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Isolation, identification and computational studies on *Pseudomonas aeruginosa sp.* strain MPC1 in tannery effluent

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Abstract:
A study about isolation, identification and analysis of bacteria in waste water. Here the tannery effluent used as a sample for the entire analysis. A bacterial strain, designated MPC1 was isolated from a waste water sample collected from tannery effluent, Trichy, India and identified using a molecular approach. On the basis of the bacterial 16s rRNA gene sequence phylogeny and comparison of this gene sequence with sequence in RNA sequence database, it is considered that isolate is closely related to members of the *Pseudomonas aeruginosa Sp*. Phylogenetic and molecular evolutionary analyses were conducted using MEGA. Identification of regulatory elements and Transcription Factor with their binding sites in 16S rRNA gene of *Pseudomonas aeruginosa mpc1* was performed using BPROM tool. The sequence of 16s rRNA (*Pseudomonas aeruginosa sp* MPC 1) is submitted to Genbank in NCBI database (Ac.No-JF708077).

Keywords: *Pseudomonas aeruginosa sp.Strain MPC1*, isolation, identification, *in silico* analysis, Heavy metal resistant bacteria.

Background:
The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Microorganisms and microbial products can be highly efficient bioaccumulators of soluble and particulate forms of metals especially dilute external solutions. Microbe related technologies may provide an alternative or addition to conventional method of metal removal or metal recovery. Waste water is a significant environmental problem. Wastewaters from the industries and sewage sludge applications have permanent toxic effects to human and the environment **[5, 6]**. Cadmium (Cd) is nonessential but poisonous for plants, animals, and humans. Cadmium is one of the most toxic pollutants of the surface soil layer, released into the environment by mining and smelting activities, atmospheric deposition from metallurgical industries, incineration of plastics and batteries, land application of sewage sludge, and burning of fossil fuels. Lead (Pb) a major pollutant that is found in soil, water and air is a hazardous waste and is highly toxic to human, animals, plants and microbes **[3]**. Tanneries, oil refineries and metal industries are causing depletion of surface and ground water quality. The discharge of various sub processes of tanneries like bathing, pickling, tanning, dyeing and fat liquoring may cause water pollution severely. The pollution of a particular water body can always be link to an industry or sewage or agricultural runoff. Likewise, contamination of soil by the release of heavy metals as a result of industrial and anthropogenic activities is a threat to human health and ecosystem. Presence of heavy metals in soil is known to have potential toxic impact on environmental quality and on human health via ground water and surface water **[12, 15]**. The genus Pseudomonas includes many species of environmental, clinical, agricultural, and biotechnological interest **[9]**. *Pseudomonas aeruginosa* is a ubiquitous environmental bacterium that is one of the top three causes of opportunistic human infections. A major factor in its prominence as a pathogen is its intrinsic resistance to antibiotics and disinfectants **[1, 7]**.

Methodology:
Isolation of bacteria:
The bacteria were identified based on colony characteristics, gram staining methods and by various biochemical tests as given by Bergey's (1984) Manual of Determinative Bacteriology.

16s rRNA SEQUENCING:
Isolates are sub cultured on agar plate. A single colony was obtained from a fresh subculture and resuspended in master mixture. A 380-bp fragment of the 16S rRNA (universal primer) used as the target. For 50µlreaction, 1µlof200mMdNTPs, 5µl of Reaction buffer (TriswithMgcl2) "10pMforwardprimerRW01 (FP) 5'AACTGGAGGAAGGTGGGGAT-3'" "10pMReverseprimerDG74 (RP) 5'AGGAGGTGATCCAACCGCA-3'" 1.2u/µl of Tag Polymerase were used, amplification was carried out in Thermal Cycler PTC 200(MJ Research), and a standard three-step protocol used for all reactions, including 35 cycles for each reaction with an annealing temperature of 55°C and 10-min extension time.

Nucleotide sequencing and Molecular characterization:
The amplified product purified by Bio basic Inc, Bangalore Genei, India. Cyclic PCR carried out using Big Dye Terminator Ver 3.1. Each purified template sequenced on both strands, reaction done by Genetic Analyzer 3130(ABI System). A comparison of the 16s rRNA sequence of the strain with sequence in the non-redundant collection using blast database have been done.

