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Low Temperature Degradation of Various Substrates by Psychrotolerant *Fusarium* Spp. Isolated from Soil of Jammu City

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Abstract: Three psychrotolerant *Fusarium* spp. were isolated from soil of Jammu city. The degradation potential of these isolates for various substrates like tributyrin oil, cellulose, starch, casein, urea, pectin and gelatin was recorded at low temperature and zone of clearance was determined. All the isolates were found to be positive and showed good results for tributyrin oil degradation at 15±0.2°C. *Fusarium* sp. F10 showed higher in degradation of cellulose and starch but there was no affinity towards casein and gelatin. *Fusarium* sp. F8 showed highest lipase activity than F5 and F10 isolates. Studies such as this can be useful for the production of various important cold active enzymes for many industrial purposes.

Key words: Psychrotolerant fungi, *Fusarium* spp., cold active enzymes.

Introduction

Jammu city is located at 32.73° N latitude and 74.87° E longitude with diverse altitudes, climate and geo-morphological features resulting in the formation of different types of soils and having maximum temperature 37°C and minimum does not fall below 6°C, which favors the growth of various microorganisms including psychrotolerants. Psychrotolerants may well grow at mesophillic range with optima above 30°C or as high as 40°C, whilst retaining the capacity to grow at or close to zero [18]. Cavicchioli *et. al.,*[3] isolated many psychrotolerant fungi that can grow at around 0°C as well as grow above 20°C from Antarctica.

A wide variety of chemical compounds, several of which are most valuable pharmaceuticals, agrochemicals and industrial products can be produced by many fungal isolates. *Fusarium* is a large genus of filamentous fungi widely distributed in soil and in association with plants. There were many studies on *Fusarium* spp. with enzymatic capability [13,21], but there is no report of psychrotolerant *Fusarium* spp. showing various cold active extracellular enzymes production for degradation of organic substrates. Cold-active enzymes like amylases, cellulases, lipases, pectinases, and proteases from psychrophilic fungal strains find vast applications in the food, medicine, and detergent industries [6,14,15]. The present study has emphasized to screen the *Fusarium* spp. for capability to degrade various organic substrates at low temperature, which were isolated from Jammu city soil.

Materials and methods

Soil sample characterization

A total of twenty soil samples were collected from four different places of Jammu city such as garden (GAS), citrus orchards (COS), guava field (GFS), and brinjal field (BFS) in the month of January in sterile plastic bags with polar packs and stored at 4°C. Soil moisture contents and soil pH was determined as per the method described

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by Jackson, [10] and Akpor *et.al.,* [1] respectively.

Isolation and identification of psychrotolerant fungi

For the isolation of psychrotolerant fungi, soil samples were spread on sterilized Czapek's Dox agar with streptomycin sulfate (0.015 %) by serial dilution technique and incubated at 15±0.2°C. After 7 days of incubation the isolated colonies were counted and CFU/gm of each soil sample was calculated. The isolated fungi was then revived again on Czapek's Dox agar slants and maintained at 4°C. Pure cultures of isolated fungi were identified on the basis of their micro- and macro-morphology characters [5,7]. Micro-morphological identification was done by lacto-phenol cotton blue staining and observed under phase contrast microscope (LAS EZ version 1.5.0) both at 40X and 100X.

Characterization of fungi

The isolated fungi was grown on different agar media (Sabouraud dextrose agar, Czapek's Dox agar, Potato dextrose agar and Malt extract agar) and investigated for their accurate identification and characterization. Besides these all isolates were investigated for varied temperature, pH, and salt tolerance tests. Temperature tolerance test was done by keeping the Czapek's Dox agar medium inoculated with fungi at 10°C, pH test by maintaining medium at 9.5 pH and for salt tolerance test, 10 % NaCl was used.

Degradation of various substrates

The isolated strains were spot inoculated on respective pseudo selective agar medium having respective substrates for screening of cold active enzymes produced at 15°C as per the method described by Maharana and Ray, [15]. After five days of incubation the plates were assayed by respective methods and zones of clearing around the colonies were measured in mm. as the difference between the total diameter and the fungal colony. The zone of clearances was observed for cellulase [8], and lipase [11]. Iodine cubes were used to test the production of amylase, 15 % acidic $HgCl_2$ for caseinase and hexadecyltrimethly-ammonium bromide (1 %) was used for pectinase test. In case of gelatin liquification, inoculated medium tubes were incubated at 4°C for 15 minute to examine whether the medium was solid (negative result) or liquid (positive result). Urea hydrolysis was observed by color change from yellow to red due to the presence of ammonia produced by urease in the medium [2].

