Research Article

A Potential Strain of Keratinolytic Bacteria VIT $RSAS_2$ from Katpadi and Its Pharmacological Benefits

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ABSTRACT

Two bacterial strains VIT $RSAS_1$ and VIT $RSAS_2$ obtained from the poultry farms in Vellore were investigated for the keratinase enzyme production. The maximum amount of keratinase activity of strain $RSAS_2$ (about 104 Uml-1) was produced at 37°C when the bacterium was cultured for 432 Hrs. in broth containing feathers with initial pH of 7. The keratinase activity was observed over a wide range of pH values (pH 3 - 9) and varying substrate concentration. It was optimal at pH 7 – 8 and 0.5 mg substrate /µl enzyme respectively. The synthesis of nanoparticles was also monitored. Silver nanoparticles were synthesised using the keratinase enzyme produced by the bacterial strain. Production of silver nanoparticles was monitored by UV- visible spectroscopic analysis. The peak was observed between 400-450 nm indicating their presence. Additionally the antibacterial activity of nanoparticles was checked against *E.coli* and *Staphylococcus aureus* which can be used for various research and pharmacological studies.

Keywords: *Escherichia coli,* Keratinase, silver nanoparticles, *staphylococcus aureus.*

INTRODUCTION

It is estimated that nearly 24 billion chickens are killed yearly (which is increasing per year) and leads to production of nearly 4 billion pounds of feathers as a waste from commercial (large and small scale) poultry industries around the world. Naturally a feather takes 3 to 4 years to get degraded due to solid structure of keratin protein. Disposal of feather waste is a major problem because simple dumping in the ground leads to the soil pollution and burning it adds to the SO2 and CO2 content in the environment and causes air pollution. This mammoth size of discarded feather, apart from polluting the soil or air, also causes various human ailments including chlorsis, mycoplasma and fowl cholera.

Feathers are composed of keratin which is an insoluble protein and not easily degraded by trypsin, pepsin and papain, the resistance mainly due to its structure(tightly packed protein chains in α-helix (α-keratin) and β-sheet (β-keratin)and cross-linking by disulfide bridges in cystine residues)[1, 2]. Keratin is also major constituents of skin, hair, feathers, wool, and nails.[3]

These feathers can be used for various commercial, beneficial processes like production of useful protein and amino acid (cysteine, serine, methionine etc) but its use is restricted to dietary components in animal feedstuffs due to poor digestibility [1].

Physical and chemical treatments which are currently used to increase the digestibility of feather keratin consume large amounts of energy and also destroy certain amino acids, thus yielding products of poor digestibility and decreased protein quality[1, 4-7]. An alternative to the above is microbial degradation by bacteria producing keratinase enzyme. Keratinases (E.C. 3.4.99.11), are peptidases which are capable of using keratin as substrate. [2]

Keratinolytic activity has been reported for various bacterial genera, such as Bacillus [8-10], Thermoanaerobacter[11], etc and also by fungal species [12,13] and actinomycetes[14,15] with the enzyme produced both by submerged[17, 18] as well as in solid state fermentation.[19,1,20]

The microbial degradation of the poultry waste is an environment friendly biotechnological process, through which one can convert the abundant waste into low-cost, nutrient-rich animal feed[21, 22]. Additionally Keratinolytic enzymes have applications in enzyme research, the detergent industry, medical, cosmetic industry , textile manufacturing, and leather industries; they can also be used in prion degradation and as pesticides and production of biodegradable films, glues and foils and also as nitrogenous fertilizer for plants. [2, 12]

Nanobiotechnology is a robust field of emerging opportunities in the arena of medicine and pharmacology, now a days different nanomaterial are synthesised with different substrates and novel methods but the poor fidelity and high cost of the process are the major problems to take care off. Biological synthesis of nanoparticles has shown a very promising solution for this problem. Therefore a biological method of production of nanoparticles is extensively studied with nanoparticles being synthesised using plants[24], fungi [25-27], bacteria and yeast respectively. The nanoparticles are used in many fields a few being electronic, sensor technology, biomedical (biological labelling, in oncology) etc. Further, the synthesized nanoparticles were characterized by UV-Vis spectroscopy. The colour change from yellow to reddish brown confirmed the formation of nanoparticles.[28]

Silver was of particular interest due to its distinctive physical and chemical properties. Silver Nanoparticles are used in antimicrobial agents, textile industries, water treatment, sunscreen lotions etc.[23, 29]

In this study we wish to isolate keratinase producing organism from the areas of Vellore and Katpadi and check the production of Ag using the same.

MATERIALS AND METHODS

Preparation of substrate

Chicken feathers collected from nearby poultry farm (near Vellore, Tamil Nadu) were used as substrate. Feathers were washed several times with distilled water and subsequently dried initially in sunlight followed by drying in hot air oven at 50°C. Following the above step feathers were pre-treated in chloroform: methanol (1:1) solution for 48 hrs followed by drying at approx 40°C and storage at 4°C.

