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Full Length Research Paper

Isolation and molecular identification of a *Serratia* strain from domesticated tree shrew (*Tupaia belangeri*) skin infectious site in Yunnan, China

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In this study, a *Serratia* strain from an infectious skin site of domesticated tree shrew was isolated and the main characterization of the isolate using a variety of genotypic and phenotypic criteria was determined. The strain was finally identified as *Serratia grimesii* on the basis of its small subunit ribosomal RNA (16S rRNA) gene sequence.

Key words: *Serratia*, tree shrew, 16S rRNA gene.

INTRODUCTION

The genus *Serratia* belongs to the family Entero-bacteriaceae and consists of the recognized species: *Serratia marcescens*, *Serratia liquefaciens*, *Serratia ficaria*, *Serratia rubidaea*, *Serratia fonticola*, *Serratia odorifera*, *Serratia plymuthica*, *Serratia grimesii*, *Serratia proteamaculans*, *Serratia quinivorans*, *and Serratia entomophila* (Eisenstein, 1990; Grimont and Grimont, 1978). All species except *S. entomophila* have been isolated from clinical samples, and *Serratia* species are regarded as significant pathogens to which a variety of infections including peritonitis, pneumonia, sepsis and wound infections have been attributed (Berg et al.,2000; Grohskopf et al., 2001; Boulton et al., 1998; Roth et al., 2000; Kampf et al., 2004).

The tree shrews are small animals belonging to the family *Tupaiidae*, mainly found in Southern China, India and Southeast Asia. The 17 known species of tree shrews are classified in the genus *Tupaia*, family *Tupaiidae*. Among them, Northern tree shrew *Tupaia belangeri* is a typical specie. Tree shrews are now seen as possible susceptibility of tree shrews (*Tupaia belangeri*) and their models for medical and biological research. Due to the hepatocytes to infection with human hepatitis B virus (HBV) *in vivo* and *in vitro*, these animals have been used to establish human hepatitis virus-induced hepatitis models. As these animals are phylogenetically close to primates in evolution and have a well-developed visual system and color vision in some species, they have been utilized to establish myopia models. However, the tree shrews holds significant promise as research models and great use could be made of these animals in biomedical research (Novacek, 1992; Cao et al., 2003).

In natural or domesticated state, tree shrew *T. belangeri* has proved to be infected with several bacteria, viruses and parasites (Bahr and Darai 2001; Tidona et al., 1999; Saitou and Nei, 1987). In this report, we describe the isolation and identification of a *Serratia* strain from the skin infection site of an adult *T. belangeri* tree shrew.

MATERIALS AND METHODS

Animal and bacterial isolate identification

A domesticated adult tree shrew caged in Kunming Medical University, the Center of Experimental Animals, Yunnan, China, was found to have the skin infection on its lower back. The animal is indifferent, hair-stiff, and inactive, the infectious site is ulcerative and exudative.

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Table 1. Phenotypic characteristics of *Serratia* strain TS26-8.

Characteristics	Test results
Motile rod	+
Pigment	-
Catalase	+
H_2S poroduction	+
Gas from D-glucose	+
DNase	+
Gelatin hydrolysed	+
Tween 80 hydrolysed	+
Chitin hydrolysed	+
Acids from	
L-Arabinose	+
D-Melibiose	+
D-Raffinose	+
D-Xylose	+
Carbon source use	
4-Aminobutyrate	+
Caprate	+
Caproute	+
Caprylate	+
L-FucoseTyrosine	+
N-acetylglucosamine	+
cis-Aconitate	+
Citrate	+
d-Fructose	+
d-Galactose	+
d-Galacturonate	+
Myo-inositol	+
Maltose	+
Maltotriose	+
d-Mannitol	+
d-Mannose	+
d-Ribose	+

Sample was taken by using sterile cotton swabs to suck the exudates in the infectious site. Swab was thoroughly obtained by rotating the swab to ensure that a good inoculum was gotten.

