INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 20, No. 6, p. 88-94, 2022

RESEARCH PAPER OPEN ACCESS

Isolation, molecular identification and phylogenetic analysis of *Staphylococcus sciuri* isolated from the gut of mulberry silkworm cadaver

Janga Sathish, Gudimalla Prashanthi, Kuntamalla Sujatha*

Sericulture Unit, Dept of Zoology, Kakatiya University Warangal-506009, Telangana, India

Key words: *Bombyx mori,* Cadavers, 16s RNA, Phylogenetic tree, *Staphylococcus sciuri,* Chromoslite.

http://dx.doi.org/10.12692/ijb/20.6.88-94 Article published on June 14, 2022

Abstract

Sericulture is a highly viable industry capable of generating substantial, gainful employment for the people. Bacteria Flacherie is the most serious disease of *Bombyx mori*; identification of bacterial isolates was done using cultural, morphological and biochemical characteristics. But in the present study, identification of the bacterial isolates from the silkworm cadavers was carried out with modern techniques like 16s RNA gene sequence analysis. On the basis of multiple sequence alignment and the phylogenetic tree constructed by Chromoslite (version 1.2) showed that the bacterial strain isolated as clustered from mulberry silkworm cadavers was the closest homology with *Staphylococcus sciuri* with 100% similarity and length of 1550 bp.

* **Corresponding Author:** Kuntamalla Sujatha ✉ k.sujatha8900@gmail.com

Introduction

Sericulture is avillage-based industry, a subsidiary occupation that is taken upon a large scale for providing gainful and continuous income not only to rural but also in semiurban and urban areas during the favorable Indian seasons.

Sometimes, the production can decline due to many technical and non-technical problems. One such technical problem is silkworm diseases (Ravikumar Sundaram *et al.*, 2009). The biotic and abiotic factors influence cocoon production qualitatively and quantitatively, while the abiotic factor may affect the growth and development of silkworms and predispose the silkworm to biotic causes, i.e., infectious diseases which leads to low cocoon crop production, the extent of crop loss due to bacterial diseases is 10-15%. The bacterial silkworm extrudes pathogens along with gut tissue and fecal matter into the rearing environment,which forms a source of diseases that may be due to unhygienic environmental conditions, improper bed cleaning practices leading to secondary contamination in the rearing room.

Since 1949, many serological techniques such as NCP,CPV and DNV rabit antiserum and anti DNV, monoclonal antibodies have been conducted by many scientists (Guoping, G Xi-jie1981) for the identification, detection and localization of viral diseases by means of agar double diffusion and indirect enzyme antibody (IIP method) diagnostics. For detection pebrine diseases/ protozoan diseases. Indirect immune florescence method, latex agglutinization, enzyme antibody method techniques were used while serological techniques were used for silkworm pathogenic fungi.

With modern molecular biological techniques usage in silkworms, pathogen detection methods have been entered into the molecular level in the silk industry. One such technology is PCR which was used to detect Bm2NPV and DNV from silkworm feces. PCR technology was also used to detect *Vairimorphanecatrix* and *Pleistophoraanguillarum* in Tasar culturing for discriminating different sources of Nosema (B.Bebitha *et al.*, 2016). Fluctuation in abiotic factors could lead to crop loss (Christri and Schaf *et al.*, 1990, Samson *et al.*, 1990).

Materials and methods

*Isolation and molecular identification of silkworm pathogen from B.mori*L.

In our study, we have concentrated on the bacterial disease of silkworm digested tract; the bacteria that induced flacherie in silkworm include *Bacillus sp, Streptococcus, Staphylococcus sps, Pseudomonas sps* etc. (Rahul *et al.*, 2019, Kloos *et al.*, 1976, Selvakumar and Datta, 2013). During the rearing that was carried out from 2015 to 2021, the silkworm showed the following flacherie disease symptoms.

The worms infected with bacterial diseases showed low appetite, are sluggish in nature body motionless, irregular moulting, short end gut, loss of capsing capacity, posterior region shrunken, chain shape litter pellets (Samson *et al.*,1990).

