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Evaluation and Decomposing Capability of Isolated Microbial Cultures through Enzymatic Activities

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ABSTRACT: Solid waste management is one of the global issue creating environmental, social and health problems because of its mismanaged disposal. Cold tolerant microbes have been found to enhance the decomposing process of the solid waste. Jammu Kashmir and Ladakh being one of the colder places in India have the highest possibility of having cold tolerant microbes that can enhance the decomposition process of the waste. In order to find the most efficient cold tolerant microorganism, the present investigation was undertaken during 2020-21 to isolate and characterize such microbes from Gurez and Ladakh regions for prompt decomposition of the waste so as to enhance the nutrient status of the manure. From the last two years (2020-21), a total of twenty seven (27) Lactic acid bacterial isolates, eighteen Actinomycetes isolates, twenty *Pseudomonas* sp. and twenty one *Bacillus* sp. were isolated from Gurez valley. Among these only nine (09), eleven (11), seven (07), and eleven (11) were cold tolerant microorganisms of Lactic acid bacteria, Actinomycetes, *Pseudomonas* sp. and *Bacillus* sp. respectively which were characterized by morphological and biochemical approach based. Qualitative and quantitative screening for different enzymatic activities was also carried out. Different selected isolates of lactic acid bacteria, actinomycetes, *Pseudomonas* sp. and *Bacillus* sp were selected for compatibility among one another based on their high enzyme activity. Five (05) different cold tolerant microbial consortia were developed based on their compatibility test and were utilized for *in-vitro* experiments at Faculty of Agriculture, Wadura and from *in-vitro* experiments, on the basis of improved nutrient status and enzyme activities, only 3 consortia were selected for Gurez (Izmarg).

Keywords: Waste, cold tolerant, enzymatic activity, decomposition.

INTRODUCTION

Composting is the process of decomposition of different organic solid wastes. Composting is a known system for rapid stabilization and humification of organic matter (Adani *et al.,* 1995) and is an environmentally friendly and economically alternative method for treating solid organic waste (Huang *et al.,* 2006). Many bacteria and actinomycetes help in carrying out the decomposition process. An extensive work on cold tolerant microbes was lacking in Jammu and Kashmir and only some work has been taken up more recently (Baba *et al.,* 2021; Ajaz *et al.,* 2019, 2018). A more comprehensive work on the characterization and different species of cold tolerant microorganisms prevalent in cold tolerant regions of Jammu and Kashmir and Ladakh has rarely been studied in earlier works. The current work gives more detailed information of microorganisms that are prevalent in cold tolerant regions of Jammu Kashmir and Ladakh with detailed information on different bacterial species and actinomycetes in general.

Microorganisms degrade organic matter and produce various products such as carbon dioxide and water during decomposition process (Holmer *et al.,* 1997). During this process heat and relatively stable organic compounds (humic substances) are produced as well. During decomposition process different microbial communities predominate at various composting phases. For example initial decomposition is carried out by mesophilic microorganisms, which rapidly biodegrade the soluble and easily degradable compounds. Though many artificial measures have been developed, but improving composting efficiency is still a key issue. During composting, process readily degradable organic matter is used by microorganisms as a source of Carbon and Nitrogen. The end product of compost consists of transformed, slowly degradable compounds, intermediate breakdown products and the cell walls of dead microorganisms, which are classified together as humic substances (HSs).The significance of humic acid to soil fertility, ecology and structure, as well as its beneficial effects on plant growth, has resulted in an increased use of compost as an

amendment to soils and also as substitute substrate for peat in container media (Chen *et al.,* 1992; Avimelech *et al.,* 1993).The results on the inoculation of different composting processes is highly found in literature (Biey *et al.,* 2000; Ichida *et al.,* 2001; Baheri and Meysami 2002; Barrena *et al.,* 2006). Now it is clear enough that inoculation can have a positive effect on composting, especially in the first thermophilic stage of the process (Tiquia *et al.,* 1997; Bolta *et al.,* 2003).

In most of the previous cited works only routine parameters of the composting process are profiled and compared between inoculated and non-inoculated treatments. Moreover, only an inoculum dosage is usually tested. Psychrophilic (cold-loving) or psychrotolerant (cold-adapted) micro-organisms are found inhabiting at various places such as in low temperature environments of the earth, polar regions, high and steep mountains, glaciers, deep oceans, the upper atmosphere, refrigerated appliances and the surfaces of plants and animals living in cold environments, where temperatures never exceed 5°C. The potential of psychrophiles and psychrophilic enzymes have been reviewed by Cavicchioli *et al.* (2002); Deming (2002); Feller and Gerday (2003); Georlette *et al.* (2004). Many psychrophiles live in biotopes having more than one stress factors, such as low temperature and high pressure in deep seas (piezo-psychrophiles), or high salt con-centration and low temperature in sea ice (halo-psychro-philes).

