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Development of microbial consortia of cold tolerant microbes for solid waste management

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Abstract
Solid waste management has become a global issue owing to various environmental, social and health problems associated with its disposal. The two capital cities of the state, Srinagar and Jammu, generate 770 tonnes of solid waste daily and in absence of proper scientific disposal methods, this waste is emerging as major source of air, water and soil pollution. Stringent efforts are required to find methods and means for disposal of these wastes. Although there are a vast array of methods for disposal but, the microorganisms that require low temperatures for conversion of solid wastes into manure particularly in the temperate regions shall go a long way to find round the year solution for this problem. Since Kashmir valley has cold temperatures during most parts of the year, for that purpose we tried to combine cold tolerant microorganisms and earthworms for solid waste decomposition with a view to enhance nutrient status of the manure.

During our research program, a total of twenty two Lactic acid bacterial isolates, Nineteen Actinomycetes isolates, eighteen *Pseudomonas* sp. were isolated from Gurez. 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. Qualitative and quantitative screening for different enzymatic activities was also done. On the basis of their high enzyme activity five isolates of lactic acid bacteria, seven of actinomycetes, five of *Pseudomonas sp.* were selected for compatibility amongst one another. Five different cold tolerant consortia were developed on the basis of compatibility test and were utilized for *in-vitro* experiments at Faculty of Agriculture, Wadura. From *in-vitro* experiments, on the basis of improved nutrient status and enzyme activities, three (CTC1, CTC2, CTC3) among five consortia were selected for *in-vivo* experiments at Gurez (Izmarg). CTC1 was combination of LG9 *Psychrobacter* sp., AG6 (*Streptomyces* sp.), PG9 *(Pseudomonas* sp.), CTC2 contained LG18 (*Lactobacillus* sp.), AG2 (*Streptomyces* sp.), PG9 (*Pseudomonas* sp.) and CTC3 contained LG9 (*Psychrobacter sp.*), AG13 (*Streptomyces* sp.), PG5 (*Pseudomonas* sp.).Over all, CTC1 gave best results i.e, available nitrogen(1.29%), available phosphorus(0.77%), available potassium (0.42%) from Gurez.

Keywords: Cold tolerant microbes, Lactic acid bacteria, *Pseudomonas* sp, Actinomycetes, Enzyme activity, Gurez

1. 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 various types of diseases. 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 wastes. 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.

Psychrophilic (cold-loving) or psychrotolerant (cold-adapted) micro-organisms are found inhabiting the low temperature environments of the earth, including polar regions, high mountains, glaciers, ocean deeps, shallow subterranean systems (*i.e.* caves), the upper atmosphere, refrigerated appliances and the surfaces of plants and animals living in cold environments, where temperatures never exceed 5 °C. The potentials of psychrophiles and

psychrophilic enzymes have been reviewed by Cavicchioli *et al.* (2002) [6], Deming (2002) [8], Feller and Gerday (2003) [10] and Georlette *et al.,* (2004) [11]. 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 concentration and low temperature in sea ice (halo-psychro-philes).

The biological treatment of these wastes appears to be most cost effective and carry a less negative environmental impact. This process of biological treatment of wastes is also known as Composting. It is a self-heating, aerobic solid phase biodegradative process of organic materials under controlled conditions, which distinguishes it from natural rotting. It has clearly been established that composts have the potential to protect the soil against erosion (Bazzoffi, and Pellegrini, 1973), to enhance the soil water retention, to reduce soil compactibility, to decrease soil acidity, to enhance soil biochemical and biological activity and to establish a sound soil ecological equilibrium. Additionally, composts can protect plants from soil or seed borne pathogens. Hence, compost can be considered as a much-needed soil conditioner with generally positive crop yield effects. The exploitation of the metabolic versatility of microorganisms is advantageous in biological waste treatment but the actual number of degraders of a target compound in a mixed culture may only represent 5-10% of the microbial community. To understand how microorganisms may be manipulated and exploited to reduce the frequency of such breakdowns and shorten start-up times of biological waste treatment, the important bacterial strains actively involved in the degradation of food waste were isolated and screened (Baffi *et al*. 2005)

Considering tremendous importance of biodegradable solid waste decomposition under temperate condition and very less work having been done in Kashmir till now, the aim of development of consortia was mainly focused on the behavior of main microbiological parameters and of indigenous microorganisms during the composting of solid wastes.

