First report of isolation of *Aeromonas taiwanensis* from India

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Abstract

Genus *Aeromonas* consists of facultative anaerobic, Gram negative Bacilli which are primary environmental inhabitants worldwide. A recently reported strain of the genus, *Aeromonas taiwanensis*, was found while studying the presence of infectious marine microbes in a lacustrine wetland in India, making this the first isolation report from the country.

Keywords: Aeromonads, *Aeromonas taiwanensis*, bacterial isolation, infectious diseases, infectious strains, isolation report
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To the Editor,

Aeromonas is a widely heterogeneous genus well known for Gram-negative, facultative anaerobic rods as its constituent bacilli, which are non-sporulating, non-encapsulated, oxidase positive and generally motile by means of flagella [1–3]. Such bacterial species are residents of various aquatic and terrestrial ecosystems, ubiquitously waterborne, but also found in soil and food sources [2,4,5]. Aeromonads have also been widely associated as gastrointestinal infectious agents, particularly in immunocompromised individuals [1,6–8]. There are 32 recognized *Aeromonas* genospecies, but *Aeromonas taiwanensis* is a comparatively new species, reported only in the past decade as a wound contaminant from two Taiwanese individuals (A2-50^T = LMG 24683^T = CECT 7403^T). So far, only five reports of environmental isolates of this strain have been presented worldwide. The aim of this study was to determine the infectious microbes and potential sources of disease present in the lacustrine wetland of Ropar (Punjab, India), by performing biochemical, physiological and morphological characterization. The source of this study, Ropar Wetland, is a man-made freshwater riverine and lacustrine wetland at an elevation of 275 m above mean sea level and is situated at a 31°01′ N latitude and 076°30′ E longitude.

The media and reagents used for all studies were procured from HiMedia Laboratories Mumbai, India). Quinic acid was purchased from Spectrochem Pvt Ltd (Bombay, India). Samples were manually collected from aquatic soil sediments of Ropar Wetland in sterile plastic bottles and stored at room temperature. Bacterial growth was studied by inoculating their decimal serial dilutions on nutrient agar medium containing ferric quinate at 30°C for 48 hours to imitate the known optimal growth conditions for *Aeromonas* species. Colour of colonies was set as the primary screening and cytochrome c production was set as the secondary screening for colony selection, followed by purification of isolates through repeated sub-culturing. After optimizing cultivation parameters, cell responses to variation in cell microenvironment were determined. The isolates were tested for change in temperature, pH, salt concentration, sulphide production, indole production, motility, catalase activity, Methyl Red, Voges–Proskeur test and citrate utilization using appropriate positive and negative controls. The genotypic identification and phylogenetic analysis were carried out using 16S rDNA gene sequence analysis. From these studies, the most prominent bacterial colonies found after purification were beige opaque in colour and tested positive for oxidase production and negative for Gram stain. Physiologically, optimum growth was observed at 30°C after 48 hours at a pH of 7.0 to 7.2 [2], whereas no growth was observed at 4°C. No growth occurred at extreme acidic pH and very little growth was observed at pH 10. Variation of salt (ferric quinate) concentration did not drastically affect the CFU of isolate. The strain tested positive for motility, catalase production, Methyl Red test, but negative for H_2S production, Voges–Proskeur test, citrate utilization, and indole production, which is in accordance with the reported data. Genotypic analysis of the purified isolates revealed ten sequences producing significant alignments that showed close association with *Aeromonas taiwanensis* strain TIL_AUN_30 (Accession No. KT998825.1), and *Aeromonas taiwanensis* strain A2-50 (Accession No. NR_116585.1); checked using the maximum likelihood method (Fig. 1).