The known weight of gut tissues from different silkworms was taken into a glass homogenizer and centrifuged at 3000rps for 3min and re-centrifuged after washing with distilled water accordingly procedure mentioned by Poinar *et al.*, 1984 from the primary cultures using the serial dilution technique, the pure culture at 10^{-3} was used for further molecular identification technique.

16 s rRNA gene sequence analysis for identification of bacteria

Different techniques have been used to analysed the bacterial community structure of gut tissue of silkworm cadaver by different methodologists; all these approaches relied on the cultivation of microorganisms of interest from 1980 new standards for identifying bacteria on the basis of phylogenetic relationships of bacteria by comparing table part of genetic code.

The gene sequence bacteria identification was based on the work done by Paul. D.Vos *et al.*, 2009 and Hott *et al.*, 1994.

Today, rRNA approach is a powerful tool used in the classification and identification of bacteria, where the comparison of complete rRNA gene sequence has been used for the taxonomic relationship when compared to the traditional identification of bacterial strain on the basis of morphological characterization and biochemical test. This approach has emerged as a significant genetic technique for the identification of purely described, rarely isolated phylogenetically aberrant strains and also for recognition of new non-cultured bacteria. The 16sRNA sequence is about 1550 bp long and it is composed of interspecific polymorphism of 16s RNA genes was amplified using PCR in a thermal cycle which was purified using exonuclease-I-Shrimp alkaline phosphate (exo-SAP)(Darby *et al.*,2005).

The purified amplifications were by Sanger method in ABI 3500XL genetic analyzer (life technologies, USA) and sequencing of the files(.ab1) was further analyzed by BLAST with closest culture sequence retrieved from NCBP database that finds regions of local similarity between sequencing (15).

The phylogenetic relationship of the bacterial strain isolated from the gut tissue of mulberry silkworm larvae was analyzed with other closely related bacterial spp in the gene bank. Each isolate was closely related to bacterial spp that were present in the gene bank which was obtained in the FASTA format. Each isolate is reported for the first five to ten observed in the said database. These sequences that were collected from the BLAST were checked for multiple sequence alignment (MSA) T-coffee tool from EBI and the data that was saved analyzed using CLC sequence viewer and then converted into Nexus format; finally the phylogenetic tree was developed.

Results

Today molecular techniques are powerful tools used for the classification and identification of bacteria, where the comparison of almost complete rRNA gene sequence has been used for taxonomic relationships. 16s rRNA used for studying bacterial phylogeny is about 1550 bp which is composed of both variable and conserved visions with sufficient interspecific polymerization of 16s rRNA genes which is the common genetic marker used for no of regions purified amplifications were sequenced by Sanger method in ABI 3500XL genetic analyzer. Sequencing files (ab1) were edited using CHROMSLITE (version 1.2) and further analyzed by BLAST (table-2) with the closest culture sequence retrieved from the National Centre for Biotechnology Programme database.

Table 1. Sequencing results in tabular form.

Sr.No	Strain	Aim	Primer	NCBI BLAST (Type Strain)	Remarks
1	SEQ179-SEKU02-NC040419	Identification of 16S RNA gene ++ Strain received From Prof. Dr .Edla Sujatha Kakatiya University	800R_1542R (1333bp)	LS483305 *Staphylococcus sciuri subsp. Sciuri* strain NCTC12103 Genome assembly. Chromosome :1 Identities : 1328/1333 (99%) Direct submission ++ NR_025520 *Staphylococcus sciuri* strain DSM 20345 16S ribosomal RNA. Partial sequence identities: 1328/1333 (99%) Direct Submission ++ NR_041327 *Staphylococcus sciuri subsp. carnaticus* 16S ribosomal RNA. Partial sequence Identities: 1327/1333 (99%) Int.J.Syst.Evol.Microbiol.57 (PT1). 25-30 (2007)	Strain showed closest homology with *Staphylococcus sp* (Closer to *sciuri*)

On the basis of multiple sequence alignment and phylogenetic analysis by CHROMOSLITE (version 1.2), the below sequence, as shown in the FASTA format, exhibited 99% similarity with *Staphylococcus scuiri* with an E value of 0.00.