Considering this tremendous importance of biodegradable solid waste decomposition under temperate condition and very less work done in Kashmir, the aim of this study was mainly focused on the behavior of main microbiological parameters and of indigenous microorganisms during the composting of agricultural solid wastes.

Adoptingproficient psychrozymes in bioremediation have various advantages such as in cold climatic environments it is difficult for the whole cell to face additional challenges, so utilization of cold-active enzymes may help in meeting clean-up standards in a short time (Miri *et al.,* 2019). Because of low temperatures in cold environments, it is challenging for microorganisms to meet clean-up standards for bioremediation as it consumes more time (Miri *et. al.*, 2019; Margesin *et al.,* 2003). Many cold-adapted microbes such as *Pseudomonas* sp. and *Pseudoalteromonas* are capable of degrading petroleum hydrocarbons (Lin *et al.,* 2009). Baba *et al.* 2021 showed that out of a total 110 bacterial isolates, only 13 bacterial isolates showed the ability of potassium solubilization at lower temperature conditions (0°C, 1°C, 3°C, 5°C, 7°C, 15°C and 20°C) under in-vitro conditions. Ajaz *et al.* (2019) characterized a total of twenty two Lactic acid bacterial isolates, Nineteen Actinomycetes isolates, eighteen *Pseudomonas* sp. from Gurez and out of these only nine, eleven and seven were cold tolerant microorganisms of Lactic acid bacteria, Actinomycetes and Pseudomonas sp. respectively were characterized morphologically and biochemically. Similarly. Ajaz *et al.* (2018) collected 10 samples from Agricultural waste dump sites from various areas of Ladakh and 12 isolates of bacteria were isolated using Nutrient Agar medium. The optimal cultural conditions, microbiological characteristics, biochemical characteristics, antagonistic and synergistic activities within the strains and production of extracellular enzymes of the bacterial strains were documented. Colonies were isolated, cultured and characterized by gram staining and biochemical tests. Six isolates were found to be gram negative while 4 were gram positive. All isolates were positive for amylase, cellulose.

MATERIAL AND METHODS

Study area. Gurez valley (34.63 N 74.833 E) is one of the coldest place in J&K with good yield of organic production of different crops and five (05) sites were selected for the experiment purpose. Four (04) samples of biodegradable wastes (soil mixed with waste) were collected from four (04) different locations and then composited into one sample per site.

Qualitative screening for enzymatic activity. All the pure cultures of collected isolates were screened for enzyme activities like amylase, protease, cellulase and xylanse on selective media (Bernfeld, 1955). All the bacterial species isolates were screened for the amylase enzyme, as per modified protocol of Bertrand *et al.* (2004). The pure bacterial culture was inoculated on Starch agar medium (Prepared in g/l with the following composition KNO_3 0.5, K_2HPO_4 1.0, $MgSO_4$. $7H_2O$ 0.2, $CaCl_2$ 0.1, $FeCl_3$ traces, potato starch 10.0, pH 7.2.). The plates were incubated inverted at 10^oC for 48 hrs. The plates were then flooded with the Lugol's iodine solution, and then washed with the distilled water. The colonies that were able to synthesize the amylase showed a clear zone around the colony.

Cellulase. Cellulose is the most abundant macromolecule and the enzyme cellulase is responsible for breaking it in its constituents. The colonies were screened for this enzyme as per modified protocol of Goel and Wood (1978) on the cellulose agar medium (1.0% peptone, 1.0% cellulose, 0.2% K_2HPO_4, 2% agar, 0.03% $MgSO_4.7H_2O$, 0.25% $(NH_4)_2SO_4$, pH 7). The plates were incubated inverted at 10^oC for 48 hrs. After incubation for 48 hours, cellulose agar plates were flooded with 1% congo red and allowed to stand for 15 min at room temperature. 1M NaCl was used for counterstaining the plates. Clear zones were observed around growing bacterial colonies indicating cellulose hydrolysis. The bacterial colonies having the clear zone were taken positive colonies.