Isolation of microorganism producing keratinase enzyme

The soil samples are collected from nearby poultry farm (near Vellore, India) and sites where feathers are dumped near Katpadi (Vellore, India). 1 gm of sample was suspended in 10 ml of sterilized water and after diluting to an extent of 10-7, an aliquot of 100 µl was spread on the nutrient agar plate., hair baiting technique is used for screening of feather degrading bacteria[9]; just in this case instead of hairs sterilized feathers were used.[20].

Primary Screening on Skim milk agar plates

Bacteria forming isolated (degrading feathers) were inoculated onto skim milk agar plates and incubated at 37°C for 24 h. Strains that produced clearing zones in this medium were selected [8].

Sub culturing

The organism screened from above obtained agar plates(step 2) was sub - cultured by continuously growing the bacterium in basal broth medium (4days at 37°C, 120rpm) and subsequently streaking on basal agar medium (2% agar, 2 days 37°C).

Keratinase assay

The assay is described below in figure 1.

Influence of pH

The effect of pH on keratinolytic activity was determined between pH 4 and 11. the enzyme solution was pre-incubated in each buffer at room temperature for 1h, and the residual activity was then determined using the standard enzyme assay. The various buffers used were as follows

1. Citrate buffer (pH 4-6)
2. Phosphate buffer (pH 6-8)
3. Tris-HCl (pH 9-11)

Production of silver nanoparticles

The production media containing the enzyme was centrifuged at 12000 rpm for 5 min and the supernatant containing the enzyme is used for the production o the silver nanoparticles adding it to the reaction vessel containing AgNO3 (0.1g/l) the reaction is carried out in bright condition for 72 hrs. The bioreduction of Ag ions in solution was monitored by sampling the aqueous solution (2 ml) and measuring absorbance spectrum (200-900 nm).

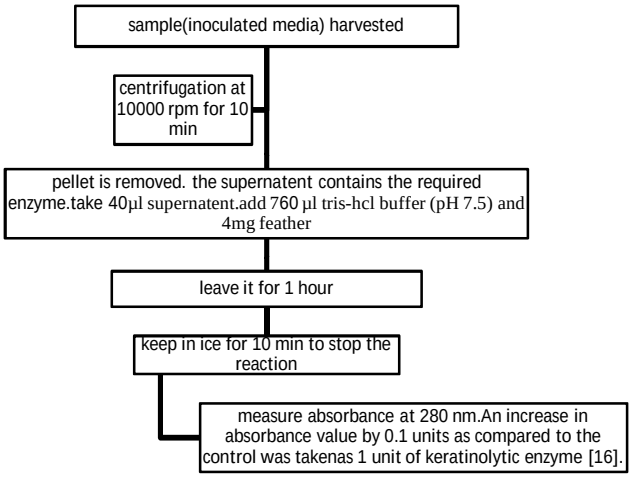

Figure 1: keratinase assay

RESULTS

Comparison of enzyme activity of the two strains

The two strains of keratinolytic bacteria obtained are monitored for a period of 20 days. It was obtained from the observation that the strain 2(RSAS 2) has more enzyme activity than strain 1(RSAS1). The strain 2 is considered for further investigation. RSAS 2 shows max. Enzyme activity on the 18th day whereas the strain 1 shows maximum activity on the 20th day (28.24 U/ml). (Fig. 2)

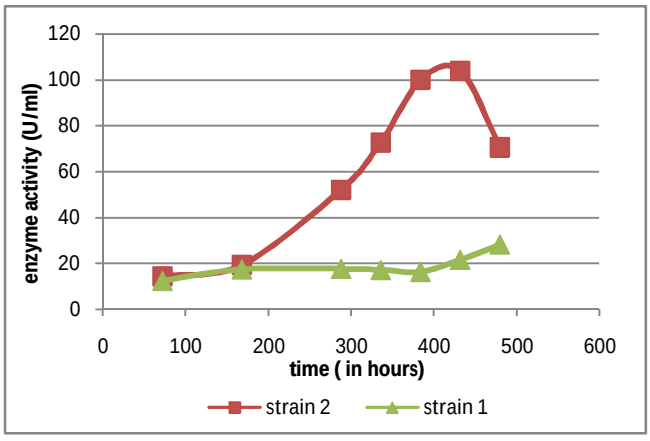

Figure 2: Comparison of the enzyme activity of 2 strains of bacteria

Primary screening using milk agar

Strains produced small yet visible clearing zones in the medium indicating that the bacteria is a keratinolytic bacteria (fig. 3).

