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A Population Study of Psychrophilic *Actinomycetes* Isolated from Rothang Hill-Manali Soil Sample

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Total thirteen Psychrophillic actinomycetes colonies were isolated from ice point region. Among these twelve are facultative psychrophile and the strain designated RH-2 is obligatory psychrophile. All the isolated Actinomycetes are morphologically distinct on the basis of pigment production, colour of spore, types of mycelium and biochemical properties. Thirteen isolates were assigned in to the genus Micromonspora (4) Streptomyces (5) Microploy spora (1) Intrasporangium (1) Dactylsporangium (1) Plaromomospora (1). Among these, 12 isolates are capable to produce both aerial and substrate mycelium where the strain RH2 produce substrate mycelium only.

Key words: Cryophiles, Sudanblack, Mycelium, DAP and SCNA.

Psychrophiles or cryophiles are extremophilic organisms that are capable of growth and reproduction in cold temperature.the environment they inhabit, are ubiquitous on earth, as a large fraction of our planetary surface experience temperature lower than 15^0C. Psychrophiles are characterizes by lipid cell membrane chemically resistant to the stiffeing caused by extreme cold and often create protein antifreeze to keep theire internal space liquid and protect theire DNA even in below water freezing point (Richard morita and Craig moyer, 2004). Actinomycetes represented as prolific secondary metabolite producers represents a ubiquitous group of microbe widely distributed in natural ecosystem around the world and especially significant for their role in the recycling of organic matter (Srinivasan, 1991).

Rare actinomyetes are become an increasingly attractive source in the search for new antibiotic to fight drug resistance (Lazzarini *et al.,* 2000) the diversity of terrestrial actinomycetes has been of extraordinary significance in several area of science and medicine. The soil represent an environment for microbial discovery (Hala *et al*., 2007) the actinomycetes have provide many important bio active compounds of high commercial value and sre being routinely screened for new bio active substance (Okami *et al*., 1988). About 61% of all bioactive compounds were obtained from Steptomycetes genus and also from other rare (non Streptomycetes) actinomycetes (Monachava *et al*., 2002) the majority of actinomycetes are free living, saprophytic and colonizing plants. Actinomycetes population has

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been identified as one of the major group of population which may vary with soil type (Kuster, 1968).

Isolation of Actinomycetes

One gram of air dried soil was where collected at ice point of Manali on December 2008 was suspended in 9 ml of 1.5% phenol solution for 30 minutes to kill fast-growing soil bacteria. A soil suspension (0.1 ml) was spread onto Actinomycetes isolation agar containing cycloheximide at final concentrations of 10 mg/ml, respectively. Plates were incubated at 12^0C for 20 days. Actinomycete isolates were maintained on Starch Casein Nitrate Agar.

Media and culture identification

Starch casein agar medium [g/l:starch 10, casein 0.3, KNO_3 2, NaCl 2, K_2HPO_4 2, $MgSO_4.7H_2O$ 0.05, $CaCO_3$, 0.02, $FeSO_4$ $7H_2O$ 0.01 and agar 18; and Actinomycetes agar (Himedia, Mumbai) supplemented with Cycloheximide (Himedia, Mumbai) 10 mg/ml] was used for the study of cultural characters of isolated actinomycetes (Kuster and Williams, 1964). Purified isolates of actinomycetes were identified using morphological and cultural characteristics by the methods as described in the International Streptomyces Project (ISP) (Shirling and Gottlieb, 1966). The morphology of the spore bearing hyphae with the entire spore chain, the structure and arrangement of the spore chain with the substrate and aerial mycelium of the actinomycetes were examined the method described by Williams *et al.,* (1989). The method is slightly modified with slide culture then slides are stained with sudanblack latter examined under Nikon photomicroscope. Colour of spore mass was visually estimated by using the colour chart (Pridham, 1965). The similar colonies are differentiated with the help of biochemical characters.

