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Characterization of Proteases produced by the thermophilic (keratinophilic) *Microsporum gypseum* (Kota, Rajasthan)

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Abstract
The selected isolates of *Microsporum gypseum* was improved for the production of proteases, an enzyme that grows in a casein-based basal salt medium. The sample was processed in the laboratory, and it was found that the most noteworthy creation was seen on seventh day of hatching and recorded the temperature at 55^0C, the ideal temperature for its maximum production on pH 8. At a concentration of 50 mM/ml in the reaction mixture, it was observed that Mg^{2+} and Na^+ ions increased protease activity; however, when the concentration was increased to 100 mM/ml, most of the ions, with the exception of Mg^{2+} and Na^+, acted as inhibitors of the enzyme's activity. The greatest inhibitory impact was seen within the sight of particles Ag^{2+}, Hg^{2+} and Cd^{2+}. At different solvent concentrations, the enzyme activity changes. At 50 mM/ml, aniline and acetaldehyde increase the enzyme's activity, but formaldehyde (4.6 U/ml) and amyl alcohol (5.5 U/ml) have the greatest inhibitory effects. All solvents, with the exception of aniline, decrease the activity at 100 mM/ml.
Keywords: *Microsporum gypseum, Protease, Keratinophilic, Metal ions, Enzyme activity.*

INTRODUCTION

In the microorganisms, different types of proteolytic enzymes are available which plays physiological capabilities like summing up protein assimilation to explicit controlled processes [1,2]. Proteases are the most important enzymes. About 60% of them are used in industries like baking, brewing, detergents, cheese making, meat tenderization, the production of digestive aids, and removing silver from photographic films [3,4]. The protease enzyme typically hydrolyses bulky protein molecules into smaller ones that the cell can actively absorb. Protease enzyme can be obtained from microbial sources like fungi, which meet industrial requirements, as well as from some sources like animals and plants. *Microsporum gypseum,* an opportunistic pathogen, was chosen for this study to enhance the production of protease enzyme, which advances the eco-friendly technology trend and helps replace the chemical hydrolysis process that was used in bioremediation, leather treatment, and pharmaceutical manufacturing [5]. As a result, the thermophilic fungal isolates of *Microsporum gypseum*, which was chosen for this procedure, is crucial to this study's goal of increasing protease production. Keratinases are the most important proteinases as a key proteolytic enzyme produced by these dermatophytes and they are well known to hydrolyze both ‘soft’ (cytoskeletal materials in epithelial tissues, containing up to 1% sulphur) and ‘hard’ (protective tissues in hairs and nails, containing up to 5% sulphur) keratins [6]. Hence, in the past few decades, a number of research projects have been focused on the activities of keratinases and their role in the virulence of dermatophytes such as *Trichopyton* and *Microsporum* [7,8]. The present study was also focused on the isolation and Characterization of the Proteases produced by the thermophilic (keratinophilic) fungi *Microsporum gypseum.* These studies have great importance in the management of cycling of keratin biomass from the environment.

MATERIALS AND METHODS

Fungal isolate:

Microsporum gypseum was isolated from the vicinity of a barbershop, from where keratin-rich soil samples were obtained using the hair bait method from Kota city of Rajasthan. One set of isolates was pure cultured on Sabouraud dextrose agar at 28^0C, and the other set was stored in the refrigerator at 4^0 C for future use [9].

Extraction of enzyme:

According to Upadhyay *et al.* [10], the enzyme was extracted. After autoclaving 15 milliliters of basal

salt medium containing one percent casein, *Microsporum gypseum* was inoculated, and crude enzyme extract was obtained by centrifuging the culture supernatant at 4^0 C for five minutes at 10,000 revolutions per minute. For the purpose of a subsequent experiment, the obtained extract was stored at -20^0C [11].