Material and methods

1) Survey and Sample Collection

For survey Gurez was selected and from each area five sites were selected. Four samples of biodegradable wastes (soil mixed with waste) were collected from four different locations and then composited into one sample per site. So, five composite samples were collected in sterile zip-lock plastic bags maintaining aseptic conditions, stored at 4 °C and marked according to their source and site. The collected samples were brought to the Laboratory, for isolation of cold tolerant microorganisms. Purposive method of sampling was adopted during the collection of samples (Table 1).

2. Isolation of cold tolerant microorganisms

The following groups of cold tolerant microorganisms which constitute effective microbial organisms for solid waste management were isolated by standard procedures by Cappuccino and Sherman, 1992.

a) Lactic acid bacteria b) Actinomycetes c) *Pseudomonas* sp.

3. Morphological and biochemical characterization of bacterial isolates:

All the bacterial isolates were examined for the colony morphology, cell shape and gram reaction as per the standard procedures given by Anonymous (1957) [1] and Barthalomew and Mittewer (1950) [2]. Colony morphology was studied with the help of magnifying lens and cell shape of the isolates under microscope. The bacterial isolates were biochemically characterized by catalase, oxidase, urease (Zaved *et al*., 2008) [23].

1. Gram Staining Technique (Gram, 1884):

2. Screening of bacterial species for biochemiccal tests

A) Catalase test(Blazevic and Ederer, 1975) [5]
B) Oxidase test(Cappuccino and Sherman, 1992) [5]
C) Urease test (Cappuccino and Sherman, 1992) [5]

3. Qualitative screening for enzymatic activity:

a) Cellulase

Cellulose is the most abundant macromolecule and the enzyme responsible for breaking it in its constituents is Cellulase. The colonies were screened for this enzyme as per modified protocol of Goel and Wood (1978) [12] 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 ^{0}C 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.

b) Protease

All the collected isolates were screened for Protease activity, as per modified protocol of Hayashi *et al.* (1967) [14]. 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 $^{\circ}$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.

c) Xylanase

All the isolates were screened for Xylanase activity (Roy and Habib, 2009) on the xylan agar media. The plates were incubated inverted at 10°C for 48 hrs (Plate-7). 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.

4) Development and activation of microbial consortium/ consortia

Prior to microbial consortium development, the compatibility of the selected cold tolerant microorganisms/ bacterial isolates was checked by carrying compatibility test(s). The compatible microbial isolates were selected accordingly for the development of consortium/ consortia including taking other parameters in consideration.

The microbial consortium/ consortia was prepared by mixing 3grams each of molasses and fish extract in 100 ml of

distilled water. The pH of the broth was adjusted to 7 with 0.1 NaCl / 0.1N NHCl. 1ml of actinomycetes culture was be added to the broth and continuously shaken at 120 rmp at 30 ^{0}C for another 2 days. Then 1ml each of lactic acid bacteria, *Pseudomonas* and *Bacillus* was added to the broth and shaken at 120 rmp at 30 ^{0}C for another 2 days. The consortium was stored for further studies.

A mixture of solution was prepared by 16 litres of chlorine free pure water mixed with 3 kgs of Jaggery and autoclaved at 10 lbs. for 10 min. to dissolve Jaggery. The solution was cooled at room temperature for 2 hrs and 1 litre of cold tolerant microbial solution was added into it. Then the mixture was poured into a clean plastic container and air was released and sealed. It was then placed in a cool dark place and for 10-14 days to get a good fermentation. After the fermentation process, a white layer with sweetish sour odour which is a characteristic feature of cold tolerant microbial solution and the product was ready.

Solid waste management using cold tolerant consortia

Agricultural waste (Randomized)

Agricultural wastes included green grasses, straw, vegetable wastes, legume crop wastes, weeds etc. and a composite sample of agricultural wastes was prepared for treatment with different CT consortia as per design.