Table 2. NCBI-BLAST in hits (top 5-10).

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Staphylococcus sciuri subsp. sciuri strain NCTC12103 genome assembly, chromosome: 1	2431	14576	100%	0.0	99.62%	LS483305.1
Staphylococcus sciuri strain DSM 20345 16S ribosomal RNA, partial sequence	2431	2431	100%	0.0	99.62%	NR_025520.1
Staphylococcus sciuri subsp. carnaticus strain GTC 1227 16S ribosomal RNA, partial sequence	2425	2425	100%	0.0	99.55%	NR_041327.1
Staphylococcus sciuri subsp. carnaticus gene for 16S rRNA, partial sequence, strain: GTC 1227	2425	2425	100%	0.0	99.55%	AB233331.1
Staphylococcus sciuri subsp. rodentium strain GTC 844 16S ribosomal RNA, partial sequence	2420	2420	100%	0.0	99.47%	NR_041328.1
Staphylococcus sciuri subsp. rodentium gene for 16S rRNA, partial sequence, strain: GTC 844	2420	2420	100%	0.0	99.47%	AB233332.1
Staphylococcus fleurettii strain GTC 1999 16S ribosomal RNA, partial sequence	2375	2375	100%	0.0	98.87%	NR_041326.1

>1542RC_Seq 179_SEKU 02

CAGTGTAGCATTGCAAAGAGGTTTCTTCCGGATTC
GGTAAAACTCTGTTGGTTA
AGGGAGGAAACCAAAATTTTGTTAAGTACTGAACC
AAAGTTCTTGACGGTACC
TAATCCAGGAAAGGCCCACCGGGCTAATTACGTGC
CCAAGCAGCCGCCGGGTA
ATTACGGTAAGGTGGCAAGCGTTTATTCCCGGAAA
TTATTTGGGCGTAAAGCG
CGCGTAGGCGGTTTTCTTTAAGTCTGATGTGAAAG
CCCACGGCTTCAACCGTGG
AGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAA
GAGGAGAGTGGAATTCC
ATGTGTAGCGGTGAAAATGCGCAGAGATATGGAGG
AACACCAGTGGCGAAGG
CGGCTCTCTGGTCTGTAACTGACGCTGATGTGCGA
AAGCGTGGGGATCAAACA
GGATTAGATACCCTGGTAGTCCACGCCGTAAACGA
TGAGTGCTAAGTGTTAGG
GGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTA
AGCACTCCGCCTGGGGAG
TACGACCGCAAGGTTGAAACTCAAAGGAATTGACG
GGGACCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGA
ACCTTACCAAATCTTGAC
ATCCTTTGACCGCTCTAGAGATAGAGTCTTCCCCTT
CGGGGGACAAAGTGACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAG
ATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTA
AGTTGGGCACTCTAAGTT
GACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGTCAAATCATCATGCC
CCTTATGATTTGGGCTACACACGTGCTACAATGGAT
AATACAAAGGGCAGCGA
ATCCGCGAGGCCAAGCAAATCCCATAAAATTATTCT
CAGTTCGGATTGTAGTCT
GCAACTCGACTACATGAAGCTGGAATCGCTAGTAA
TCGTAGATCAGCATGCTA
CGGTGAATACGTTCCCGGGTTTTTGTACACACCGCC
CGTCACACCACGAGAGTT
TGTAACCCCCGATTTCCCTGGAGTAACCTTTTAGGA
GCTAGCCGTCGAAGGTGG
GACAAATGATTGGGGTGAAGTCGACACCAATGTGT
TAAAAG

