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Enzymatic Profiling of Actinomycetes Isolated From Soil Samples of Chitwan

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ABSTRACT

This study explores the isolation and characterization of actinomycetes from soil samples in Chitwan, Nepal, focusing on their enzymatic activities and potential industrial applications. Actinomycetes, a group of Gram-positive, filamentous bacteria, are known for their ability to produce a variety of bioactive compounds and industrially significant enzymes. Sixteen soil samples were collected from different areas, resulting in the isolation of 31 actinomycete strains. These isolates were characterized macroscopically and microscopically, followed by biochemical and physiological tests to assess their enzyme production and tolerance to temperature and salt concentrations. The study found that many isolates produced enzymes such as amylase, cellulase, gelatinase, lecithinase, and urease. Additionally, the isolates demonstrated varying degrees of tolerance to different temperatures and salt concentrations. The findings underscore the rich potential of actinomycetes from Chitwan for industrial applications, particularly in producing robust enzymes with desirable properties.

Keywords: Actinomycetes, Enzymes, Soil Microorganisms, Industrial Applications.

Introduction

Many microorganisms are recognized for their ability to produce a wide array of natural compounds that have considerable industrial applications. These organisms are prolific sources of bioactive substances that can be harnessed in pharmaceuticals, agriculture, and biotechnological processes. Soil microorganisms, in particular, have been extensively utilized for their ability to produce diverse compounds such as enzymes and bioactive secondary metabolites. Among these microorganisms, actinomycetes are a crucial group, recognized for their commercial importance in producing enzymes. Actinomycetes are Gram-positive, filamentous bacteria with a morphology resembling that of fungi. They are renowned for their capacity to produce a vast array of secondary metabolites, making them one of the most promising microorganisms for generating bioactive molecules (Sapkota et al., 2020). They produce nearly half of the known bioactive metabolites, underscoring their significance in industrial fields (Budhathoki & Shrestha, 2020).

Actinomycetes are not only important for their enzyme-producing capabilities but also for their ability to produce various enzymes with industrial applications. These enzymes are vital in processes such as biodegradation, composting, plant growth promotion, and humus formation. They also play a crucial role in the agricultural sector by decomposing plant biomass and enhancing soil mineral recycling (Dhanasekaran et al., 2016).

Recognizing their vast potential, the continuous screening of actinomycetes for new bioactive compounds is essential. The Chitwan district in Nepal, with its rich biodiversity and unique climatic conditions, is an excellent source for isolating diverse actinomycetes strains. This research focuses on isolating actinomycetes from soil samples in Chitwan and examining their enzymatic activities, aiming to discover new bioactive compounds that could have significant industrial applications (Lekhak et al., 2018).

The demand for industrial enzymes with robust properties such as thermostability and pH tolerance is ever-increasing. Actinomycetes are known for producing a wide range of enzymes with these desirable properties, which can be utilized in various industries, including pharmaceuticals, food and beverages, and biotechnology (Mukhtar et al., 2017). However, there is a need for systematic screening and characterization of actinomycetes from diverse environments to identify strains that produce novel enzymes.By exploring the unexplored habitats of Chitwan, this study aims to fill the gap in the search for novel actinomycetes strains capable of producing industrially significant enzymes.

The successful extraction and commercialization of these metabolites could significantly impact the economy of Nepal, providing affordable solutions for various industries. Moreover, the findings from this study could lead to further research and the discovery of new bioactive compounds, enhancing the global repository of effective industrial enzymes (Shweta, 2012).

Materials and Methods

Study Area- This study was conducted in Chitwan District, Nepal, from March to July, 2023. Soil samples were collected from different areas, including Narayanghat, Ramnagar, and Baseni.

Sample Size- Sixteen soil samples were collected from different parts of Chitwan District.

Sample Collection- Soil samples were collected from a depth of 5 to 10 centimeters, targeting areas known for high microbial activity. Using a sterile spatula, the samples were placed in clean polyethylene bags, labeled with detailed identification information, and tightly sealed to prevent moisture loss or contamination. The samples were then promptly transported to the microbiology laboratory for further analysis.

Sample Processing

Isolation of Actinomycetes- The isolation of actinomycetes was carried out in the Microbiology Laboratory of Balkumari College using the spread plate technique on Starch M-Protein Agar. One gram of soil was dissolved in 10 ml of sterile distilled water and subjected to serial dilution up to 10^-3. Aliquots of 0.1 ml from the 10^-2 and 10^-3 dilutions were spread onto Starch M-Protein Agar plates and incubated at 28°C for two weeks (Kaur & Teotia, 2019). Pure colonies were obtained by picking typical actinomycete colonies and streaking them onto fresh agar plates, followed by incubation at 28°C for one week.

Characterization of Actinomycetes

Macroscopic Characterization- Isolated actinomycetes were observed for macroscopic characteristics such as the color of aerial mycelium, diffusible pigments, colony texture, size, elevation, and opacity.

Microscopic Characterization- Microscopic characterization was done using Gram staining. Smears were prepared from colonies, stained, and observed under an oil immersion microscope.