Biodegradation of solid wastes

***In vitro* biodegradation of agricultural waste**

The agricultural wastes were spread layer after layer of 5cm thick in the plastic buckets of uniform size and regarded as plates and treatment of CT consortia were sprayed after every layer @ 2 litres per 10 KGS of waste separately. This layering process was repeated up to the mouth of the buckets and then sealed with a cover. Different treatments were used as:

Treatments

T0: Control
T1: CTC1
T2: CTC2
T3: CTC3
Replications: 03 Design: CRD

Plastic buckets of uniform size were taken a 5 cm thick base layer containing waste mixture was spread and activated microbial consortium @ 2 litres/10 Kg of waste sprayed over this layer. A second layer of solid waste about 10 cm thick was spread over the previous layer and also sprayed with the activated microbial consortium. This layering process was repeated to the height of bucket and finally sealed with cover.

Physico-chemical analysis of the waste samples

The methods employed for the estimation of Physico-chemical properties of the decomposed waste was done as per procedure adopted by Dipti & Anthappan (2012) [9]. The nutrient status of the waste samples was analyzed for the following properties as per standard procedures:

pH and electrical conductivity

The soil PH and electrical conductivity were determined in 1:2.5 soil: water suspension using pH Meter (Micro PH. System-361., systronics) and conductivity meter-306 (Systronics) as per Jackson (1973) [15].

Estimation of organic carbon

Organic carbon content of the samples was analysed by rapid titration method of Walkley and Black (1934).

Calculations

$$\%\ Organic\ carbon = (B - S)X\ N\ X\ 0.003\ X\ \frac{100}{weight\ of\ Sample\ (Oven\ Dried)}$$

Where,
B = ml of std. 0.5 N ferrous ammonium sulphate required for blank.
S = ml of std. 0.5 N ferrous ammonium sulphate required for sample.
N = Normality of std. ferrous ammonium sulphate (0.5N)
Organic matter = Organic Carbon x 1.724 (Correction factor).

Available nitrogen

The available nitrogen was determined by alkaline permanganate method (Subbiah and Asija, 1956). The available N was calculated using formula:

$$Percentage\ of\ Available N = \frac{(25 - X) \times 0.00028 \times 100}{10} = e$$

Wherein 10 is Weight of soil taken in g, 25 is the volume of 0.02N H_2SO_4 taken in ml, X is the volume of 0.02N NaOH used (titrate value) in ml and 25 –X is the volume of 0.02N acid used for ammonium absorption. 1ml of 0.028N H_2SO_4 = 0.00028 g of N or 0.28 mg N
Available nitrogen in soil (ppm) = Percentage of available N×10,000 = f
Available N in soil (Kg/ha = f × 2.24

Available Phosphorus

The available phosphorus in the samples collected from the target wastes was estimated by Olsen's method.

Calculation

Convert ppm concentration in filtrate to concentration in the sample:
ppm P in sample = ppm P in filtrate x 20
P (lb/acre) in sample = ppm P in filtrate x 40
P (kg/ha) = P (ppm) in filtrate x 20 x 2.24

Available Potassium

Available potassium content of the samples was estimated by Flame photometry method (Stanford & English, 1949).

Calculation

K (ppm) = Reading from graph ug K / ml in extract (R) x 5 x Dilution Factor (*Df*)

$$K\ \left(\frac{kg}{ha}\right) =\ R\ x\ 5\ x\ 2.24\ x\ Dilution\ Factor\ (Df)$$

K_2O^+ (kg/ha) = R x 11.20 x 1.2
K_2O = K x 1.2
i.e. K = K_2O x 0.83

Statistical analysis

The data collected/ recorded for various parameters were subjected to Statistical analysis using standard statistical procedures as followed by Gomez and Gomez (1984) [13] and Statistical Software *Statistica* AG (Statsoft USA) licensed to Faculty of Agriculture, SKUAST-Kashmir were utilized for analysis of data.

