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ISOLATION AND SCREENING OF PSYCHROPHILIC YEAST FROM GUREZ VALLEY OF J&K

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ABSTRACT

Psychrophilic are called as true extremophiles, psychrophilic yeast thrive in cold-environments where temperature remains below 20°C. Gurez valley is covered with high snow-capped Himalayas thus creating extreme cold climatic conditions and habitat for psychrophilic yeast. Three yeast strains PYG3, PYG5 and PYG6 were isolated from the soil samples collected from Gurez valley. These isolates showed luxurious growth at 15°C when screened for growth characteristics at 4°C, 10°C, 15°C, 20°C, 25°C and 30°C respectively for 10 days. Yeast strains couldn't grow normally above 20°C so defining psychrophilic nature of isolated yeast. On wide range of pH (3, 5, 7, 9) all three isolates PYG3, PYG5 and PYG6 showed positive growth. Among three psychrophilic yeast PGY3 shoowed best growth below 20°C and optimal growth was observed at 15°C. After screening isolates for extracellular enzyme activities on media PYG3, PYG5 and PYG6 possess different enzyme activities like amylolytic activity, cellulase activity, chitinase activity and xylanase activity. Enzymes (Cold-active enzymes) secreted by psychrophilic yeasts at low temperature possess immune biotechnological applications. Theses enzymes can be used in food processing industries, juice industries, leather and starch industries and pharmaceutical industries. Isolation of psychrophilic yeast outside Polar Regions and Antarctica will help in exploring application of psychrophiles and their cold active enzymes.

Key words: Cold-active enzymes, Extracellular enzymes, Gurez valley, Psychrophilic yeasts.

I. INTRODUCTION

Gurez is a valley coming under Indian Himalayan Region (IHR) and is surrounded by snow-capped mountains. Soils of the Gurez valley possess diverse microbial fauna; however no systematic study has been carried out so far on microbial diversity of the soils. There is high possibility to explore diverse microbial population with special features of cold-tolerance. Psychrophilic and thermophilic groups have attracted considerable attention because of their ability to produce extremozymes with enormous biotechnological potential [1]. Cold environments are extensively distributed on the Earth that includes the Polar Regions, mountains, glaciers, forests and deep-sea environments. It has been reported that psychrophilic enzymes isolated from psychrophilic

yeasts exhibit high activity at low and moderate temperatures and offer possible economic benefits [2]. Psychrophilic yeasts play an essential role in nutrient cycling and biomass production processes in cold ecosystems [3]. Psychrophilic microorganisms and their cold shock and cold-acclimation proteins and enzymes possess a host of biotechnology applications [4]. Among different groups of extremophiles, psychrophiles (cold-loving) are attracting much attention due to various industrial as well as biotechnological applications. Due to valuable applications of psychrophilic yeasts it is important to explore their potentiality outside Antarctica and Polar regions. It has been found that psychrophilic yeasts grow at temperatures below 5°C and exhibit no growth above 20°C [5], whereas thermophilic yeasts grow between 25°C - 45°C and thermophilic bacteria grow above 50°C [6, 7]. Psychrophilic yeasts and their cold-active enzymes (amylases, proteases, lipases, pectinases, phytases, lactases etc.) own numerous potential uses for industries [8]. Microorganisms mostly isolated and studied from cold environments, the majority consists of bacteria, while yeasts constitute a minor proportion [9]. Our knowledge about psychrotrophic yeasts are mainly the results of study on the yeast of permanently cold environments such as Antarctica and Arctic regions. Antarctica has been preferred by scientists and researchers for study of diversity of psychrophilic microorganisms. Due to this reason diversity of Antarctic psychrophilic yeasts has been deeply reviewed [10, 11, 12]. But other than Antarctica different parts of world having cold-climatic conditions are now studied and explored for microbial diversity. Likewise psychrophilic yeast *Cystofellobasidium capitatum* SPY11 has been isolated from the soil of northern region of India, Kashmir valley and it was able to grow up to 20 °C above which it couldn't grow normally [13]. In recent research work carried out by researchers it was found that 43 % of Antarctic yeast isolates were assigned to undescribed species [14], this redirects the deficiency of knowledge regarding cultivable yeasts that colonize the Antarctic soils.

This research work was designed to isolate psychrophilic yeast from Gurez valley of Jammu & Kashmir. Yeasts were screened for extracellular enzymatic activities at low temperatures. This piece of work will help in exploring microbial soil health of Gurez as well as potential applications of psychrophilic yeasts and their enzymes secreted at low temperatures.

