Yeast Isolates From Diverse Sources For Cold-Active Polygalacturonase And Amylase Production

K. Divya, P. Naga Padma

Abstract: Cold–active polygalacturonase and amylase producers were screened using enrichment culture technique. The diverse sources screened were cold stored spoilt fruits and vegetables from different local super markets, market waste dumped soils, fruit waste dumped soils, mountain soils and Himalayan soils. About sixty yeasts showing pectinolytic activity were isolated by ruthenium red plate assay. Eight yeasts with higher zones of pectin hydrolysis were selected and tested for cold-active polygalacturonase and amylase production. The cultures were tested for cold active pectinase and amylase enzyme activity by dinitrosalicylic acid (DNS) method. The cultures were grown at both room temperature (20-25 °C) and cold temperatures (5°C) but the cold active enzyme activity was tested at 5°C. Highest cold-active pectinase producing yeast culture with good cold-active amylase activity was selected for further study. Thus the present cold-active polygalacturonase producer with amylase activity could have better application in fruit juice clarification and so could be a potential isolate.

Keywords: Amylase, cold-active enzyme, *Geotrichum sps,* polygalacturonase, ruthenium red, screening, yeasts.

1 INTRODUCTION:

Pectinases are depolymerizing enzymes that degrade pectic substances present in middle lamella and primary cell walls of plant tissues [8]. Pectinases have wide spread applications in food industry for clarification of fruit juices, wines [1], [23] coffee and tea fermentations [23]. Cold-active enzymes from psychrophiles offer potential economic benefits as substantial energy can be saved during commercial production by fermentation. The production of pectinolytic enzymes has been widely reported in bacteria and filamentous fungi [17] but enzymes are produced as a mixture of enzymes (pectinases). Yeast pectinases being mostly single enzymes and not a mixture are preferable and also for the fact that yeast is a GRAS organism [15]. Cold-active enzymes are attractive for usage in fruit juice industry as colder conditions hamper spoilage and favour milder conditions that avoid changes in organoleptic and nutritional properties [14]. Yeasts are preferred as production strains for their short fermentation cycles and also because they can be efficiently immobilized by entrapment method and used for continuous fermentations. The main aim of the present study was to selectively isolate pectinolytic yeasts with amylase activity from diverse sources like cold stored fruits, cold soils etc. With interest in cold-active enzyme producer with growth at room temperature, a potential isolate was selected from the primary pectinolytic isolates. It was also tested for amylase production as indigenous fruits suitable for juice clarification like mango, sapota, guava etc need use of these enzymes in combination [19], [20].With current stress on fruit production and processing in an agro based economy like India the need for such cold active enzymes is highly demanding as it is important to keep temperatures low during extraction and clarification to retain the organoleptic properties and hence the present study focused on it.

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Diverse pectin rich sources like refrigerated fruits and vegetables fruit /vegetable dumped cold soils and cold soils were collected in sterile polythene bags.

2.2 Primary screening:

Serially diluted soil samples/pectin rich sources were inoculated on modified yeast extract peptone dextrose (YEPD) agar enriched with 1% pectin. Plates were incubated at RT (20-25°C) and 5°C for 24 to 72 hours. Pectinolytic primary isolates were screened using ruthenium red plate assay. Positive pectinolytic isolates were detected based on clear (colour less) zones of pectin hydrolysis around the colonies. Pectinolytic yeasts having clearing zones more than 1cm were selected for secondary screening (Fig 3).

2.3 Preliminary identification studies:

The isolates were identified based on cultural, microscopic and biochemical studies. Cultural identification was done by growth on YEPD plates and their colony characteristics, microscopic identification, Gram's reaction and morphology of cells. Biochemically identification was done by tests like sugar fermentation, antibiotic resistance, utilization of compounds etc.

2.4 Secondary screening:

Efficient cold active pectinase producers were further screened by broth studies for enzyme production. The pectinolytic enzyme was assayed by measuring the D-galacturonic acid released from polygalacturonic acid as substrate by DNS method.