Results

Lactic acid bacteria (LAB)

Out of the total twenty two lactic acid bacterial isolates obtained from the collected samples, only nine were isolated at 10° C and were selected for further analysis as cold tolerant microbes, rest that exhibited growth 28± 2 °C were discarded. The results are given in Tables 2 and 3. These lactic acid bacterial isolates were purified, characterized, identified and maintained for further use. The selected lactic acid bacterial isolates were coded on the basis of their location e.g., LG 1 to LG 22 for Gurez.On the basis of morpho-biochemical characters the genera of lactic acid bacteria identified were *Psychrobacter* sp., *Lacto Bacillus sp* and the results are presented in Tables 4,5,6 and 7

4.1.2 Actinomycetes

Nineteen actinomycetes cultures were isolated from the samples analyzed. All the isolates were further checked for their growth at 28± 2 °C for one week and out of Nineteen isolates only eleven were cold tolerant and were further analysed and rest of the isolates were discarded. The predominant isolates of actinomycetes were purified, characterized, identified and maintained for further use. The selected actinomycetes isolates were coded on the basis of their location e.g., AG 1 to AG 19 for Gurez. On the basis of morpho-biochemical characters the probable genera of actinomycetes were *Micropolyspora sp.*, *Nocardioides sp.*, *Dactylsporangium sp and Streptomyces sp*. The results are presented in Tables 8, 9, 10, 11 and 12

4.1.3 Pseudomonas sp.

Eighteen *Pseudomonas sp.* were isolated from the samples analysed. All the isolates were further checked for their growth at 28± 2 °C for one week and out of eighteen only seven were cold tolerant and were further analysed and rest eighteen were discarded. The predominant isolates of *Pseudomonas* sp. were purified, characterized, identified and maintained for further use. The selected *Pseudomonas sp.* isolates were coded on the basis of their location e.g., PG 1 to PG 18 for Gurez. The results are presented in Tables 13,14,15,16 and 17

4.2 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, 11 from actinomycetes and 07 from *Pseudomonas sp* were then examined for the qualitative enzymatic tests. Out of 09 lactic acid bacteria, five isolates exhibited negative enzyme activity i.e., LG and LG12 while as LG 6 was only amylase positive, LG14,LG16 and LG22 were cellulase and xylanase positive. Rest all were positive for all the enzymes. Out of 09 isolates of actinomycetes AG15, AG16 and AG19 exhibited only amylase positive.AG9 was only xylanase positive, AG2 and AG14 were amylase and xylanase positive, AG11 were cellulase and protease positive. Rest AG4, AG6, AG13, AG18 and AG20 expressed all four enzymes. Out of 07 isolates of *Pseudomonas sp.* PG1 and PG7 showed negative enzyme activity.PG10 was only cellulase positive, PG18 was amylase and cellulase positive,PG3 was cellulase and protease positive. Rest PG5 and PG9 exhibited all enzymes positive.

4.2.1 Quantitative enzyme assay

The isolates that showed qualitative enzymatic tests positive were than examined for quantitative enzymatic tests. The isolates that overall, showed high enzyme activity were LG18, AG6, PG9 from Gurez (Tables 18,19 and 20)

4.3 Development and activation of microbial consortia

Cold tolerant bacterial consortium was developed by using various compatibility tests like antagonism, cross streak assay and spot test. To study the antagonistic properties of single isolates LG18, LG9,LG5,LG6,AG3, AG4, AG6, AG13, AG18, PG6, PG9, PG20 were used. Syntrophic relationships between different organisms have been demonstrated in several microbial ecosystems. Therefore, mixed inoculants (combination of microorganisms) that interact synergistically were currently being devised, which yield better and quick results (Table 21)

The selected bacterial isolates which showed compatibility with one other were accordingly selected for the development of five different consortia. For the preparation of consortia, the colony forming unit per milliliter (Cfu ml^{-1}) of actinomycetes, lactic acid bacteria, *Pseudomonas* sp. were kept as 1.0 x 10^6,3.0 x 10^8, 2.0 x 10^7 respectively. Composition of different consortia is presented in Table 22

A mixture of 3 kgs of Jaggery in 16 litres of chlorine free pure water was autoclaved at 10 lbs psi. for 10 minutes and cooled at room temperature for 2 hours. One litre of CT consortium was added into it in a clean plastic container, sealed air tight and placed in a cool dark place for 10-14 days for good fermentation. A white layer with sour odour of the CT consortia was a characteristic feature of the activated product for further use.

