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Identification and Molecular Characterization of Myxobacterial Diversity From Soils of Ganges Riverbank

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Abstract

Myxobacteria, a soil bacterium which grows with a unique swarming pattern, produces fruiting bodies under the stress conditions. As this bacterium naturally synthesises the bioactive compounds, it has gained recognition from the pharmaceutical industries. The study was conducted for isolating the myxobacteria from the Ganges riverbank, Banaras, Uttar Pradesh. Myxobacteria were identified from their morphology and growing pattern. Two pure strains (Vmyxo01 and VMyxo02) were isolated from the soil sample by visualizing the unique and specific properties of Myxobacteria, characterization on the basis of colony formation, observing the fruiting bodies under the stereomicroscope. Further confirmation of the species was done by 16S rRNA analysis.

Keywords: Fruiting bodies, Ganga Riverbank, Myxobacteria, 16S rRNA, Swarming pattern.

Introduction

Myxobacteria are gram-negative rod-shaped bacteria which grow on soil rich in the organic matter of alkaline or neutral pH [1]. The unique behaviour of the vegetative cells to grow in gliding pattern, formation of a swarm and the development of fruiting bodies under starvation conditions separates myxobacteria from others [2].It has been reported that it can synthesise a diverse number of secondary metabolites which have distinct composition and function [3]. Myxobacteria are even coined as "pharmaceutical factories" [4]. Thus, exploration has taken pace for identifying different strains and secondary metabolites of great clinical importance.

The reports have suggested that to date; only 10% of the myxobacterial species have been isolated from the environment [5]. Several reports are available about the occurrence of the myxobacterial strains in the various parts of the world such as *Stigmatella sp.* and *Archangium gephyra* from India, *Chondromyces crocatus, Stigmatella erecta,* and *Chondromyces apiculatus* from Australia, *Archangium gephyra*, *Myxococcus fulvus,* and *Myxococcus virescens* from Brazil and many other species from different parts of the world [6, 7, 8]. As a result, there is a need for extensive research for the isolation of the myxobacteria from different niches of nature as it is of great industrial importance.

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The natural environment for the myxobacteria is the soil of pH in the range of 5-8 which is rich in humus content and are mainly found in soil where the medical dump gets accumulated [9]. Extensive research has explored different parts of the ecosystem. However, no reports were documented from the riverbank of the Ganges. In the present study, two myxobacterial colonies were isolated from the soil near the riverbank of the Ganges. The characterisation of myxobacterial strains was done based on their morphology and the growth pattern.

Materials and Methods

Collection of Samples

Soil samples were collected from the riverbank of the Ganges in Banaras, UP (Latitude - 25° 19' 18.0624" N and Longitude -82° 59' 14.2404" E). The soil sample were collected aseptically in the sterile polythene bag and transported to the laboratory for further investigation.

Identification and Isolation of Myxobacteria

For the isolation of the myxobacterial strains, the Dung baiting method was used in which the soil sample was placed on the petri plates containing Casitone media. The sterilised rabbit dung was then put on the soil and incubated at 28ºC for 7-14 days. On the growth of white-coloured patches on the dung pellet, a small amount was scraped-off and inoculated into casitone broth and incubated at 28 ºC for 14 days. Thus, suspension cultures obtained were then spread-plated on the casitone agar. The inoculated casitone petri-plates were incubated at 28 ºC for 7-14 days and were observed for swarming pattern and gliding growth on the periphery of the plate. Moreover, the confirmation of the myxobacteria was done by viewing the swarm growth under a stereomicroscope for visualising the fruiting bodies [10, 11].

Characterisation by 16S rRNA

Isolation of Myxobacterial DNA

The inoculated culture of positive isolates showing the swarming growth were harvested and centrifuged at 12,000g for 10 minutes. After centrifugation, the pellet was suspended into 500µl of extraction buffer, followed by addition of 70µl of 10% SDS, and 12µl of proteinase K (20mg/ml), and 7µl of RNase. The contents of the tube were mixed by gentle inversions and incubated at 37ºC for one hour. After incubation, equal volumes of Phenol: Chloroform: Isoamyl alcohol (25:24:1) reagent was added, mixed by repetitive inversion and centrifuged for 15 minutes at 12000g. The aqueous top-layer was transferred to a fresh tube, and 0.7

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volume of isopropanol (chilled) along with 1/10 volume of ammonium acetate was added, for precipitating DNA. Then, the tubes were centrifuged at 12000g for 20 minutes. The supernatant obtained was discarded, and the pellets were washed with 70% ethanol. After washing, the tubes were centrifuged for 25 minutes at 12,000g. The pellets were kept undisturbed for air drying. After that, the pellet was re-suspended in 200µl TE buffer. The extracted genomic DNA was confirmed by running it on the 0.8% agarose gel [12].

Preparation and Investigation of 16S rRNA

The primer Myxo2F (5'-AMG ACG SGT AGC TGG TCT- 3') and 1492R (5'- TAC CTT GTT ACG ACT T-3') were used for amplification of extracted genomic DNA by 16S rRNA ribotyping in a thermal cycler. A 20µl of the reaction mixture was prepared consisting of 10µl of molecular grade water, 2.5µl of 10X buffer, 1.5 µl each of forward and reverse primer (10pmol), 1.5 of dNTPs (10mM), 2µl of template DNA and 1µl of Taq Polymerase (1U/ µl). The 36 cycles were programmed in a thermal cycler as follow: where initial denaturation was done at 94ºC for 3 minutes, rest 36 cycles followed the program in the following order: 94ºC for 1 minute, 43ºC for 30seconds, 72ºC for 1 minute and at the end the final extension was done at 72ºC for 5miutes. After that, it was held at 4ºC. The amplification of the DNA was then confirmed by running it on 1.2% agarose gel [13].

