Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 868-872
Received: 13-07-2018
Accepted: 14-08-2018

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Identification and genetic characterisation of *Serratia liquefaciens* isolated from textile effluents

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Abstract
Textile industries were the major source of environmental pollution In terms of quality as well as quantity. The textile industries consume rich amount of water (200 m^3/ton of product) out of which around 90% is appeared as wastewater. Textile dye effluents contain dye as the major pollutants which were not only recalcitrant but also imparts intense colour to the waste effluent. Bacteria were isolated from textile dye effluents. Morphological and biochemical characterization was done to identify isolates and was found to be *Serratia liquefaciens* in preliminary. The identification of bacteria was confirmed by 16S rRNA sequencing. They were identified as *S. liquefaciens*. The sequences were deposited in NCBI and accepted the accession number was KU041531. The present investigation was conducted to identification and genetic characterisation of *S. liquefaciens*.

Keywords: Textile effluents, *Serratia liquefaciens*, Sequencing technique, GENBANK

Introduction
Water is so important for life that we cannot imagine life without it**.** In today's life, rapid industrialization and urbanization realized the arrival of boundless measure of waste into nature. Water is critical for survival and nearness of life on planet earth. The waste water is released from the textile industries, that misuses are going into the water bodies, it is one of large cause of environment risk (Kaur *et al.,* 2010; Ahmed *et al.,* 2012) [1-2]. The textile industries produce effluents that contain several types of chemicals that are toxic and mutagenic and/or carcinogenic (Brown and DeVito 1993) [3]. Textile effluents contain dyes, heavy metals and bacteria. Over the millennia, microbes have adapted to extremely diverse environments, and developed an extensive range of new metabolic pathways or library of catabolic enzymes (Butler and Mason 1997; Ellis, 2000) [4-5].

Serratia appears to be a ubiquitous genus in nature, and ten species are currently recognized (Grimont & Grimont, 1992) [6]. *Serratia* species have been isolated from water, soil, animals (including man) and from the surfaces of plants (Grimont & Grimont, 1992). *Serratia liquefaciens* is an organism rarely encountered in clinical practice. It belongs to the genus *Serratia* and the family Enterobacteriaceae [7]. It is widely distributed in nature, including river water [8], mineral, spring and table water [9], domestic sewage [10], fish, minced meat and pasteurized milk or cream [11].

Preliminary identification of the bacterial isolate regarding its morphological, biochemical characteristics' and decolourisation activity as well as the genetic level identity gives valuable information with regard to the further application of strain for different purposes [12]. The right taxonomic position of an environmental isolate could be accurately assigned to identify its novelty, this could only be done by the use of advanced molecular techniques (Yumoto *et al.,* 2001) [13]. 16S rRNA gene sequence analysis is the most commonly used method for identifying bacteria or for constructing bacterial phylogenetic relationships (Woese, 1987; Vandamme *et al.,* 1996; Joung & Cote, 2002) [14-16]; however, its usefulness is limited because of the high percentage of sequence similarity between closely related species (Ash *et al.,* 1991; Martı´nez-Murcia *et al.,* 1992; Christensen *et al.,* 1998) [17-19]. The DNA sequence of the16S rRNA gene has been determined for an extremely large number of species. RNA structure plays an important role in the life cycle of bacteria and provides the ability to understand evolution and stability (Zuker, 1989) [20]. Several reports have been available on RNA secondary structure of genes. The 5S rRNA sequences from different bacteria have been used previously for the generation of secondary structure (Singh and Somvanshi, 2009) [21]. The scope of this study was to identify and characterize a *Serratia* strain isolated from Tirupur textile dye effluents.

Materials and Methods

Sample collection

Textile dye effluent sample was collected from Tirupur textile industry, Tamil Nadu, India. 5 litre volumes precleaned and rinsed polythene water canes and 500 ml BOD bottles were used for collection of water samples for the analysis of physicochemical characteristics. Samples were protected from direct sun light and immediately transported to the laboratory for further analysis.