Enzyme activity:

Riffel et al., [12] method was used to determine protease activity, with a few modifications. After mixing 1.0 ml of the extracted crude enzyme with 1.0 ml of the substrate solution (0.1 M sodium phosphate buffer containing 1.0% casein, pH 7.4), the mixture was incubated for 60min at 37^0C. The reaction was stopped by adding 3.0 ml of 5% trichloroacetic acid. The mixture was kept at room temperature for 10 minutes before being filtered using Whatman filter paper no.1. The filtrate's absorbance was measured at 280 nm in comparison to blank [10].

Protease activity is affected by a variety of parameters, and incubation times: Effect of incubation periods:

The action of chemical was read up for 10 brooding days. As previously mentioned, 1.0 ml of substrate was mixed with 1.0 ml of crude enzyme for 60 minutes at 37^0C, and spectrophotometric activity was measured against a blank [10].

Effect of pH and temperature:

Casein hydrolysis aids in determining how pH and temperature affect protease activity when the pH range is between 3 and 11. The optimal enzyme temperature was found by incubating the reaction mixtures at the following temperatures: The effects of metal ions: 10^0C, 15^0C, 20^0C, 25^0C, 30^0C, 35^0C, 40^0C, 50^0C, 55^0C, 60^0C, and 65^0C [11].

Effect of metal ions:

By determining the nature of the enzyme's active sites, the presence of metal ions affects the enzyme's activity. During this investigation, various salt ions are measured at rates of 50mM/ml and 100mM/ml in the reaction mixture. Ag^+ (AgNO3), Zn^{+2} (ZnSO4), Cd^{+2} (CdCl2), Mg^{+2} (MgCl2), Mn^{+2} (MnCl2), Cu^{+2} (CuSO4), Hg^{+2} (HgCl2), K^+ (KCl), Ca^{+2} (CaCl2), Co^{+2} (CoCl2), Na^+ (NaCl) and Fe^{+2} (FeCl3) are the metal ions that are utilized [11].

Solvents' effects:

The effects of various solvents on enzyme activity were investigated in order to identify the enzyme's catalytic site. The solvents are joined with the response blends at the pace of 50mM/ml and 100mM/ml. the solvents which was utilized for this were formic corrosive, ethanol, carbon tetra chloride, formaldehyde, amyl liquor, toluene, chloroform, aniline, acetaldehyde, and acidic corrosive.

RESULTS AND DISCUSSION

The bench culture of basal salt medium aides in assurance of time span expected for the protease discharge and corruption of casein by *Microsporum gypseum.* The crude extra cellular enzyme extract was extracted from culture supernatant at the conclusion of incubation. The catalyst creation improves during cell development and arrives at its most extreme level 54.95 U/ml on the seventh day of hatching, after this, decline in the movement of chemical happens. It had been seen from our analysis that in the event when defatted hairs got utilized as a test of casein, the creation of catalyst begun. After tenth day of hatching since the keratin was present in the culture it was served as hard substrate because of which the slack period of cell cycle increases, and comparative perception was recorded by Raju *et. al.,* [13].

The pH is the most impacting factor for enzyme activity. The enzyme's activity was affected by pH values that are either very high or very low. According to fig. 2, the enzyme activity increased from 3.0 to 8.0 before decreasing. The basic pH assumes a significant part in silver recuperation from visual film or in bioprocessing of utilized X-beam [14]. The organism's growth is linked to the effect that temperature has on the production of protease. For development, the ideal temperature is enormously affected by the way of life whether it is thermophilic or mesophilic. According to Figure 3, the optimal temperature for protease activity is 55^0C, or within the thermophilic range. The current

perception is proven by Letourneau *et. al.,* [9] while concentrating on *Streptomyces species* keratinolytic action.