II. METHODOLOGY

2.1 Soil sampling and yeast isolation

Yeast was isolated from soil samples collected from different locations of Gurez valley of Jammu & Kashmir. Gurez valley is located at an Altitude of 8000ft (2400m) from sea level with an elevation of 34.38 North Latitude and 74.56 East Longitude (34.6333°CN 74.8333°E). The valley experiences extreme cold-climatic conditions where temperature ranges from -25°C to 30°C. Soil samples were carried to laboratory in cold container under sterile condition. By following dilution plate method and soil sprinkle method isolation of yeast were carried out [15]. Initially the samples were culture on Malt Agar and Potato Dextrose Agar plates at 6°C for 30 days. Purification of yeast isolates was done by single cell isolation method and the yeast cultures were maintained at 6°C in refrigerator for further study.

2.2 Growth characteristics of yeast isolates

Isolated yeast cultures were screened for their growth features in response to change in temperature. Yeast isolates were subculture on freshly prepared Potato Dextrose Agar plates and the plates were incubate at different temperatures 4°C, 10°C, 15°C, 20°C, 25°C and 30°C for 10 days respectively. Similarly effect of pH on growth character of isolates was screened at pH 3, pH 5, pH 7 and pH 9 on Potato Dextrose Agar plates. The yeasts isolates were screened for their psychrophilic (growth below 20°C and exhibit no growth above 20°C) and psychrotolerant (grows above 20°C) character.

2.3 Morphological characteristics

Yeast isolates were cultured under specific atmospheric conditions (temperature and pH) to assess different morphological characters including colony size, shape, colour, and colony texture, growth pattern and cell shape.

2.4 Extracellular enzyme activity

Extracellular enzyme assays were performed on solid YM medium and medium were supplied with the appropriate substrate for enzyme activity. The plates were incubated at the optimal growth temperature of the yeast isolates, and the enzyme activities were determined. Yeasts were screened for amylase, cellulase, chitinase and xylanase activities.

2.4.1 Amylolytic activity: The yeast isolates were grown in medium containing 0.2% soluble starch. The plates were flooded with 1ml of iodine solution [16].

2.4.2 Cellulase activity: Yeast cells were grown in medium supplemented with 0.5% carboxymethyl cellulose (CMC) [17]. Later plates were flooded with 1mg/ml of Congo red solution, which was poured off after 15 min. The plates were then flooded with 1M NaCl for 15min [18].

2.4.3 Chitinase activity: Yeast isolates were sub-cultured and incubated in medium containing 2.5% purified chitin [16].

2.4.4 Xylanase activity: The cells were grown in medium supplemented with 0.5% xylan [19].

III. RESULTS

3.1 Isolation and screening of yeast strains

Seven yeast morphotypes were isolated from the soil samples collected from Gurez valley of Jammu & Kashmir region (Fig 1). Among seven yeast isolates three were purified and sub-cultured due to their psychrophilic growth characteristics. Morphological features of isolated psychrophilic yeast (PYG3, PYG5 and PYG6) were observed including colony colour, texture, and cell shape and growth pattern (Table 1). Psychrophilic yeasts shows fast growth at temperature of 10°C or lower, but cannot survive at temperature above 20°C whereas, psychrotolerant yeast strains showed fast growth rate above 20°C up to 40°C [20]. The three potent isolates namely PYG3, PYG5 and PYG6 were selected on the basis of fast growth below 20°C optimally growing

temperature of 15°C. These isolates possess wide range of pH tolerance and other important growth characteristics (Fig 2). That makes them more efficient and potent than other yeast isolates.

3.2 Effect of temperature and pH on growth

For further clarification and observation of growth features of psychrophilic yeast PYG3, PYG5 and PYG6. Isolates were sub-cultured on Potato Dextrose Agar plates and plates were incubated at different temperatures 4°C, 10°C, 15°C, 20°C, 25°C and 30°C for 10 days. PYG3 was showing luxurious growth below 20°C followed by PYG5 and PYG6 respectively (Table 2). This pattern of growth defines the character of psychrophilc yeast and it was also observed that yeast morphotypes grow at wide range of pH after incubating at pH 3, pH 5, pH 7 and pH 9 respectively (Table 3). The ability of isolates to grow at low temperatures and on wide range of pH makes them efficient for biotechnological applications.

3.3 Extracellular enzyme activity

Three yeast isolates were screened for amylolytic activity, cellulase activity, chitinase activity and xylanase activity at optimal growth temperature of 15°C. All three isolates PYG3, PYG5 and PYG6 showed extracellular enzyme activities at optimal temperatures and secreted cold-active enzymes. Yeast isolates showed amylolytic activity which was defined as a clear halo around the colony on a purple background. Positive cellulase activity was observed as a clear halo around the colony on a red background was formed. Similarly chitinase activity was indicated directly by the presence of a clear halo around the colony. Yeast strains PYG3, PYG5 and PYG6 possess xylanase activity which was specified by a clear halo around the colony. Secretion of different cold-active enzymes by PYG3, PYG5 and PYG6 makes them industrially important and effective source of psychrophilic enzymes and can be used in different low temperature processing's.

