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First report of isolation of opportunistic human pathogenic gut bacteria *Staphylococcus cohnii* from grubs of *Brahmina coriacea* (Hope) (Coleoptera, Scarabaeidae, Melolonthinae) from Himachal Pradesh, India

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ABSTRACT: *Staphylococcus cohnii* (Schleifer and Kloos) an opportunistic pathogen for humans, is reported for the first time its presence in grubs of *Brahmina coriacea* (Hope) (Coleoptera, Scarabaeidae, Melolonthinae). The cellulolytic gut bacteria were isolated from different populations of *B. coriacea*, collected from different parts of Himachal Pradesh, India. The isolated *S. cohnii* from the grub population of Shillaroo location recorded maximum cellulolytic index. The isolated bacteria were identified using morphological, biochemical, and 16S rRNA gene sequencing approaches.

KEY WORDS: Cellulolytic, pathogen, 16S rRNA

The scarabaeoids are a large and distinct group of highly specialized beetles that are easily recognized by their lamellate antennae and high degree of polyphagy (Mehta *et al*., 2010). While their larvae (white grubs) are among the most destructive soil pests, adults are the most frequent leaf chafers. *Brahmina coriacea* (Hope) (Coleoptera, Scarabaeidae, Melolonthinae) an invasive pest of potato and apple agro-ecosystem in north-western India is found in the mid- and high-hill regions of the districts of Mandi, Kullu, Chamba, Kinnaur, Solan, and Sirmaur in the state of Himachal Pradesh. It is a polyphagous pest causeing severe harm by feeding the leaves, fruits of forest trees, their nurseries, vegetables, lawns, and field crops (Chandel *et al*., 1997). It is reported in temperate, subtropical, and tropical regions of Uttarakhand (Singh *et al*., 2003). *Staphylococci* belong to CoNS group (Coagulase-negative Staphylococci), which are opportunistic pathogens in humans, animals and other non-human primates (Kloos and Wolfshohl, 1991). Some of the species are reported to be normal microbiota of human skin and mucous membranes, which are associated with blood stream infection, endocarditis and meningitis (Garza-González *et al*., 2011; Soldera *et al*., 2013).

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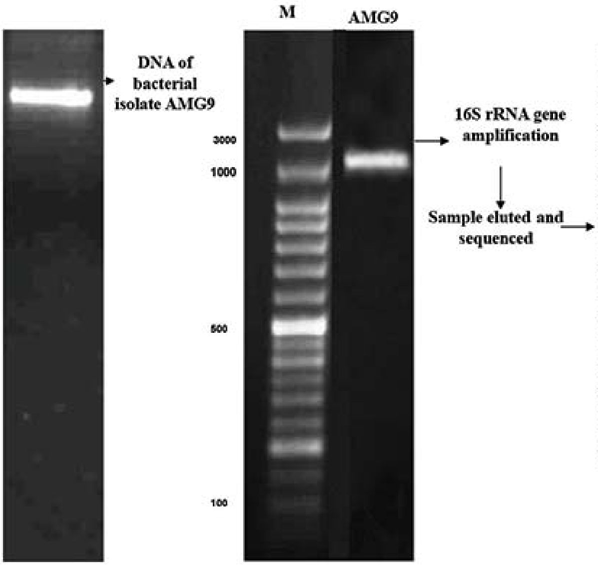