Effect of various metal ions and solvent:

The enzyme's activity is influenced by a variety of metal ions. According to a number of studies, the enzyme activity of metal ions was affected at a concentration of 50 mM/ml of the reaction mixture. While Zn^{2+}, Mn^{2+}, Co^{2+}, Ca^{+}, K^{+}, and Cd^{2+} have a 60-70% impact on the relative activity, the metal ions Mg^{2+} and Na+ increase the enzyme activity. With the exception of Mg^{2+} and Na^{+}, all ions exhibit inhibitory effects as the concentration of the mixture is increased to 100 mM/ml. The ability of Hg^{2+}, Ag^{+}, and Cd^{2+} to bind irreversibly with the protease enzyme molecule, which results in the permanent inhibition of catalytic activities, was shown in fig. (5) to have a strong inhibitory effect. As a result, the protease reaction is complete and unaffected by addition or increase in substrate concentration [15]. Amyl alcohol and formaldehyde, among solvents, greatly inhibit enzyme activity at a concentration of 50 mM/ml; acetic acid and toluene, on the other hand, partially inhibit enzyme activity at the same concentration. On the other hand, when the concentration of the reaction mixture is increased to 100 mM/ml, all solvents, with the exception of aniline, decrease enzyme activity Fig (4). The present study demonstrated that the enzyme protease produced by *Microsporum gypseum* fungi was capable of producing enzyme protease in batch culture with an active pH of 8.0. These enzyme proteases are required for the production of enzyme-based detergents and are implicated in the hydrolysis of waste containing keratin and leather industries [14]. The ideal temperature noticed was 55^{0}C. The thermophilic proteases are valuable in some execution due to their high bearableness to temperature which brings about quicker response rates, undeniable level in solvency of nongaseous responses and items and lessening in the microbial pollution frequency by mesophilic creatures. The fact that Mg^{2+} and Na^{+} were responsible for the activity suggests that proteases can be used in industry [16].

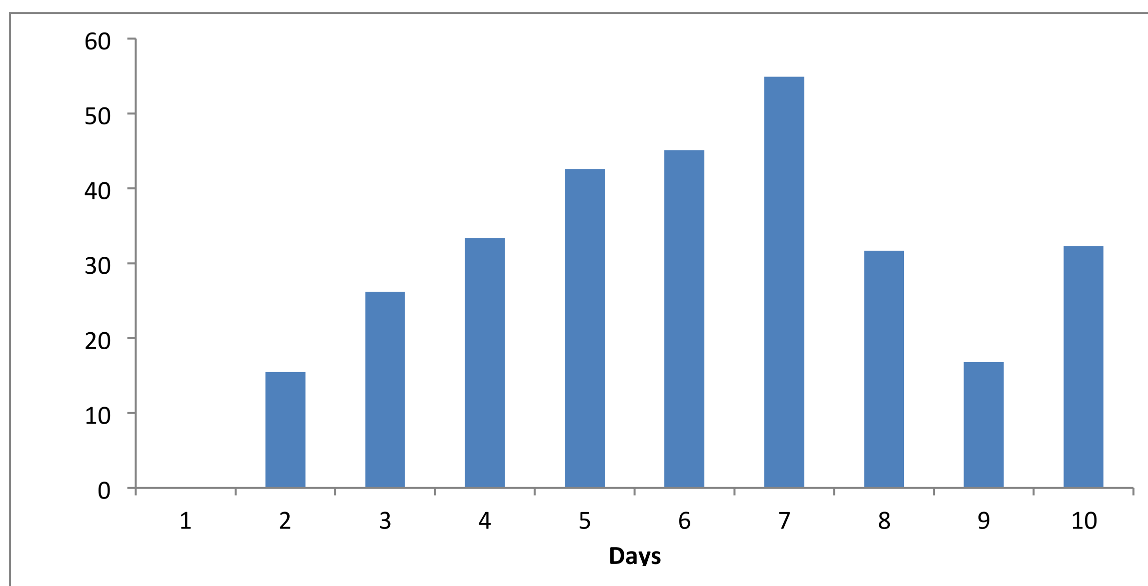

Fig 1. Effect of Various incubation periods on protease production of *Microsporum gypseum*.

