



# Phylogenetic Analysis Of Paenibacillus Dendritiformis Genotype From Water Samples Of Southern Tamilnadu Lakes.

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## ABSTRACT

The primary focus of this paper is to separate microscopic organisms from ecological samples, for example, trash squander, street side strong squanders, lake and stream water tests. The samples were collected for investigating phylogenetic relationship among a wide range of organisms present in southern Tamilnadu lake areas in India. The lake water isolates of anaerobic organisms like Paenibacillus spp in lab culture was monitored for growth and checked for any distinction in development or distribution pattern in the natural habitats. Besides investigating the potential beneficial use of Paenibacillus spp in agriculture, recently their importance is increasingly focused in exploiting the use of its exopolysaccharides (EPS) biocomposite material in biotech industries. The separated microorganisms were distinguished for its identity of gram positive or negative types as well as its motility. Later sequenced for its genome and phylogenetic tree was derived. In the present study, the Paenibacillus dendritiformis was isolated and genome sequenced and the phylogenetic tree was investigated by comparing with other bacterial species in lake water.

**Keywords:** Biocomposites, lake water samples, phylogenetic, Paenibacillus and bacterial genome

## **Introduction:**

Paenibacillus is a gram positive anaerobic bacteria widely distributed in almost all ecological samples like water, air, soil, sewages, clinical samples etc., The members of genus Paenibacillus can exist in natural habitats as spores for prolonged periods which are resistant to cold, heat and disinfectants. Ash et al reported this species in 1993 based on its RNA gene sequence analysis (1-3). Bacillus anthracis belonging to this genus are known to cause food poisoning causing systemic infections and other species are rarely known to have clinical significance and found in clinical specimens as contaminants. Previous reports elucidated the phylogenetic structure of this bacillus genus through small subunit rRNA sequencing methods. Such studies observed that genus Bacillus is heterogeneous and needs further taxonomical studies to ascertain its status in phylogenetics.

The exopolysaccharides (EPS) of Paenibacillus spp have gained recent attention as a source of biomaterials and large quantity of EPS was reported to be extracted from wasp honeycombs (4,5). Such EPS molecule was found to have structure as a linear linked fructan or levan polymer with high biocompatibility which might find importance in biocomposite material. Several species of Paenibacillus family were reported been identified in polar regions and Antarctic soils, where spore were subjected to highly varied temperatures, UV radiation and low water content. (6-8) In identification of such a variety of species, it is necessary to perform genome based comparative study those species, study genome content scaling and compare to samples isolated from other habitats. Such studies

will provide insights into survival mechanisms under extreme weather changes. In temperate *Paenibacillus* spp. the mechanisms of survival for growth through protein coding sequences (CDSs) were earlier investigated related to their population growth. Such studies also indicated that CDSs are present in fewer levels and have lower paralogous content which is indicative for genome content scaling. A few studies identified *Paenibacillus* spp in milk samples and highlighted its role in milk spoilage (9-11). In genetic analysis whole-genome sequencing (WGS) and average nucleotide identity by BLAST (ANIb) analyses were performed for the identified isolates to represent its difference in its genus classification. However, the link between phenotypes and genetic diversity was not clearly elucidated in earlier reports.

The family of bacterial Spp. belonging to rhizobacteria have potential application in agriculture as they found importance as beneficial to the plant by direct and indirect mechanisms. The *Paenibacillus polymyxa* has earlier been reported to involve in plant growth possibly through producing phytohormones like indole-3-acetic acid (IAA) (12-14). Other strains like *P. polymyxa* SQR21 and E681 were reported to reduce plant diseases and *P. polymyxa* P2b-2R involved in nitrogen fixation (15,16). Extensive comparative genomic analysis indicated *P. polymyxa* gene cluster express innate immunity associated antimicrobial peptides. The beneficial role of plant growth and antibacterial activity was previously demonstrated using *P. polymyxa* CR1 and such studies revealed that they mediate nitrogen fixation and produce growth promoters. Therefore, *P. polymyxa* CR1 was reported to show multiple beneficial traits improving bio-economy through controlling pathogens and plant growth promotion (17-18).

The family of *Paenibacillus* Spp being widely present in soil sample will possibly contribute to plant growth by several mechanisms. In agricultural requirements, phosphorous is known to be another key component required in early plant development. Phosphorous is known to occur in soil as insoluble mineral complexes (19-21). The contribution of *Paenibacillus* Spp in soil phosphorous is yet to be determined. A few studies investigated the role of *nif* genes of *Paenibacillus* Spp using recombinant *E. coli* strain and observed its role of nitrogen fixation in such experiments. Other species like *P. dendritiformis* might be a social microorganism: when cultured under controlled conditions that copy common habitats like debilitating surfaces, being essential for an enormous agreeable, the microbes can more readily go after food assets and be ensured against antibacterial assaults (22-24).