Cell wall analysis

The isomers of Diaminopimelic acid and whole cell organism sugars were examined using standard procedures described by Hasegawa *et al*. (1983). 5 Mg dry weight of sample was hydrolysed in 1ml of 6N HCl and kept at 100^0 C for 18 hr then filtrated, evaporated and dried three times on a boiling water bath to remove HCl and taken up in 0.3ml for differentiating isomers of Diaminophimilic acid (DAP) 10 µl of hydrolysate were spoted on paper for descending chromatography**.**

Biochemical properties

All the isolates are Charecterised by IMViC, Urease, catalase, Oxidase and Utilization of carbohydrates such as Arabinose, Xylose, Sucrose, Maltose, Mannose and Inostol.

RESULTS AND DISCUSSION

The soil sample had a pH 7.2 with the organic matter and total nitrogen about 26.4% and 12.4% respectively. Totally thirteen actinomycetes were isolated on Actinomycetes

Table 1. Growth at different temperature of isolated *Psychrophilic actinomycetes*

Strain Code	Growth at 0°C	Growth at 15°C	Growth at 35°C
RH1	Negative	Positive	Positive
RH2	Positive	Positive	Negative
RH3	Negative	Positive	Positive
RH4	Negative	Positive	Positive
RH5	Negative	Positive	Positive
RH6	Negative	Positive	Positive
RH7	Negative	Positive	Positive
RH8	Negative	Positive	Positive
RH9	Negative	Positive	Positive
RH10	Negative	Positive	Positive
RH11	Negative	Positive	Positive
RH12	Negative	Positive	Positive
RH13	Negative	Positive	Positive

isolation agar and designated in to RH-1 to RH-13(Rot hang hill). Except RH-2 all other strains are grown well under 15°c and 35°c. The RH-2 does not grown at 35°C hence it known to be an obligatory psychrophile (Table 1). Based on their types of mycelium, hyphae and spore the isolated strains are placed under the genus Micromonaspora, Intrasporangium, Planomonaspora, Streptomyces and Dactysporangium (Table 2). All the isolated strains grown very well on SCNA medium. The growth on actinomycetes agar comparatively

Table 2. Study of spore and morphology of isolated Psychrophilic actinomycetes

Strain Code	Type of mycelium	Spore mophology	Possible genus
RH1	AM/SM	Rarely branched,septate hyphae,monospore	Micromonospora sp
RH2	SM	Long vegitative cells	Dactylsporangium sp
RH3	AM/SM	Oval intergallery vesicle, zygospore	Intrasporangium
RH4	AM/SM	Irrugulr branched, non fragmented,rarely septate	Planpmonospora
RH5	AM/SM	Spiral chain of spores	Streptomyces sp
RH6	AM/SM	Medium length chain of spores	Streptomyces sp
RH7	AM/SM	long chain spore	Streptomyces sp
RH8	AM/SM	Rrarely branched spiral spore	Streptomyces sp
RH9	AM/SM	Septate hyphe with Monospore	Micromonospora sp
RH10	AM/SM	Septate hypahe with monospore	Micromonospora sp
RH11	AM/SM	Long chain spore	Streptomyces sp
RH12	AM/SM	Rarely branched, Fragmented hyphae, chains of spore.	Micropolyspora sp
RH13	AM/SM	Monospore,Septate hyphae	Micromonospora sp

Table 3. Growth of isolted Psychrophilic actinomycetes on Actinomycetes agar and Starch Casein Nitrate Agar

Strain code	Strain	Growth on actinomycetes agar	Growth on starch casein agar
RH1	A1	Light ash, powdery, slightly rough, pale yellow pigmentation(non diffusable)	Greenish grey, powdery, rough, yellowish green pigmentation(non diffusable)
RH2	A2	Mucoid, white, no pigmentation	Mucoid, white, no pigmentation
RH3	A3	White, powdery, moist, no pigmentation	Ash, powdery, pale yellow pigmentation
RH4	B	Chalky white, powdery, pale red wine, diffusable	Ash, powdery, diffusable brown pigment
RH5	C1	Lightgreenish ash to white, rough, no pigmentation	Dark greenishash, powdery, no pigmentation
RH6	C2	Grey, powdery, slightly rough, no pigmentation	Grey, fine powdery, blackish brown pigmentation
RH7	D	Sandal white, powdery,no pigmentation	Sandal, powdery, no pigment
RH8	E	Ash, powdery, no pigmentation	Grey, powdery, brown pigmentation
RH9	F	Dull white, , non diffusable pale yellow pigment	Grey, powdery, yellowish brown,diffusable
RH10	G	White fine powdery, pinkish red wine pigmentation	Whitish ash,powdery, diffusable red
RH11	H	Fine powdery, sandal white, no pigmentation	Dark ash, powdery, no pigmentation
RH12	I	White powdery, , non diffusable pale yellow pigment	Grey, powdery, diffusible yellowish brown pigmentation
RH13	J	Whitish ash, pale yellow pigment, non diffusable	Dark grey, powdery, pink pigmentation

