

Original Research Article

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Characterization of Psychrophilic Bacteria Involved in Solid Waste Decomposition under Temperate Conditions

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ABSTRACT

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A total of 10 samples were collected 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, xylanase and protease production. It was found that bacterial isolates produce chemical(s) inhibitory to other bacterial strains including both gram positive and gram negative bacteria. These results have increased the scope of finding agro -industrially important bacteria from agricultural waste dump sites and these isolates could be vital source for the discovery of industrially useful enzymes/molecules.

Introduction

The word “waste” refers to useless, unwanted or discarded materials which are no longer considered of sufficient value and are thrown away. When these wastes are not properly handled or disposed off they cause pollution / contamination leading to pathological conditions (World Bank, 1998). But if utilized properly they can be turned into products of high economic value. Due to increasing population pressure on the land, and the ever-increasing loads of waste generated every

minute of the day, it has become difficult for government agencies to cope up with the challenge of handling the enormous quantities of the waste. Massive quantities of biodegradable solids are also generated in the form of aquatic and terrestrial weeds, leaf litter, and crop wastes. If left unharvested, the weeds seriously pollute and deplete the land and the water resources. In developing countries, leaf litter and crop waste is often burnt in the open air to generate fertilizer in the form of ash, but this not only destroys a great deal of carbon and other nutrients but is

also a source of air pollution and global warming. Disposal of agricultural wastes after the processing of crops (e.g. maize stalks, rice husks, foliage, etc.) is another problem in our country. There is a wide variety of applications for this residue, ranging from simple combustion on an open fire to complex energy production processes that use this waste as a fuel stock (CPCB, 2006). The agricultural waste usually contains the remains of plants, crops, fruits and the plant litter. Agricultural waste can be composted by the use of the microorganisms to produce the natural fertilizers rich in the nutrients. The manure produced after the agricultural waste composting can be used for making the land fertile by using it as a natural fertilizer.

A diverse range of psychrophilic microorganisms, belonging to gram-negative bacteria (*Pseudoalteromonas*, *Moraxella*, *Psychrobacter*, *Polaromonas*, *Psychroflexus*, *Polaribacter*, *Moritella*, *Vibrio* and *Pseudomonas*), gram-positive bacteria (*Arthrobacter*, *Bacillus* and *Micrococcus*), archaea (*Methanogenium*, *Methanococcoides* and *Halo-rubrum*), yeast (*Candida* and *Cryptococcus*) and fungi (*Penicillium* and *Cladosporium*) have been isolated from cold environments. These psychrophiles are able to degrade a wide range of polymeric substances such as starch, cellulose, xylan, pectin, chitin, protein and lipid and produce enzymes like amylase, cellulase, xylanase, pectinases, chitinase, protease and lipase, respectively (Cavicchioli *et al.*, 2002; Deming, 2002; Feller and Gerday, 2003; Georlette *et al.*, 2004).

Considering tremendous importance of biodegradable solid waste decomposition under temperate condition there is an immense possibility to screen effective bacterial strains from waste dump sites with valuable applications. To cope up with the demand for new organisms with properties of production

of unique enzymes/ molecules for agro-industrial application and waste degradation there have been a constant effort in isolating novel bacteria from diverse environment. Accordingly, the present study was aimed to investigate bacterial strains from waste dump sites with the ultimate objective of waste degradation and discovering novel bioactive compounds for agro- industrial application

Materials and Methods

Study Area

Soil sample was collected from agricultural waste disposal sites of Ladakh (latitude 34°10'12"N and longitude 77°34'48"E).

Collection of samples

Ten waste samples were collected from 5 agricultural waste disposal sites of Ladakh. Sample (soil mixed with waste) was collected in sterile zip-lock plastic maintaining aseptic conditions, stored at 4 °C and marked accordingly to their source and location. The collected samples were brought to the laboratory for isolation of soil bacteria and the moisture content and pH of sample were documented.

Isolation of bacteria from waste samples

Serial dilution techniques were used for the isolation of bacteria. In this technique sample suspension was prepared by adding soil mixed with waste (1g) was added to 10 ml of sterile water (the stock) and shaken vigorously for at least 1 minute. The dilute was then sedimented for a short period. Sterile dilution blanks were marked sequentially starting from stock and 10-1 to 10-4. One ml from the stock was transferred to the 10-1 dilution blank using a fresh sterile pipette. One ml from the 10-1 dilution was transferred to the 10-2 tube for each succeeding step then from the 10-2 to the

10⁻³, then from the 10⁻³ to the 10⁻⁴. From each dilution tube 0.1 ml of dilution fluid was transferred into Nutrient Agar culture media and incubated at 10 °C for 24 hours. After successful growth of microorganisms the pure cultures of bacteria were sub-cultured in NA slants; incubated at 4 to 10°C to achieve vigorous growth.

