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LATION, IDENTIFICATION AND MORPHOLOGICAL ARACTERISTICS AND THEIR PHOSPHATE UBILIZING ACTIVITY OF SOIL ALKALPHILIC TIONOMYCETES FROM AGRICULTURAL SOIL AT NAR CRATRE

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ABSTRACT

An alkaliphlic actinomycetes was sequestered from a desert soil sample of lonar, Dist. Buldhana. The isolate was detected to produce, white, grey, milky white (cotton) colour colonies are obtained from soil sample. These strain produced aerial and substrate mycelium comprising of chain or smooth spore. The colonical growth of strain varied from yellow to grey. All the isolate were later purified and imperiled to a few phosphatic enzymatic screening. Result indicate that number of isolates showed the ability to solubilize phosphate.

RODUCTION

nomycetes are gram- positive Bacteria viewing a entous development like fungi.They are aerobic and nsively extent in nature. Actinomycetes are iologically miscellaneous group, as evident by their uction of frequent extra cellular enzymes and by the sands of metabolic yields they produce. Actinomycetes A are rich in G+C content with GC% of 57-75%. They n in dry alkaline soil Actinomycetes have been well wn for the making of secondary metabolites. Many biotics are currently and used such as streptomycin, itamycin and erythromycin are the product of inomycetes. The acinomycetes are important not just he pharmacological industries but also the agriculture. ntification of actinomycetes using microscopic hniques alone was not enough to confirm inevitability. logical methods would be best method to indentify tinomaycetes to their type. After isolating an inomycetes it is primarily acknowledged on the basis morphological characters so has to have preliminary termination of genus. Microbial natural products have en one of the major incomes for detection of novel drugs. nong the potential sources of natural products, bacteria ve been proven to be a prolific source with a surprisingly all group of taxa accounting for the vast majority of mpounds discovered. Of the 22,000 recognized bacterial condary metabolites, 70% are produced by tinomycetes, and two thirds of them are contributed by e genus *Streptomyces* (Subramani and Aalbersberg, 012). Unlike bacteria, actinomycetes are unique in their orphology with widespread diverging substrate and erial mycelium bearing chain of arthrospores. The bstrate mycelium and spores can be pigmented, which makes them most colourful and attractive among microbes. On agar plates they form lichenoid, leathery or powdery colonies.

MATERIALS AND METHODS

Method for collection of soil sample

Soil sample were composed about 15 cm below superficial of the soil. All soil sample Were collected casually from agriculture Research Center, Lonar. Each sample was occupied from 5-15 cm penetration of the soil by using serried degraded metal tube (30 cm length). Soil samples were assorted and sieved to remove stones, leaf, stem and roots. Then, samples were crammed in cleaned and sterile plastic bags, established and stored at 4°c until analysis.

Microbe isolation and Enumeration from soil sample media

Soil samples were air dehydrated for 1 week previous isolation. This helps in decreasing the populace of gram negative bacteria. Soil suspension method described by OSKAY et.al (2004) was used, where 19 of the soil. Sample were occupied and mix with 100 ml of sterile distilled water.

The soil suspension was stunned dynamically under room temperature on an orbital shaker at 200 rpm for 1hr. 200 u of the soil suspension were pipette and lawn on to Agar.

Isolation of Alkaliphilic Actinomycetes strain from soil sample

Media

The alkaliphilic actinomycetes strains were sequestered using alkaliphlic actinomycetes medium and nutrient agar medium. Alkaliphlic actinomycetes medium was confined,0 .2g sodium casinate,0.010g L-aspergine,0.4g sodium propionate,0.050g dipotassium phosphate, 0.1g

ium sulphate, 0.001g ferrous sulphate, 1.5g agar- g glycerol and PH was adjusted to 9.0

n method

t method

h composed sample, 0.2g of soil samples were over the surface of media. Then plates were d at 30-35°c for one week. The isolated colonies cked and respread on designated media.

ilution technique

nycetes were isolated from the soil samples by ilution plate technique (10^{-3}to10^{-6}) .One milliliter was taken from each dilution and spread evenly surface of the discriminatory isolation media and d. Plates encompassing pure cultures were stored rther examinations. Isolates were used in the g experiments.

terization of actionomycetes

logical and Cultural Characterization

inomycetes include abundant order of bacteria, xhibit wide morphological and physiological Morphological, physiological and biochemical gs of the strains were studied as per International yces Project (Shirling and Gottlieb, 1966) and manual of systematic bacteriology (Williams et

).