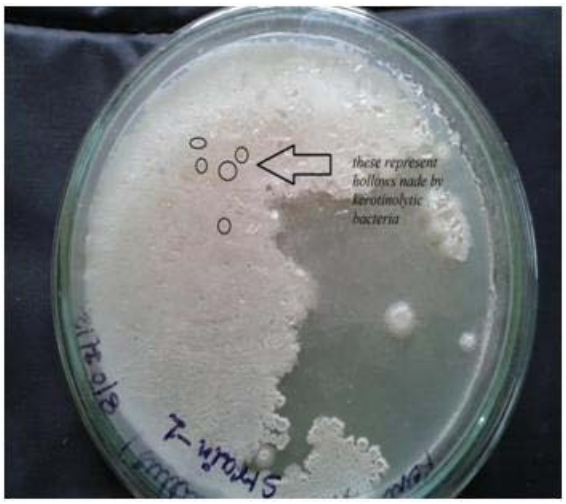

Figure 3: primary screening using milk agar

Influence of the ph and substrate concentration on enzyme activity

Keratinase activity was observed to be optimal at pH 7 – 8 and 0.5 mg substrate /µl enzyme respectively. (Fig. 4 and fig. 5)

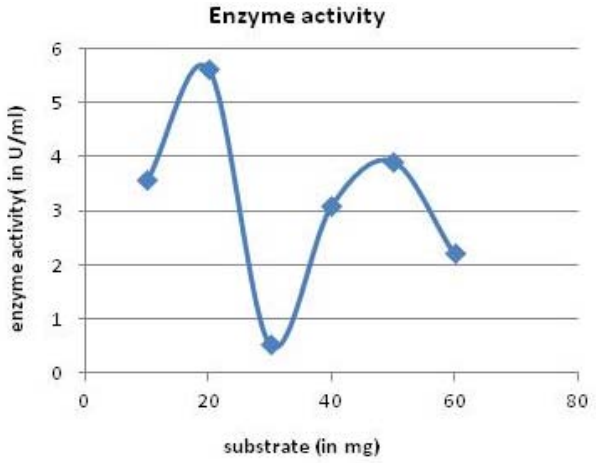

Figure 4: Influence of the substrate concentration on the enzyme activity.

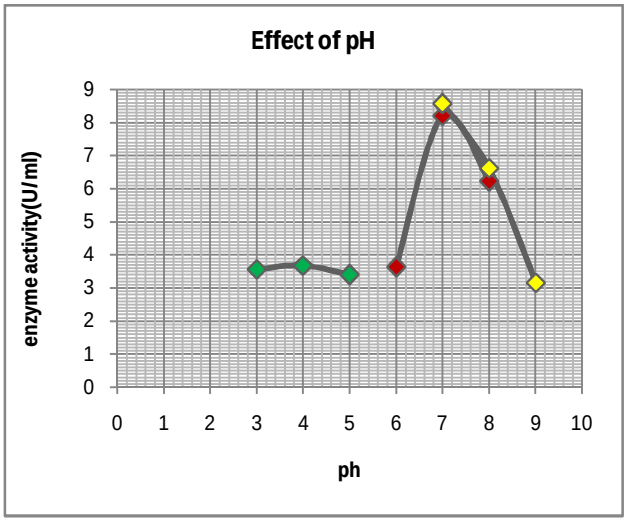

Figure 5: Influence of the pH on the enzyme activity (♦- citrate buffer (pH: 3-5).♦- phosphate buffer (pH: 6-8) ♦- Tris-HCl buffer (pH: 7-9))

Production of silver nanoparticle

In bright conditions within 72 hrs the colour of flask changes from white to dark brown due to formation of silver nanoparticles. (Fig 6)

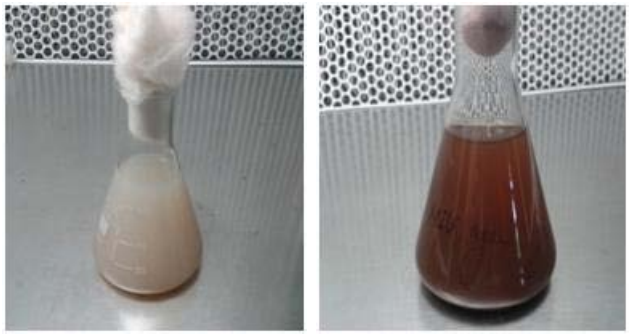

Figure 6: (left to right) the $agno_3$ solution at the beginning of the 72 hours, the $AgNO_3$ solution at the end of 72 hrs.

DISCUSSION

During this research we found that keratinase enzyme produced by strain 2 has more enzyme activity which may further be increased by genetic engineering techniques.[30] it can be used in various applications such as extraction of amino acid which can be used as food supplements. It can be used to produce nanoparticles which may serve many applications pertaining to many spheres such as drug delivery, environmental biotechnology.

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