Microbiological and biochemical characteristics of isolated bacteria

Gram stain was performed to observe the cellular morphology and gram nature of the bacteria and biochemical characterization of the strains were also carried out. The biochemical tests of Amylase production; Protease production; cellulase production; and xylanase tests were performed.

Optimization of growth condition

Three semi-solid media as NA (Nutrient Agar), BCDA (czapek dox agar medium (Basic)) and ACDA (Czapek Dox Agar medium (Acidic)) were used to optimize the cultural media of isolated bacteria. The pH were adjusted to 5.2, 6.5, 7.2, 8.9 and 10.2 in NA medium; 2.2, 3.2, 4.0, 5.5 and 6.9 in BCDA medium; and 7.1, 7.6, 9.1, 10.06 and 12.10 in ACDA medium. For optimization of incubation period and temperature the culture plates were incubated at 10, 15 and 20°C for 6-72 hours.

Extracellular enzyme production

All the isolated bacterial strains were screened qualitatively for the production of four important enzymes such as protease, cellulase, amylase and xylanase. The Petri plates were incubated overnight at 10 to 15°C. Then the plates were flooded with indicator solution and the development of clear zone around the growth of organism was considered positive for enzyme activity.

Results and Discussion

Cultural characteristics of bacterial isolates

In our study, G1, G2, G3, G4, G5, G6, G7, G8 and G9 bacterial strains were isolated in culture media. Czapek dox agar and Nutrient agar were selected to determine the best suitable media for ensuring massive growth of the isolated strains. Czapek dox agar (BCDA) was suitable for massive growth of G3, G4, G8 and Nutrient agar (NA) medium was suitable for massive growth of G1, G2, G5, G6, G7, G9.

Visual and microscopic observation was used to characterize the selected strains. Details of the colony features of the bacteria are noted (Table 1). Gram staining is an old and reliable method for observing the bacteria. Gram negative bacteria were decolorized by alcohol, losing the purple colour of crystal violet. Gram positive bacteria did not decolorize and remained purple (Fig 5 and 6).

In the present investigation, G1, G2, G3, G4, G5, G6, G7, G8 and G9 -these 9 bacterial strains were isolated and the microbiological characterization was carried out.

Different biochemical tests were also performed for the 9 isolates to know their biochemical characteristics. Details of the biochemical characters of the bacteria.

Screening and evaluation of isolated microbial cultures for their decomposing capability through enzymatic activities

Qualitative enzyme assay

Selection of potential cold tolerant microorganisms was done on the basis of enzymatic activities (cellulases, protease, amylase, and xylanase) at low incubation temperature.

Fig.1 Amylase test



Fig.2 Cellulase test

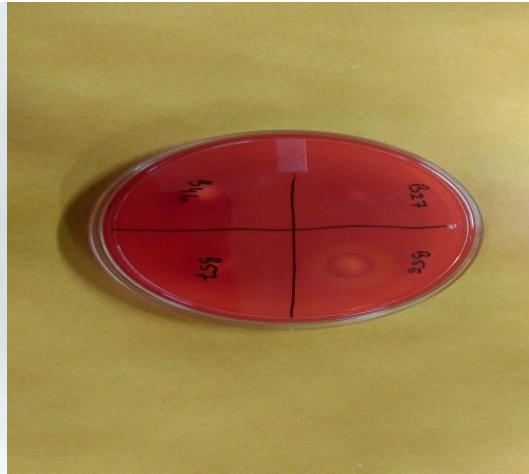


Fig.3 Protease test



Fig.4 Xylanase test



Fig.5 Gram Positive

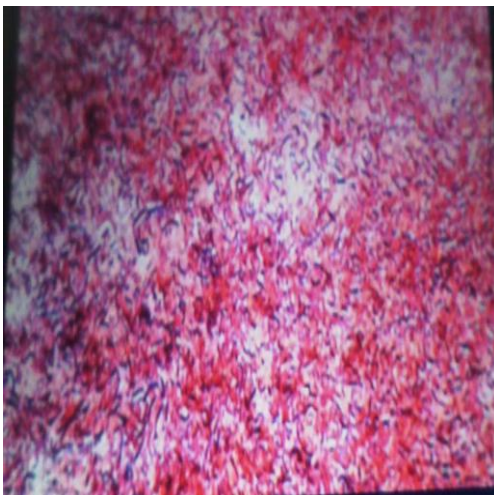


Fig.6 Gram Negative



Table.1 Cultural characteristics of bacterial isolates

Strain number	Color of colony	Colony feature	Cell feature	Cell feature
		Nature of colony	Gram nature	Shape
G1	White	irregular	Gram positive	Bacilli
G2	cream	Round shape, transparent	Gram positive	Short bacilli
G3	Cream	Irregular transparent	Gram positive	Bacilli
G4	Cream	Round shaped, shiny	Gram positive	Bacillibacilli
G5	White	Irregular	Gram positive	Diplobacilli
G6	Cream	Irregular, convex appearance, smooth and shining	Gram negative	Rod shaped
G7	Cream	Round shaped	Gram negative	Cocco bacilli
G8	cream	Thin, even growth	Gram positive	Rod or coccus shaped
G9	Greenish black	Radiating colonies	Gram positive	irregular