slower than the growth on SCNA (Table 3). The isolates are differentiated based on their cell wall composition and biochemical properties (Table 4).

The study shows the presences of the presence of rare genera in the psychrophilic region were not reported in the previous study by any one is Planomonospora and Micropolyspora. The predominant genus ever studied in the actinogroup is Streptomycetes is also reported by our study. Nearly five *Streptomycetes* species were isolated. The second most isolated genera is Micromonospora. The genus Streptomycetes and Micropoluspora produce similar short spores and differentiated by fragmenting vegetative mycelium and short chains of spore on aerial and substrate mycelium produced by Microplyspora (Thirumachalar and Sukapure, 1964). The isolates Dactylsporangium, micropolyspora and planomonospora are very rare isolated actinomycetes and may produce noval biologically active metabolites. differentiation of isolates in a genus was noted by the colour of spore and it biochemical properties. The geneus Streptomycetes and Micromonospora having species are differing each other noted by their morphology.

Among the four Micromonospora sp (RH1,RH9, RH10 and RH13) only two species are starch hydrolysis positive but all the 5 of Streptomyces are having ability to utilze starch.

Table 4.1. Bio chemical properties of isolated Psychrophilic actinomycetes

Strain	Grams stain	AFB	Catalase	Oxidase	Starch	Urease	Indole	MR	Vp	Citrate
RH1	+	-	+	+	+	+	++	+	++	-
RH2	+	-	+	+	-	-	++	+	+	-
RH3	+	-	-	-	++	+	++	-	-	-
RH4	+	-	-	-	++	-	++	+	-	-
RH5	+	-	+	+	+	-	++	+	++	-
RH6	+	-	-	+	++	+	++	++	++	-
RH7	+	-	-	+	++	-	++	+	-	-
RH8	+	-	+	+	++	-	++	-	-	-
RH9	+	-	-	-	-	+	++	+	+	-
RH10	+	-	+	+	-	+	++	-	++	-
RH11	+	-	-	+	++	+	++	+	++	-
RH12	+	-	-	+	++	-	++	++	+	-
RH13	+	-	-	+	++	+	++	++	++	-

Table 4.2 . Utilization of various carbohydrates by isolated Psychrophilic actinomycetes

Strain Code	Arabinose	Mannose	Maltose	Lactose	Inositol	Sucrose
RH1	-	++	++	++	+	++
RH2	-	++	-	++	++	++
RH3	+	++	++	++	++	++
RH4	-	+	-	++	++	++
RH5	-	+	-	++	++	++
RH6	-	+	+	++	++	++
RH7	-	+	++	++	++	++
RH8	-	++	-	++	++	++
RH9	-	++	++	++	++	++
RH10	+	-	++	++	++	++
RH11	+	+	-	++	++	++
RH12	-	+	++	++	++	++
RH13	-	+	++	++	++	++

+ : weakly positive ++ : Positive- -: Negative

All the isolated Micromonospora sp are urease positive. Isolated Streptomyces are shows difference in urease utilization. Only RH6 and RH 11 Streptomyces shows Urease positive and rest of the three Stretomyces are urease negative. The differentiation of species among the genus can be done by theire morphological and physiochemical properties.

Among the four different novel isolated Psychrophilic *Actinomycetes* such as Dactylsporangium sp (RH2), Intrasporangium sp (RH3), Planamonospora sp (RH4) and Micropolyspora sp(RH12) the genus Dactylsporangium only starch negative in nature. Among this four genera genus *Planomonaspora* is the only urease positivae genera.

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