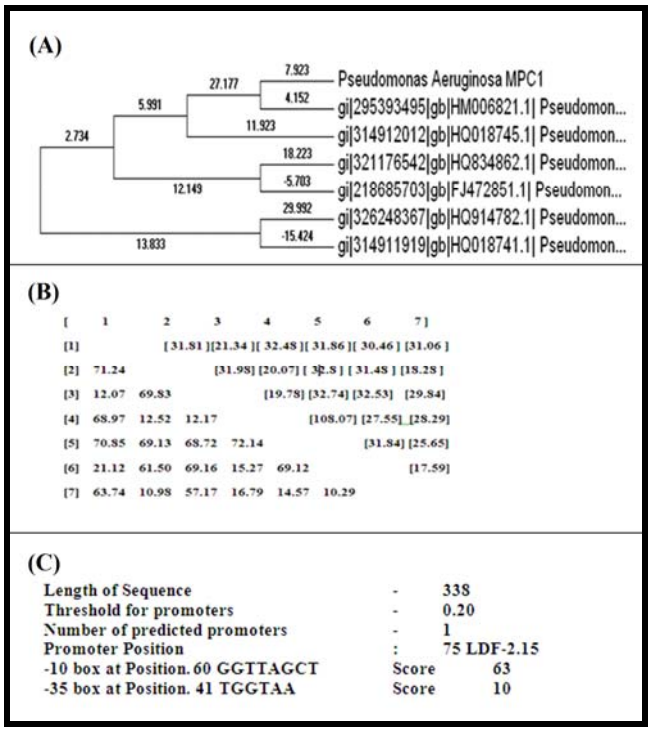

Figure 1: Computational Studies on *Pseudomonas aeruginosa MPC1 sp.*: **(A)** Evolutionary relationships of different strains of Pseudomonas *aeruginosa sp with pseudomonas aeruginosa* MPC1; **(B)** Estimates of evolutionary divergence between sequences; **(C)** Prediction of Promoter position using BPROM.

Computational studies on Bacterial isolates:
The occasional exceptions to the usefulness of 16S rRNA gene sequencing usually relate to more than one well-known species having the same or very similar sequences. Computational studies have evolved to include the creation and exploration of sequence alignments, the estimation of sequence divergence, the reconstruction and visualization of phylogenetic trees, and the testing of molecular evolutionary hypotheses. To understand more information about 16s rRNA sequence, the study is extended to identification of promoter position **(Figure 2)** and secondary structure prediction **(Figure 3) [2, 14, 15, 20]**.

Discussion:
The isolates obtained were identified by morphological, and biochemical characteristics **(Table 1 see Supplementary material)**. **[10, 11, 13, 16]**. The evolutionary history was inferred using the Neighbor-Joining method **[1, 2, 8, 13]**. The optimal tree with the sum of branch length = 112.96911444 is shown in **Figure 1**. The evolutionary distances were computed using the Maximum Composite Likelihood method **[16, 17, 18, 19]** and are in the units of the number of base substitutions per site **(Table 2 see Supplementary material)**. Phylogenetic analyses were conducted in MEGA4 **[4, 8]**. Standard error estimate(s) are shown in the plot (D=44.63; S.E=23.21) and were obtained by a bootstrap procedure. The probability of rejecting the null hypothesis that sequences have evolved with the same pattern of substitution, as judged from the extent of differences in base composition biases between sequences. A Monte Carlo test was used to estimate the *P*-values, which are shown below the diagonal. *P*-values smaller than 0.05 are considered significant the estimates of the disparity index per site are shown for each sequence pair above the diagonal. The difference in base composition bias per site is indicate the larger differences in base composition biases than expected based on evolutionary divergence between sequences and by chance alone.

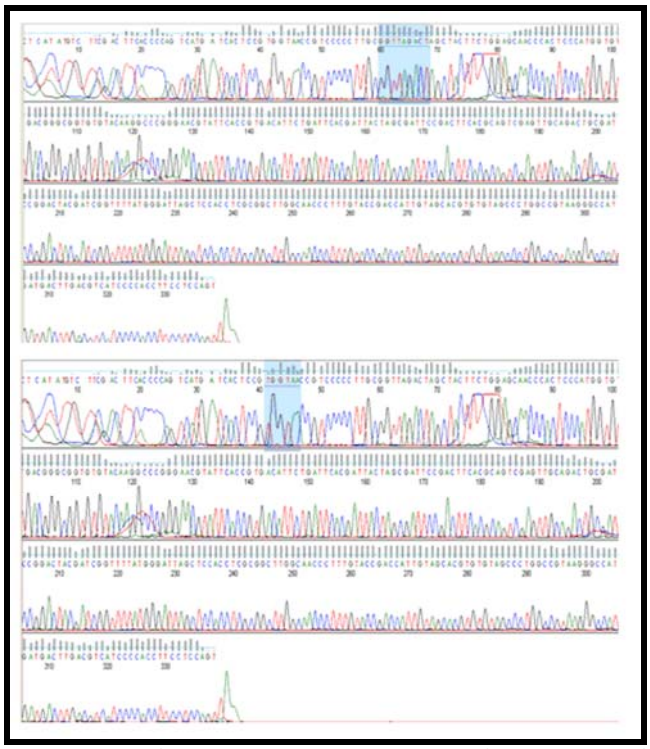

Figure 2: Result of Chromatogram with upstream region of 16S rRNA gene of *Pseudomonas aeruginosa strain MPC 1*, Promoter position are -10 box at pos. 60 **GGTTAGACT**; Score 63; -35 box at pos.41 **TGGTAA**; Score 10 is highlighted.

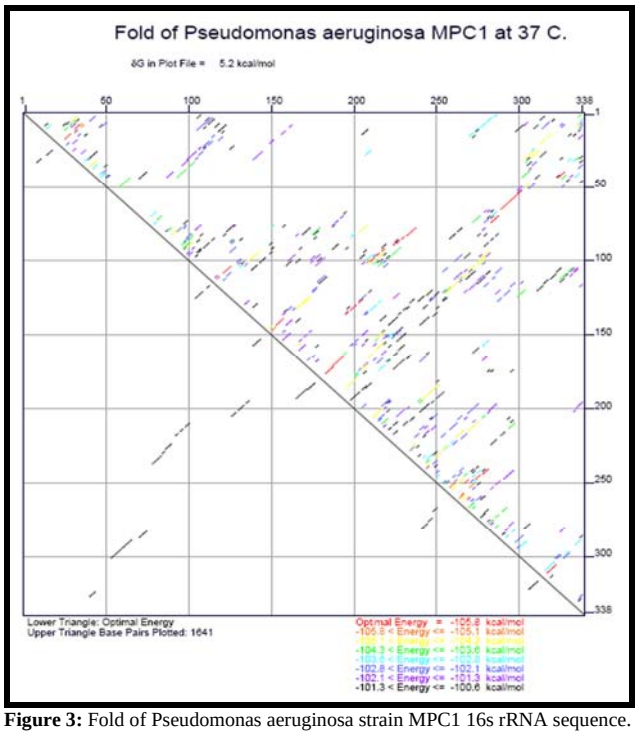

Figure 3: Fold of Pseudomonas aeruginosa strain MPC1 16s rRNA sequence.

Conclusion:
The nucleotide frequencies are 0.234 (A), 0.213 (T/U), 0.238 (C), and 0.316 (G). The transition/transversion rate ratios are k_1 = 14.587 (purines) and k_2 = 16.07 (pyrimidines). The statistic is calculated for numbers of synonymous and nonsynonymous substitutions per site, respectively. The variance of the difference was computed using the bootstrap method. There were a total of 75 codons and positions counted by Nei-Gojobori method. The Tajima test statistic was estimated for the 7 strains to identify the segregating sites. The relationship between the two estimates of genetic variation at the DNA level, namely the number of segregating sites: 326 and the average number of nucleotide differences estimated: 4.839163 from pairwise comparison and mutation are occurred in random, is investigated. Upstream region of 16S rRNA gene of *Pseudomonas aeruginosa strain MPC 1*, Promoter position are -10 box at pos. 60 GGTTAGACT; Score 63; -35 box at pos.41 TGGTAA; Score 10. As well as secondary structure and plot have predicted, which reveals the fold information of 16s rRNA gene sequence.

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Supplementary material:

Table 1: Results observed from the morphological and biochemical test

S.No	Bacterial isolates	Colony morphology	Appearance	Gram reaction	Motility	Indole Test	Methyl Red Test	VP Test	Citrate test	Starch hydrolysis Test
1	Isolate 1	Light green color	Watery appearance	Rod -	+	-	-	-	+	-
2	Isolate 2	Yellow color	Colloidal	Cocci +	+	+	+	-	+	+
3	Isolate 3	White color	Gummy appearance	Cocci +	+	+	+	-	+	+
4	Isolate 4	White color	Colloidal	Cocci +	+	+	+	-	+	-
5	Isolate 5	White color	Colloidal	Cocci +	+	+	+	-	+	+
6	Isolate 6	Red color	Colloidal	Cocci +	+	-	+	-	+	-

Table 2: Maximum composite likelihood estimate of the pattern of nucleotide substitution

	A	T	C	G
A	-	1.23	1.38	26.67
T	1.36	-	22.16	1.83
C	1.36	19.79	-	1.83
G	19.78	1.23	1.38	-