

A *Flavobacterium lindanitolerans* Strain Isolated from the Ascites Sample of a Chinese Patient with EV71 Virus Infection

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Abstract

A strain of *Flavobacterium lindanitolerans* isolated from a sick child's ascites was described. The 16S rRNA gene of the strain was 100% identical to that of *Flavobacterium lindanitolerans* which was first identified in India in 2008. It was first described that the isolate required X factor (Hemin) for growth in the optimal conditions of 37 °C with 5% CO₂. The isolate produced indole and H₂S. It did not present hemolytic feature on blood agar.

Key words: *Flavobacterium lindanitolerans*; 16S rRNA; Biology

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INTRODUCTION

In 2008, a novel species of *Flavobacterium*, *Flavobacterium lindanitolerans* sp. nov was isolated from soil samples from a waste site highly contaminated with hexachlorocyclohexane in Ummari village, India^[1]. Here, we describe a second strain of this species, which was isolated from a human ascites sample. The strain was named as *F. lindanitolerans* CN-1.

CASE PRESENTATION

A 5-year-old child admitted to a hospital with serious symptoms of high fever and decompensation. He deteriorated rapidly and developed fatal pulmonary edema and hemorrhage. The child died on 20 April 2008 after 24 h in hospital. The patient was diagnosed with EV71 virus infection on 24 April 2008 by reverse-transcriptase polymerase chain reaction (RT-PCR) and sequence determination assays^[2]. The ascites specimen was inoculated onto

Columbia blood agar and Chocolate agar respectively. One isolate was obtained on chocolate agar at 37 °C and 5% CO₂. X factor (Hemin) was required for growth. The isolate did not present hemolytic on blood agar. The colony morphology initially had a translucent or transparent appearance and after 48 h, they became cream–yellow in color and had a diameter of 1.5 mm (Figure 1).



Figure 1. Colony morphology of *F.lindanitolerans* CN-1 strain. The cells were cultured on Chocolate agar for 48 h (see the description in text).

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When observed by light microscopy, cells were Gram-negative, non-sporulating, aerobic, non-flagellated short rods, which were motile by gliding, 0.5 μm in diameter, and 1.8 μm in length, occurring singly, in groups of parallel cells or in short chains (Figure 2).

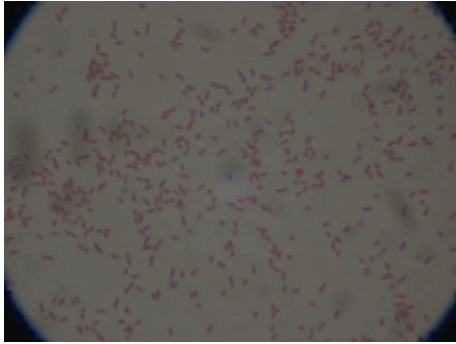


Figure 2. *F.lindanitolerans* CN-1 strains were observed by light microscopy. Cells were Gram-negative, non-sporulating, aerobic, non-flagellated short rods, which were motile by gliding, 0.5 μm in diameter, and 1.8 μm in length, occurring singly and in groups of parallel cells (magnification $\times 1000$).

ISOLATE IDENTIFICATION

The biochemical reaction characteristics of the strains were tested for the glucose fermentation reaction, enzymatic activity and production of indole by using APINH, API 20E, API Coryne, and API ZYM (bioMerieux SA) systems. There was a positive reaction for catalase and a negative reaction for oxidase. The strain produced indole and H_2S . And It did not ferment D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-saccharose, D-melibiose, Amygdaline, L-arabinose, D-fructose, D-maltose, D-xylose, D-galactose, D-lactose, D-ribose, and Glycogen. Enzyme activity was detected for Alkaline phosphatase, Esterase C4, Esterase lipase C8, Leucine arylamidase, Valine arylamidase, Cystine arylamidase, α -chymotrypsin, Acid phosphatase, and Apthol-AS-biphosphohydrolase. No enzyme activity was detected for Penicillinase, Lipase C14, L-arginine dihydrolase, L-lysine decarboxylase, L-arginine dihygrolase L-ornithine decarboxylase, Urease, Alkaline phosphatase, Proline phosphatase, γ -glutamyl transferase, Pyrolidonyl arylamidase, α -glucosidase, and Gelatinase. There was no utilization of Trisodium citrate. DNA was extracted from the ascites sample. The 16S rRNA gene sequencing was amplified by using the universal

primer pairs 16S1 (5'-TCCTACGGGAGGCAGCAG-3') and 16S2 (5'-CCCGGAACGTATTCACCG-3')^[3]. The amplified product was purified and used for sequencing by the primers 16S1 and 16S2. The stretch of the 16S rRNA gene sequence obtained (1 045 bp) was checked manually for quality and gaps. Similarity searches were conducted by using the sequence match tool of the Ribosomal Database Project (http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp) and the BLAST program of the National Centre for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>). The 16S rRNA gene sequence of this strain showed 100% similarity with that of *F.lindanitolerans* IP-10T by comparison with sequence data published in GenBank. The nucleic acid sequences of the 16S rRNA gene of the genus *Flavobacterium* was downloaded from the GenBank website. A set of primers that were species-specific for *F. lindanitolerans* was designed by the DNASTar software to detect *F.lindanitolerans* by PCR. The primers were F-lin-F (5'-AGGGCTTGAATTGCCG-3') and F-lin-R (5'-TCCATCAGCGTCAATACG-3'). The predicted DNA was 116 bp for *F.lindanitolerans*. An amplicon of 116 bp was amplified from the DNA from the ascites sample. The phenotypic, chemotaxonomic, and genetic data were unambiguously consistent with those described previously for *F.lindanitolerans*. Therefore, the species was named as *F.lindanitolerans* CN-1.

DISCUSSION

To the best of our knowledge, this is the first description of the *F.lindanitolerans* isolated from clinical specimens. Because the recovery of this organism from humans has not been described previously, its contribution to disease appears to be variable. It is possible that the organism was simply a contaminant derived from environmental and/or water sources because *Flavobacterium* spp. are often isolated from soil, water and fish^[1]. Further studies are required to elucidate its clinical relevance. This report also highlights that identification of this bacterium by 16S rRNA sequencing, is ambiguous.

We also described the biological characteristics of *F.lindanitolerans* CN-1 strain in detail. The NCBI GenBank accession number for the 16S rRNA gene sequence of *F.lindanitolerans* CN-1 strain was GQ121368.1.

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