The sample was transferred to the sheep blood agar culture dish for pathogenic coccus cultivation and nutrient agar culture dish and MacConkey agar for pathogenic rod bacteria growth and differentiation by touching the swab to the agar surface in a single spot. The Petri dish was incubated at 32°C and blood agar at 37°C for 48h. Pure cultures were obtained by streaking the isolated colonies to suitable agar dishes and used in further experiments. Biochemical tests were selectively done according to the standards in Bergey's Manual of Systematic Bacteriology (second ed) and The Prokaryotes: A Handbook on the Biology of Bacteria (third ed). The isolated colonies were differentiated by colonial character, pigment, staining, bacterial morphology and selective biochemical tests.

Phylogenetic study

In spite of the tentative biochemical identification of strain TS26-8 as *S. liquefaciens* complex, the difficulties in precise phylogenetic positioning of Serratia strain TS26-8 motivated us to perform a detailed phylogenetic study based on 16S rDNA. DNA (deoxyribonucleic acid) was extracted from cultures using a Takara Genome DNA Extraction Kit (Takara, Dalian, China) according to the manufactures' specifications. DNA purity was evaluated by OD260/280.

PCR (polymerase chain reaction) amplification and sequencing of isolate 16s rRNA gene was carried out using a Takara 16s rDNA bacterial Identification kit. Five microliters (5 µl) of the extracted DNA was amplified with primers F27(5′-AGAGTTTGATCM TGGCTCAG-3′) and R1492(5′-TACGYTACC-TTGTTACGACT-3′), generating a PCR product corresponding to nucleotide positions 27 to 1492 of the *Escherichia coli* 16S rDNA sequence. All reactions were carried out in 50 ml volumes, containing 12.5 pmol of each primer, 200 mM of each dNTP, 2.5 ml of 103 PCR buffer (100 mM Tris-HCl, 15 mMMgCl2, 500 mM KCl; pH 8.3), and 0.5 U of *Taq* DNA polymerase (Roche Diagnostics, Mannheim, Germany) and increased to 50 ml with sterile water. PCR was performed in a Takara PCR machine (Takara, Dalian, China) with the following program: 5 min denaturation at 94°C, followed by 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, 1.5 min extension at 72°C, and a final extension step of 5 min at 72°C. Five microliters (5 µl) of PCR products was visualized by electrophoresis in 2% (wt/v) agarose gels and with ethidium bromide (0.5 mg/ml) staining. To verify the presence of appropriate sized amplicons, the PCR products were subjected to electrophoresis in 1% agarose gels according to standard methods.

Products of the correct size were purified with a TakaRa Agarose Gel DNA Purification Kit Ver.2.0 and sequenced in both directions using an ABI Prism DNA sequencing kit and an ABI Model 377 sequencer (PE Biosystems, ABI). The bacterial 16S rDNA sequences obtained were then aligned with known 16S rDNA sequences in Genbank using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information, and percent homology scores were generated to identify bacteria. Bacteria with 16S rDNA sequences >98% similarity were considered to be of the same phylotype. Each 16S rDNA sequence was compared by using the BLAST alignment program with data available from GenBank at the National Institutes of Health. The computer alignment provides a list of matching organisms, ranked in order of similarity between the unknown sequence and the sequence of the corresponding organism from the database. The percentage and absolute number of matched base pairs from each BLAST match were reported.

Bacterial 16S rDNA sequences were also aligned with near neighbor sequences using the Clustal W program and phylogenetic relationships were inferred using a maximum likelihood algorithm (Phylip package). Phylogenetic trees were constructed by the neighbour-joining method (Saitou and Nei 1987) using the PHYLIP version 3.6 software package (Felsenstein, 2008).

RESULTS AND DISCCUSSION

Pure bacteria cultures from infectious skin site of tree shrew (*T. belangeri*), which were tentatively identified as *Serratia* by colonial character, pigment, stain and bacterial morphology, was tentatively identified as *Serratia* sp. based on phenotypic analysis and designated Serratia TS26-8. The results of the phenotypic analysis of this isolate, in comparison to type strains of the nine Serratia species most closely related to *S. liquefaciens* complex, are summarized in Table 1.