The colony forming unit of cold tolerant microbes in CTC1 viz lactic acid bacteria, actinomycetes, *Pseudomonas* sp. on seventh day when checked was 5.0 x 10^8, 3.0 x 10^6,3.0 x 10^7 Cfu ml^{-1} respectively and was found maximum on the fifteen day of activation as 7 x 10^8, 6.0 x 10^6, 7.0 x 10^7 respectively Cfu ml^{-1} respectively. In CTC2 consortium, lactic acid bacteria, actinomycetes, *Pseudomonas* sp on seventh day when checked was 4.0 x 10^8, 2.0 x 10^6,4.0 x 10^7 Cfu ml^{-1} respectively and was found maximum on the fifteen day of activation as 8 x 10^8, 8.0 x 10^6, 6.0 x 10^7 and 7.0 x 10^8 respectively Cfu ml^{-1} respectively. In CTC3 consortium, lactic acid bacteria, actinomycetes, *Pseudomonas* sp on seventh day when checked was 5.0 x 10^8, 2.0 x 10^6, 3.0 x 10^7 Cfu ml^{-1} respectively and was found maximum on the fifteen day of activation as 8 x 10^8, 5.0 x 10^6, 6.0 x 10^7 respectively Cfu ml^{-1} respectively. In CTC4 consortium, lactic acid bacteria, actinomycetes, *Pseudomonas* sp on seventh day when checked was 4.0 x 10^8, 5.0 x 10^6,4.0 x 10^7 Cfu ml^{-1} respectively and was found maximum on the fifteenth day of activation as 6 x 10^8, 7.0 x 10^6, 6.0 x 10^7 respectively Cfu ml^{-1} respectively. Similarly, In CTC5 consortium, lactic acid bacteria, actinomycetes, *Pseudomonas sp* on seventh day when checked was 4.0 x 10^8, 5.0 x 10^6, 5.0 x 10^7 Cfu ml^{-1} respectively and was found maximum on the fifteenth day of activation as 7 x 10^8, 7.0 x 10^6, 7.0 x 10^7 respectively Cfu ml^{-1} respectively and the results are presented in Table 23.The biochemical characteristics of five different consortia formed are presented in Table 24.

4.4. Solid waste management using CT consortium

4.4.1) *In vitro* biodegradation of agricultural waste

Agricultural waste was treated with five selected consortia CTC1,CTC2,CTC3,CTC4 and CTC5 under controlled conditions to select the best consortia. Various parameters studied are described as under:

4.4.1.1 Estimation of temperature and pH

The temperature of the composting waste material was measured after every three days and pH at an interval of seven days throughout the composting period.

During application of different selected consortia for the biodegradation of agricultural wastes at 10±2 ºC the initial temperature of the agricultural waste was 10 ºC which respectively increased to 44 ºC on 21st day, 46 ºC on 27th day and 47 ºC on 30th day and gradually decreased to 22 ºC, 19 ºC and 23 ºC on 70th day in case of CTC1,CTC2 and CTC3. Similarly, in case of CTC4 and CTC5 the initial temperature of agricultural wastes was 10 ºC which showed increasing trend up to 47 ºC and 43 ºC on 21st day and then a gradual decrease up to 20 ºC on 70th day of composting process respectively. However, the temperature in the control treatment (No CT used) increased from 10 ºC to 34 ºC on 33rd day and then gradually decreased up to 18 ºC on 70th day. The results are presented in Table 25

4.4.1.2. Variation in pH

Perusal of the data presented in Table 31 indicates that the initial pH values of all the treatment levels sharply increased which ranged from 6.4 to 6.9. It was found that in case of trials laid at 10± 2 ºC, the pH value in treatment control, CTC1, CTC2,CTC3,CTC4 and CTC5 reached up to 7.3 (on 14th day), 7.4 (on 14th day), 7.4 (on 14th day), 8.4 (on 14th day), 7.6 (on 14th day) and 7.5 (on 21st day) and showed decreasing trend towards the end to 6.7, 6.9, 6.8, 6.5 and 6.5 respectively. The results are shown in Table 26