Table.2 Biochemical characteristics of isolates

Strain number	Amylase	Cellulase	Xylanase	Protease
G1	+	-	+	-
G2	+	+	+	+
G3	-	-	-	+
G4	-	+	+	-
G5	+	+	-	-
G6	-	+	+	+
G7	+	-	-	-
G8	-	+	+	+
G9	-	-	-	+

40 isolates that were classified into different groups on the basis of morphological features and gram's staining were then examined for the qualitative enzymatic tests.

Out of 40 isolates only 9 isolates showed one or other test positive and rest 31 colonies were discarded for further analysis (Table-2).

Quantitative enzyme assay

9 isolates that showed qualitative enzymatic tests positive were then examined for quantitative enzymatic tests.

Identification of most efficient cold tolerant microorganisms

On the basis of quantitative enzymatic isolates obtained belonged to genera *Actinomycetes*, *Alcaligenes sp.* *Lactic acid bacteria* and *Bacillus*. The results of the present investigation entitled "Isolation and Characterization of cold tolerant microorganisms involved in solid waste decomposition through enzymatic activities"

Described in the preceding chapter have been discussed here under the following headings:

Enzymatic activity of isolated bacteria

Amylase synthesizing bacteria

The isolates were screened for the amylase activity and isolates G2, G5, G7 exhibited the maximum amyolytic activity. The isolates were compared with one another on the basis of the amylase production and the maximum enzymatic activity per unit time in the *in vitro* experiments. The production was found to be maximum after 96 hrs of inoculation; however further increase in the incubation period showed an unsupportive effect on the enzyme production. The decrease in the enzyme activity may have been due to depletion of the nutrients or due to the accumulation of the byproducts of the cellular metabolism (Ali, 1992; Gupta *et al.*, 2008). The results suggested that these isolates produced extracellular amyolytic enzymes and were further selected for the treatment of the solid wastes (Fig 1).

Cellulase synthesizing bacteria

Screening of the bacterial isolates for their Cellulase activity was carried out by the hydrolysis of the substrate in the basal salt medium and the isolates G₂, G₄ and G₈ exhibited maximum the Cellulase enzyme activity. The isolates were compared with one another on the basis of the Cellulase production and the maximum enzymatic activity per unit time in the *in vitro* experiments. The decrease in the enzyme activity might be due to depletion of the nutrients in the medium which stressed the bacterial physiology resulting the inactivation of the secretary machinery (Nochuer *et al.*, 1993) (Fig 2).

Protease synthesizing bacteria

The protease secreting isolates were screened and the isolates exhibited an immense enzyme

activity. Among the isolates compared with one another G₂, G₃, G₆, and G₈ exhibited maximum Proteolytic activity. The maximum enzymatic activity was based on the experiments carried under *in vitro* conditions (Gupta and Lorenz, 2002). The production was found to be maximum after 120 hrs of incubation; and further increase in the incubation period had a negative effect on the enzyme production. The decrease in the enzyme activity may have been due to depletion of the nutrients or due to the accumulation of the byproducts of the cellular metabolism (Mabrouk *et al.*, 1999) (Fig 3).

Xylanase synthesizing bacteria

The isolates were screened for the soluble xylanase activity in the medium containing the substrate in proper proportions. On the basis of the comparison with one another the isolates G₁, G₂, and G₆ exhibited maximum Xylanase activity per unit time in the *in vitro* experiments. The production was found to be maximum after 84 hrs of inoculation; however further increase in the incubation time showed a negative effect on the enzyme production. The decrease in the enzyme activity may have been due to depletion of the nutrients or due to the accumulation of the byproducts 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 (Fig 4).

Agricultural wastes are residues from the growing and processing of raw agricultural products are non-product outputs of production and processing and may contain material that can benefit man. These residues are generated from a number of agricultural activities and they include cultivation, livestock production and aquaculture. These wastes when managed properly through the application of the knowledge of agricultural

waste management systems such as the “3Rs” can be transformed into beneficial materials for human and agricultural usage.

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