Results and Discussion

Isolation, Purification and Confirmation of Myxobacteria

The exploration of the myxobacteria has been started in various areas of the world that are yet unexplored. Soil samples were collected from the different parts of the Ganges riverbank for the isolation of myxobacterial strains. The myxobacterial strains were isolated with the help of Dung-baiting method illustrated in Fig. 1 [14].

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Fig. 1: Casitone Agar plate showing white-coloured patches on rabbit dung pellets, an indicator of myxobacterial growth.

The white-coloured patches on the rabbit-dung pellets were collected and transferred to casitone broth for obtaining the suspension culture of myxobacterial strains, yielding two myxobacterial strains named Vmyxo01 and Vmyxo02. The suspension culture of these strains was plated on the casitone agar for isolating the pure colonies, as illustrated in Fig. 2. The swarm growth on the periphery of the Petri plate (as illustrated in Fig. 2) confirmed that strain isolated was of myxobacteria. These isolates showed a similar growth pattern of swarm formation, as shown by family *Myxococcaceae* [15].

Fig. 2: Casitone Agar plates showing the swarming growth pattern of myxobacterial strains Vmyxo01 (Left) and VMyxo02 (Right)

Further, the results were validated on observing the fruiting bodies under the stereomicroscope, as shown by the Myxobacterial strains illustrated in Fig. 3 [16].

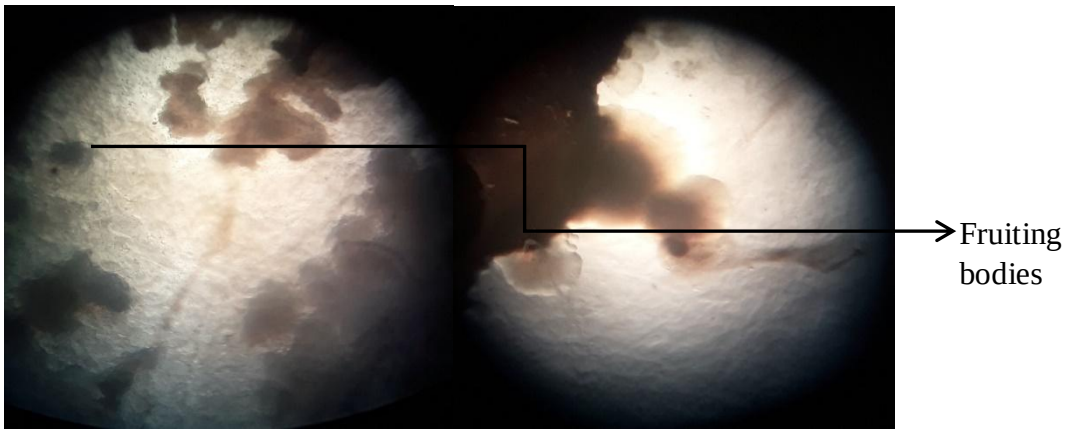

Fig. 3: Microscopic examination of Myxobacteria under a stereomicroscope at 4X magnification Vmyxo01 (Left) and VMyxo02 (Right).

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Isolation of Myxobacterial DNA

The genomic DNA of myxobacterial strains was isolated according to the described above procedure. After the isolation, it was confirmed by running the isolated DNA samples on the 0.8% agarose gel, as illustrated in Fig. 4.

Fig. 4: 0.8% Agarose Gel showing Genomic DNA extracted from VMyxo01 and Vmyxo02. (Well 1: Uncut Lambda DNA, Well 2: VMyxo01, Well 3: VMyxo02)

PCR Amplification of 16S rRNA Gene

Thus, on verifying the isolation of genomic DNA of the myxobacterial strains, the 16S rRNA gene was amplified by using the Myxo2F and 1492R primers. The amplified DNA was then run on 1.2% agarose gel. The PCR amplified DNA was determined to be approximately 1200bp by running the 1kb DNA ladder, which is depicted in Fig. 5.

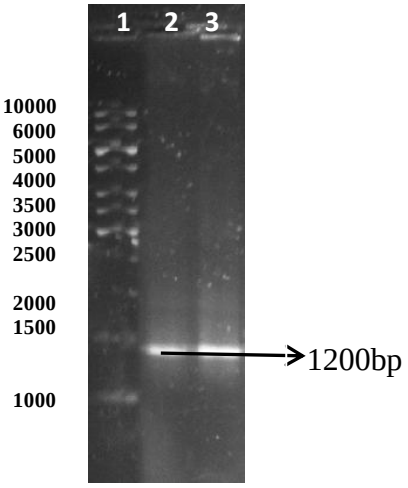

Fig. 5: 1.2% Agarose gel showing the PCR amplification of 16S rRNA. Well 1: 1kb DNA ladder; Well 2: VMyxo01; Well 3: VMyxo02

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Conclusion:

In the study, the myxobacterial strains were isolated from the soil samples collected from the banks of the Ganges in Uttar Pradesh, India. The myxobacteria are gram-negative bacteria that show swarming growth and fruiting bodies. The unique swarm-formation of the myxobacteria makes them different from other bacteria. Under a stereomicroscope, the fruiting bodies of the myxobacteria are visible. The genomic DNA was extracted from the myxobacteria isolates, and PCR amplification using myxobacteria-specific primers indicated that the isolates indeed belonged to the myxobacteria. Myxobacteria are well-established to produce biologically active secondary metabolites, including some effective antibiotics. Many strains of myxobacteria have been identified that produce the metabolites with anti-tumour activity. These potentials make myxobacteria a unique yet potential source for novel metabolites, which warrants more studies on myxobacterial diversity as well as the production of secondary metabolites.

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Conflict of Interest

The authors declare no conflict of interest.

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