4.4.1.3. Nutrient status

Nutrient status like organic carbon, available Nitrogen, available phosphorus and available potassium of the *in vitro* trials were estimated before treatment application and after thirty five and seventy days and data are depicted in Tables 427. It is evident from the data that CTC1, CTC2 and CTC3 showed overall maximum decrease in organic carbon and maximum increase in available nitrogen, available phosphorus and available potassium followed by CTC4 and CTC5.The three consortia namely CTC1, CTC2 and CTC3 were selected for further analysis under *in-vivo* conditions.

Discussion

1. Isolation of cold tolerant microorganisms:

The results obtained regarding the Isolation of cold tolerant microorganisms are discussed as under (plate 1)

1.1. Lactic acid bacteria

Lactic acid bacteria is an important group of bacteria being placed in group 19 with important biochemical characters that is gram's reaction, catalase negative, carbohydrate utilizing, casein hydrolysis as authenticated in Bergey's manual of Determinative Bacteriology (7th Edn). In our study, we isolated twenty two isolates from Gurez which were further processed and only nine were most promising cold tolerant isolates of lactic acid bacteria as of *Lactobacillus sp* Some of the lactic acid bacterial isolates also showed high enzyme activity.

Our results corroborate with that of Savard *et al.* (2002) [18] who found *Lactobacillus sp* most promising for starch degradation. Several researchers have reported that *Lactobacillus sp* has an ability to control fungi, produce more lactic acid and is effective for cellulose degradation (Pitt and Hocking, 1997; Jay, 2000; Strom *et al.*, 2002) [16, 20].

1.2. Actinomycetes

Actinomycetes have been frequently isolated from soil, water and mulberry rhizosphere. In our study, we isolated nineteen isolates from Gurez and Ladakh which were further processed and only eleven were most promising cold tolerant isolates of Actinomycetes and through biochemical, morphological and microscopic observations, the probable genus was identified as *Streptomyces sp*. Some of the Actinomycete isolates also showed high enzyme activity.

Actinomycetes could utilize a wide range of carbon sources which included glucose, arabinose, mannitol and sucrose and was thus included in cold tolerant microbial consortium. Inoculation of cellulolytic microorganisms such as Actinomycetes rapidly decrease the hemicelluloses and cellulose content in paddy straw composting. Streptomycetes strains decompose lignocelluloses. Our results corroborate with Kurt and Buyukalaca, (2010)

1.3 Pseudomonas sp.

Pseudomonas sp have a widespread occurrence in soil, water and plant seeds such as dicots etc. *Pseudomonas sp*. play an important role in decomposition of organic materials, especially in the early stages of decomposition when moisture levels are high. *Pseudomonas sp.* are also known as decomposer bacteria.

In our study, we isolated eighteen isolates of *Pseudomonas sp* from Gurez and Ladakh which were further processed and only seven were most promising cold tolerant isolates and were also studied by visual observation of colony, microscopic observation and biochemical tests. Bacterial growth depends upon various physiochemical conditions such as media, pH, temperature, incubation period, carbon source, etc. Our results corroborate with Berkeley and Campbelt (1972) [3]. *Pseudomonas sp* are also known as decomposer bacteria and are able to degrade cellulose faster (Crawford 1978; Thilagavati *et al*, 2006) [7] so they were used in preparation of cold tolerant consortia also.

2.Effective microbial consortium

2.1 Compatibility test

The compatibility of lactic acid bacteria, actinomycetes, *Pseudomonas sp* and *Bacillus sp*ecies was tested by dual and consortia inoculations *in vitro* and were found to be coexisting and complimentary. The consortia was developed only after knowing their compatibility. All the organisms were growing without interference probably because of non existence of any antagonistic effect among them. In these combinations, it is found that there is no antagonistic effect. There was only mutual or beneficial and synergistic effect among the organisms (Nandi *et al*., 2000).