ates were streaked on to 1) Starch casein agar 0.0 g; casein 1.0 g; K2HPO4 0.7 g; KH2PO4 0.3 O4.7H2O 0.5 g; FeSO4.7H2O) Glycerol ne agar (ISP 5) (L-asparagine 1.0 g; glycerol 10.0 O4 1 g; seawater 1 L; agar 20.0 g; pH 9.2

ip Culture technique

ip culture is an important tool for learning the orphology of filamentous actinomycetes under pted circumstances. Spore chain morphology, ation of substrate mycelium, aerial mycelium, d amount of spores in spore chain etc. can be udied by this method. The isolates were inoculated rine actinomycete broth and incubated at 28 °C ys. Plates containing Casein starch peptone yeast ract agar medium (Casein 3.0 g; maize starch peptone 1.0 g; yeast extract 1.0 g; malt extract 2HPO4 0.5 g; sea water 1 L; pH 7.4; agar 20 g) anized. Sterile cover slips 3-4 were implanted at of45°C into the agar medium. A loopful of spore ion of actinomycetes was dispensed at the ion of the medium and cover slip. The plates were d at 28°C for 4-8 days. The cover slips were at intervals of 2-4 days and were observed under ver and oil immersion objectives. Morphology of ycelium, substrate mycelium, organization of ous hyphae, their morphology (straight, flexuous, aped) were recorded according to ISP (Nonomura, irling and Gottlieb,

ected actinomycetes were considered by ogical and biochemical tests [7]. Morphological mprises of macroscopic and microscopic es The mycelium structure, color arrangements ; on the mycelium and colors of colonies were d and compared with Bergey's manual of ative bacteriology.

MORPHOLOGICAL CHARACTERISTICS

Morphological characteristics of the strains were considered according to Methods of Shirling and Gottlieb,(1966); Bergey's manual of determinative Bacteriology (Holt et al.,1994) and . The morphology Of mycelial structures, spore chains and spore surface was observed with a light microscope x 100 (circa 2000, Wolfe ,USA) and Various tests (pigmentation of substrate mycelium , diffusible pigments were performed for the characterization of the actinomycetes isolate.

Following are some plates showing growth of actinomycetes on actinomycetes isolation medium.

The spore chain morphology of actinomycetes developed in coverslip observed under high power and oil immersion objectives exposed four types of spore chain morphology. The most prominent spore chain morphology was the spiral one, and 34% of the cultures exhibited spiral spore chain (mostly verticillate type) followed by 28% revealing rectiflexibiles (straight to flexuous) and 13.9% retinaculiaperti (open hooks, loops or spirals with one to two turns) spore chain morphology. (Fig.2.3 and Fig. 2.4) Remaining 23.9% of the isolates unveiled long chain of spores with zigzag fragmenting hyphe

Screening of phosphate solubilizing alkaliphilic actinomycetes

Alkaliphilic actinomycetes were inoculated on starch casein agar medium encompass 2% of tricalcium phosphate as a sole phosphorus source for selective screening of actinomycetes which have capability to release inorganic phosphate from tricalcium phosphate. inoculated plates were incubated at 30 degree temp for 7-10 days. after incubation period plates perceiving zone of clearance around the colonies which displays the degree of phosphate solubilization.

RESULT AND DISCUSSION

Isolation of alkaliphilicactinomycetes and distinguished their morphological and physiological characteristics.A total of 4 isolates were isolated from the soil samples on the actinomycetesisolation medium and 2 isolates on the starch casien agar.Aerial mycelium colours were observed for the detection of strain varieties in the different soil samples from dissimilar areas of lonar .colour type were more plentiful in isolates grown actinomycetes isolation mediumthan in isolates grownup on starch casein agar medium .This medium used for the observation of spores and sporophores development in actinomycetes it was noted as medium for characterizing actinomycetes colony and the colouralteration after the development of sporulation Regardless the media differences .This data provided that all the isolates are alkalipilic organisms. Alkaliphilic organism is recognized to be able to grow optimally at PH above 9, usually between 10 &12 .Nonethless,it cannot grow or grow slowly at the near neutral PH value 6.5.zone of clearance around the colonies which indicates the degree of phosphate solubilizationi.ealkaliphilicactinomycetesdisplay phosphate solubization activity .The appearance of colonies ranged from concentric, wrinkled, uinbonate, and chrysanthemum (radial furrows) type (Fig. 2). The spore mass colour of actinomycetes is considered taxonomic