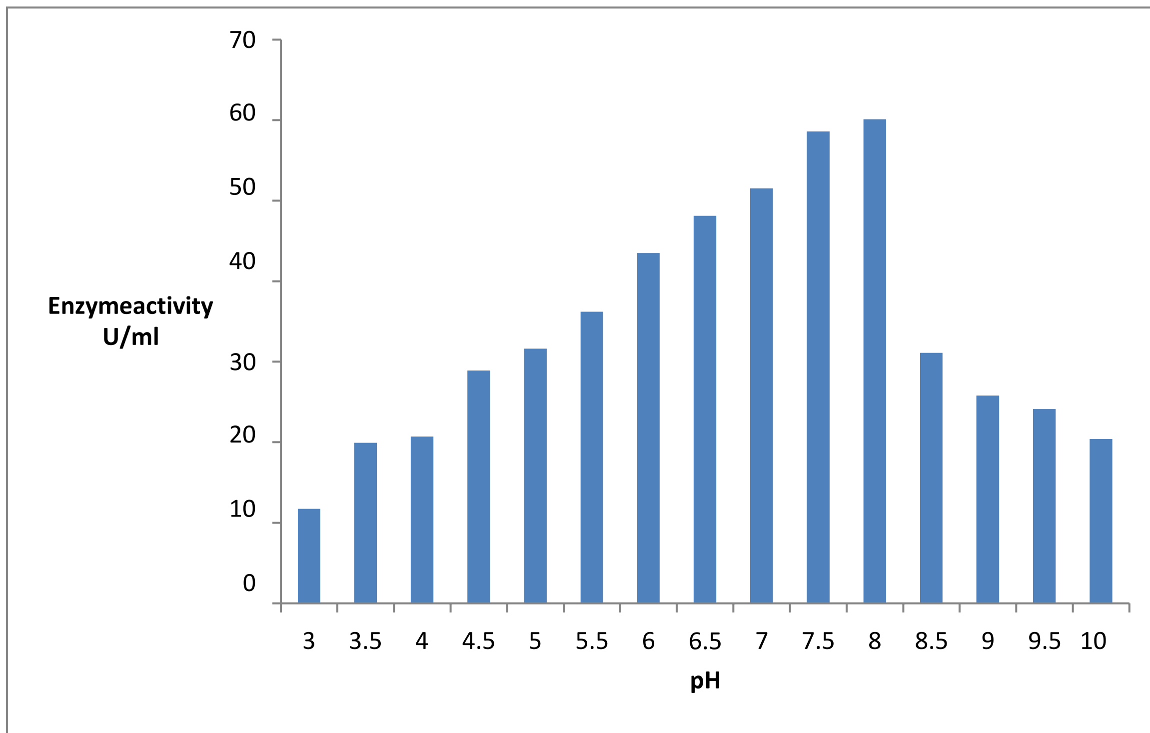

Fig 2. Effect of Ph on protease activity of *Microsporum gypseum.*

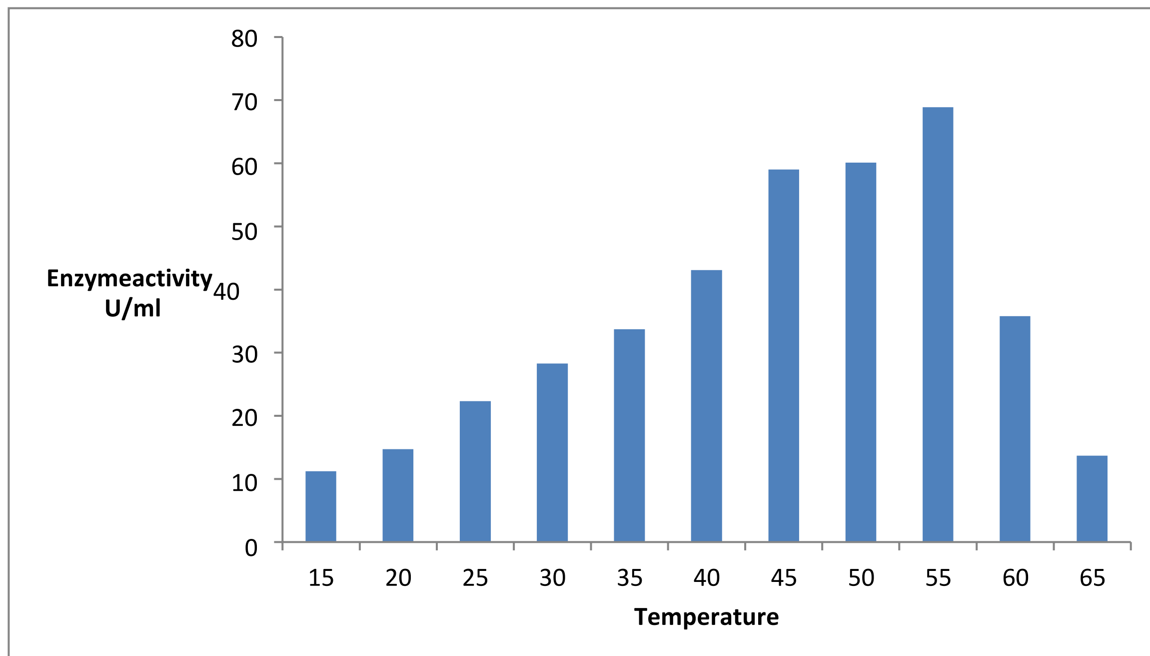

Fig 3. Effect of temperature (^{0}C) on protease activity *Microsporum gypseum*

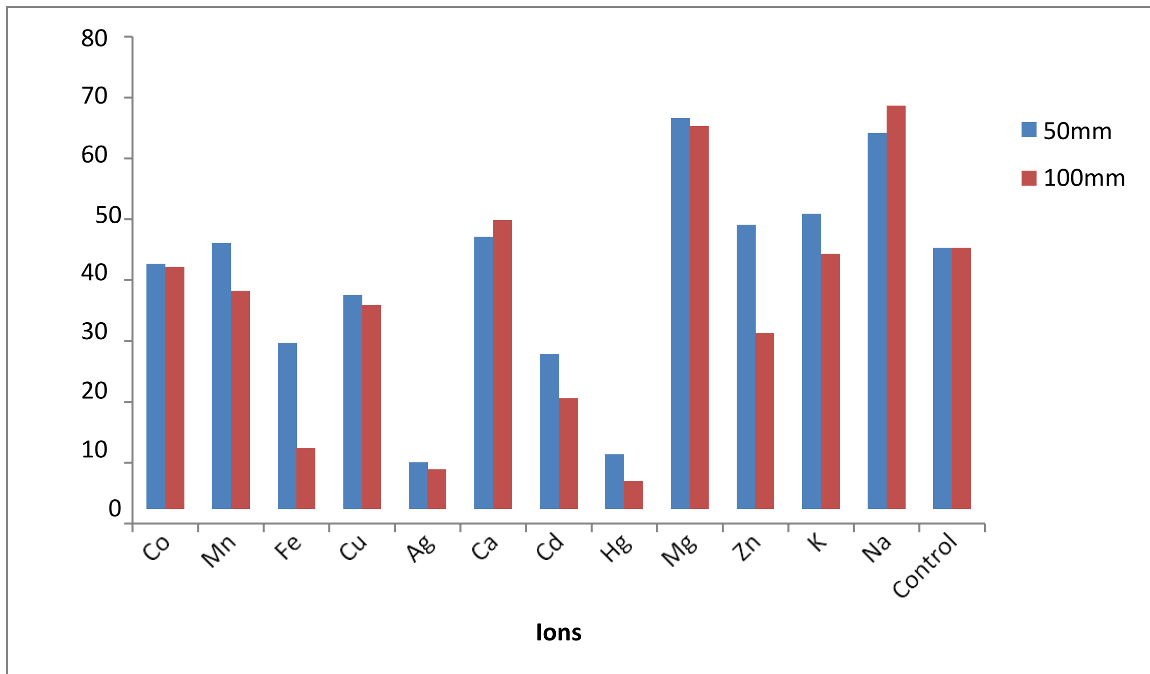

Fig 4. Effect of solvents on protease activity of *Microsporum gypseum.* Control(A), Ethanol(B), Acetaldehyde(C), Aniline(D), Toluene(E), Formaldehyde(F), Carbon