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Culturable bacterial diversity and hydrolytic enzymes from drass, a cold desert in India

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Bacterial diversity of composite soil sample of drass was explored and screened for various hydrolytic enzymes. About 600 bacterial strains were isolated using six different growth media, that is, R2A, nutrient agar, King's B media, tryptic soy agar, Luria-Bertani agar and minimal media (100 isolates picked randomly from each media). These bacterial isolates were further differentiated on the basis of colony/cell morphology analysis, pigmentation and growth patterns. The 99 selected strains were subjected to amplified ribosomal DNA restriction analysis and the representative isolates from each cluster were chosen for 16S rRNA gene sequencing. Phylogenetic analysis led to the identification of 40 bacteria, grouped into three major phyla, Proteobacteria, Actinobacteria and Firmicutes differentiated into 17 different genera. These representatives were also investigated for hydrolases at low temperature (4-30°C). All the isolates secreted one or the other hydrolytic enzyme, that is, esterase (90%), lipase (80%), protease (32.5%), amylase (20%), cellulase (17.5%). These results indicate that culturable bacteria in soil of Drass could serve as an ideal candidate region for enzyme bioprospecting.

Key words: Pigment, drass, cultivable bacteria, phylogenetic diversity, enzyme production, soil.

INTRODUCTION

Microorganisms in the cold environments have received increasing attention during the past decade as they play a major role in food chains and biogeochemical cycles of these environments (Margesin and Miteva, 2011). Diverse bacteria have been recovered from polar environments such as Arctic and Antarctic. However, diversity in polar regions differ from several high-altitude regions such as the Himalayan ranges due to seasonal variations in temperature that results in different physical and biochemical properties. Studies on non-polar environments particularly Himalayan region have been largely carried out on glaciers and snow samples (Pradhan et al., 2010; Shivaji et al., 2011). There are very few reports on bacterial diversity of Himalayan hilly terrains. Microbes inhabiting these cold environments are extensively prospected for unique adaptabilities of their enzymes (de Pascale et al., 2008). Cold adapted enzymes have high catalytic efficiency and unique specificity at low and moderate temperatures, signify-cantly at higher rate than the mesophilic counterparts (Gerday et al., 1997). These enzymes offer economic benefits through energy savings as they wipe out the requirement for expensive heating step. Due to their distinctive properties, these enzymes have also

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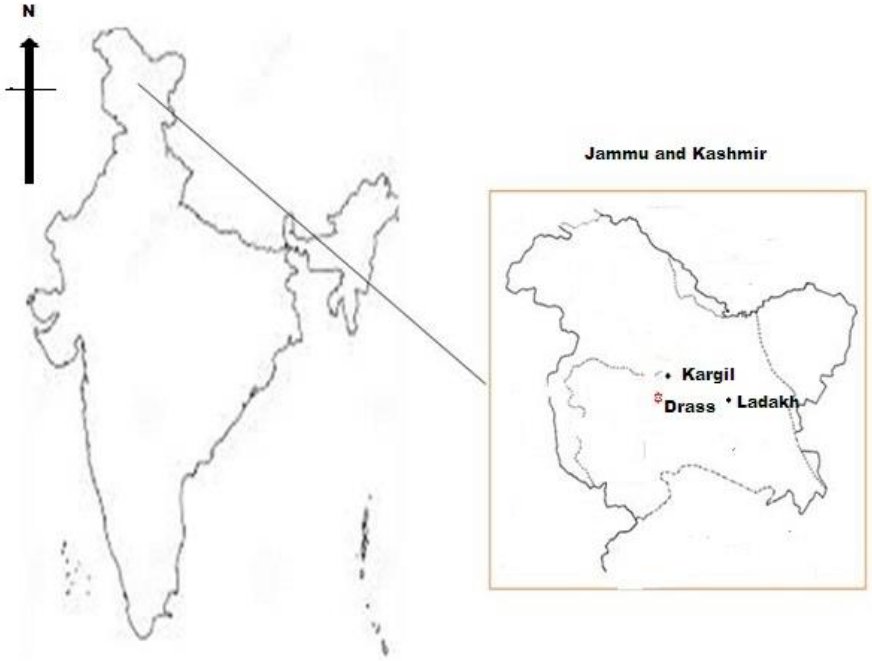

Figure 1. Outline map of Jammu Kashmir map showing the location of Drass (ladakh, J&K).

gained much attention regarding their potential for industrial and biotechnological applications, e.g cold adapted proteases are well suited in waste management in cold environments where the degradation capabilities of endogenous micro-flora are reduced due to low temperatures (Pulicherla et al., 2011). In industries, for dehairing of hides and skins using psychrophilic proteases not only save energy but also reduce the impacts of toxic chemicals used in de-hairing (Joshi and Satyanarayana, 2013). Amylases are one of the important industrial enzymes that have wide range of application such as food processing, fermentation and pharmaceutical industries. Cellulases are used in laundry detergents for exhibiting color brightness and removing the soil from cotton fibers, bio-polishing of fabric giving the finishing look of the product and producing stone washed look of denims (Aygan and Arikan, 2008; Sarvanan et al., 2013). Cellulases are gaining additional consideration in the enzyme market owing to their ability in the degradation of lignocellulosic biomass into biofuels and other products (Zhang et al., 2011). Lipases/esterases hold important position in the world enzyme market. The commercial use of lipases of cold origin is a billion dollar business. Psychrophilic lipases have attracted attention for synthesis of organic substances due to their inherent greater flexibility, whereas the activity of mesophilic and thermophilic enzymes are severely impaired by excess rigidity. These have great value for bioremediation and are widely used for degrading hydrocarbons present in contaminated soil (Aislabie et al., 2000; Paniker et al., 2006).

In the present study, an effort has been made to explore the bacterial diversity towards bioprospecting for hydrolytic enzymes from composite soil sample of Drass, located at 34.428152°N, 75.75118°E. It starts from the base of the Zojila pass (the Himalayan gateway to Ladakh), a trans-Himalayan region that separates the western Himalayan peaks from the Tibetan plateau (Figure 1). It is situated 60 km west of Kargil on the road to Srinagar with an average elevation of 3,280 m (10,764 ft) and experiences an altitude-influenced subarctic climate. The subarctic climate is characterized by long, usually very cold winters, and short, cool to mild summers. Winters start from mid-October and lasts in mid-May with temperature −22°C (-8°F) to as low as -45°C (-49°F) at the height of winter. Summers start in June and lasts till early September, with average temperatures near 15°C (59°F) and little precipitation. Annual precipitation is almost entirely concentrated in the months of December to May when Drass gets about 360 mm (14 inches) of snow.

MATERIALS AND METHODS

Collection of soil sample and sampling site

The soil samples (10) were collected from different regions of Drass mountains at 34.45°N, 75.77°E in North Himalayan range (J&K, Ladakh) during May 2010 and pooled into one composite sample. The soil was collected 1 cm deep into the earth by digging and collected in aseptic plastic bags (Shivaji et al., 2011). Hands, trowels were treated with 70% ethanol immediately before use. The samples were transported to the laboratory in ice and stored at -

20°C.

Enumeration and isolation of heterotrophic bacteria

One gram of sample was aseptically weighed and homogenized in 9 ml sterile physiological water (0.86% NaCl) by vortexing vigorously. Six different media namely R2A, Nutrient agar, King B agar, Tryptic soy agar, Luria-Bertani agar Minimal media were used to isolate bacteria by plating10^{-2} and 10^{-4} soil dilution with saline. All the growth media used in the present study were purchased from Himedia Pvt. Ltd India (Cat no. ≠ M962, M001, M1544, GM1151, M512, M290 respectively) and prepared according to the instructions given by the manufacture. The plates were incubated for 4-5 days in incubators at 4, 10, 20, 30°C temperature and the CFU/g of the soil was calculated.

Morphology and molecular identification

Preliminary taxonomic characteristics of the isolated bacteria were determined by colony morphology, pigment colour, growth pattern and biochemical analysis (Hamid et al., 2003). Pure cultures were cryopreserved in 50% glycerol at -80°C (New Brunswick, Effendorf). Genomic DNA was extracted by Hipura kit (Himedia, cat no.≠ MB505). Universal bacterial primers, namely Bac8f (AGTTTGATCCTGGCTCAG) & Univ529r (ACCGCGGCKGCTGGC) based on *Escherichia coli* positions, were used to amplify internal fragments of 16S rRNA gene that amplify ~500 bp (Fierer et al., 2007). PCR products were analyzed by electrophoresis on 1.5% agarose gel, followed by staining with ethidium bromide and visualization under UV light. The amplified PCR products were purified with a PCR product purification kit (Himedia cat no. ≠ MB512). ARDRA of the PCR products was done using restriction enzymes ALu I and Hha I to screen for duplicacy (Moreno et al., 2012). The unique bacterial isolates were sent for Sanger's DNA sequencing to Scigenome Labs Pvt. Ltd. (Cochin, India). For identification of closest relatives, sequences were compared to 16S sequences available in the GenBank (http://blast.ncbi.nlm.nih.gov) databases by BLASTn. The phylogenetic tree was constructed by MEGA 5 (http://www.megasoftware.net). The sequences that showed less than 98% homology with the reported sequences in the database were reamplified by bac8f (5-AGAGTTTGATCCTGGCTCAG-3) and 1492r (CGG TTA CCT TGT TAC GAC TT) corresponding to *E. coli* positions 8 to 27 and 1492 to 1509, respectively to amplify ~1500 bp region (Yong et al., 2011).

Diversity measures

OTUs at the 3% distance and Shannon-Wiener index (H) were calculated using Fastgroup11 tool (http://fastgroup.sdsu.edu/).

