Non-contiguous finished genome sequence and description of *Streptococcus varani* sp. nov.

S. Bakour[1], J. Rathored[1], C. I. Lo[1,2], O. Mediannikov[1,2], M. Beye[1], C. B. Ehounoud[1], P. Biagini[3], D. Raoult[1,2,4], P.-E. Fournier[1] and F. Fenollar[1,2]

1) *Aix-Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, Inserm U1095, Faculté de médecine, Marseille cedex 05, France,* 2) *Campus international UCAD-IRD, Dakar, Senegal,* 3) *Equipe Emergence et Co-évolution Virale, UMR 7268 – Aix-Marseille Université/ EFS / CNRS, IHU Méditerranée Infection, Marseille, France and* 4) *Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdul Aziz University, Jeddah, Saudi Arabia*

Abstract

Strain FF10^T (= CSUR P1489 = DSM 100884) was isolated from the oral cavity of a lizard (*Varanus niloticus*) in Dakar, Senegal. Here we used a polyphasic study including phenotypic and genomic analyses to describe the strain FF10^T. Results support strain FF10^T being a Gram-positive coccus, facultative anaerobic bacterium, catalase-negative, non-motile and non-spore forming. The sequenced genome counts 2.46 Mb with one chromosome but no plasmid. It exhibits a G+C content of 40.4% and contains 2471 protein-coding and 45 RNA genes. On the basis of these data, we propose the creation of *Streptococcus varani* sp. nov.

Keywords: Culturomics, genome, *Streptococcus varani*, taxonogenomics, *Varanus niloticus*
Original Submission: 5 February 2016; **Accepted:** 14 March 2016
Article published online: 19 March 2016

Corresponding author: P.-E. Fournier, URMITE UM63, CNRS7278, IRD198, INSERM1095, Faculté de Médecine, Aix Marseille Université, 27 boulevard Jean Moulin, 13385 Marseille cedex 5, France
E-mail: pierre-edouard.fournier@univ-amu.fr

Introduction

The genus *Streptococcus* contains 113 recognized species and 22 subspecies (http://www.bacterio.net/streptococcus.html) as of 5 February 2016. These species are Gram-positive cocci, chain-forming, facultative anaerobes and catalase negative [1]. Many of them are associated with human or animal hosts. Previous studies have reported that a large number of *Streptococcus* species colonize the oral cavities of humans and animals [2]. Among oral streptococci isolated from animals, we note: *Streptococcus ursoris* isolated from the oral cavities of bears [1], *Streptococcus orisratti* isolated from the surface of the lower molars of Sprague-Dawley rats [3], *Streptococcus oriloxodontae* isolated from the oral cavities of elephants [4], and *Streptococcus mutans* isolated from dental plaque [5].

Recently, with next-generation sequencing technology able to sequence whole genomes in a short time, and mass spectrometric analysis of bacteria, we have had easy access to genetic and proteomic information [6]. Therefore, we propose a polyphasic approach combining genomic properties in combination with matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) spectra and phenotypic characteristics to describe new bacterial species. The strain FF10^T (= CSUR P1489 = DSM 100884) was isolated from the oral cavity of the Nile monitor (*Varanus niloticus*) in Dakar, Senegal.

Here, we present a summary classification and a set of features for *Streptococcus varani* sp. nov. strain FF10^T (= CSUR P1489 = DSM 100884), including the description of its complete genome and annotation. These characteristics support the circumscription of the species *S. varani*.

http://dx.doi.org/10.1016/j.nmni.2016.03.004

Classification and Features of the Strain

Strain identification

In 2014, a sample was collected from the oral cavity of a lizard reptile named 'Nile monitor' (*Varanus niloticus*) in Dakar, Senegal, and stored at −80°C. In October 2014, the strain FF10^T (Table 1) was isolated from this sample by cultivation on 5% sheep blood-enriched Columbia agar (BioMérieux, Marcy l'Etoile, France) at 37°C with 5% CO_2.

