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Research Article

Isolation and Identification of Bacteria from Stocking Pond of Bhategaon Fish Culture Center

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ABSTRACT-

This study carried out to evaluate the isolation and identification of bacteria from water sample of stocking pond of Bhategaon fish culture center. Isolation of bacteria is carried out from the water sample. The isolated bacteria is cultured on nutrient agar plate , the selected distinct colony is then utilized for preliminary identification by gram staining and the further analysis is completed using 16S r RNA sequencing. The bacterium was identified as *Pseudomonas hibiscicola* by 16S r RNA sequencing with accession number AB021405.

Keywords- Bacteria, *Pseudomonas hibiscicola*, colony.

[I] INTRODUCTION-

Fish is a vital source of food for people and contributes about 60% of the world's supply of protein. 60% of the developing countries derive 30% of their annual protein from fish [1].Fish has been one of the main foods for humans for many centuries and still constitute an important part of the diet in many countries [2]. The advantages of fish as a food are its easy digestibility and high nutritional value [2]. Fish should be viewed not only as food, but also as ready source of income in the small holders farming sector [3]. The fishes are readily susceptible to microbial attack particularly bacteria [4]. The bacteria from fish only become pathogen when fishes are physiologically unbalanced. Nutritionally deficient or there are other stressors i.e.- poor water quality, overstocking which allow opportunistic bacterial infections to prevail [5]. The bacteria are transmitted by fish that have made contact with other diseased fish. Bacterial fish diseases and infections are very common and one of the most

difficult health problems to deal with [6]. Bacteria can enter in the fish body through the gills or skin or it can stay on the surface of the body [6]. The speed with which a product spoil is also related to the initial microbial load on the product: the higher the count, sooner spoilage occurs [4].

[II] MATERIALS AND METHODS-

Water samples were collected from the Bhategaon fish culture center of stocking pond.

2.1 ISOLATION OF BACTERIA-

Isolation of the bacteria was carried out from the water sample using sterilized wire loop directly on the nutrient rich medium i.e. – Nutrient agar media with the streaking plate method. Incubate the plates at 37°c for 48 hrs. The cultured isolated colony then selected for further morphological and biochemical identification.

2.2 MORPHOLOGICAL CHARACTERIZATION-

The pure colony thus isolated was characterized on the basis of following characteristics. i.e. Morphological characteristic- shape, size, margin, color, opacity, consistency, motility and Gram nature. [7]

Preliminary identification was carried out on the basis of above characteristics. Further identification was carried out using 16S r RNA sequencing (partial) of the bacteria in the laboratory at MTCC Chandigarh.

2.3 DNA SEQUENCING OF 16S R RNA GENE FRAGMENT-

The 16S r RNA purified PCR product (100ng concentration) was subjected for the sequencing using ABI DNA 3730 XL sequencer. (Applied Biosystem Inc.). Sequencing of 16S r RNA gene of the bacterial isolate was done from both the direction. The sequence obtained subjected to BLAST search and the bacterial species were determined. (Basic Local Alignment Search Tool) Blast is a web based program that is able to align search sequence to thousands of different sequences in a database and show the list of top matches. This program can search through a database of thousands of entries in a minute. BLAST [8] performs its alignment by matching up each position of the sequences in the database. The most similar sequences are listed in the result in Table no. (1)

Rank	Name	Strain	Accession	Pairwise Similarity (%)	Diff/ Total nt
1	*Pseudomonas hibiscicola*	ATCC 19867 (T)	AB021405	99.72	4/1433
2	*Pseudomonas geniculata*	ATCC 19374(T)	AB021404	99.36	9/1410
3	*Stenotrophomonas maltophilia*	MTCC 434(T)	JALV01000036	99.3	10/1433
4	*Pseudomonas beteli*	ATCC 19861(T)	AB021406	99.08	13/1413

5	*Stenotrophomonas chelatiphaga*	ICB 89(T)	FJ748683	99.02	14/1433
6	*Stenotrophomonas chelatiphaga*	LPM-5 (T)	EU573216	98.46	22/1433
7	*Stenotrophomonas rhizophila*	DSM 14405 (T)	CP007597	97.56	35/1433
8	*Stenotrophomonas koreensis*	TR6-01 (T)	AB166885	97.28	39/1433
9	*Stenotrophomonas ginsengisoli*	DCY01 (T)	DQ109037	97.28	39/1433
10	*Stenotrophomonas panacihumi*	MK06 (T)	GQ856217	97.07	42/1433

Table no. 1 EZ Taxon result.

