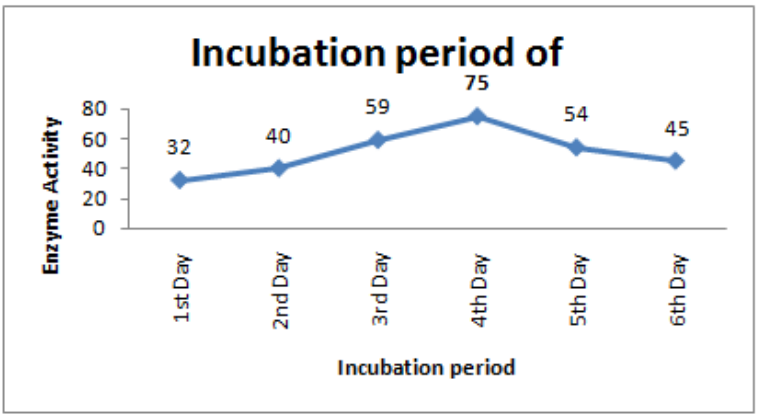
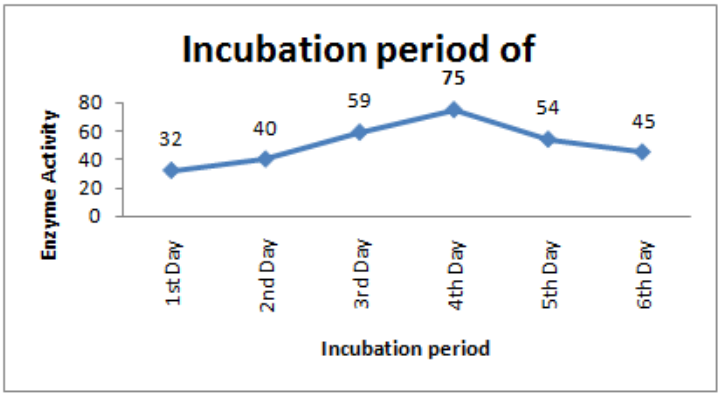


Fig. 8



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