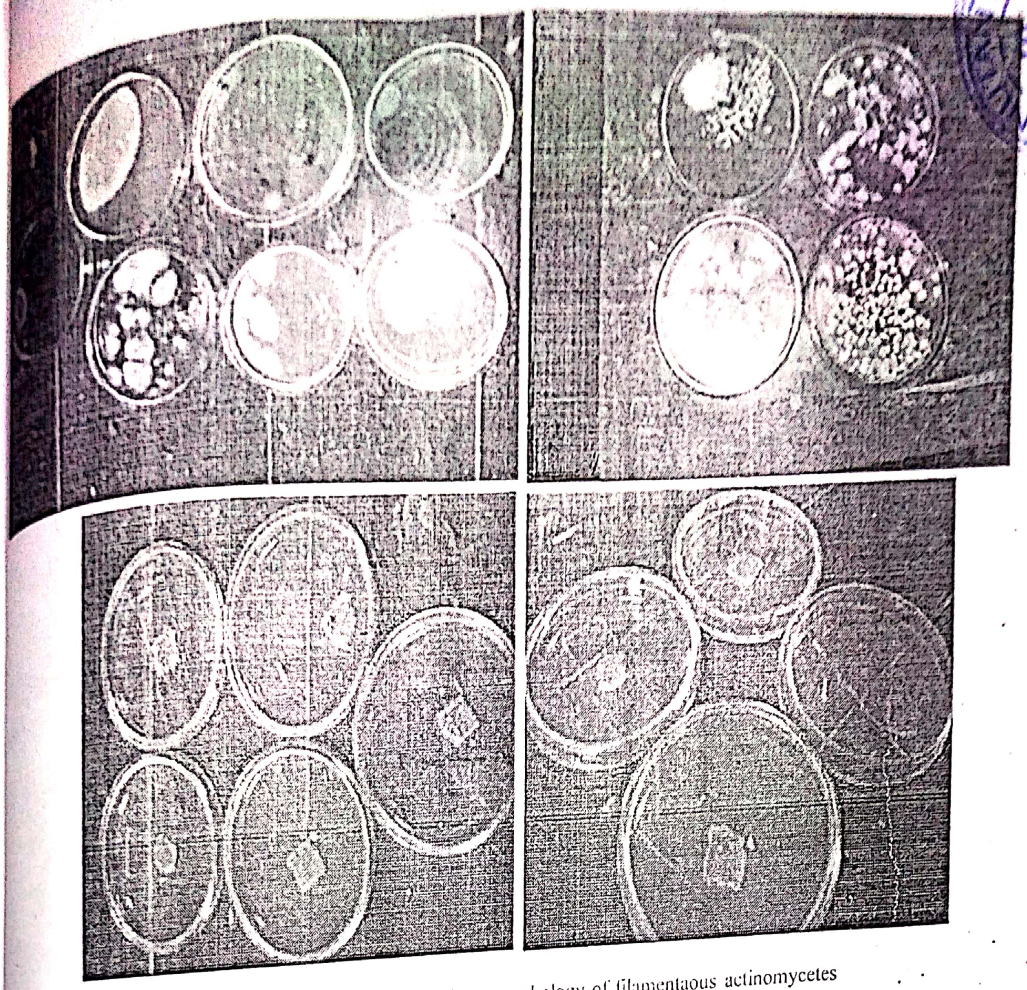

Fig. 1. Cover slip techanique for micro morphology of filamentaous actinomycetes

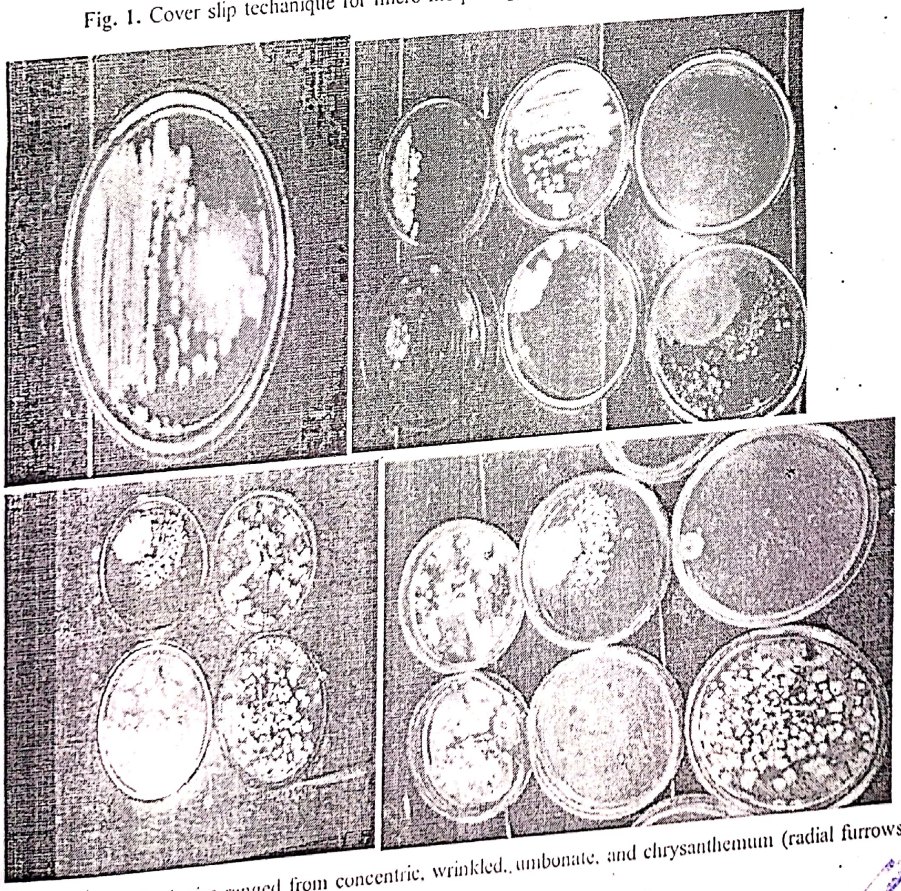

Fig. 2. The appearance of colonies ranged from concentric, wrinkled, umbonate, and chrysanthemum (radial furrows) type

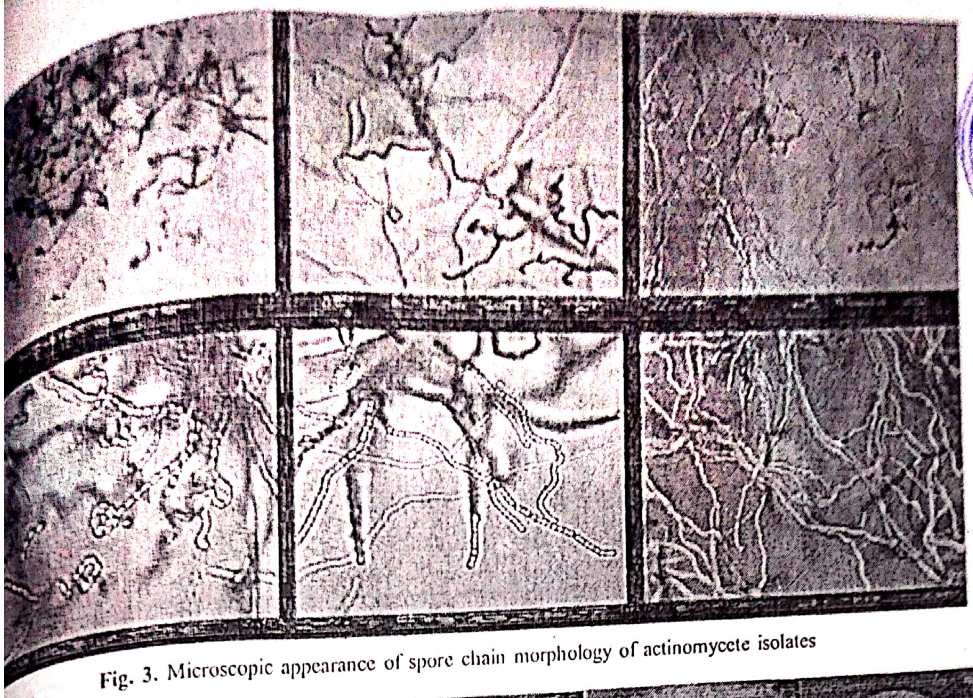

Fig. 3. Microscopic appearance of spore chain morphology of actinomycete isolates

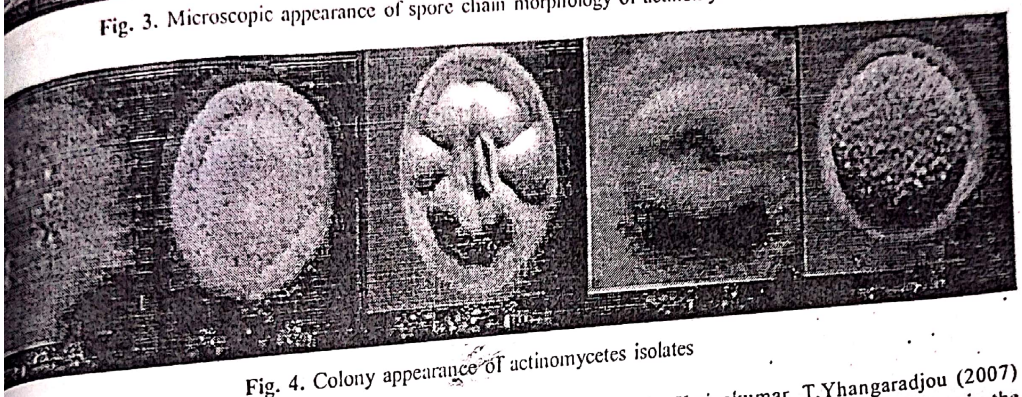

Fig. 4. Colony appearance of actinomycetes isolates

for grouping of actinomycetesin addition, park 991) reviewed that neutrophilessterptomyces re able to cultivate between PH 5.0 & 9.0 with growth close to neutrality. The soil samples d in this study were sandy and alkaline (range 76& 8.80) . Isolation of actinomycetes from sandy been done with various media.

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