Screening for hydrolytic enzyme

Agar medium containing appropriate substrate and 1.5% agar (w/v) were inoculated with freshly grown cultures and incubated at 4, 10, 20, 30°C for 48 h. Different substrates for example 0.4% soluble starch (w/v), 0.4% (*w/v*) carboxymethylcellulose, 0.4% (w/v) tributyrin, olive oil (1%) and casein (0.4% w/v) were used for screening amylases, cellulases, esterase, lipases and proteases (Gangwar et al., 2009). For amylase and cellulase activity, incubated plates were developed by flooding the plates with iodine solution (1%) and washing with normal saline. For screening lipases, syringe filtrated olive oil (1%) and a florescent dye rhodamine B (0.001% w/v) was added to the autoclaved cooled growth medium with vigorous stirring. The plates containing bacterial cultures were observed for an orange fluorescence under UV light at 350 nm (Ranjitha et al., 2009).

RESULTS AND DISCUSSION

The present study is a first attempt to isolate and characterize the heterotrophic bacteria from soil of Drass, using different culturing conditions. Drass is the second coldest place in world after Siberia and its bacterial diversity (both cultivation dependent and independent) has not been unexplored so far. It is an established fact that cultivation based technique harvest only 1% of the bacteria and cultivation independent metagenomic techniques catalogue majority of the diversity. However, cultivation dependent conventional isolation techniques were employed as the aim of the present study was to isolate bacteria with hydrolytic activity that can be used commercially subsequently.

Bacterial isolation and characterization

Both oligotrophic and nutrient rich media were selected to obtain maximum cultivable bacteria. About 600 isolates were randomly selected (100 each from six different media: Nutrient agar, LB agar, King's B agar, TSA, Minimal media, R2A agar) used in the study. Since the average summer and winter temperature varies between 4-30°C, the bacteria were isolated within this temperature range. The growth pattern of individual bacterial culture were studied and placed into psychrophilic (4–20°C), psychrotrophic (4-30°C), and psychrotolerant mesophilic (4–37°C), mesophilic (25-40°C) groups (Sahay et al., 2013) (Table 1). Maximum bacterial load (including pigmented and non-pigmented 5.0± 0.07x 10^6 CFU/ml at 30°C CFU/ml was obtained using NA (Table 2) but maximum number of pigmented bacteria 2.9±0.17x10^6 were obtained with R2A media (Table 2). Pigment production was intense at 4°C and decreased with increase in incubation temperature which is in accordance with earlier studies on bacterial diversity of Puruogangri ice core (Zhang et al., 2008) and Himalayas (Venkatachalam et al., 2015). R2A is an oligotrophic medium and allows cultivation of many pigmented bacteria in particular that will not readily grow on fuller, complex organic media. R2A has been used to isolate bacteria from various cold environments e.g glaciers (Foght et al., 2004), marine surface waters (Agogue et al., 2005), ice cores (Zhang et al., 2008) and Antarctic soils (Dieser et al., 2010; Peeters et al., 2012). The pigments produced by these bacteria are reported to be carotenoids and has been co-related with cold adaptation of microorganisms by many workers (McDougald et al., 1998; Cho and Tiedje, 2000; Daniela et al., 2012; Mojib et al., 2013).

Diversity measures

Diversity indices were used to compare between the

Table 1. Taxonomic affiliations and phenotypic characterization of bacteria isolated from soils of Drass (J&K, Ladakh) determined by sequencing of 16S rRNA.

Close representative Organisms/ group	Phylum	Accession no.	Media used	Temperature range (°C)	Colony description
Pseudomonas vranovensis Dr1	Gammaproteobacteria	KF555604	King,s B	Psychrotolerant mesophilic	Pale yellow
Pseudomonas putida Dr2		JX978885	King,s B	Psychrotolerant mesophilic	Pale yellow, transparent
Pseudomonas fuscovaginae Dr5		JX978887	King,s B	Psychrotolerant mesophilic	Pale yellow, transparent
Pseudomonas stutzeri Dr12		KF555605	R2A	Psychrotolerant mesophilic	Yellow, wrinkled
Pseudomonas mandelii Dr13		JN088486	Kings B	Psychrotrophic	Yellow, smooth
Pseudomonas psychrotolerans Dr17		KF555606	R2A	Psychrotrophic	Yellow, wrinkled
Pseudomonas brenneri Dr29		KF555610	Kings B	Psychrotrophic	Yellow
Pseudomonas frederiksbergensis Dr27		KF555608	LB	Psychrotrophic	Pale yellowish
Acinetobacter calcoaceticus Dr4	Alphaproteobacterria	JX978886	LB	Psychrotolerant mesophilic	White, slimy
Acinetobacter spp. Dr11		JX978891	LB	Psychrotrophic	cream
Acinetobacter radioresistens Dr25		JX978884	NA	Psychrotolerant mesophilic	cream
Serratia proteamaculans Dr7		JX978888	LB	Psychrotolerant mesophilic	White, slimy
Pantoea agglomerans Dr31		KF555611	R2A	Psychrotolerant mesophilic	Dark yellow
Pantoea agglomerans Dr46		KM188063	R2A	Mesophilic	Yellow
Paracoccus marcusii Dr32		KF555612	R2A	Psychrotolerant mesophilic	orange
Bacillus safensis Dr6	Firmicutes	KF682429	LB	Mesophilic	White Opaque
Bacillus cereus Dr8		JX978889	LB	Mesophilic	Cream slimy
Bacillus atrophaeus Dr14		JX978892	NA	Mesophilic	Brownish black, rough
Bacillus simplex Dr18		JN088488	NA	Psychrotolerant mesophilic	Cream slimy
Bacillus spp. Dr22		JN088491	NA	Psychrotrophic	Cream
Bacillus vallismortis Dr38		KF555618	LB	Mesophilic	Black
Bacillus thuringiensis Dr45		KF555624	NA	Psychrotrophic	Off-white
Staphylococcus equorum Dr34		KF555614	LB	Psychrotolerant mesophilic	White
Sporosarcina psychrophila Dr35		KF555615	MM	Psychrotolerant	Beige, shiny
Sporosarcina psychrophila Dr41		KF555620	R2A	Psychrotrophic	Brownish
Exiguobacterium sibiricum Dr19		JX978893	MM	Psychrotrophic	Orange, smooth
Exiguobacterium undae Dr28		KF555609	R2A	Psychrotrophic	Light orange
Planomicrobium koreense Dr24		JX978895	TSA	Psychrotolerant mesophilic	Orange
Arthrobacter agilis Dr16	Actinobacteria	JX978896	R2A	Psychrotrophic	Rose red, smooth
Arthrobacter crystallopoietes Dr37		KF555617	R2A	Psychrotrophic	Light yellow
Mycetocola reblochoni Dr23		HE774268.1	R2A	Psychrotrophic	Yellow, smooth
Mycetocola reblochoni (Dr42)		KF555621	TSA	Psychrotolerant mesophilic	Light yellow
Kocuria Polaris (Dr20)		KF682428	R2A	Psychrotrophic	Red and smooth
Kocuria rosea (Dr33)		KF555613	R2A	Psychrotrophic	Dark pink