Part of the 16S rDNA gene sequence of strain TS26-8 was amplified and analysed (GenBank Accession No. FJ914227). Figure 1 shows a neighbour-joining phylo-

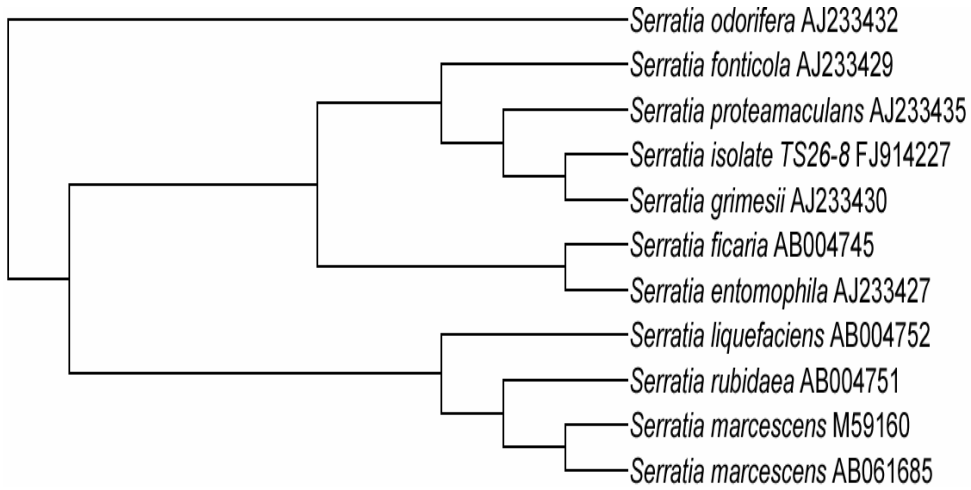

Figure 1. Phylogenetic tree derived from a comparison of the 16s rDNA sequences of *Serratia* sp. by the PHYLIP method. Bacterial 16S rDNA sequences were aligned with near neighbor sequences using the Clustal W program and phylogenetic relationships were inferred using a maximum likelihood algorithm (Phylip package). Phylogenetic trees were constructed by the neighbour-joining method using the PHYLIP version 3.6 software package. GenBank accession numbers are shown. The position of the sequence derived from tree shrew sample is included.

genetic tree based on the alignment of the nearly complete 16S rDNA gene sequence of strain TS26-8 with 16S rDNA sequences of the 10 described *Serratia* type strains available in GenBank and EMBL databases. The 16S rDNA sequence similarity between strain TS26-8 and the 9 described *Serratia* species ranged between 99.82% and 95.63%, with the highest similarity to *S. grimesii* (99.82%) and *S. proteamaculans* (99.18%) and the lowest to *S. rubidaea* (95.79%) and *S. marcescens* (95.63%).

Rapid advances in DNA sequencing technology have led to a major change in the way prokaryotes are classified. Sequence analysis of highly conserved regions of the bacterial genome, such as the small subunit rRNA gene, now provide us with a universal method of estimating the evolutionary relationships among all organisms. Such gene-based phylogenetic classifications have led to many new discoveries about prokaryotes that were not reflected in the classical classification. Phylogenetic classification is now broadly accepted as the preferred method of representing taxonomic relationships among prokaryotes. Many taxa based on shared phenoltypic features may be quite distinct from one another based on phylogenetic evidence (Petti, 2007; Clarridge, 2004).

In conclusion, a pathogenic *Serratia* strain TS26-8 from an infectious tree shrew skin site was isolated and a comparative characterization of it with the type strains of 10 most closely related *Serratia* species was performed. Phenotypically, the isolate could not be clearly assigned to any of the described *Serratia* species. Furthermore, these observations add new phenotypic and genotypic information to the *Serratia* genus, thus contributing to a more precise phylogenetic classification of *Serratia* strains.

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Abbreviations:

DNA, Deoxyribonucleic acid; **rRNA,** ribosomal ribonucleic acid; **RNA,** ribonucleic acid; **rDNA,** recombinant deoxyribonucleic acid; **PCR,** polymerase chain reaction.

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