>800RC_ Seq 179_SEKU 02
CGAACAGATGAAGAAGCTTGCTTCTCTGATGTTTA
GCGGCGGACGGGTGAGTA
ACACGTGGGTAACCTACCTATCAAGACTGGGATAA
CTCCGGGAAACCGGGGCT
AATACCGGATAATATTTTGAACCGCATGGTTCAATA
GTGAAAGACGGTTTCGG
CTGTCACTTATAGATGGACCCGCGCCGTATTAGCTA
GTTGGTAAGGTAACGGCT
TACCAAGGCGACGATACGTAGCCGACCTGAGAGGG
TGATCGGCCACACTGGAA
CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCA
GTAGGGAATCTTCCGCAA
TGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGT
GATGAAGGTCTTCGGATC
GTAAAACTCTGTTGTTAGGGAAGAACAAATTTGTT
AGTAACTGAACAAGTCTT
GACGGTACCTAACCAGAAAGCCACGGCTAACTACG
TGCCAGCAGCCGCGGTAA
TACGTAGGTGGCAAGCGTTATCCGGAATTATTGGG
CGTAAAGCGCGCGTAGGC
GGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCA
ACCGTGGAGGGTCATTGG
AAACTGGGAAACTTGAGTGCAGAAGAGGAGAGTG
GAATTCCATGTGTAGCGG
CGTTTCCAGAGATATGGAGGAACACCAGTGGCGAA
GGCGGCTCTCTGGTCTGT
AACTGACGCTTGATGTCAGGGCTATTG
>800RC_1542RC_ Seq 179_SEKU 02
ACAGATGAAGAAGCTTGCTTCTCTGATGTTTAGCG
GCGGACGGGTGAGTAACA
CGTGGGTAACCTACCTATCAAGACTGGGATAACTC
CGGGAAACCGGGGCTAAT
ACCGGATAATATTTTGAACCGCATGGTTCAATAGTG
AAAGACGGTTTCGGCTGT
CACTTATAGATGGACCCGCGCCGTATTAGCTAGTTG
GTAAGGTAACGGCTTACC
AAGGCGACGATACGTAGCCGACCTGAGAGGGTGAT
CGGCCACACTGGAACTG
AGACACGGTCCAGACTCCTACGGGAGGCAGCAGTA
GGGAATCTTCCGCAATGG
GCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGAT
GAAGGTCTTCGGATCGTA
AAACTCTGTTGTTAGGGAAGAACAAATTTGTTAGT
AACTGAACAAGTCTTGAC
GGTACCTAACCAGAAAGCCACGGCTAACTACGTGC
CAGCAGCCGCGGTAATAC
GTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGT
AAAGCGCGCGTAGGCGGT
TTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCG
TGGAGGGTCATTGGAAA
CTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAAT
TCCATGTGTAGCGGTGAA
AATGCGCAGAGATATGGAGGAACACCAGTGGCGAA
GGCGGCTCTCTGGTCTGT
AACTGACGCTGATGTGCGAAAGCGTGGGGATCAAA
CAGGATTAGATACCCTGG
TAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTA
GGGGGTTTCCGCCCCTTAG
TGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGG
AGTACGACCGCAAGGTTG
AAACTCAAAGGAATTGACGGGGACCCGCACAAGCG
GTGGAGCATGTGGTTTAA
TTCGAAGCAACGCGAAGAACCTTACCAAATCTTGAC
ATCCTTTGACCGCTCTAG
AGATAGAGTCTTCCCCTTCGGGGGACAAAGTGACA
GGTGGTGCATGGTTGTCG
TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
CAACGAGCGCAACCCTTA
AGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGT
TGACTGCCGGTGACAAAC
CGGAGGAAGGTGGGGATGACGTCAAATCATCATGC
CCCTTATGATTTGGGCTA
CACACGTGCTACAATGGATAATACAAAGGGCAGCG
AATCCGCGAGGCCAAGC
AAATCCCATAAAATTATTCTCAGTTCGGATTGTAGT
CTGCAACTCGACTACATG
AAGCTGGAATCGCTAGTAATCGTAGATCAGCATGC
TACGGTGAATACGTTCCC GGGT

Sequence analysis

Total 1550 bp partial 16s rRNA was retrieved in FASTA format and subjected for BLAST search in GENBANK, results showed that the test organism was similar to *Staphylococcus sciuri*with 99% similarity with E value 0.00.