2.5 Polygalacturonase/Amylase production:

Submerged fermentation studies for enzyme production were carried out in 250ml Erlenmeyer flasks containing 50ml YEPD broth with 1% commercial pectin/ 1% starch. The flasks were inoculated with actively growing yeast cultures and incubated for 72 hours both at RT (20-25° C) and 5°C for enzyme production. The inoculum size of 2.5% containing 1x104cells/ml was used. Broth samples were collected from both 5°C and RT (20-25°C) incubated flasks at every 12hrs and assayed for enzyme activity

(Polygalacturonase/Amylase) at 5°C. Yeast isolate having both polygalacturonase and amylase activity both at RT (20-25°C) and 5°C was selected and studied for enzyme production in commercial (citrus) pectin for a period of 72 hours at 5°C.

2.6 Polygalacturonase assay:

One ml of culture broth was cold centrifuged at 4°C, 5000 rpm for 10 minutes. Supernatant was taken as enzyme source. The enzyme was assayed by measuring the D-galacturonic acid released from polygalacturonic acid as substrate by DNS method [6], [12] at 5°C as assay temperature. One unit of enzyme activity is defined as the amount of enzyme required to produce 1 µM of galacturonic acid per minute at incubated temperature (5°C).

2.6 Amylase assay:

The enzyme was assayed using 1% soluble starch as substrate. The reducing sugars liberated on incubation of enzyme with substrate were determined by dinitrosalicylic acid method [12]. One unit of amylase activity is defined as the amount of enzyme required to liberate 1 µ mole of reducing sugars (glucose equivalents) per minute.

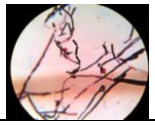
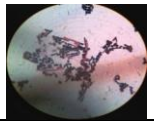
3 RESULTS:

Different pectin rich source samples when screened by enrichment culture technique gave pectinolytic yeasts in different proportions as indicated in (Fig1&2). Pectinolytic isolates that depolymerized pectin produced colourless hydrolytic zones around colonies in ruthenium red plate assay [22]. Pectinolytic organisms were detected by observing a yellow zone of hydrolysis around the colony. More than 200 different yeast isolates from diverse sources were screened for pectinolytic activity by plate assay and about sixty isolates were found to be pectinolytic. Eight isolates having hydrolysis zones more than 1cm when grown at both room temperature (20-25°C) and cold temperature (5°C) were selected (Fig3). Among the eight isolates one efficient isolate with higher pectinolytic zone at both the temperatures was selected and tested for its polygalacturonase production (Fig4).The selected isolate was subjected to identification studies. It was identified culturally morphologically and biochemically as Geotrichum sp (Table 1). Pectin being predominant component of fruit and vegetable cell walls, it promotes the growth of pectinolytic isolates hence good pectinolytic yeasts were obtained from such source samples. Yeasts are known to produce pectinases especially polygalacturonases and preferred for their shorter fermentation cycles. With interest in selection of a pectinolytic isolate with amylolytic activity the isolate was tested for amylase production using 1% and 2% starch (Fig 5). The selected isolate Geotrichum sp showed good cold-active amylase production of 130 U/ml by 36 hrs. The cold-active polygalacturonase production was also highest at 36 hours and the enzyme yield was 5 U/ml. The polygalacturonase production by Geotrichum sp was compared with various other strains of Geotrichum sp (Table 2).The enzyme production was nearly equal or more than the reported strains, but the enzymes from reported strains were produced and assayed at room temperatures but the selected isolate produced the enzymes polygalacturonase and amylase at 20- 25°C and 5°C but showed good activity at 5°C.

Table 2: Polygalacturonase production by different *Geotrichum strains*

Geotrichum sp.	Temperatures of Enzyme production and actvity Pectinase activity U/ml	Pectinase activity U/ml	Reference
Geotrichum klebahnii ATCC 42397	Production30°C and activity at 30°C	15	[16]
Geotrichum candidum ATCC 34614	Production 30°C and activity at 30°C	0.290	[24]
Geotrichum candidum ATCC 28129	Production30°C and activity at 30°C	0.057	[24]
Geotrichum klebahnii ATCC 42397	Production 30°C and activity at 30°C	10-11	[21]
Geotrichum sps	Production 20-25°C activity at 5°C	5	Present study

Table 1: Comparision of cultural , microscopic and biochemical characteristics of Yeast 5(Geotrichum sp) with selected pectinolytic yeast [10]