Morphological and Biochemical Characterization

The bacterial isolate was further studied for its morphology including gram staining and biochemical observations which includes the colony size, color, shape, diameter, elevations, and whether opaque, transparent or translucent. The results were compared using Bergey's manual (1984) [22].

DNA isolation

Five ml of overnight culture were washed and suspended in TE buffer, PH 8.0. Genomic DNA was isolated as per the method of Schmalenberger [23].

PCR conditions

All the reaction mixtures contained 1X PCR buffer (10 mM Tris HCl, pH-9.0, 50 mM KCl and 0.01% gelatin); 100 mM concentration of each dNTPs and 0.75 unit of Taq polymerase. The final concentration of $MgCl_2$ was adjusted to 1.5 mM in PCR-Ribotypng. In PCR-ribotyping $MgCl_2$ concentration was adjusted to 3 mM. Each primer concentration was 20 pmol for PCR-Ribotypng. PCR-ribotyping was carried out according to the method of Franciosa .The primers for the amplification of DNA spacer regions between the 16S-5S genes were F (50 -TTG TAC ACA CCG CCC GTC A-30) and R (50 -GCT TAA CTT CCG TGT TCG GTA TGG G-30). The amplification was carried out by after initial denaturation at 94 1C for 2 min, followed by 35 cycles at 94 1C for 1 min, 35 1C for 1 min and 72 1C for 2.5 min, with a ram time of 2 min between 35 and 72 1C; a final extension was performed at 72 1C for 5 min. Agarose gel electrophoresis.

Agarose gel electrophoresis

The PCR products (10 ml) were separated by electrophoresis on a 1.5% agarose gel containing ethidium bromide (0.5 mg/ml) at 100 V for 1 h. in 1X TBE (Tris-Boric acid-EDTA) buffer. The gel images were digitized through UV gel image acquisition camera (Kodak, Japan).

16S r RNA sequencing analysis

The PCR amplified DNA was sequenced in ABI 3730 DNA Analyzer (Applied Biosystems). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment required editing of the obtained sequences were carried out using Geneious Pro v5.

Phylogenetic analysis

The 16s rDNA sequences of *B.a* isolates aligned with the sequences of similar species which were retrieved from the Gene bank database. The sequences were converted in to FASTA format. The retrieved sequences were fed with ClustalW for multiple sequence alignment to observe sequence homology. Evolutionary tree was inferred by using the neighbour-joining method [24]. Dendrogram was constructed by neighbour-joining method using PHYLIP software package.

Results and Discussion

Table 1: Morphological Characterization of *S. liquefaciens*

S. No.	Characterization	*S. liquefaciens*
1	Grams Staining	Gram negative
2	Shape	Rods
3	Motility	+

Table 2: Biochemical Characterization of *S. liquefaciens*

S. No.	Characterization	*S. liquefaciens*
1	Catalase	+
2	Oxidase	+
3	Nitrate reductase	-
4	Starch	-
5	Casein	+
6	Gelatin	+
7	Urea	-
8	MR	+
9	Indole	-
10	VP	-
11	Gas production	-
12	Citrate	+

Morphological and biochemical Characters

In the present investigation morphological features were observed for the isolates grown on Nutrient agar medium (Figure 1). Bacterial isolates were rod shaped. Motility characterisation was observed under the compound microscope. *S. liquefaciens* is a gram negative bacteria was also observed by microscope. Because bacteria do not accept the gram stains (Table 1). Colony morphology and Gram staining is one of the basic microbial techniques used to group the bacteria. The present results revealed that 90% of the strains were Gram negative which is in agreement with previous studies. It is generally observed that most of the bacteria isolated from water are Gram negative (Berry *et al.*, 2006) [25].