Phylogenetic tree

Phylogenetic relationship of the bacteria isolated from gut tissue of mulberry silkworm was analyzed

with partial 16s rRNA sequence of closely related bacterial species were matched under multiple sequence alignment using T- coffee tool it was found that the test organism was under the group of *Staphylococcus* species and showed the closest homology with *Staphylococcus sciuri*.

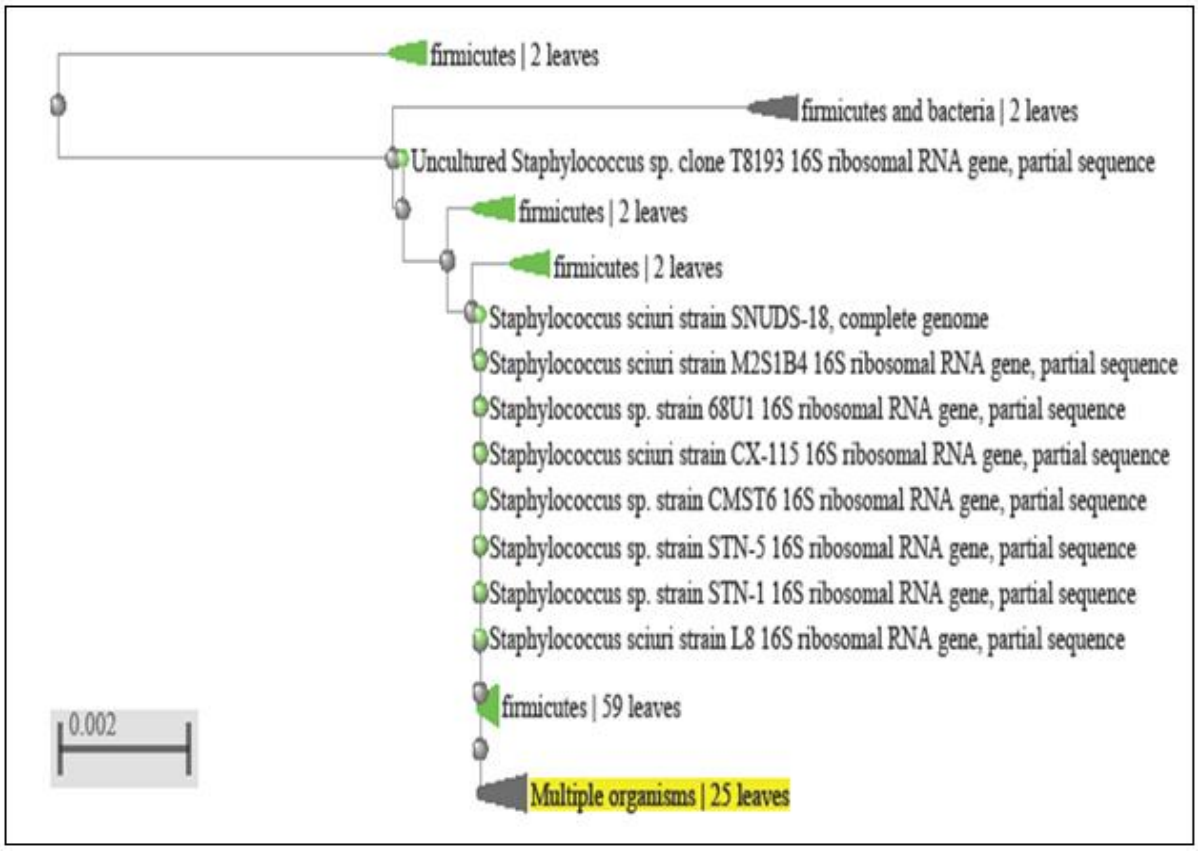

Fig. 1. The similarity of the tested organism is based on the 16s rRNA sequence.