Table 1. Contd.

Rhodococcus erythropolis (Dr10)	JX978890	MM	Psychrotrophic	Pale yellow, slimy
Rhodococcus qingshengii (Dr21)	JX978894	R2A	Psychrotolerant mesophilic	Light pink
Rhodococcus erythropolis (Dr36)	KF555616	MM	Psychrotrophic	White, very slimy
Citricoccus alkalitolerans (Dr40)	KF555619	R2A	Psychrotolerant mesophilic	Light yellow
Dietzia schimae (Dr43)	KF555622	TSA	Psychrotolerant mesophilic	reddish orange
Micrococcus luteus (Dr44)	KF555623	NA	Mesophilic	Yellow

NA, Nutrient agar; LB, Luria-Bertani agar; MM, minimal media; TSA, tryptic soy agar.

Table 2. Bacteria load (pigmented and non-pigmented) on six different media at different temperatures.

Growth media	C.F.U at 4°C		C.F.U at 10°C		C.F.U at 20°C		C.F.U at 30°C	
	(P)	(NP)	(P)	(NP)	(P)	(NP)	(P)	(NP)
R2A	0.5±0.15 x10^6	0.2±0.25 x10^6	1.2±0.05 x10^6	0.8± 0.02x10^6	2±0.09 x10^6	0.8± 0.02 x10^6	2.9±0.17x10^6	1.2±0.0 3x10^6
NA	0.07± 0.02 x10^6	0.13 ± 0.02 x10^6	0.1±0.01x10^6	0.7±0.2x10^6	0.1± 0.01 x10^6	0.9 ± 0.03x10^6	1.0± 0.01x 10^6	4±0.06 x10^6
LB	0.18±.10 x10^6	0.22±0.05x10^6	0.4± 0.02x10^6	1.6±0.06 x10^6	0.6± 0.01x10^6	2.4±0.1x10^6	0.9± 0.07x10^6	3±0.10 x10^6
Kings B	0.3±.10x10^6	0.1±0.07 x10^6	0.4± 0.16x10^6	0.2 ±0.13x10^6	0.6 ±0.3 x10^6	0.2± 0.05 x10^6	1.4±0.05x10^6	0.8±0.02 x10^6
TSA	0.2± 0.10x10^6	0.3± 0.12x10^6	0.3± 0.02x10^6	0.6±0.03x10^6	0.5±0.02x10^6	1.5±0.05 x10^6	1.0 ±0.13x10^6	2.3±0.10 x10^6
MM	0.2± 0.09 x10^6	0.1± 0.05 x10^6	0.4 ±0.15x10^6	0.1± 0.05x10^6	0.6± 0.3 x10^6	0.2±0.1 x10^6	1.8± 0.040 x10^6	0.4±0.005x10^6

C.F.U counted as cells/ml; Experiments were conducted in triplicates and the data are expressed as mean± SD.

communities obtained by using different media. More community complexity was found using R2A media (Figure 2 and Table 3). Overall Shannon-Wiener index (H) was 3.2, that is in accordance with previous reports from Himalayan bacterial diversity (Pradhan et al., 2010; Shivaji et al., 2011, Yadav et al., 2014).