To identify the strain, MALDI-TOF MS protein analysis was performed as previously described [7] using a Microflex spectrometer (Bruker Daltonics, Leipzig, Germany). The 12 FF10^T spectra were imported into the MALDI BioTyper software (version 2.0, Bruker) and analysed by standard pattern matching (with default parameter settings) against the main spectra of 6252 bacteria. From the resulting scores, the tested species may or may not be identified compared with the instrument's database; a score $\geq$ 2 with a validly published species enables identification at the species level; a score $\geq$ 1.7 and < 2 allows identification at the genus level; and a score < 1.7 does not enable any identification. For strain FF10^T, the scores obtained were lower than 1.306, suggesting that our strain was not a member of any known species. The reference mass spectrum of *S. varani* strain FF10^T was added to our database (Fig. 1). A gel view comparing the spectrum of strain FF10^T with those of other *Streptococcaceae* species is shown in Fig. 2. The bacterium was identified using 16S rRNA PCR coupled with sequencing, as previously described [8]. Strain FF10^T exhibited 96% 16S rRNA sequence similarity with *Streptococcus minor* strain DSM 17118 (GenBank accession number: AY232832), the phylogenetically closest bacterial species with standing in nomenclature-validated *Streptococcus* species (Fig. 3). This value was lower than the 98.7% 16S rRNA gene sequence threshold recommended by Stackebrandt and Ebers to delineate a new species without carrying out DNA–DNA hybridization [9]. *Streptococcus varani* strain FF10^T 16S rRNA accession number from GenBank Sequence Database is LN810501.

TABLE 1. Classification and general features of *Streptococcus varani* strain FF10^T

Property	Term	References
Classification	Domain *Bacteria*	[25]
	Phylum *Firmicutes*	[26,27]
	Class *Bacilli*	[28,29]
	Order *Lactobacillales*	[29,30]
	Family *Streptococcaceae*	[31,32]
	Genus *Streptococcus*	[31,33,34]
	Species *Streptococcus varani*	
	Type strain FF10^T	
Gram stain	Positive	
Cell shape	Cocci	
Motility	Non-motile	
Sporulation	Non-spore forming	
Temperature range	Mesophile	
Optimum temperature	37°C	
Carbon source	Unknown	
Habitat	Lizard	
Salinity	Unknown	
Oxygen requirement	Facultative anaerobe	
Biotic relationship	Free living	
Pathogenicity	Unknown	
Geographic location	Dakar	
Sample collection	October 22, 2014	
Latitude	13.7167	
Longitude	−16.4167	
Altitude	51 m above sea level	

Growth characterization

Different growth temperatures (25°C, 30°C, 37°C, 45°C and 56°C) were tested. Growth was obtained between 25 and 37°C, with optimal growth at 37°C. Growth of the strain was tested also under anaerobic and microaerophilic conditions using GENbag anaer and GENbag microaer systems, respectively (bioMérieux), and under aerobic conditions, with or without 5% CO_2. Strain growth was observed under anaerobic and microaerophilic conditions but optimal growth was observed under aerobic conditions. Colonies were translucent and yellow with a regular surface, haemolytic on 5% sheep blood-enriched Columbia agar (bioMérieux), and approximately 1 mm in diameter. A motility test was negative. Cells were Gram-positive cocci, unable to form spores (Fig. 4), and with mean diameter of 0.6 µm (range 0.4–0.8 µm) and mean length of 1.3 µm (range 0.7–1.9 µm) (Fig. 5).

Biochemical characterization and antibiotic susceptibility

This bacterium, FF10^T, exhibits neither catalase nor oxidase activities. Using an API ZYM strip (bioMérieux), positive reactions were observed for alkaline phosphatase, esterase, esterase-lipase, lipase, leucine arylamidase, acid phosphatase, β-glucosidase, β-galactosidase, α-mannosidase, and α-fucosidase. Negative reactions were noted for cystine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, α-glucosidase, α-galactosidase, β-glucuronidase, naphthol-AS-BI-phosphohydrolase, and *N*-acetyl-β-glucosaminidase. Using an API 20 NE strip (bioMérieux), positive reactions were observed only for the esculin hydrolysis test whereas negative reactions were observed for nitrate reduction, urease, indole production, arginine dihydrolase, glucose fermentation, arabinose, mannose, mannitol, *N*-acetyl-glucosamine, maltose, gluconate, caprate, adipate, malate, citrate, phenyl-acetate assimilation, and gelatine hydrolysis.

Using API 50 CH strip (bioMérieux), negative reactions were observed for the fermentation of glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-αD-xylopyranoside, methyl-αD-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin,