

Isolation of Bacteria from Soil sample for Exo-Polysaccharide production

A. Gowsalya, V. Ponnusami and K.R. Sugumaran

School of Chemical and Biotechnology, SASTRA University, Thanjavur – 613402,
Tamilnadu, India.

*Corres.author: sugumaran@biotech.sastra.edu

Abstract: For bacterial isolation, the soil samples were collected from farmland at Gandarvakottai, Pudukkottai district. Microorganism was separated by serial dilution method. Each of the isolates were experimented for the morphological characteristic like shape, gram nature and arrangement of cells, motility etc. Enzymatic activities were tested by biochemical characterization. The name of *Alcaligenes aquatilis* GDRSGP was confirmed by 16s rRNA sequencing. Accession no of isolated organism was KJ486573.

Keywords: Bacterial isolation; 16s rRNA sequencing; *Alcaligenes aquatilis*.

Introduction

Soil contains varieties of microorganism including bacteria that can be established in any natural environment. Bacteria are the most important and abundant microorganism which is present in surrounding environment. These are very small, unicellular, primitive and non chlorophyll containing microorganism. Dilution method is one of most important method to isolate the soil bacterium which allows the list of living cells in the soil¹. An enzymatic activity of one bacterium differs from another bacterium. Biochemical test is used to differentiate among the other bacteria². 16S rRNA gene sequencing studies the bacterial taxonomy and phylogeny⁵

Alcaligenes sp are motile gram negative soil bacterium which can be able to produce exo poly saccharide⁶ such as curdlan and welan gum. In this work we focused to isolate the exo polysaccharide producing soil bacterium⁹.

Materials and Methods

Soil sample collection

For bacterial isolation, 10 g of soil was collected from different area within pudukottai district. Soil sample were collected from upper layer of the farmland where maximum population of microorganism was concentrated. 5 g of soil sample was collected by using clean and dry sterile spatula in a clean polythene⁴.

Pure culture

For reducing microbial population, 1 g of soil was dissolved in 10 ml of sterile distilled water to make soil suspension. Serial dilution was carried out for getting isolated single colony. In this research, nutrient medium was used for bacterial growth. 28 g of nutrient agar was dissolved in 1000 ml distilled water and

sterilized in autoclave for 15 min at 121°C. Streaking plate method was used to get single colonies of pure culture³.

Sample inoculum

1 ml of 10⁻⁵ dilution of soil suspension was poured and spreaded over the nutrient agar plates by using sterile L rod. After incubation for 24 hrs at 37°C, mucous colonies were formed over the plates. Every 4 months interval, isolated bacteria was recultured which was identified on basis of Bergey's Manual of Systematic Bacteriology.

Nature of isolated bacteria

Gram staining was used to determine the nature of the bacterium. Colonies grown on nutrient agar where gram stained as per the procedure explained by Todar *et al*⁸. Bacterial motility was done by hanging loop method. Few drops of liquid culture were placed onto the cover slip in sterile condition. Depression slide was taken and the concave portion over the drop was pressed the slide onto the cover slip. The slide was inverted quickly to keep from disrupting the drop. Then the motility was examined under microscope at 40 X magnification.

Biochemical Tests

Biochemical test such as indole test, sugar utilization test, methyl red test, citrate utilization Test, voges proskauer test, starch hydrolysis, catalase test, casein hydrolysis were carried out to find the enzymatic activity of isolated organism^{4,2}

Bacterial sequencing

An isolated bacterium was sent for bacterial sequencing to Bhat Biotech India Private Limited at Bangalore. The genomic DNA was isolated and its 16s rRNA gene was amplified using universal primer in a Master cycle[®] Thermocycler. Programs followed were Initial denaturation of DNA strands at 94°C for 2 min, annealing with primers at 55°C for 1 min and extension at 72°C for 10 mins. About 1500 bp PCR product was purified to remove unincorporated dNTPS and primers before sequencing using PCR purification kit (Norgen Biotek, Canada).

Sequencing analysis of 16s rDNA genes

Amplified strands were sequenced by DNA sequencer -3037xl DNA analyzer using BigDye[®] terminator v3.1 cycle sequencing kit. Aligned sequences were converted to dendograms using sequence analysis software version 5.2. These sequences were compared with the sequences in NCBI database using BLASTIN. The most similar sequences were matched by E core and aligned by CLUSTAL W2 for multiple alignments. Finally phylogram was constructed using MEGA5 software⁷.