Sequence

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AGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGC
ACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCT
GTTTGATCCCCACGCTTTCGCACATCAGCGTCAGTTACAGA
CCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCT
GCGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCT
GCACTCAAGTTTCCCAGTTTCCAATGACCCTCCACGGTTGA
GCCGTGGGCTTTCACATCAGACTTAAGAAACCGCCTACGC
GCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTT
CTGATTAGGTACCGTCAAGACGTGCACAGTTACTTACACAT
TTGTTCTTCCCTAATAACAGAGTTTTACGATCCGAAGACCT
TCATCACTCACGCGGCGTTGCTCCGTCAGGCTTTCGCCCAT
TGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGA
CCGTGTCTCAGTTCCAGTGTGGCCGATCACCCTCTCAGGTC
GGCTACGTATCGTTGCCTTGGTAAGCCGTTACCTTACCAAC
TAGCTAATACGGCGCGGGTCCATCTATAAGTGATAGCAAAA
CCATCTTTCACTTTAGAACCATGCGGTTCTAAATGTTATCCG
GCATTAGCCCCGGTTTCCCGGAGTTATTCCAGTCTTATAGGT
AGGTTACCCACGTGTTACTCACGCGTCCGCCGCTAACGTCA
AAGGAGCAAGCTCCTTATCTGTTCGCTCGACTTGCATGTAT
TAGGCACGCCGCCAGCGTTCATCCTGAGC
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Fig. 1 Molecular identification of bacterial isolate AMG9 based on 16S rRNA amplification (Lane M, DNA marker; Lane1, AMG9)

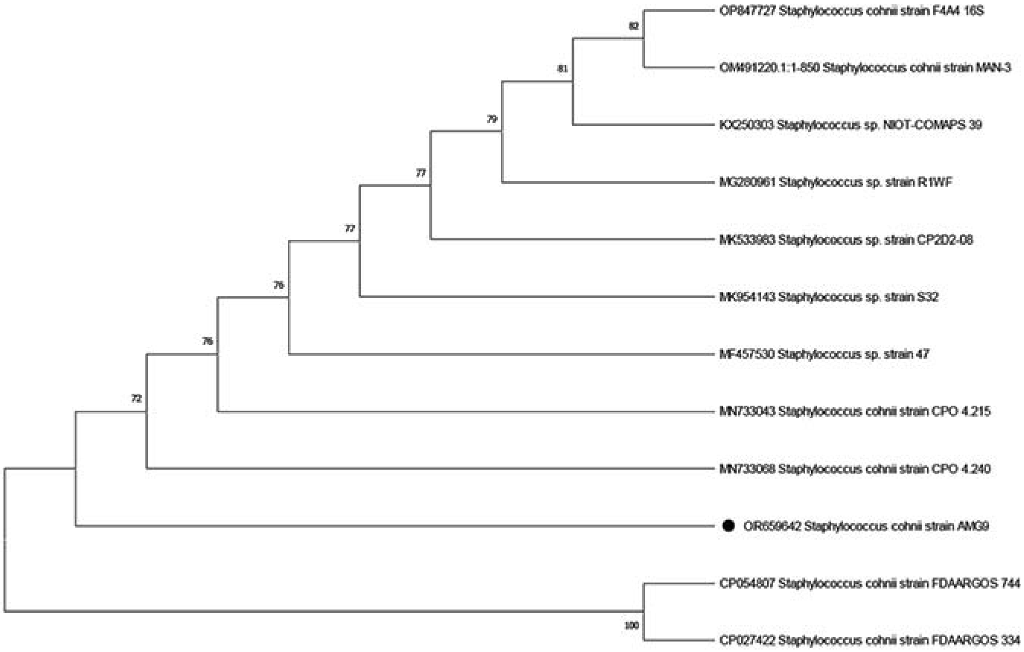

Fig. 2 Phylogenetic tree constructed by using 16S rRNA gene sequences, showing distant relationship of *S. cohnii* strain AMG9 with other strains available from NCBI database (MEGA X)

Some CoNS make biofilm, which is composed of polysaccharides, proteins, and DNA (Götz, 2002).

The grubs of *B. coriacea* were collected from different parts of Himachal Pradesh and identified by examining their raster pattern (Thakur *et al.*, 2022). The grubs were dissected under laminar air flow and bacterial isolation was done on the nutrient agar media. The bacterial isolates were categorized by morphological, biochemical and molecular methods.