Protease. All the collected isolates were screened for Protease activity, as per modified protocol of Hayashi *et al.* (1967).The pure cultures of the isolate were inoculated on the skim milk agar media with the

following composition. Skim milk salt agar pH 7.0 1) Salt solution, $MgSO_4.H_2O$ 10.0 g, $KNO_3$2.0 g, Sodium chloride 250.0 g, Ferric citrate traces, Neopeptone 5.0 g, Glycerol 10.0 ml, Agar 20.0 g, 2). Reconstituted skim milk (10% solids) 100.0 ml and autoclaved at for 10 min. Reconstituted skim milk (10% solids) was mixed with Salt solution. The plates were incubated inverted at 10° C for 48-72 hrs. After incubation the presence of clear halos around the colonies secreting the protease was considered to be positive for protease. In case of negative colonies no clear zone was observed.

Xylanase. All the isolates were screened for Xylanase activity (Roy and Habib 1986) on the xylan agar media. The plates were incubated inverted at 10°C for 48 hrs (Plate 1). After incubation, Xylan agar plates were flooded with 1% congo red and allowed to stand for 15 min at room temperature. 1M NaCl was used for counterstaining the plates. Clear zones were observed around growing bacterial colonies indicating xylanase activity. The bacterial colonies having the clear zone were taken positive colonies.

Quantitative screening for enzymatic activity. The bacterial species differ in the genomic complexity and hence have the effect on their physical and chemical properties. Different bacterial strains secrete the soluble enzymes in the surrounding medium with varying activities. The surroundings play a great role in effecting the properties of the enzymes. We tried to identify the bacterial species with the best enzyme activities. The following methodology was adopted for isolating the species with the best overall enzymatic activities.

Quantitative determination of amylolytic activity

Extraction of enzyme from bacteria (recovery of amylase). The selected strains of isolates were grown in 50 ml of 1% of starch medium placed in Erlenmeyer flask of 250 ml capacity and placed in a shaker-cum-incubator operated at 120 rpm at 10°C for 24hrs. The extracellular enzyme solutions were obtained by centrifugation at 5000 rpm for 20 min using a high cold speed centrifuge. The supernatant obtained was collected and used as enzyme source and used in enzyme assays.

Determination of enzyme activity. Amylase activity was assayed as per the method of Miller (1959) by pipetting 1 ml of culture extract "enzyme" into test tubes containing 1 ml of 1% soluble starch in citrate phosphate buffer having a pH of 7.0. The reducing sugars liberated were estimated by the 3, 5-dinitrosalicyclic acid (DNSA) method.

The reaction mixture was incubated in a water bath at 10°C for 30 min. A blank consisting of 1 ml of soluble starch in citrate-phosphate buffer (pH 7.0) was also incubated in a water bath at the same temperature and time with the other test tubes. The reaction was terminated by adding 1 ml of DNSA reagent in each test tube and then immersing the tubes in a boiling water bath for 5 min after which they were allowed to cool and 5 ml of distilled water was added. The absorbance for all the test tubes was measured at 540 nm with spectrophotometer (BIO-RAD).

Calculation of enzyme activity. The units of amylolytic enzymes were calculated in terms of saccharifying activity with the help of following formula:

$$\text{Units/ml Amylase} = \frac{\mu\text{mole Glucose released}}{\text{Molecular weight of glucose}} \times \frac{\text{Total volume of assay (ml)}}{\text{Time of assay (in minutes)}} \times 1000$$

One unit of enzyme activity (IU) referred to the amount of enzyme required for the formation of one micromole of product (glucose) ml^{-1} min^{-1} under the assay condition.

Quantitative determination of Cellulolytic activity

Extraction of enzyme from bacteria (recovery of Cellulase). The isolates were grown at 10°C for 3 days with rotary shaking at 120 rpm in 50 mL of Cellulose liquid medium in Erlenmeyer flask of 250ml capacity. The cell free supernatant containing the crude extracellular enzyme was collected after centrifugation of the culture at 7000 g at 4°C for 20 min. The crude enzyme was kept in 4°C in refrigerator for further analyses.

Determination of enzyme activity. Cellulase activity was determined Forouni and Gunn (1983) by incubating 1mL of supernatant with 1mL of 1% amorphous cellulose in 0.02M citrate phosphate buffer (pH 7.0) at 15°C for 30min. After incubation, the reaction was terminated by adding 5mL of 3, 5-dinitrosalicylic acid (DNS) reagent to 2mL of reaction mixture and then immersing the tubes in a boiling water bath for 5 min after which they were allowed to cool and 5 ml of distilled water was added. The absorbance for all the test tubes was measured at 540 nm on a spectrophotometer (BIO-RAD) using glucose as standards. The enzymatic activity of total Cellulase was defined in international units (IU).