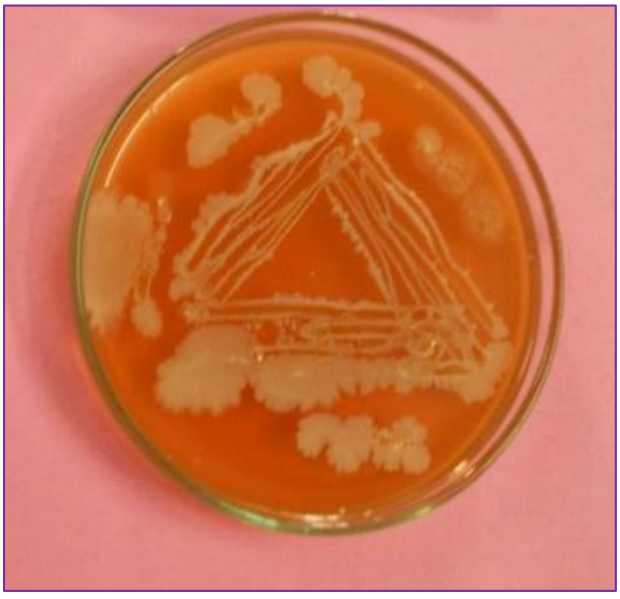
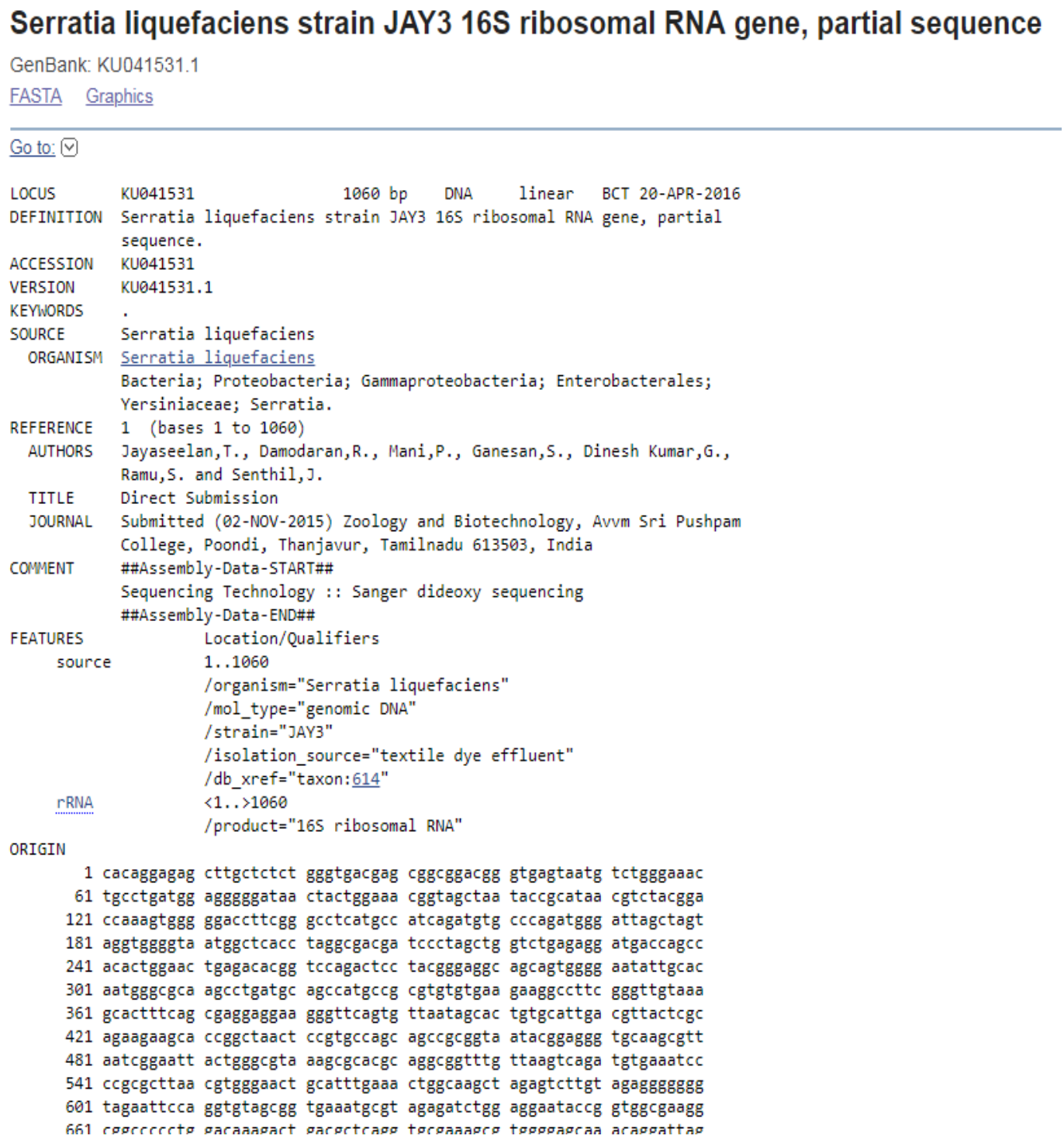