Results and discussion

A total of twenty soil samples collected from different places of Jammu city were investigated for physico-chemical parameters and microbiological study. The pH values of all sampling sites were near to the neutral and soil moisture content varied with sites (Table 1). Garden soil showed maximum psychrotolerant fungal load (average 5×10^3 CFU/gm) at 15°C than other soil samples. Soil physico-chemical parameters were found to influence the fungal distributions and population variation at various levels of significance. Soil pH showed negative but significant correlation (r = -0.46) with fungal load where as insignificant with soil moisture (r= 0.28).

Table 1. Study of physico-chemical parameters and psychrotolerant fungal load

Sites	No. of samples	Soil pH[a]	Soil moisture content (%)[a]	CFU/g[a]
GAS	5	7.30	21.00	5.00×10^3
COS	5	6.95	22.99	3.66×10^3
GFS	5	6.88	18.432	4.66×10^3
BFS	5	7.72	23.90	3.00×10^3

[a] Results were average of five soil samples of each sites

$p_{(soil\ moisture,\ soil\ pH)} < 0.01$ (significant), $p_{(soil\ pH,\ CFU/gm)} < 0.01$ (significant)

$p_{(Soil\ moisture,\ CFU/gm)} < 0.01$ (significant).

A total of 25 psychrotolerant fungal isolates were obtained of which 20 % were identified as *Fusarium* spp. Characterization of Fusarium spp. was conducted at 15°C and the result is presented in Table 2. These Fusarium spp. were screened for cold active enzymes i.e. protease, lipase, amylase, cellulase, gelatinase, urease and pectinase production at 15°C (Table 3) The *Fusarium* sp. F8 was regarded as good producers of cold lipolytic and pectinolytic enzymes than others. *Fusarium solani* are known to be good lipase producers having optimum pH at 7.25 and temperature 25°C as investigated by Maia *et.al.,* [16] and Poulsen *et.al.,* [19].

The tested *Fusarium* spp. were resistant to fluconazole except F10 and other two were susceptible to amphotericin B. However, the most active agent was nystatin. The above result is in accordance with the results of Pujol, *et.al.,* [20].

A strain of *Fusarium oxysporum* was isolated by Moataza *et.al.,* [17] which showed lipase activity at 20°C and the optimum was 30°C. Antarctic fungi have been evaluated for extracellular enzyme activity including cellulase, amylase, and pectinase and fungi studied were *Fusarium lateritium, Aspergillus aculeatus, A. flavus, A. niger, Mucor, Myrothecium* and *Penicillium* [9]. Many *Fusarium* spp. were already discussed for the pectinase production [12,21]. The present results are in accordance with the above mentioned reports. Caseinase was produced solely by *Fusarium* sp. F5 and F10 was the only producer for cold active cellulase and amylase. All the three were positive for urease production where as isolate F10 was unable to liquefy gelatin. Chary and Reddy [4], investigated two species of *Fusarium* for starch degradation.

It can be concluded that these isolates can be used for the production of cold active enzymes which can be utilized for making detergents for cold washing and numerous potential applications in biotechnology processes. Psychrotrophs are preferred for enzyme production than psychrophiles and mesophiles because industrial fermentation proceeds at ambient temperature.

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Table 2. Characterization of *Fusarium* spp. at 15°C

Characters		*Fusarium* spp.		
		F5	**F8**	**F10**
Macroscopic	Colony growth	Fast	Medium	Slow
	Texture of mycelium	Floccose	Cottony	Cottony
	colony color	White	White	White
Microscopic	Hyphae	Septate	Septate	Septate
	Microconidia	Slightly curved	Curved	Canoe shape
	Macroconidia	Fusiform	Fusiform	Fusiform
Salt tolerance (10 % NaCl)[a]		+	-	-
pH tolerance (9.5)[a]		+	+	+
Growth at 10°C[a]		+	+	+
Antibiosis[b]	Fluconazole	R	R	R
	Amphotericin B	S	S	R
	Nystatin	S	S	S

[a] + growth,
- no growth
[b] R, resistant
S, susceptible

Table 3. Low temperature degradation of various substrates at 15°C

Substrates	*Fusarium* spp. F5	F8	F10
	Zone of clearance (mm.)[b]		
Casein	1	0	0
Tributyrin oil	5	11	1
Cellulose	0	0	4
Starch	0	0	2
Pectin	2	6	1
Gelatin[a]	+	+	-
Urea[a]	+	+	+

[a] + Degradation positive, - degradation negative;
[b] Zone of clearance= (total diameter including clearance - fungal diameter) mm.

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