Calculation of enzyme activity. The units of Cellulosic enzymes were calculated in terms of saccharifying activity with the help of following formula:

$$\text{Units/ml cellulase} = \frac{\mu\text{ mole Glucose released}}{\text{Molecular weight of glucose}} \times \frac{\text{Total volume of assay (ml)}}{\text{Time of assay (in minutes)}} \times 1000$$

One unit of enzyme activity (IU) referred to the amount of enzyme required for the formation of one micromole of product (glucose) ml^{-1} min^{-1} under the assay condition.

Quantitative determination of Protease activity. Extraction of enzyme from bacteria (recovery of Protease). The selected strains of isolates were propagated at 10°C for 24 hrs with rotary shaking at 120 rpm in 50 mL of skimmed milk medium in Erlenmeyer flask of 250ml capacity. The extracellular enzyme solutions were obtained by centrifugation at 5000 rpm at 4°C for 20 min using a high speed centrifuge. The supernatant obtained was collected and used as enzyme source and used in enzyme assays.

Determination of enzyme activity. Protease activity was assayed as per the method of Hayashi *et al.* (1967) by pipetting 1ml of culture extract into test tubes containing 1 ml of 0.65% casein solution in 50 mM potassium phosphate buffer and citrate phosphate buffer having a pH of 7.0. They were mixed by swirling and incubated for 10-30 minutes at 15°C. After incubation, 5 ml of TCA (110 mM) reagent was added to each tube to stop the reaction. Tubes were incubated at 15° C for 30 minutes.

After incubation, centrifugation was done at 5000 rpm for 10 minutes to remove any insolubles from the samples. To all of the vials 5ml of sodium carbonate was added, and followed by 1 ml of Folin's reagent. The vials were then mixed by swirling, incubated at 15°C for 30 minutes and the absorbance of samples was measured by a spectrophotometer at 660nm.

Calculation of enzyme activity

$$\text{Units/ml protease} = \frac{\mu\text{mole tyrosine equivalents released}}{\text{Volume of enzyme used (ml)}} \times \frac{\text{Total volume of assay (ml)}}{\text{Volume used in colorimetric determination}} \times \text{Time of assay (in minutes)}$$

One unit of enzyme activity (IU) is the amount of enzyme required for the formation of one micromole of product (tyrosine equivalents) ml^{-1} min^{-1} under the assay condition.

Quantitative determination of Xylanase activity

Extraction of enzyme from bacteria (recovery of xylanase). The isolates were grown at 10°C for 3 days with rotary shaking at 120 rpm in 50 ml xylan liquid medium in Erlenmeyer flask of 250ml capacity. The cell free supernatant containing the crude extracellular enzyme was collected after centrifugation of the culture at 7,000 g at 4°C for 20 min. The crude enzyme was kept in a refrigerator for further analyses.

Xylanase activity was assayed using birchwood xylan (0.5%) as substrate and the amount of reducing sugar released was determined by DNSA (Dinitrosalicylic acid) method.

Determination of enzyme activity. Xylanase activity was determined as per the method described by Miller (1959) incubating 1 ml of supernatant with 1 ml of 0.1% Xylan in 0.02 M citrate phosphate buffer (pH 7.0) at 15°C for 30 min. Dinitrosalicylic acid reagent (DNSA) was added to the mixture in the test tube to terminate the reaction. The test tubes were incubated in boiling water bath for 20 minutes to develop maximum color and then cooled to room temperature and absorbance was recorded against blank at 540 nm. One unit of xylanase activity was expressed as the amount of enzyme required to produce 1 µmol of reducing sugar (xylose equivalent) in 1 minute.

$$\text{Units/ml xylanase} = \frac{\mu\text{mole xylose equivalent)}}{\text{Molecular weight of xylose}} \times \frac{\text{Total volume of assay (ml)}}{\text{Time of assay (in minutes)}} \times 1000$$

RESULT

Qualitative screening for enzymatic activity. Selection of potential cold tolerant microorganisms was done on the basis of enzymatic activities (cellulases, protease, amylase and xylanase) at low incubation temperature. The nine isolates of lactic acid bacteria, eleven from actinomycetes, seven from *Pseudomonas* sp. *and* eleven of *Bacillus* sp were then examined for the qualitative enzymatic tests. Out of nine lactic acid bacteria, five isolates exhibited negative enzyme activity i.e., LG3, LG12, LG14, LG16 and LG22 while as LG 5, LG 6, LG 9 and LG 18 showed amylase positive reactions, LG5, LG9 and LG 18 showed cellulase, Protease and xylanase positive results while as and LG14, LG16 and LG22 showed cellulase and xylanse positive results. Rest showed negative results for all the enzyme reactions (Table 1 & Plate 1).