Phylogenetic analysis of 16S rDNA sequences of isolates

Bacterial isolates were screened for duplicacy by colony/cell morphology analysis, pigmentation, conventional biochemical tests that narrowed the 600 isolates into 99 isolates. These selected isolates were subjected to 16S rRNA gene amplification followed by restriction digestion with Alu I and Hha I. On the basis of ARDRA profiling, representative isolate from each cluster were sequenced and the nucleotide sequences were deposited in the NCBI GenBank database (Accession numbers: JX978884-JX978891, JX978892-JX978896, JN088486, JN088488, JN088491, KF555604-KF555606, KF555608-KF555624, KF682428, KF682429 and HE774268, KM188063). The nearest phylogenetic neighbor of all the 40 representative isolates were identified through BLAST analysis of the 16S rRNA gene sequences against nucleotide database available in the National Centre for Biotechnology Information (NCBI) (Table 1).

Drass isolates represented both Gram-positive and negative heterotrophic bacteria belonging to three major phylogenetic groups organized into three clusters, Proteobacteria (37.5%), Firmicutes (32.5%) and Actinobacteria (30%) (Figure 3). Proteobacteria dominates (37.5%) the culturable bacterial diversity of Drass with Gammaproteobacteria (35%) as the dominant class represented by genera *Pseudomonas, Acinetobacter, Serratia* and *Pantoea.* Pseudomonads represented the dominant genera among Gammaproteobacterium. Alphaproteobacteria is however represented by single genera, that is, Paracoccus (Dr32) (Figure 3). The results are in accordance with the previous studies on Himalayan that reports Firmicutes, Actinobacteria and Proteobacteria as the most common phylum (Shivaji et al., 2011).

Bacterial isolates showed 99% similarity with the reference sequences in the Genbank except for Dr 46 that showed 96% similarity with *Pantoea agglomerans* (Figure 4). DNA-DNA hybridization

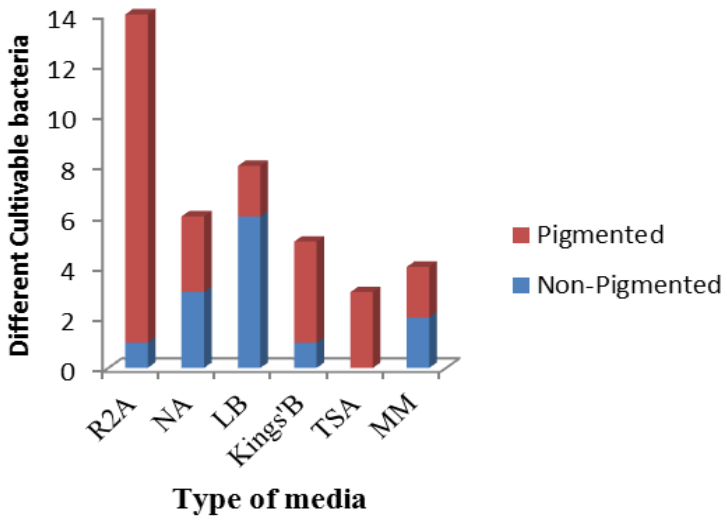

Figure 2. Diversity of pigmented and non-pigmented bacteria on six different media. NA, Nutrient agar; LB, Luria-Bertani agar; MM, minimal media; TSA, tryptic soy agar.

Table 3. OTUs and Shannon-Wiener index represented by bacteria on different growth media.

Growth media	OTUs	Shannon-Wiener index
R2A	14	2.54
LB	8	2.07
NA	6	1.79
Kings B	5	1.60
Minimal media	4	1.38
TSA	3	1.09

NA, Nutrient agar; LB, Luria-Bertani agar; MM, minimal media;TSA, tryptic soy agar.

will be carried with close relatives to confirm and publish as novel species. The bacteria isolated and characterized from Drass soil have been reported from other cold environments also. The genera *Arthrobacter, Bacillus, Sporosarcina, Rhodococcus, Pseudomonas* were reported in the culturable bacterial diversity of Pindari glacier (Shivaji et al., 2011). The genera *Acinetobacter, Bacillus, Pseudomonas* were reported in the culturable bacterial diversity of Kafni glacier (Srinivas et al., 2011). The genus *Arthrobacter* is the dominant bacteria in Qinghai-Tibet Plateau permafrost (Zhang et al., 2007), *Brevibacterium* and *Acinetobacter* are present in abundance in Dry Valley soils of Antarctica (Cary et al., 2010), *Planomicrobium, Mycetocola, Rhodococcus, Sporosarcina* have been reported from Himalayan soils in India and Nepal (Venkatachalam et al., 2015) and an Arctic glacier (Reddy et al., 2009). *Exiguobacterium* (Gram positive and facultatively anaerobic) have been repeatedly isolated from ancient Siberian permafrost (Rodrigues et al., 2009). Members of genera *Exiguobacterium* are adapted to long-term freezing at temperatures as low as -12°C where intracellular water is not frozen and grow at subzero temperatures, displaying several feature of psychrophiles, such as membranes composition. Genus *Pantoea* (Selvakumar et al., 2008; Venkatachalam et al., 201**5**), *Dietzia* (Mayilraj et al., 2006), *Staphylococcus* and *Citricoccus* (Yadav et al., 2015) have been reported from Indian Himalayas. Members of the genus *Paracoccus* have been reported from Qinghai-Tibet Plateau permafrost (Zhu et al., 2013).