Fig 1: *S. liquefaciens* culture on Petri plate



Serratia liquefaciens strain JAY3 16S ribosomal RNA gene, partial sequence

GenBank: KU041531.1

FASTA Graphics

Go to:

```
LOCUS       KU041531               1060 bp    DNA     linear   BCT 20-APR-2016
DEFINITION  Serratia liquefaciens strain JAY3 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   KU041531
VERSION     KU041531.1
KEYWORDS    .
SOURCE      Serratia liquefaciens
  ORGANISM  Serratia liquefaciens
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Yersiniaceae; Serratia.
REFERENCE   1  (bases 1 to 1060)
  AUTHORS   Jayaseelan,T., Damodaran,R., Mani,P., Ganesan,S., Dinesh Kumar,G.,
            Ramu,S. and Senthil,J.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-NOV-2015) Zoology and Biotechnology, Avvm Sri Pushpam
            College, Poondi, Thanjavur, Tamilnadu 613503, India
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            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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      121 ccaaagtggg ggaccttcgg gcctcatgcc atcagatgtg cccagatggg attagctagt
      181 aggtggggta atggctcacc taggcgacga tccctagctg gtctgagagg atgaccagcc
      241 acactggaac tgagacacgg tccagactcc tacgggaggc agcagtgggg aatattgcac
      301 aatgggcgca agcctgatgc agccatgccg cgtgtgtgaa gaaggccttc gggttgtaaa
      361 gcactttcag cgaggaggaa gggttcagtg ttaatagcac tgtgcattga cgttactcgc
      421 agaagaagca ccggctaact ccgtgccagc agccgcggta atacggaggg tgcaagcgtt
      481 aatcggaatt actgggcgta aagcgcacgc aggcggtttg ttaagtcaga tgtgaaatcc
      541 ccgcgcttaa cgtgggaact gcatttgaaa ctggcaagct agagtcttgt agaggggggg
      601 tagaattcca ggtgtagcgg tgaaatgcgt agagatctgg aggaataccg gtggcgaagg
      661 cggccccctg gacgaagact gacgctcagg tgcgaaagcg tggggagcaa acaggattag
```

Fig 2: *S. liquefaciens* sequences in NCBI

In the present study bacterial isolates showed positive for catalase test, oxidase test, gelatin test, MR test and citrate test. Strains showed negative for nitrate reduction test, starch test, urea test, indole test, VP test and gas production (Table 2). These biochemical tests were not always conclusive. So bacterial identification were carried out by molecular level which was best and suitable.