Out of eleven isolates of Actinomycetes AG9 and AG11 showed amylase negative results while as others exhibited only amylase positive results.AG15, AG16 and AG19 exhibited xylanase negative results and rest were xylanase positive. AG4, AG6, AG11, AG13 and AG18showed cellulase and protease positive results (Table 2 & Plate 1).

Out of seven isolates of *Pseudomonas* sp.PG1 and PG7 showed negative enzyme activity. PG3 was cellulase and protease positive. PG10 and PG18 were only cellulase positive. PG3 was cellulase and protease positive, PL23 and PL25 were amylase and xylanase positive (Table 3 & Plate 1).

Out of eleven isolates of *Bacillus* sp BG1 and BG5

showed negative enzyme activity. BG7, and BG12 were positive for amylase only. BG8 and BG15 were positive for cellulase only. BG3 and BG10, exhibited all enzymes positive for all the enzymes (Table 4 and Plate 1).

Quantitative enzyme assay. The isolates that showed qualitative enzymatic tests positive were than examined for quantitative enzymatic tests. The isolates that exhibited, showed high enzyme activity were LG18, AG6, PG9 and BG3 from the Gurez valley (Table 5-8).

Table 1: Qualitative enzyme activity of cold tolerant Lactic acid bacterial isolates.

Isolate	Identified isolate	Enzyme activity (IU/ml)			
		Amylase	Cellulase	Protease	Xylanase
LG3	*Psychrobacter* sp.	-	-	-	-
LG5	*Psychrobacter* sp.	+	+	+	+
LG6	*Psychrobacter* sp.	+	-	-	-
LG9	*Psychrobacter* sp.	+	+	+	+
LG12	*LactoBacillus* sp.	-	-	-	-
LG14	*Psychrobacter* sp.	-	+	-	+
LG16	*Psychrobacter* sp.	-	+	-	+
LG18	*LactoBacillus* sp.	+	+	+	+
LG22	*Psychrobacter* sp.	-	+	-	+

Table 2: Qualitative enzyme activity of cold tolerant actinomycetes.

Isolate	Identified isolate	Enzyme activity (IU/ml)			
		Amylase	Cellulase	Protease	Xylanase
A G2	*Streptomyces* sp.	+	-	-	+
A G4	*Streptomyces* sp.	+	+	+	+
A G6	*Streptomyces* sp.	+	+	+	+
A G9	*Streptomyces* sp.	-	-	-	+
A G11	*Streptomyces* sp.	-	+	+	+
A G13	*Streptomyces* sp.	+	+	+	+
A G14	*Streptomyces* sp.	+	-	-	+
A G15	*Micropolyspora* sp.	+	-	-	-
A G16	*Streptomyces* sp.	+	-	-	-
A G18	*Streptomyces* sp.	+	+	+	+
A G19	*Dactylsporangium* sp.	+	-	-	-

Table 3: Qualitative enzyme activity of cold tolerant *Pseudomonas* sp.

Isolate	Identified isolate	Enzyme activity (IU/ml)			
		Amylase	Cellulase	Protease	Xylanase
PG1	*Pseudomonas* sp.	-	-	-	-
PG3	*Pseudomonas* sp.	-	+	+	-
PG5	*Pseudomonas* sp.	+	+	+	+
PG7	*Pseudomonas* sp.	-	-	-	-
PG9	*Pseudomonas* sp.	+	+	+	+
PG10	*Pseudomonas* sp.	-	+	-	-
PG18	*Pseudomonas* sp.	+	+	-	-

Table 4: Qualitative enzyme activity of cold tolerant *Bacillus* sp.