Extracellular hydrolytic enzyme activity

Cold-active enzymes from microbial sources have potential applications in biotechnology, agriculture and medicine (Feller, 2007; Tropeano et al., 2012; Moreno et al., 2013). The representative isolates were screened for their extracellular hydrolytic enzyme activity viz., esterase, lipases, protease, amylase and cellulose.

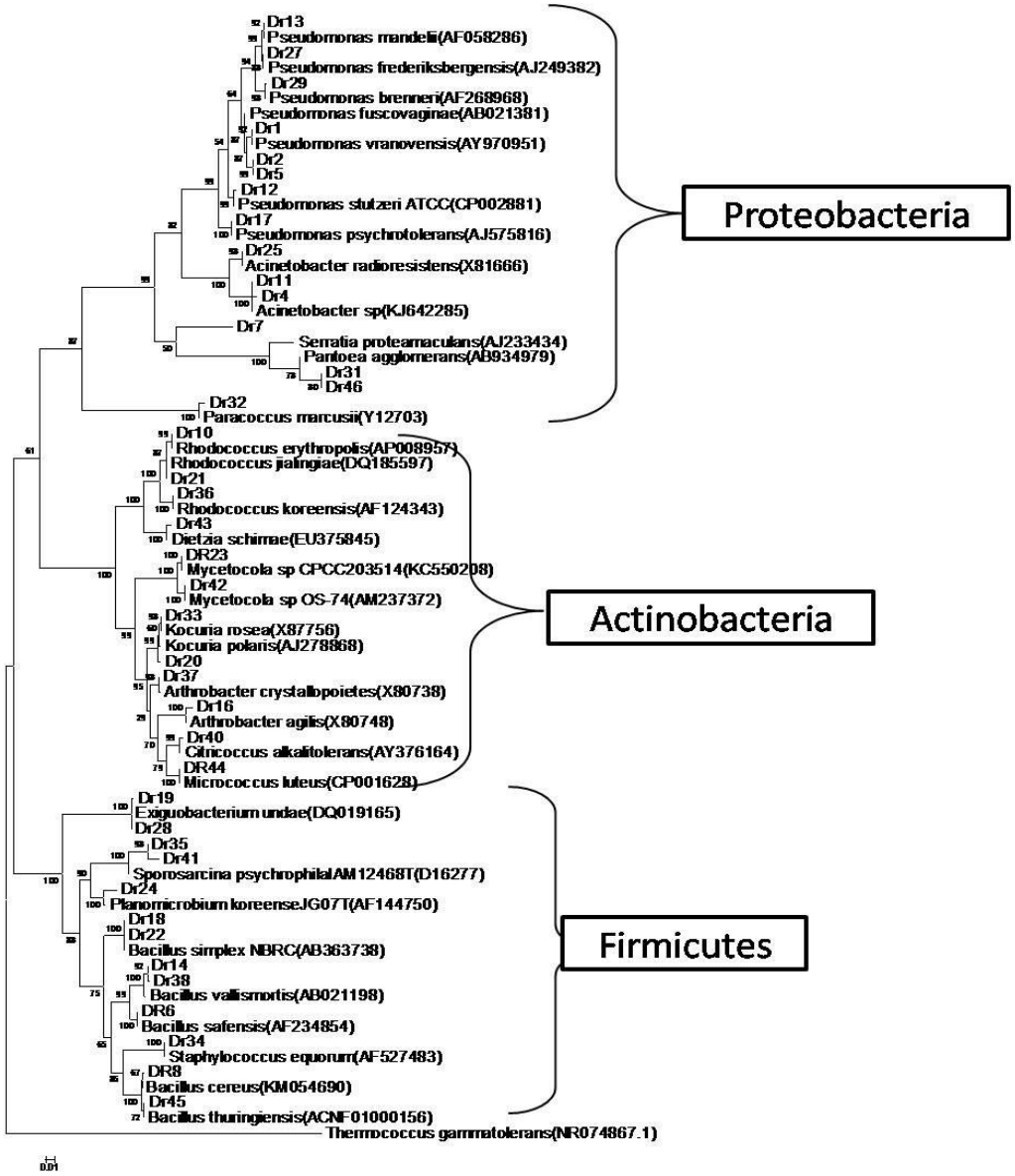

Figure 3. Maximiun likelihood phylogenetic tree of 16S rRNA gene from a soil sample of Drass (J&K, Ladakh). Bar, 0.01 substitutions per nucleotide position. *Thermococcus gammatolerans* was used as an outgroup