After isolation, identification and screening of microorganisms, the already effective isolates of various microbial groups were mixed in different proportions to prepare microbial consortia with the names of CTC1, CTC2 and CTC3 and their microbial and physico-chemical characteristics were noted accordingly. The consortia comprises of lactic acid bacteria which enhance the breakdown of organic matter such as lignin and cellulose. *Pseudomonas sp* which releases bioactive compounds which act on the sewage and precipitates or detoxifies the metal. Actinomycete which produces antimicrobial substances from amino acids derived from organic matter for suppressing harmful fungi and bacteria and similar results shown by Monica *et al*., (2011)

3. Biodegradation of solid wastes (*in vitro*)

Agricultural waste was treated with five selected consortia CTC1, CTC2, CTC3, CTC4 and CTC5 under controlled conditions to select the best consortia. The results of various parameters studied are as under (Plate 2):

3.1 Temperature

The temperature during the aerobic composting is a microbiological phenomenon that shows a lot of fluctuation (Lo *et al.,* 1993). The composting process under aerobic conditions can be divided into three major cycles, a mesophilic-heating phase, a thermophilic phase and a cooling phase (Mustin, 1987). The temperature shows increasing trend during the first three weeks of the composting, during this phase the temperature increases and reaches about 40 to 50°C, due to the biodegradation of the organic compounds (Mustin, 1987; Castaldi *et al.,* 2005). At the end of the experiment the temperature shows a gradual fall, probably due to the depletion of the organic matter and during this phase the C/N ratio on different windrows tends to stabilize, and the temperature shows a steady state from there onwards (Daiz *et al.,* 1993).

The contribution in maximizing the temperature during the experiment by cold tolerant consortia CTC1, CTC2 and CTC3 (47, 47and 41°C on 30th day of biodegradation) in agricultural waste could be due to the high proportion of the degrading microorganisms. The temperature at the end of the experiment was results of Daiz *et al.* (1993).

3.2. pH

The pH of the composts is usually neutral to slightly alkaline probably due to the natural buffering of the humus. Throughout the degradation of the Solid wastes the pH showed variation from neutral to slightly alkaline and the results corroborated with the observations of Thompson and Troeh (1978) who reported that the compost was to some extent alkaline in nature and had a striking buffering capability. However the controls showed a slight change in the pH and remained largely neutral. Comparatively the composts that were produced by the treatment of the varying concentration of the isolates their pH was slightly alkaline.

In Cold tolerant consortia (CTC1, CTC2 and CTC3) treated waste the pH was observed maximum 7.18 as compared to their respective controls where it was 7.01 in agricultural and municipal wastes respectively.

4 Chemical characteristics

4.1 Carbon content

The decomposition rate of the compost materials can also be measured by observing the percent reduction in the organic carbon with time. From our observations, CTC2 treated wastes exhibited the maximum decline in carbon up to 36.73 from Gurez followed by CTC3 (36.90) and CTC1 (37.1) respectively towards the end of decomposition. Such a decrease in carbon content is due to the utilization of the organic matter by the organisms involved in its decomposition. Actinomycetes become active in the degradation process of the organic matter in the mesophilic phase (Fergus, 1964; Chang and Hudson, 1967). Results of this study have shown that the organisms present in CTC2 treated wastes in the mesophilic phase were more active in the decomposition process than in the thermophilic phase. The rate of decomposition of the organic matter ultimately depends upon the capability of microorganisms to break down the material under the changing environmental conditions. Similar results were also reported by Morus (2003) while working on decomposition of waste.

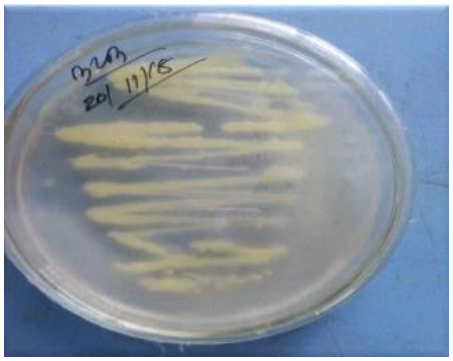
These observations corroborated with the results of Fergus (1964) and Chang and Hudson (1967). According to Bishop and Godfrey (1983), one third of the carbon utilized by organisms combines with nitrogen in the living cells while remaining is released as carbon dioxide.