Isolate	Identified isolate	Enzyme activity (IU/ml)			
		Amylase	Cellulase	Protease	Xylanase
BG1	*Bacillus* sp.	-	-	-	-
BG3	*Bacillus* sp.	+	+	+	+
BG5	*Bacillus* sp.	-	-	-	-
BG7	*Paeni Bacillus* sp.	+	-	-	-
BG8	*Bacillus* sp.	-	+	-	-
BG10	*Bacillus* sp.	+	+	+	+
BG12	*Bacillus* sp.	+	-	-	-
BG13	*Bacillus* sp.	-	+	+	-
BG15	*Paeni Bacillus* sp.	-	+	-	-
BG17	*Bacillus* sp.	+	-	+	-
BG19	*Bacillus* sp.	+	+	-	-

Table 5: Enzymatic activities of isolated cold tolerant Lactic acid bacteria.

Isolate	Identified isolate	Enzyme activity (IU/ml)			
		Amylase	Cellulase	Protease	Xylanase
LG3	*Psychrobacter* sp.	0.00	0.00	0.00	0.00
LG5	*Psychrobacter* sp.	0.20	0.12	0.15	0.14
LG6	*Psychrobacter* sp.	0.15	0.00	0.00	0.00
LG9	*Psychrobacter* sp.	0.22	0.13	0.14	0.10
LG12	*LactoBacillus* sp.	0.00	0.00	0.00	0.00
LG14	*Psychrobacter* sp.	0.00	0.06	0.00	0.06
LG16	*Psychrobacter* sp.	0.00	0.04	0.00	0.02
LG18	*LactoBacillus* sp.	0.26	0.14	0.15	0.12
LG22	*Psychrobacter* sp.	0.00	0.06	0.00	0.10

Table 6: Enzymatic activities of cold tolerant Actinomycete isolates.

Isolate	Identified isolate	Enzyme activity (IU/ml)			
		Amylase	Cellulase	Protease	Xylanase
A G2	*Streptomyces* sp.	0.16	0.00	0.00	0.14
A G4	*Streptomyces* sp.	0.25	0.13	0.10	0.14
A G6	*Streptomyces* sp.	0.32	0.19	0.21	0.21
A G9	*Streptomyces* sp.	0.00	0.00	0.00	0.06
A G11	*Streptomyces* sp.	0.00	0.12	0.01	0.10
A G13	*Streptomyces* sp.	0.30	0.16	0.19	0.19
A G14	*Streptomyces* sp.	0.12	0.00	0.00	0.13
A G15	*Micropolyspora* sp.	0.07	0.00	0.00	0.00
A G16	*Streptomyces* sp.	0.01	0.00	0.00	0.00
A G18	*Streptomyces* sp.	0.22	0.11	0.16	0.14
A G19	*Dactylsporangium* sp.	0.12	0.00	0.00	0.00

Table 7: Enzymatic activity of cold tolerant *Pseudomonas* isolates.

Isolate	Identified isolate	Enzyme activity (IU/ml)			
		Amylase	Cellulase	Protease	Xylanase
PG1	*Pseudomonas* sp.	0.00	0.00	0.00	0.00
PG3	*Pseudomonas* sp.	0.00	0.12	0.01	0.00
PG5	*Pseudomonas* sp.	0.24	0.15	0.12	0.15
PG7	*Pseudomonas* sp.	0.00	0.00	0.00	0.00
PG9	*Pseudomonas* sp.	0.32	0.16	0.20	0.19
PG10	*Pseudomonas* sp.	0.00	0.10	0.00	0.00
PG18	*Pseudomonas* sp.	0.03	0.06	0.00	0.00

Table 8: Enzymatic activity of cold tolerant *Bacillus* sp.

Isolate	Identified isolate	Enzyme activity (IU/ml)			
		Amylase	Cellulase	Protease	Xylanase
BG1	*Bacillus* sp.	0.00	0.00	0.00	0.00
BG3	*Bacillus* sp.	0.31	0.18	0.22	0.28
BG5	*Bacillus* sp.	0.00	0.00	0.00	0.00
BG7	*PaeniBacillus* sp.	0.13	0.00	0.00	0.00
BG8	*Bacillus* sp.	0.00	0.10	0.00	0.00
BG10	*Bacillus* sp.	0.29	0.18	0.20	0.24
BG12	*Bacillus* sp.	0.11	0.00	0.00	0.00
BG13	*Bacillus* sp.	0.00	0.06	0.01	0.00
BG15	*Paeni Bacillus* sp.	0.00	0.08	0.00	0.00
BG17	*Bacillus* sp.	0.11	0.00	0.19	0.00
BG19	*Bacillus* sp.	0.10	0.11	0.00	0.00

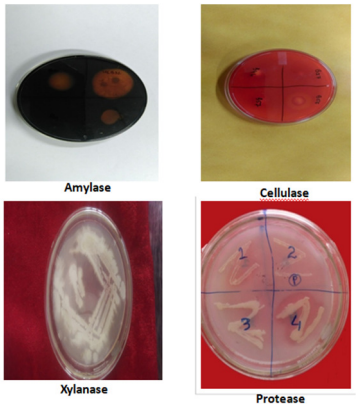

Plate 1: Enzymatic Activities of Cold Tolerant isolates.