Interestingly, 90% of the isolates were esterase producers and out of them 80% were lipase producers, 32.5% were protease producers, 20% were amylase producers and 17.5% are cellulose producers. The comparative profile of the hydrolytic enzymes produced by bacterial isolates of Drass has been represented (Figure 5). *Arthrobacter agilis* Dr16 and *Kocuria Polaris* Dr20 were the best esterase producers and the enzymes produced were active at low temperatures (10°C). *Mycetocola reblochoni* Dr23 and *Planomicrobium koreense* Dr24 though best protease producers, do not degrade skimmed milk below 20°C. *Bacillus cereus* Dr8 and *Acinetobacter radioresistens* Dr25 were multiple hydrolases producer at low temperatures (10°C). The results clearly indicated that these enzymes can be characterized for exploitation at industrial level. Gangwar and coworkers 2009 worked on the bacterial diversity isolated from soil samples from the western Himalayas, India and reported that 62% of the bacterial isolates produced lipase followed by protease, 54%, Amylase 28% and only 11% have cellulase activity (Gangwar et al., 2009). *Pseudomonas, Bacillus, Arthrobacter, Exiguobacterium, Mycetecola, Pantoea, Acinetobacter* and *Serratia* have been identified as hydrolytic enzyme producer in previous study on Himalayas (Salwan et al., 2010; VenKatachalam et al., 201**5**). Although, in addition other genera especially *Kocuria, Rhodococcus* and *Planomicrobium* isolated in our study also showed hydrolytic enzyme activity.

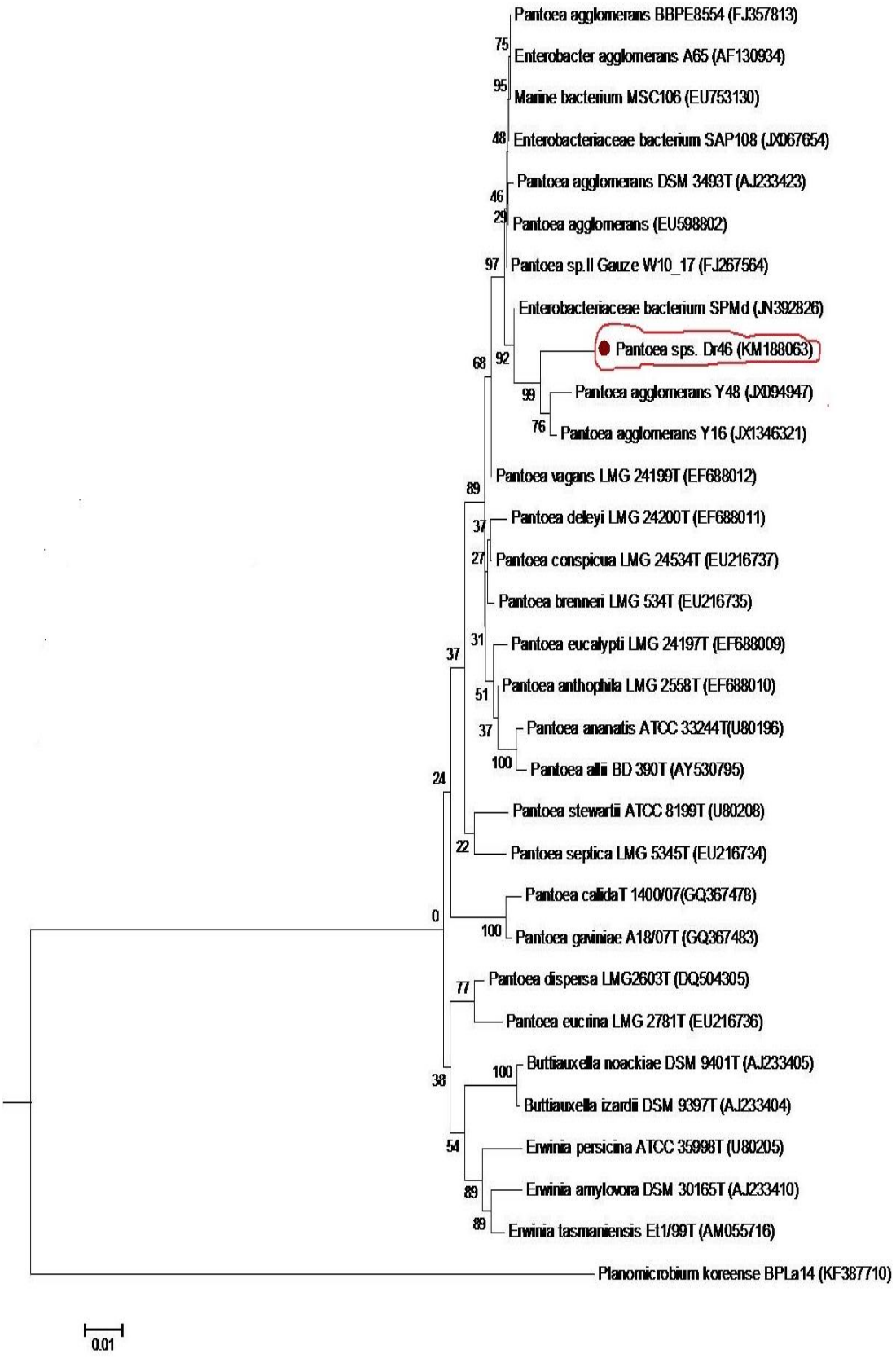

Figure 4. Neighbour joining tree based 16S rRNA sequences showing the positions of strain Dr46 *Pantoea* sp. and represenatives of some other taxa. Bar, 0.01 substitutions per nucleotide position. *Planomicrobium Koreense* BPLa14 was used as an outgroup.