Characters	*Geotrichum candidum*	Yeast 5
Colony Morphology	White, velvety colony, Flat and spreading colony with a central umbo.	White, Flat colony and dry colony.
Gram's Reaction/Microscopic view		
Presence of Pseudo mycelium/ Germ tube	Present	Present
Shape of the cell	Cuboidal	Cuboidal
Growth at 25°C	+	+
Growth at 37°C	-	Slow
Fermentation of Glucose	+	+
Galactose	+	+
Sucrose	+	+
Maltose	+	+
Lactose	-	-
Nitrate utilization	-	-
Production of acetic acid	-	-
Gelatin liquefaction	-	-

4 DISCUSSION:

Good pectinolytic isolates including yeasts can be obtained from pectin rich sources like cold stored spoilt fruits, vegetables and market waste dumped soils as pectin promotes their growth as a carbon and energy source [2]. There are reports of yeasts as polygalacturonase producers [15], [3], [4]. Use of a chromogen like ruthenium is efficient in screening of pectinolytic isolates as the chromogen specifically binds to free carboxyl groups in pectin giving a red colour and a colourless halo in absence of pectin and so pectin hydrolysis can be clearly identified [22]. Higher pectinolytic zone indicates high pectinolytic activity and so a strain with comparatively highest zone of pectin hydrolysis was selected for study. The selected pectinolytic isolate was tested for amylolytic activity as fruit juice clarification needs activity of pectinases and amylases. The isolate being isolated from pectin and starch rich source showed good cold-active polygalacturonase and amylase activity(Fig 4& 5). The present isolate is a significant one as it can produce the cold active enzymes commercially at RT (20°-25°C) and use them for clarification of cold stored fruit juices so that the organoleptic properties of juices can be retained along with their increased shelf life. Use of cold active enzymes for fruit juice clarification is not only cost effective at industrial scale[5], [11], [14] but also commercially significant for an agro-based economy like India with fruits like mango both with high production and export potential. This also gives scope for labour intensive fruit processing industries in a populated country like India.

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6 FIGURES:

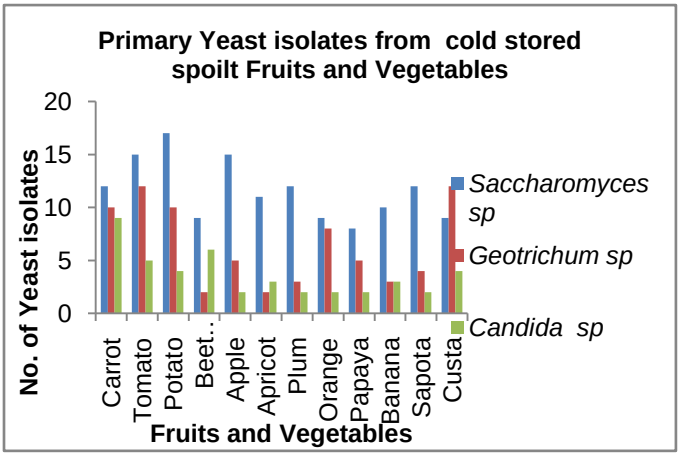

Fig 1: Different types of Yeasts isolated from cold stored spoilt fruits and vegetables.

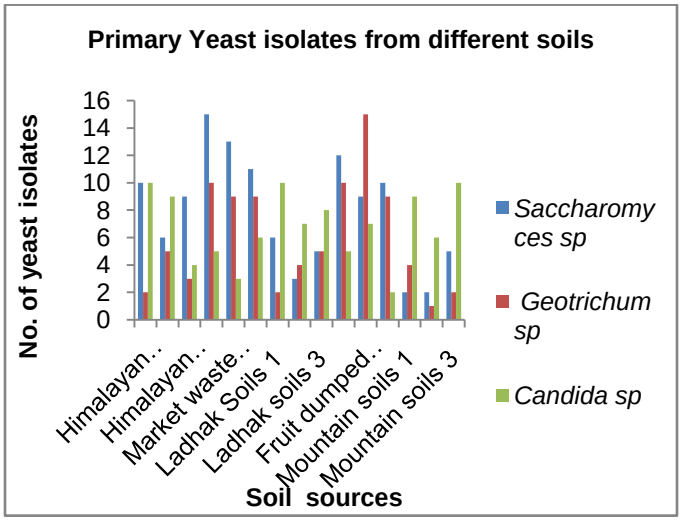

Fig2: Different types of yeasts isolated from different cold soil sources and fruit dumped soils