The *P. dendritiformis* display a few unmistakable physiological and hereditary attributes along with  $\beta$ -galactosidase-like movement causing states to show blue for X-gal plates and multidrug resistance (MDR) (counting septrin, penicillin, kanamycin, anti-infection drug, ampicillin, antibiotic medication, spectinomycin, streptomycin and mitomycin C (24-28). The results showed that square measure grown-up on surfaces in Petri dishes indicated many fold higher medication opposition contrasted with development in fluid media. This unequivocal obstruction is accepted to result to a surfactant-like fluid front that really frames a chose design on the Petri plate. Another study isolated antimicrobial compounds like polymyxin A (P1 and P2) from *P. dendritiformis* strain PV3-16 from soil samples. Biosurfactant like compound were identified in strain CN5 of *Paenibacillus dendritiformis* family isolated from wood treatment plant soil (29,30). In the present study we focused in investigating phylogenetic relationship of commonly distributed *P. dendritiformis* from soil bacterial samples to determine their potential beneficial role in agriculture.

## Materials and Methods:

### Soil sample collection

The samples were collected from different ecological environments both the liquid and soils were collected in sterile disposable plastic containers with the aim that they are not contaminated with different microorganisms and the generally introduced organisms are not affected. Microorganisms inside the collection contain parasites, protozoa and green growth. Notwithstanding the significant areas, the other significant natural elements are a remarkable gathering known as infections that can contaminate eukaryotic or prokaryotic organisms. Infections have no metabolic limit and depend on contaminated hosts to create viral parts that self-gather. Like infections are the viroids, which are irresistible single-abandoned RNAs that can contaminate plants. At last, we talk about prions, which are irresistible proteins that can obliterate cerebrum tissue—the contagious spongiform encephalopathy (TSE) infections (31,32).

## Bacterial culture

Petri dishes are regularly used to make agar plates for microbial examinations. The dish was somewhat loaded up with agar and a combination of growth substances that might incorporate supplements, blood, salts, carbs, colors, pointers, amino acids or anti-toxins. When the agar cools and hardens, the dish is fit to be incubated ("plated") with an organism loaded sample (33-34). Later in agar plates, the samples were filled in the dish to give hosts to the sample inoculation. The cultures were incubated for microbial growth and poorly grown cultures were incubated longer. The sterilized agar plate was wrapped with parafilm and afterward positioned upside down in the incubator this gives ideal temperature to development of the microorganisms.

## Gram Staining

Gram staining was a typical procedure used to separate two rapid growing microbe's dependent on their diverse cell divider constituents. The gram stain technique recognizes gram positive and gram negative collection by shading red or violet.

## Extraction of bacterial DNA

DNA was extracted from the cultured samples using normalized salting-out technique. Little bacterial colonies were set in 1.5 mL tubes independently and afterward added 500  $\mu$ L of solution1 and 10% of 10  $\mu$ L SDS. Homogenize the example with sterile homogenizer. Also 5  $\mu$ L of Proteinase K was added (20 mg/mL). The mixer was incubated at 55°C for 2 hrs in water bath (with intermittent blending/fast vortex) for simple absorption. Later complete processing, the samples were on ice for 10 min. To some extent dried DNA was re-suspended in 100  $\mu$ L of 1x TE buffer.

## Measurement of DNA

The concentration of the isolated DNA was checked in UV spectrophotometer (SHIMADZHU, JAPAN) by taking the optical density (OD) at 260 nm and 280 nm. The quality was checked by estimating the proportion of absorbance at 260 nm and 280 nm (260/280). The values between 1.7-1.8 demonstrated good quality DNA without protein/RNA contamination. DNA estimation was completed by the accompanying estimation: test showing 1 OD at 260 nm was identical to 50  $\mu$ g of DNA/mL. The OD of every DNA test at 260 nm was estimated and evaluated.

## Phylogenetic tree investigation

A phylogenetic tree or transformative tree is a spreading graph or "tree" showing the connections among different evolutionary species or different organisms—their phylogeny dependent on homology and contrasts in their physical or evolutionary relationships (33-34). In an established phylogenetic tree, every branch represents relatedness identifying the normal progenitor of those relatives and the edge lengths in certain trees might be deciphered as time measurements. Every branch is known as an ordered unit. Inward branches are by and large called theoretical ordered units, as they can't be straight forwardly noticed (35,36). Trees are valuable in areas of science, for example, bioinformatics, systematics, and phylogenetics. Unrooted trees show just the relatedness of the leaf branches and don't need the genealogical root to be known or assumed.

## Results and Discussion

The environmental samples were gathered from different spots in an exceptionally sterile holder. Both the fluid and strong example like soil and water from a lake water way and ocean. These examples were contained and securely kept in a sterile compartment. Then, at that point, the sample was taken with 1ml/mg it was blended in with 100ml of sterile water. With the goal that they can be sequential refined. The refined samples are sterilized with the spread plate strategy in agar petri plates where 1 $\mu$ l of the sterile sample was put in the focal point of the agar plate and spread with the assistance of L bar. The petri plate was ready with the LB broth where 100 ml purified water was taken and 2.5 gm of stock a 1 gm of agar was added and warmed. Then, at that point, it was cultured and saved for further studies (Fig1). An alternate samples of bacterial species was refined. The bacterial settlements were shaped then the DNA was isolated. Then the DNA was sequenced and confirmed as "P.Dendritiformis". The P.

dendritiformis was isolated and genome sequenced and total DNA was monitored using agarose gel electrophoresis (Fig 2).