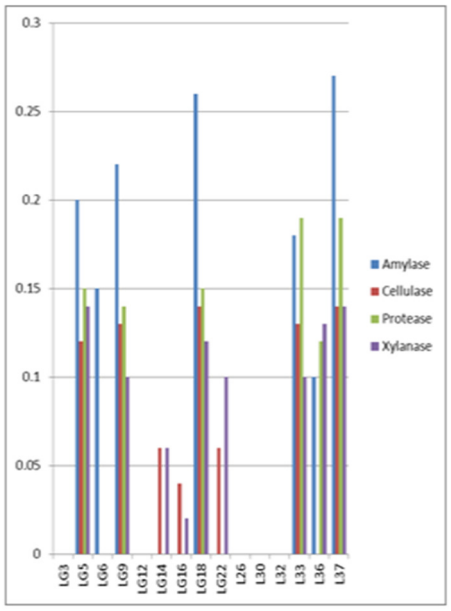

Fig. A. Enzymatic activity of isolated Lactic acid Bacteria.

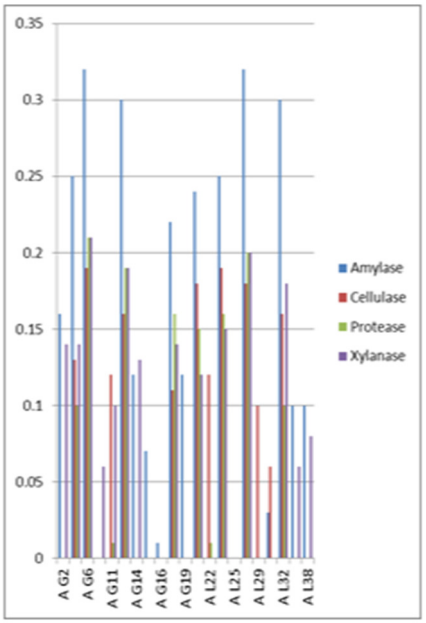

Fig. B. Enzymatic activity of isolated Actinomycetes.