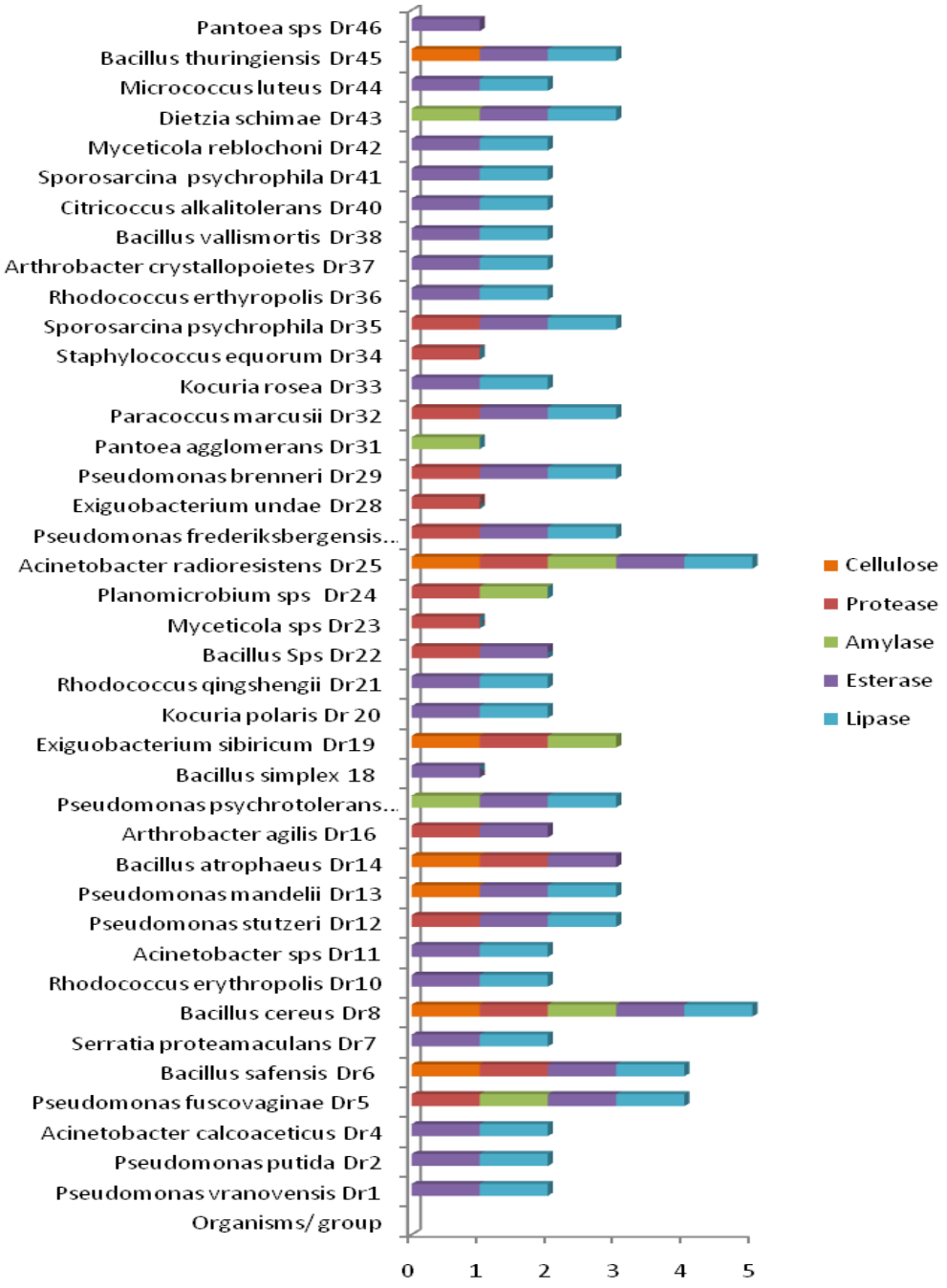

Figure 5. Enzyme profile of bacterial isolates from Drass (J&K, Ladakh).

Conclusion

Though earth is dominated by cold habitats but most of the diversity studies and bioprospecting has been done either on thermophiilic/thermotolerent or mesophillic bacteria. Recently, focus on exploring cold environments for diversity and bioprospecting has increased due to energy concerns and ecological reasons. Drass, located in the Western Himalayas, the second coldest place in India was found to be a rich source of novel bacteria and their produce. Interestingly Dr46 has low percentage similarity with the reference strain *Pantoea* and could probably be new species. The low percentage of probable novel bacteria isolated despite using six media and starting from 600 isolates suggests that more media formulation need to be tried and larger population needs to be characterized. Further screening for hydrolytic enzyme activity resulted in screening of some of the

multiple hydrolase producers for industrial use and not merely cataloguing them as is possible in the cultivation independent approach.

Conflict of interest

The authors declare that there is no conflict of interest.

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