Biochemical Characterization- Biochemical tests performed included gelatin hydrolysis, starch hydrolysis, carboxymethyl cellulose degradation, lecithin hydrolysis, and urea hydrolysis.

Physiological Characterization

Physiological characterization involved testing temperature tolerance and salt tolerance at varying concentrations and temperatures.

Results

From 16 soil samples collected across various locations in the Chitwan district, 31 actinomycete isolates were successfully obtained.

Macroscopic Characterization - The 31 isolated actinomycete colonies exhibited diverse macroscopic characteristics. The majority of colonies were grey (55%) and white (38%), with smaller proportions being black (4%) and brown (3%). The reverse side of the colonies was predominantly black or white. Most colonies had a rough, powdery texture and an average diameter of about 2 mm. They typically featured an entire, round margin, a rough, crusty elevation, and were opaque. Notably, isolates A2 and F1 differed by producing smooth, powdery colonies.

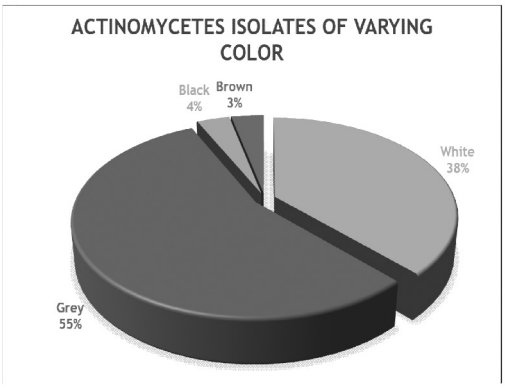

Figure 1: Actinomycetes isolates producing various colony color.

M**icroscopic Characterization of the isolates-** Gram staining was performed to observe the isolates microscopically and the results revealed that the isolates were gram-positive filamentous thread-like in appearance with unfragmented mycelium.

Biochemical and Physiological Characteristics

Substrate Hydrolysis Test- Substrate hydrolysis tests were conducted on 31 isolates to evaluate their capability to hydrolyze various substrates, including starch, carboxymethyl cellulose, gelatin, urea, and lecithin. These tests revealed that different isolates produced distinct hydrolytic enzymes such as amylase, urease, cellulase, lecithinase, and gelatinase. Notably, certain isolates like A1, A2, M1, M6, and M8 demonstrated the ability to produce all five enzymes, showing positive hydrolysis for each of the substrates tested.

Enzyme Production by Actinomycetes- Substrate hydrolysis tests were performed on 31 actinomycete isolates to determine their enzymatic capabilities. Among these isolates, 26 (83%) produced gelatinase, 15 (48%) produced amylase, 13 (41%) produced cellulase, 14 (45%) produced lecithinase, and 19 (61%) produced urease. These results highlight the diverse enzymatic potential of the actinomycetes isolated from the Chitwan District. Detailed findings on the substrate hydrolysis activities of these isolates are presented in Figure 2.

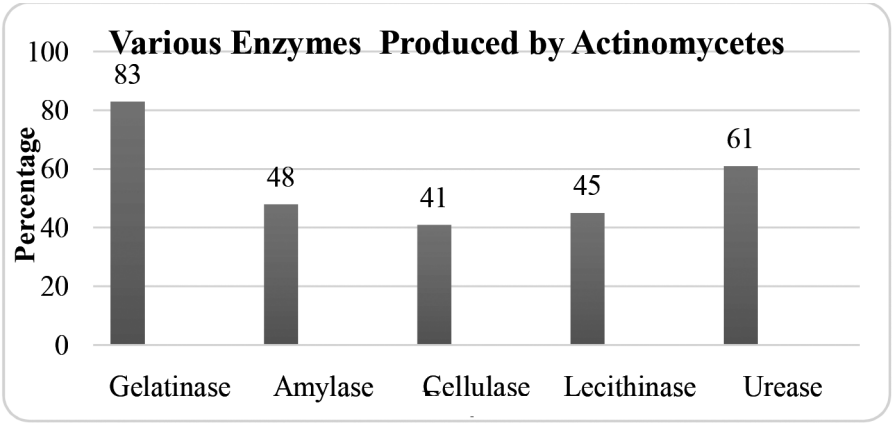

Figure 2: Various enzymes produced by isolates of actinomycetes in percentage.

Physiological Test- Temperature tolerance test, NaCl tolerance test were performed on the actinomycetes isolates. These test isolates were allowed to grow on varying salt concentration and at varying temperature .

Temperature Tolerance of Actinomycetes Isolates- Among the 31 isolated actinomycetes, all of them were capable of growing at 27 °C. The number of isolates growing at temperature 38°C and 45°C were 20 (64%) and 9 (29%) respectively. 5 isolates (16%) showed growth even at the low temperature of 10°C.