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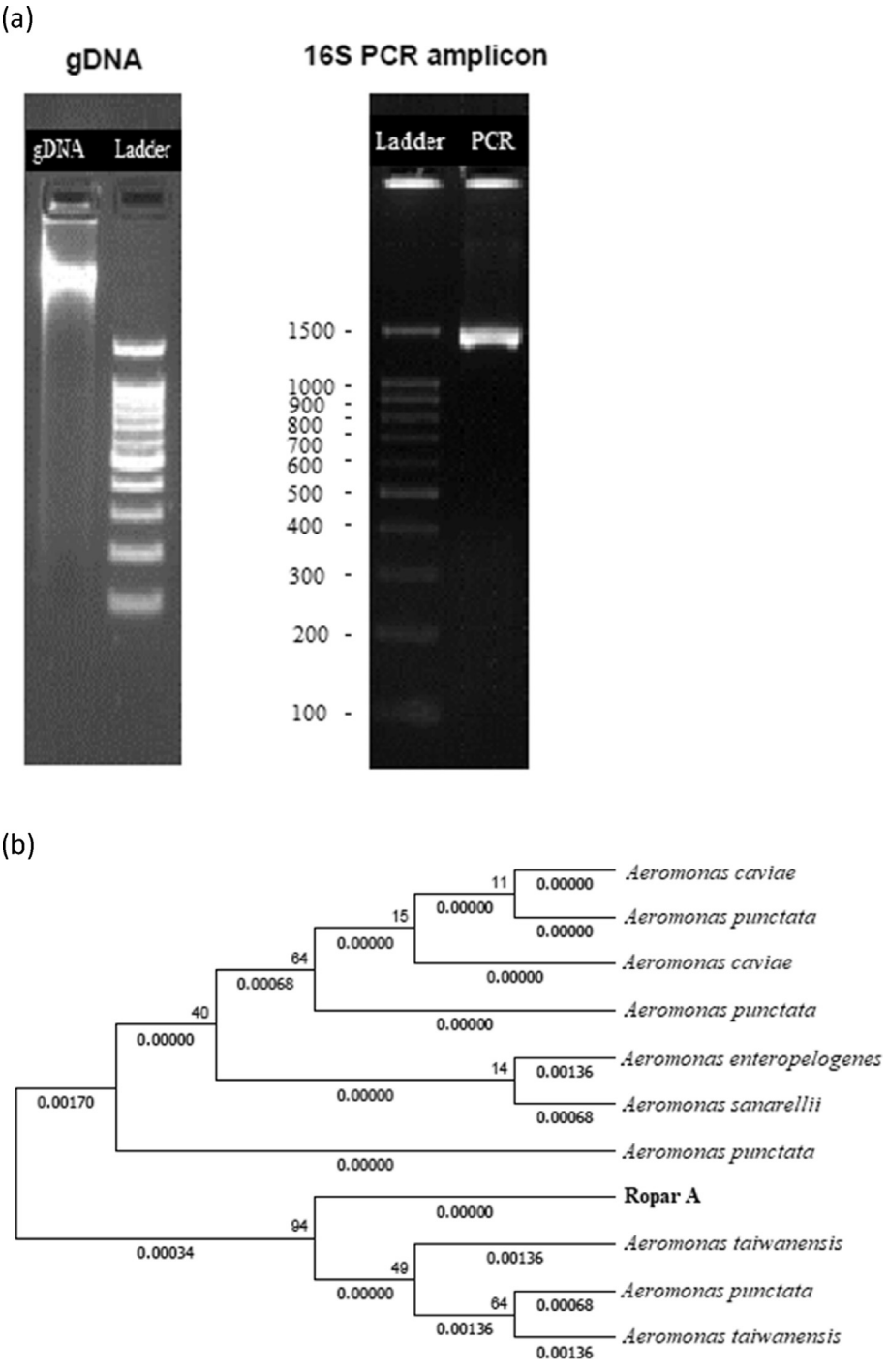

FIG. 1. (a) Genomic DNA was isolated from the culture and its quality was evaluated on 1.0% agarose gel. A fragment of 16S rDNA gene was amplified by 27F and 1492R primers. The PCR amplicon was purified to remove contaminants, and forward and reverse DNA-sequencing reactions of the PCR amplicon were carried out with forward and reverse primers using a BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic Analyzer. Consensus sequence of 16S rDNA gene was generated from forward and reverse sequence data using ALIGNER software. A single discrete PCR Amplicon band of 1500 bp was observed when a fragment of 16S rDNA gene was amplified and resolved on agarose gel. (b) The 16S rDNA gene sequence was used to carry out BLAST with the GenBank database. Based on maximum identity score, the first ten sequences were selected and aligned using a multiple alignment software program, Clustal W. A distance matrix was generated and the phylogenetic tree was constructed using MEGA 7. Molecular phylogenetic analysis revealed the ten sequences producing significant alignments. Based on analysis, ten isolates with 99% similarity were identified and the isolate showed close association with *Aeromonas taiwanensis*, checked using the maximum likelihood method. The unrooted neighbour-joining molecular phylogenetic tree is shown. (The strain under study is denoted Ropar A.)

This study concludes the existence of *Aeromonas taiwanensis* in the Ropar lacustrine wetland, which is the source of water for various domestic and agricultural tasks in the area. This highlights a potential for gastrointestinal and related infections in humans and animals and calls for higher risk management when using the water for ingestion.

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Conflict of interest

The authors declare that there is no conflict of interest and confirm that this manuscript does not infringe any other person's copyright or property rights. All authors have contributed equally to the manuscript and agreed to publication of the work.

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