16S rRNA sequencing of bacteria

After the determination of colony colour, shape and biochemical characteristics, the isolate selected by the PCR analysis was hesitantly identified as *S. liquefaciens* and it was confirmed by the 16S rRNA sequencing with help of universal bacterial primers, 16s-UP-F and 16s-UP-R. Sequence of the *S. liquefaciens* isolate showed partial 16S rRNA sequences, consisting of 1060 nucleotides which was submitted to the Gene bank of NCBI (National Center for Biotechnology Information, USA) and was obtained an accession Number (KU041531) (Figure 2). Bacteria have 1060 nucleotides were compare with already known all kinds of bacterial genomes. The comparison of the bacterial 16S rRNA gene sequence has emerged as a preferred genetic technique (Clarridge, 2004) [26]. *A. veronii* has been isolated from the septic arthritis patient and identified by 16S rDNA and PCR (Roberts, *et al.*, 2006) [27]. Therefore, 16S rRNA is a significant target to the molecular level identification. The upstream region of 16S rRNA is known to be highly conserved in species to species so this region could also be

used for the verification of the thermodynamic stability on the basis of conserved secondary structures of RNA. The sequences showed 100% resemble with the already established the species *S. liquefaciens*. The 16S rRNA gene sequences showed 100% similarity with *A. veronii* in the existing NCBI database. The sources of different strains of *A. veronii* were reported by Vijai Singh *et al.,* (2011) [28]. From this study, the isolate was confirmed as the isolate of the species *S. liquefaciens*. Phylogenetic tree was deduced from species of *S. liquefaciens* using Neighbour-joining method.

After DNA isolation and PCR reaction the amplified product was purified to remove the excess primer for sequencing and it was sequenced using the automated DNA sequencer. Sequence of the bacterial isolate showed partial 16S rRNA sequences, consisting of 1060 base pairs which were submitted to the Gene bank (National Center for Biotechnology Information, USA) and an Accession Number (KU041531) was obtained. The obtained sequences were compared with the NCBI gene bank database using BLAST search program (http;//www.ncbi.nlm.nih.gov) (Marchler–Bauer *et al.,* 2000; Pruitt *et al.,* 2005) [29-30]. Next, Phylogenetic tree was deduced from species of *S. liquefaciens* using Neighbour-joining method. The goal of this study was to evaluate the potential of 16S rRNA sequencing to rapidly identify *S. liquefaciens* in bacterial cultures.

Systematically analysed the usefulness and degree of confidence in using 16S rRNA gene sequence for identification of medically important bacterial species (Woo *et al.,* 2007, 2009) [31-32]. Drancourt *et al.,* (2000) [33] employed 99% and 97% nucleotide identity as the cut-off for species and genus identification respectively. The 16S rRNA gene sequences showed 100% similarity with *S. liquefaciens* in the existing NCBI database. The MicroSeq databases do not take into account bacteria with similar 16S rRNA gene sequences, which can confuse the identity of the bacterium. In this study revealed that out 1060 nucleotides were compare with already identified *S. liquefaciens.* Gene sequences were 100% similar to that of *S. liquefaciens.*

Conclusion

The textile dye effluents are enriched media to grow and spread microbial population. Previously, strains were identified by colony morphological and biochemical characteristic features. The conventional morphological and biochemical tests used for identification of *S. liquefaciens* are time consuming, laborious and are not always conclusive. Bacterial species have at least one copy of the 16S rRNA gene containing highly conserved regions together with hyper variable regions. The ribosomal operons mainly 16S rRNA has proven to be a stable and specific molecular marker for the identification of bacteria. 16S rRNA sequencing has a powerful capacity to rapidly identify *S. liquefaciens* and other bacterial species. The identification of bacteria was confirmed as *S. liquefaciens*. The genotyping method using 16S rRNA gene sequence is both simple and effective in strain identification. Although further studies are needed to fully evaluate 16S sequencing as a diagnostic assay, its value as a tool for rapid initial screening in outbreak investigations has been demonstrated. In future, this *S. liquefaciens* strain will be used for the Degradation process of Textile Dye effluents.

Acknowledgement

The author is very grateful to the University Grant Commission (UGC), Government of India, New Delhi, for providing financial assistance in the form of Rajiv Gandhi National Fellowship which buttressed me to carry out my research work successfully. The author is also very grateful to the Secretary and Correspondent and the Principal of A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur (Dt.) for providing the excellent infrastructure and necessary facilities to carry out my research work successfully.

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