IV. DISCUSSION

Psychrophilic yeasts are very important due to their physiological adaptation and contributions to soil fertility, and they also possess various applications in biotechnology. The properties of cold-active enzymes provide numerous avenues for industrial utility; however, specific properties may be improved through enzyme engineering. Psychrophilic yeast PYG3, PYG5 and PYG6 isolated from soils of Gurez valley retain potential of secreting different cold-active enzymes (amylases, cellulases, chitinases, xylanases) which can be used in industries. [5], reported novel psychrophilic yeast *Rhodotorula Himalayans* sp. Nov; isolated from a Roopkund lake of Himalayan mountain range. Earlier it has been reported that psychrophilic yeasts have potential of secreting novel cold-active enzymes including pectinase, lactase, amylase [21, 22, 23] which can be used in cold processing in industries. All the three yeast isolates possess psychrophilic features and they have capacity to produce cold-active enzymes. Cold-active or cold-adaptive enzymes have attracted great attention as biocatalysts because they have the ability to resist quite unfavourable reaction conditions in industry [24]. Cold-active enzymes produced by microorganisms thriving in cold environments display higher catalytic efficiency at

low temperatures and greater thermosensitivity than their mesophilic counterparts [25]. They have attracted attention of researcher for identification of novel enzymes sources. Psychrophilic yeast produces cold-active enzymes having numerous applications in textile, medical and pharmaceuticals, fine chemical synthesis, food Industry, domestic and environmental applications. So far very rear research has been carried on yeast especially in Gurez region of Jammu and Kashmir; this piece of research will explore the opportunities for the researches to isolate new and novel yeast strains having immune application in food, pharmaceutical, leather and other industries.

V. FIGURES AND TABLES

Table 1. Morphological characteristics of yeast isolates.

Isolates	Colony Color	Colony Texture	Growth Pattern	Cell shape	Optimal growth temperature
PYG3	Pink	Mucoid	Fast	Oval	15°C
PYG5	Cream	Mucoid	Fast	Elongated oval	15°C
PYG6	Yellow	Mucoid	Fast	Oval /circular	15°C

Table 2. Growth characteristics of yeast isolates at different temperatures.

Parameters	PYG3	PYG5	PYG6
Growth at 4°C	+	+	+
Growth at 10°C	++	+	+
Growth at 15°C	+++	+++	++
Growth at 20°C	++	++	++
Growth at 25°C	–	–	W
Growth at 30°C	–	–	–

+ = Good growth, ++ = Very good growth, +++ = Luxurious growth, - = No growth, W = Weak growth

Table 3. Growth characteristics of yeast isolates at different pH range.

Parameters	PYG3	PYG5	PYG6
Growth at pH 3	+	+	W
Growth at pH 5	++	++	+
Growth at pH 7	++	++	++
Growth at pH 9	++	+	++

+ = Good growth, ++ = Luxurious growth, - = No growth, W = Weak growth

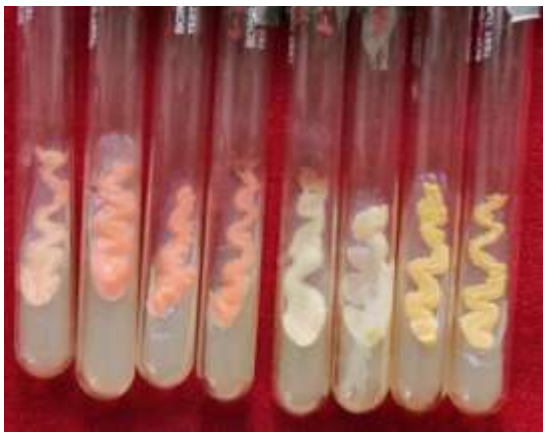

Fig 2. Isolated psychrophilic yeast in slants (from right 1-4 PSY3, 5-6 PSY5 and 7-8 PSY6).

Fig 1. Snow-capped mountains of Gurez valley.

VI. CONCLUSION

Yeast living in the extreme environmental conditions possesses high stability and ability to produce extremozymes in extremophilic conditions. Cold-active enzymes secreted by different yeast strains have immune applications in different industrial processes as well as in biotechnology and research. Even though cold-active enzymes have been derived from different sources other than microbes but due to high stability and more efficiency of microbial enzymes they take advantage upon plant and animal origin enzymes. Psychrophilic yeast are being used in food industries, pharmaceutical industries etc espacilly the enzymes they produce keep high market values in present scenario. Gurez valley observing an extreme cold environment, possess diverse range of psychrophilic microorganism especially yeasts which are potent source of industrially important enzymes. They can be used in low temperature processes and are thermo stable in character.

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