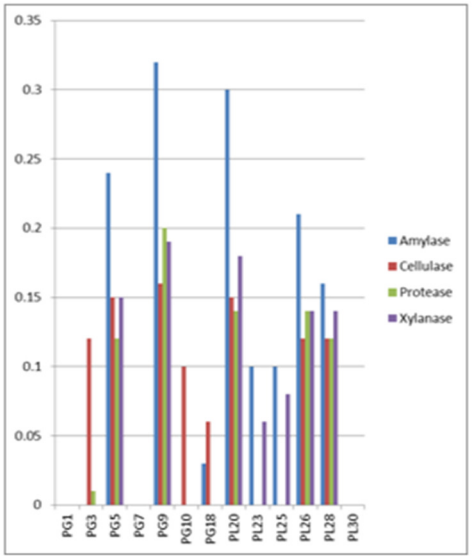

Fig. C. Enzymatic activity of isolated *Pseudomonas* sp

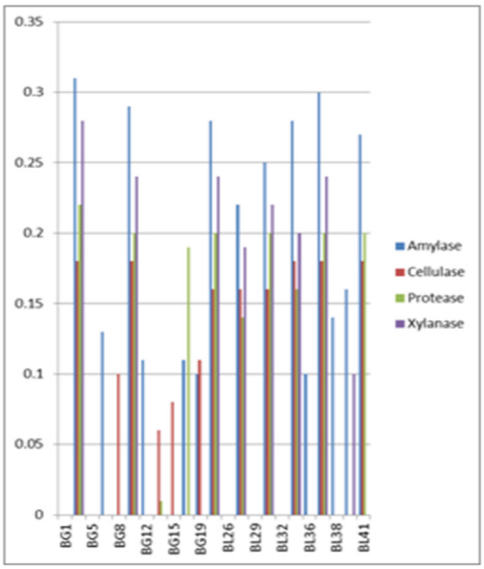

Fig. D. Enzymatic activity of isolated *Bacillus* sp

DISCUSSION

Enzymatic activities of isolated bacteria

Amylase synthesizing bacteria: The bacterial isolates were screened for the amylase activity and LG18 and LG9 isolates of lactic acid bacteria, AG4 and AG6 isolates of Actinomycetes, PG9 and PG5 isolates of *Pseudomonas* sp., BG3, BG10 and BG34 isolates of *Bacillus* sp. exhibited the maximum amylolytic activity. On the basis of the amylase production and the maximum enzymatic activity per unit time in the *in vitro* experiments the isolates were compared with one another. The production was found to be maximum After 96 hrs of inoculation, the production was found to be maximum however further increase in the incubation period exhibited an unsupportive effect on the enzyme production. Due to depletion of the nutrients or due to the accumulation of the byproducts of the cellular metabolism there could be The decrease in the enzyme activity may have been (Ali, 1992; Gupta *et al.*, 2008). The results suggested that these isolates produced extracellular amylolytic enzymes and were further selected for the treatment of the solid wastes under *in-vivo* conditions.

Cellulase synthesizing bacteria. Bacterial isolates were screened for their Cellulase by the hydrolysis of the substrate in the basal salt medium and LG5, LG 9 and LG18 isolates of lactic acid bacteria, PG5 and PG9 isolates of *Pseudomonas* sp., AG6 and AG13 isolates of actinomycetes, BG3 and BG10 isolates of *Bacillus* sp exhibited maximum Cellulase enzyme activity.

Comparison of isolates was done with one another on the basis of the Cellulase production and the maximum enzymatic activity per unit time in the *in vitro* experiments. The incubation period had a direct effect on the production of the enzyme to a certain extent, an increase in the incubation period showed an obstructive effect on the enzyme production. Due to depletion of the nutrients in the medium which stressed the bacterial physiology and there was decrease in the enzyme activity as well (Nochuer *et al.*, 1993).

Protease synthesizing bacteria. Screened protease secreting isolates exhibited an immense enzyme activity. Among the isolates compared with one anotherLG5, LG9 and LG18 isolates of lactic acid bacteria, PG5 and PG9 isolates of *Pseudomonas* sp, AG6 and AG13 isolates of actinomycetes, BG3, BG10 and BG17 isolates of *Bacillus* exhibited maximum Proteolytic activity.

Of enzymatic activity was found to be maximum after 120 hrs of incubation in *in vitro* conditions; and further if incubation period was increased it had a negative effect on the enzyme production which could be due to depletion of the nutrients or due to the accumulation of the by products of the cellular metabolism (Mabrouk *et al.*, 1999). These results suggested that isolates with high enzyme activity and were further selected for the treatment of the solid wastes under *in-vivo* conditions.

Xylanase synthesizing bacteria. The isolates were analysed for the soluble xylanase activity in the medium containing the substrate in proper proportions. On the basis of the comparison with one another the LG5 and LG18 isolates of lactic acid bacteria, PG9 isolate of *Pseudomonas* sp. AG6 and AG13 isolates of actinomycetes, BG3 and BG10 isolates of *Bacillus* sp. exhibited maximum Xylanase activity per unit time in the *in vitro* experiments.

After 84 hrs of inoculation the production was found to be maximum; however further increase in the incubation time showed a negative effect on the enzyme production which could be due to depletion of the nutrients or due to the accumulation of the by products of the cellular metabolism. The results suggested that these isolates produced extracellular Xylanase enzymes and were further selected for the treatment of the solid wastes under *in-vivo* conditions.

CONCLUSIONS

Because of the tremendous importance of biodegradable solid waste decomposition through composting especially in temperate regions, the ability of psychrophilic enzymes to catalyze reactions at low or moderate temperatures offers great industrial and biotechnological potential. Various groups of bacteria from differentsites were isolated, identified and characterized on the basis of some important morphological and biochemical properties. The most potential isolates that were isolated and identified were lactic acid bacteria isolates, Actinomycetes isolates, *Pseudomonas* sp. and *Bacillus* sp from Gurez. Different enzyme tests (quantitative and qualitative) which include Amylase, Cellulase, protease and xylanase were assessed and overall,LG18, AG6, PG9, BG3 from Gurez showed high enzymatic activity which were further analysed for compatibility tests and on that basis five different cold tolerant consortia were developed and utilized for *in vitro* and *invivo* experiments respectively for degradation of solid waste under cold tolerant conditions at Gurez valley.

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Conflicts of Interest. None.

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