tetra chloride(G), Amyl alcohol(H), Formic(I), Acetic(J).

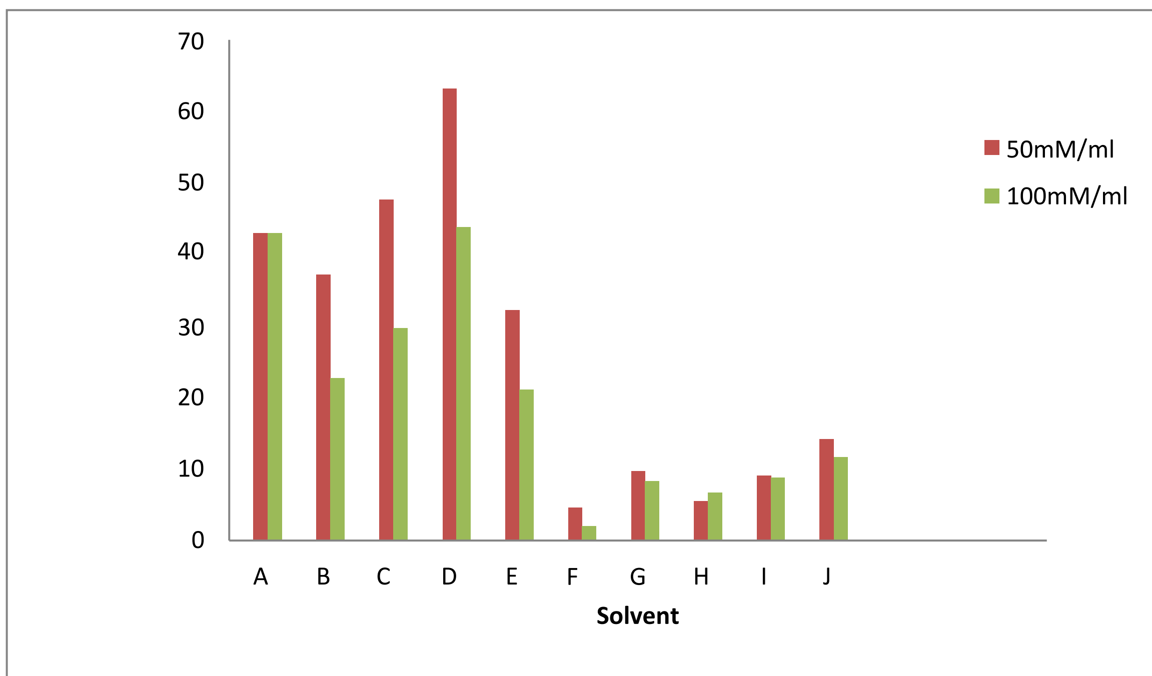

Fig 5. Effect of ions on protease activity of *Microsporum gypseum.*

CONCLUSION

The study suggested that the proteinase enzymes are produced by *Microsporum gypseum* at temperature 55^0 C and at pH 8. These enzymes are very beneficial for the management of keratin biomass in the environment. Keratinases are versatile and valuable enzymes that play important role in the degradation of keratin biomass and act similarly as the recalcitrant proteins. The need of hour is to spread awareness about the biotechnological applications of this enzyme. A diverse population of microorganism are able to produce the keratinases, and more are being discovered by the researchers every year.

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