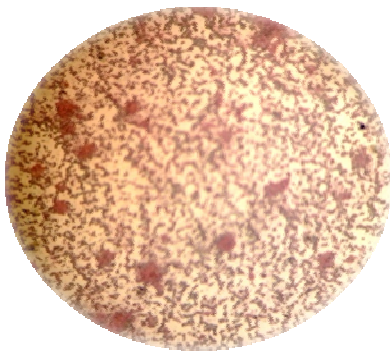
Result and Discussion

Biochemical identification

Characteristics	Observation	Interference
Gram Staining	The slide was examined under the 40X light microscope. Pink colonies were observed.	Negative
Shape	spherical-shaped bacterium	Cocci
Motility	Motility was observed under oil immersion objective lens	Possible
Indole production	Red colour was produced when adding kovac's reagent	Positive
Methyl red test	When adding methyl red indicator, the medium turned into red color. That means, Bacteria had ability to oxidize the glucose by producing high concentration of acid end products.	Positive
Voges-Proskauer test	After adding Barritt's reagent, the deep rose color was formed which was indicative of the presence of acetylmethylcarbinol	Negative
Citrate utilization test	Growth of the organism on the surface of the slant along with color formation confirmed the utilization of citrate.	Negative

Catalase test	Bubble formation was happened.	Positive
Gelatin liquefaction	After the incubation microorganism was liquefied in the medium	Negative
Starch hydrolysis	Clear zone around the growth of the organism proved the hydrolysis of starch.	Negative
Casein hydrolysis	Clear zone formation was produced around the growth of organism.	Negative
Carbohydrate Fermentation, Glucose, Fructose, sucrose	When utilizing carbohydrate, medium was turned into yellow colour which was indicated by phenol red.	Positive

Biochemical characterization of the soil isolate



GRAM NATURE



GLUCOSE UTILIZATION



CASEIN HYDROLYSIS



STARCH HYDROLYSIS



INDOLE TEST



METHYL RED



CITRATE UTILIZATION TEST



CATALASE TEST



MRVP TEST



GELATINASE TEST

Phylogenetic tree

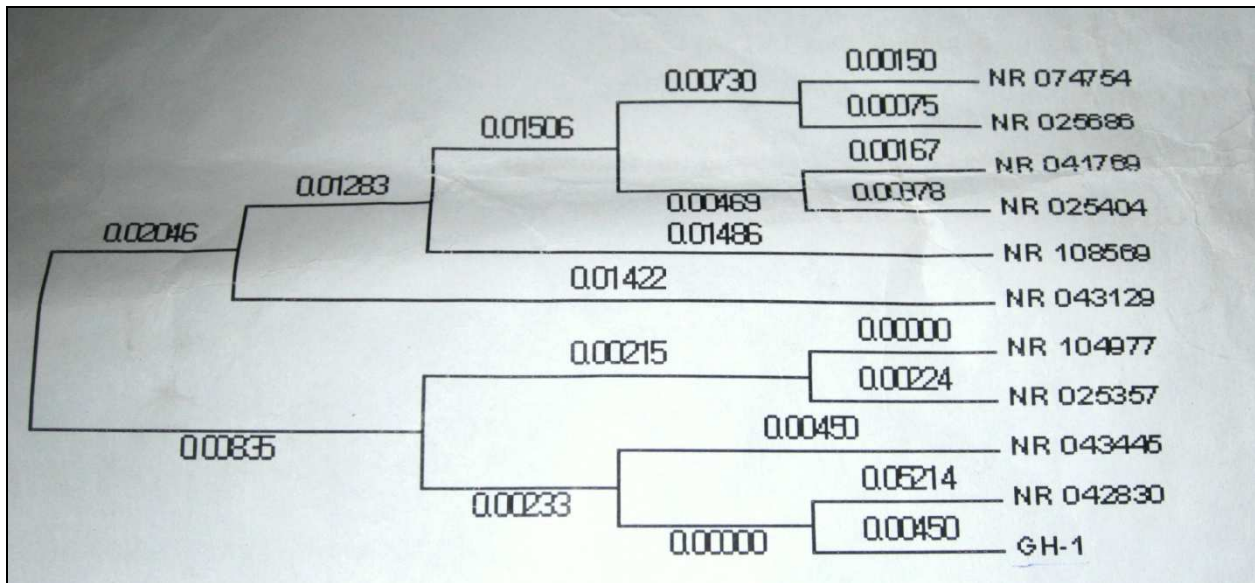


Figure 2

Tamura-Nei model was used to depict the evolutionary history. Figure 2 shows the phylogenetic tree with maximum likelihood. Trees for heuristic search were obtained using Neighbor Join and BioNJ algorithms. Matrix of pairwise distances was calculated using maximum composite likelihood method and topology having superior log likelihood value was selected. The final dataset contained 11 nucleotide sequences with a total of 1340 codon positions (1st+2nd+3rd+Noncoding). MEGA 5 software analyzed the evolutionary data. All the above analysis clarified that the isolated bacteria was *Alcaligenes aquatilis*.

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