2.4 NUCLEOTIDE SEQUENCE OF BACTERIA SAMPLE -

GTCGAACGGCAGCACAGAGGAGCTTGCTCCTTGGGTGGCGAGTGGCGGACGGGTGAGGAAT
ACATCGGAATCTACTTTTCGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACGA
CCTACGGGTGAAAGCAGGGGATCTTCGGACCTTGCGGCTGGCGGTAGGCCTAACACATGCA
ACGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGGCGAC
GATCCGTAGCTNNTCTGNGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTC
CTACTCCGTAGCTNNTCTGNGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACT
CCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACC
GCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCCGG
CTAATACCTGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGC
CGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGT
GGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGATACTGGGC
GACTAGAATGTGGTAGAGGGTAGCGGAATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCA
GGAGGAACATCCATGGCGAAAGGCAGCTACCTGGACCAACATTGACACTGAGGCACGAAAG
CGTGGGGAGCAAACAGGATTAGATACTCTGGTAGTCCACGCCCTAAACGATGCGAACTGGA
TGTTGGGTGCAATTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGT
ACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGT
GGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGA
GATGGATTGGTGCCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAA
TGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAA
GTCATCATGGCCCTTACGGCCAGGGCTACACACGTACTACAATGGTAGGGACAGAGGGCTG

CAAGCCGGCGACGGTAAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAA
CTCGACTCCAAGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTTTGTTGCACCAGAAGCAGGTAG
CTTAACCTTCGGGAGGGCGCTGCCA

2.5 16S R RNA GENE SEQUENCE AND PHYLOGENETIC ANALYSIS-

PCR was carried out by using the universal primers: 27F: 5' AGA GTT TGA TCC TGG CTC AG 3' and 1492R: 5' TAC GGY TAC CTT GTT ACG ACT T 3'. The amplification conditions were: 94°C for 3 min for the first the first step, 30 cycles comprising of 94°C for 1 min, 52°C for 45 sec, 72°C for 3 min .

The PCR product obtained was purified and sequenced by an automated DNA sequencer. The forward and reverse sequences were assembled through DNA Baser V35.0 software and submitted to Gene Bank through NCBI online sequence submission tool: Bankit. MEGA5 software was used to construct phylogentic tree. In table no. (2).

Sl. No.	Strain designation	Identity	Total nucleotides	Percent Similarity
1	Sample-1	*Pseudomonas hibiscicola*	1437	99.72%

Table no.2: The BLAST analysis of bacteria by 16 S rRNA sequencing.

[III] RESULT-

3.1 MORPHOLOGICAL CHARACTERIZATION OF BACTERIA-

The colony formed on the nutrient agar was smooth, sticky and pale yellow in color. Gram nature of the isolate was Gram negative.

3.2 IDENTIFICATION OF ISOLATES BY 16S R RNA SEQUENCING-

The nucleotide BLAST analysis of the 16S r RNA gene sequence of the bacterial colony with 16S r RNA gene sequence retrieved from NCBI gene bank showed 99.72% similarity with Pseudomonas hibiscicola strain ATCC 19867 (T) which belongs to the class Proteobacteriae and phylum Xanthomonadaceae.

Gene Bank accession no: 16S r RNA gene sequence of the bacterial sample was deposited to the NCBI Gene Bank under the accession no. AB021405.

[IV] DISCUSSION-

The result of the study revealed that Pseudomonas hibiscicola was the bacteria found in the water sample of stocking pond of the bhategaon fish culture center where Cyprinus carpio is cultured.

The members of the genus Pseudomonas are important phytopathogens and agents of the human infections while other strains and species exhibit bioremediation and biocontrol activities. Species-specific detection of Pseudomonas species in the environment may help to gain more complete understanding of the ecological significance of the microorganism.

CONCLUSION-

The pseudomonas hibiscicola bacteria was isolated from the water sample of Bhategaon fish culture centre.

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