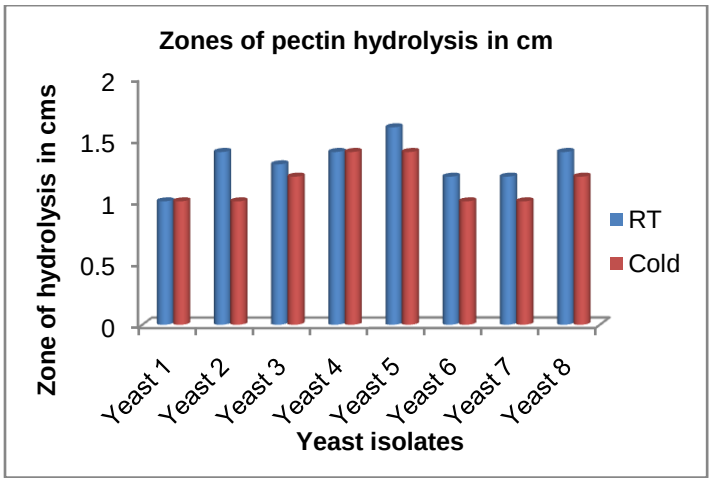

Fig3: Zones of hydrolysis by Cold active pectinase producing yeast isolates.

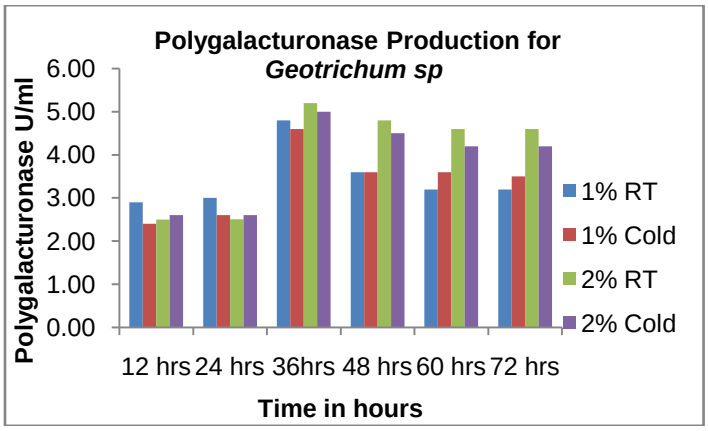

Fig4: Cold-active polygalacturonase activity of selected primary pectinolytic yeast in 1 % & 2% pectin at 5°C with growth temperature as RT and 5°C

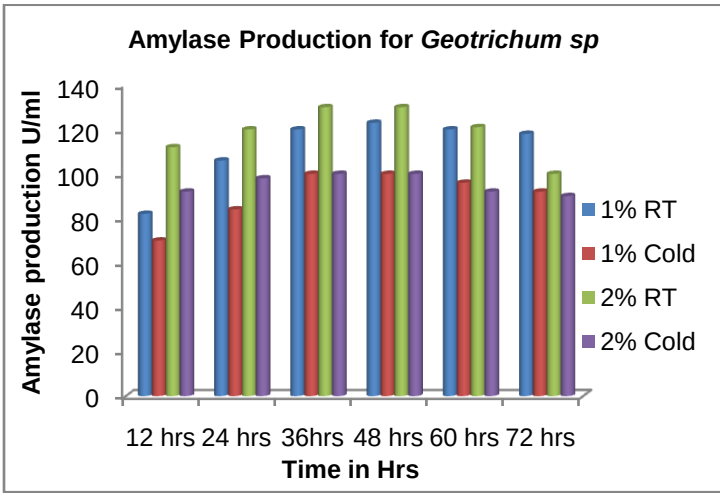

Fig5: Cold-active amylase activity of selected primary pectinolytic yeast in 1 % & 2% starch at 5°C with growth temperature as RT and 5°C.

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