4.2. Available N

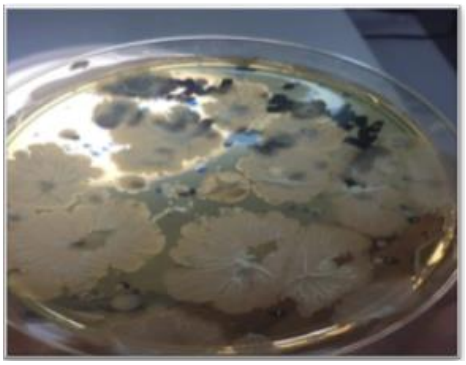
The available nitrogen content was maximum in CTC treated agricultural waste composts (1.29% from Gurez respectively), followed by their respective controls as 0.545%. It is noteworthy that the steady increase in nitrogen content was mainly because of higher loss of carbon by the metabolic reactions of aerobic microorganisms. The oxidation of the nitrogen finally gives nitrate and this would not be normally lost from the compost. Alexander (1961) has reported the presence of nitrogen fixing bacteria during microbial decomposition of wastes during the mesophilic phase which increases the nitrogen concentration to the compost. These findings also corroborate with the results of Chaudhuri *et al.* (2005).

4.3. Available phosphorus

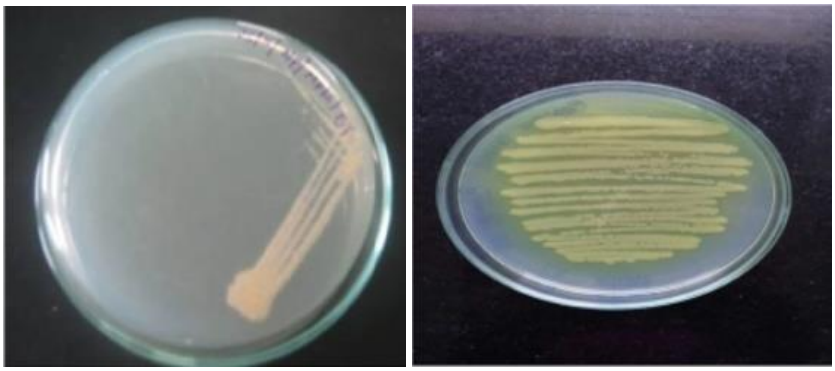
Available phosphorus content was maximum in CTC treated agricultural waste composts, 0.77% at Gurez, followed by its respective control as 0.57%. The small increase in phosphorus content is mainly due to the loss of carbon and organic matter mineralization in each compost. Arja and Mariha (1997) and Traore *et al.* (1999) put forth the similar hypothesis regarding the availability of phosphorus in composts.



Lactic acid bacterial isolate



Actinomycetes



Pseudomonas sp. *Bacillus* sp.

Plate 1: Isolation of cold tolerant microorganisms

Polybags used for *in vitro* biodegradation Pots used for *in vitro* biodegradation

In vitro assesment of pH and tempertures

Plate 2: *In vitro* technology for biodegradation of agricultural solid wastes using Cold Tolerant Consortia (CTC)

5. Conclusion

On the basis of results obtained during the present investigation, following conclusions could be drawn:

- Cold tolerant isolates of lactic acid bacteria, actinomycetes, *Pseudomonas sp* were isolated from Gurez region
- On the basis of enzymatic activities,5 isolates of lactic acid bacteria,7 of actinomycetes,5 of *Pseudomonas sp* were selected and five consortia were prepared on the basis of compatibility.
- Treatment of agricultural wastes with 5 consortia under *in-vitro* conditions indicated that cold tolerant consortia 1, 2 and 3 were more efficient.
- CTC1, CTC2 and CTC3, the three best consortia were used on agricultural waste at Gurez under *in-vivo* conditions conditions and performed better in decomposing the wastes with subsequent increase in available nutrients

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