Fig1 Isolated *P. dendritiformis* bacteria culture

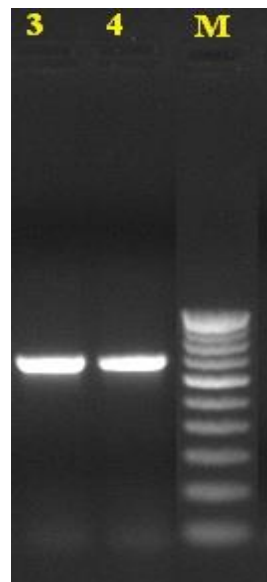


Fig.2. DNA extracted from *Paenibacillus Dendritiformis* was subjected to Agarose Gel Electrophoresis (lane 3 & 4 DNA extracted test sample and lane M DNA ladder).

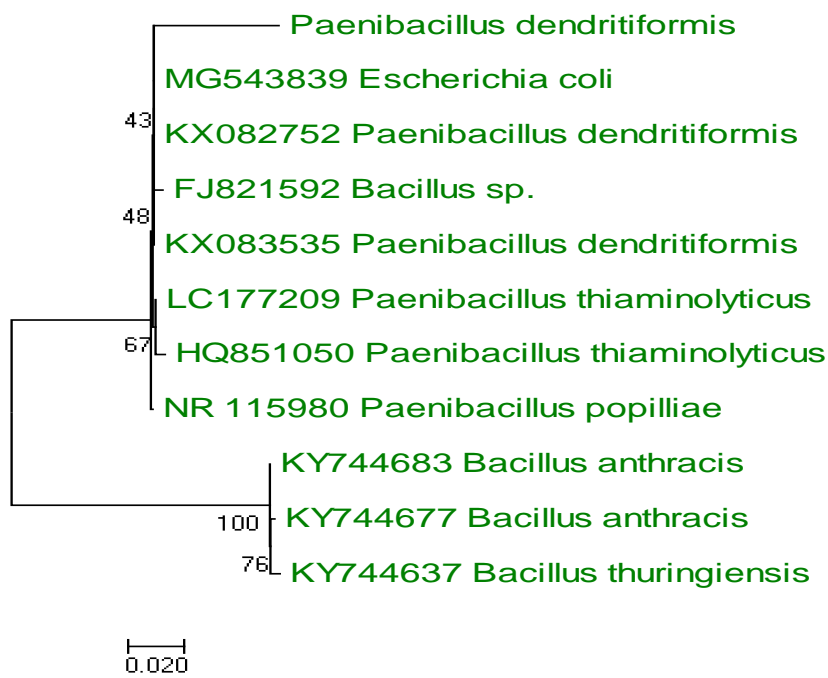


Fig. 3 : Phylogenetic tree of sequence homology of isolated *P. dendritiformis* with other bacterial species

The *P. dendritiformis* genome was sequenced and then the phylogenetic tree was analysed (Fig 3). Numerous *Paenibacillus* species can advance harvest development straightforwardly through natural nitrogen obsession, phosphate solubilization, creation of the phytohormone indole-3-acidic corrosive (IAA), and arrival of siderophores that empower iron procurement. This wide survey sums up the significant positive and adverse consequences of *Paenibacillus*: its acknowledged and imminent commitments to farming, medication, process assembling, and bioremediation, just as its effects because of pathogenicity and food deterioration. This survey additionally remembers nitty gritty data for major known *Paenibacillus* species with their areas of separation, genome sequencing ventures, licenses, and mechanically huge mixtures and proteins. *Paenibacillus* will, over the long haul, assume progressively significant parts in practical agribusiness and modern biotechnology (5-8).

The microscopic organisms more examination has been completed by isolating them from a wide scope of sources, the sort *Paenibacillus* includes bacterial species applicable to people, creatures, plants, and the climate. Numerous *Paenibacillus* species can advance harvest development straightforwardly through natural nitrogen obsession, phosphate solubilization, creation of the phytohormone indole-3-acidic corrosive (IAA), and arrival of siderophores that empower iron procurement. This wide survey sums up the significant positive and adverse consequences of *Paenibacillus*: its acknowledged and imminent commitments to farming, medication, process assembling, and bioremediation, just as its effects because of pathogenicity and food deterioration. This survey additionally remembers nitty gritty data for major known *Paenibacillus* species with their areas of separation, genome sequencing ventures, licenses, and mechanically huge mixtures and proteins. *Paenibacillus* will, over the long haul, assume progressively significant parts in practical agribusiness and modern biotechnology (5-8). The microscopic organism has been confined from the ecological examples has been observed as *P. dendritiformis*. A portion of the other investigates have demonstrated these microbes delivers a bioactive medicinal compounds which is potentially useful in several biopharmaceutical industries.

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