Quiry ID- seq-179-SEKU 02
Description – bacterial strain from the gut tissue of kolar gold mulberry silkworm cadaver
Molecular type-Nucleic acid
Query length- 1550 bp

Discussion

It is gram-positive cocci with a diameter of 0.72 to 1.2µm, non-sporing, and non-motile, which might occur singly in pairs or tetroids. When the cultural characteristics of the bacteria are taken into consideration, the colonies are circular yellowish-grey. *Staphylococcus scuiri* grows better under aerobic conditions, but pure growth is seen at 45˚C.

It also grows under anaerobic conditions of 37˚C; the media on which the growth of *Staphylococcus scuiri* is trypticase soy yeast extract agar ± 5% sheep blood, nutrient agar, P agar etc. (Kloos*et al.*, 1997). The bacteria in our experiments were isolated from the gut tissue of mulberry silkworms during their late age.

Conclusion

16s RNA gene sequencing is an important tool used to study bacterial phylogeny and taxonomy for many reasons. The phylogenetic tree constructed revealed that the newly isolated *Staphylococcus scuiri* was a close member of *Staphylococcus* species with 99% similarity with *Staphylococcus scuiri* with an E value of 0.00 and length 1550 bp.

References

Bebitha B, Mohan raj P, Mani megalai S, Mohalingam CA. 2016. Silkworm diseasedaignosis through molecular approach and their management, International journal of plant protection **9(1),** p 343-357.

Darby AC, Chandler SM, Welburn SC, Douglas AE. 2005. Aphid-symbiotic bacteria cultured in insect cell lines.Applied Environental Microbiology. **71(8),** 4833-4839.

Hott JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. 1994. Bergey's Manual of determinative bacteriology, ninth edition, Williams and wilkins, Baltimore group 17, gram negative cocci, 527-558.

Guo-Ping K, Xi-jie G. 2011. Over view of silkworm pathology in China. African journal of Biotechnology. **10(79),** p18046-18056.

Kloos WE, Schleifer KH, Smith RF. 1976. Characterization of Staphylococcus scuiri species nov and its subspecies. International journal of systematic bacteriology **26,** p22-37.

Kloos WE, Ballard DN, Webster JA, Hubner RJ, Tomasz A, Couto I, Sloan GL, Dehart HP, Fiedler F, Schubert K, de Lencastre H, Sanches IS, Heath HE, Leblanc PA, Ljungh A. 1997. Ribo type delineation and description of 497 Staphylococcus sciuri subspecies and their potential as reservoirs of 498 methicillin resistance and staphylolytic enzyme genes. International Journal of Systematic Bacteriology **499(47),** 313-323.

Paul Vos D, Garrity G, Jones D, Kreig NR, Ludwig W, Rainey FA, Schleifer KH, Whitmann WB. 2009. Bergey's Manual of systematic bacteriology **3,** the firmicules, springler, 392-426, second edition, Springler.

Poinar GO, Thomas GM. 1984. Laboratory guide to insect pathogen and parasites. Plentum press, New York, London.

Rahul K, Moamongba Saikia K, Rabha M, Siva Prasad V. 2009. Identification and characterization of bacteria causing flacherie in mulberry silkworm Bombyx mori, L. Journal of crop and weed **15(3),** p 178-181.

Samson MV, Baig M, Sharma SD, BalaVenkatasubbaiah M, Sasidharan TO, Jolly MS. 1990. Survey on the relative incidence of silkworm diseases in Karnataka. Indian Journal of Sericolgy **29,** 248-254.

Schefler WC. 1980. Analysis of variance - Part I & II. In:Statistics for the Biological Sciences, Second Edition, Addison - Wesley Publishing Company, Inc., Philippines, p 121-160.

Selvakumar T, Datta RK. 2013. Role of bacteria streptococcus faecalis & staphylococcus aureus, viruses and their combined infection in causation of flacherie under different environmental conditions. Sericologia **5(1),** 54-62, 2013.