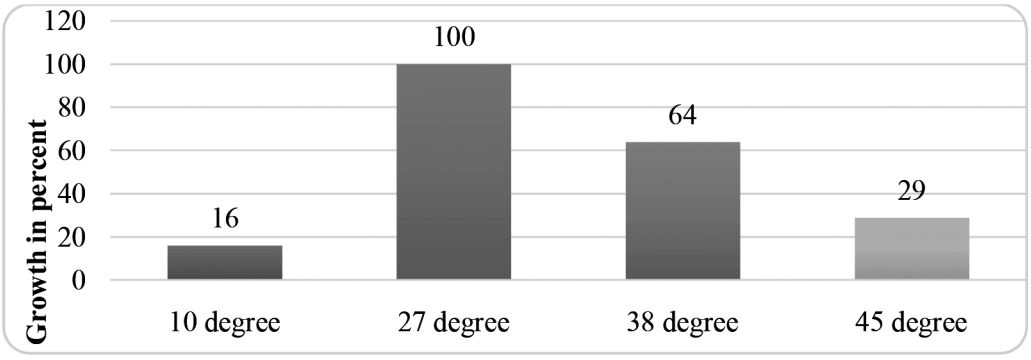

Figure 3: Actinomycetes isolates growing at varying temperature

Salt tolerance test of actinomycetes isolates- On performing salt tolerance test on given isolates, all of the isolates were capable of growing at a low salt concentration of 2.5%, 15 isolates (48%) grew at 5% salt concentration, 10 isolates (32%) grew at 7.5% Salt concentration and only 2 isolates (3%) H1 and M2 tolerated the highest salt concentration of 10%.

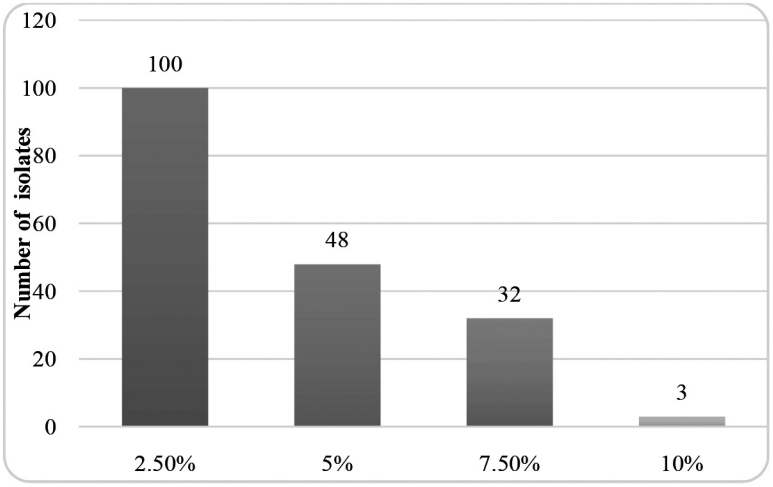

Figure 4 : Actinomycetes isolates growing at varying salt concentrations

Discussion

Actinomycetes, a group of Gram-positive bacteria, are particularly significant in this context. They bridge the gap between bacteria and fungi, displaying unique metabolic diversity and enzymatic capabilities, and producing substances with pharmaceutical applications, including valuable enzymes and antibiotics (Barka et al., 2016). Streptomyces spp., a prominent subgroup of actinomycetes, are especially notable for producing numerous antibiotics and biologically active secondary metabolites, accounting for approximately 80% of the total antibiotics produced (Waksman, 1961).

In this study, 31 actinomycetes isolates were obtained from 16 soil samples from Chitwan District. The colonies exhibited small, white, rough, glabrous, and chalky white characteristics, consistent with findings by Oskay et al. (2004). These isolates were slow-growing, aerobic, glabrous or chalky, heaped, folded, and displayed various colors of aerial and substrate mycelia, similar to observations by Ramazani et al. (2013). Additionally, all colonies possessed an earthy odor. The isolates were Gram-positive with varying degrees of mycelial branching, a characteristic also reported by Gautham et al. (2012). The pigmentation ranged from white, greyish, and black to a spectrum of colors such as brown, yellow, purple, bluish, and reddish, with melanin production causing black coloration in the medium, as observed by Sapkota et al. (2020).

Among the 31 isolates, 15 strains could hydrolyze starch, producing amylase, as demonstrated by clear zones around colonies on starch agar, similar to findings by Jassim et al. (2022) and Nithya et al. (2017). Amylases from Streptomyces spp. are crucial in various industries, accounting for approximately 25% of the global enzyme

market demand (Gupta et al., 2003). Cellulase production was observed in 13 isolates, aligning with Jassim et al. (2022), who reported 8 cellulase-producing isolates out of 33. These enzymes are essential for converting cellulose into fermentable sugars for sustainable biofuel production (Jang & Chang, 2005). 14 isolates produced lecithinase, degrading lecithin in egg yolks to release insoluble diglycerides, forming a white opaque zone, as noted by Naggar et al. (2019). Gelatinase activity was seen in 26 isolates, confirmed by clear zones on gelatin agar post-$HgCl_2$ flooding, consistent with findings by Chaudhary et al. (2013) and Roopan et al. (2019).

Physiological characterization revealed salt tolerance in all isolates at a 2.5% concentration, with decreasing growth at higher concentrations, paralleling studies by Rani et al. (2020) and Cai et al. (2009). Temperature tolerance tests showed growth at 27 ºC for all isolates, with reduced growth at higher temperatures, aligning with findings by Gayathri et al. (2013) and Agarwal et al. (2019).

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