The colony morphology of the isolate *S. cohnii* strain AMG9 (where A denotes the location and MG denotes Midgut part of alimentary canal) was circular, convex, entire, and cream in colour. The bacteria were singly arranged cocci, and showed positive results for the gram's reaction. The isolate were able to utilize carbohydrates (Lactose, Maltose, Fructose, Dextrose, Trehalose, Mannose, Inulin, Sodium Gluconate, Glycerol, Salicin, Mannitol, Rhamnose, Cellobiose), perform Esculin hydrolysis and citrate utilization, whereas showed negative results (means not able to utilize) for the Xylose, Galactose, Raffinose, Melibiose, Sucrose, L-Arabinose, Dulcitol, Inositol, Sorbitol, Adonitol, Arabitol, Erythritol, á-Methyl-D-glucoside, Melezitose, á-Methyl-D-mannoside, Xylitol, ONPG, D-arabinose, Malonate, and Sorbose carbohydrates]. *Staphylococcus cohnii* was recorded to be produce halo zones around the colony for starch hydrolysis and not able to produce urease enzyme. The bacteria were identified by direct sequencing of the obtained PCR product (Fig. 1), and by comparing it with the available sequences on the NCBI database. The *Staphylococcus cohnii* strain AMG9 (OR659642) showed 99.98 per cent similarity with the NCBI accession of *Staphylococcus cohnii* strain FDAARGOS-744. Phylogenetic tree was constructed between *Staphylococcus cohnii* strain AMG9 and its closest strains in the GenBank using neighbor-joining method of 16S rRNA sequences (Fig. 2). The dendrogram in figure showed evolutionary relationship of different strains of *S. cohnii*. The bacterial strain AMG9 showed high relatedness with the closest strains at boot-strap value ranged from 75 to 100 per cent at 1000 replicates. This is a potent cellulose degrading bacterial species which can be used for the degradation of agricultural and biological waste material. In order to manage *B. coriacea*, the bacterial microorganisms were isolated, described, and their potential for insecticidal activity was examined (Sharma, 2013). Mendoza-Otero *et al.,* (2017) reported the draft-genome sequences of two pathogenic strains of *Staphylococcus cohnii* isolated from human. One strain was reported from the blood and the other from a catheter of a male patient.

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REFERENCES

Chandel R.S., Gupta P.R. and Thakur J.R. (1997) Host preference and seasonal abundance of defoliating beetles infesting fruit trees in mid hills of Himachal Pradesh. Journal of Soil biology and Ecology 17(2): 140 –146.

Garza-González E., Morfin-Otero R., Martínez-Vázquez M.A., Gonzalez-Diaz E., González-Santiago O. and Rodríguez-Noriega E. (2011) Microbiological and molecular characterization of human clinical isolates of *Staphylococcus cohnii*, *Staphylococcus hominis*, and *Staphylococcus sciuri*. Scandinavian journal of infectious diseases 43(11-12): 930–936. doi:10.3109/00365548.2011.598873.

Götz F. (2002) *Staphylococcus* and biofilms. Molecular microbiology 43(6):1367–1378. doi:10.1046/j.1365-2958.2002.02827.x.

Kloos W.E. and Wolfshohl J.F. (1991) *Staphylococcus cohnii* subspecies: *Staphylococcus cohnii* subsp. cohnii subsp. nov. and *Staphylococcus cohnii* subsp. *urealyticum* subsp. nov. International Journal of Systematic and Evolutionary Microbiology 41(2): 284–289. doi:10.1099/00207713-41-2-284.

Mehta P.K., Chandel R.S. and Mathur Y.S. (2010) Status of white grubs in north western Himalaya. Journal of Insect Science (Ludhiana) 23(1): 1–4.

Mendoza-Olazarán, S., Garcia-Mazcorro, J.F., Morfín-

Otero, R., Villarreal-Treviño, L., Camacho-Ortiz, A., Rodríguez-Noriega, E., Bocanegra-Ibarias, P., Maldonado-Garza, H.J., Dowd, S.E. and Garza-González, E., 2017. Draft genome sequences of two opportunistic pathogenic strains of *Staphylococcus cohnii* isolated from human patients. Standards in Genomic Sciences 12: 1–9.

Sharma A., Thakur D.R., Kanwar S. and Chandla V.K. (2013) Diversity of entomopathogenic bacteria associated with the white grub, *Brahmina coriacea*. Journal of pest science 86: 261–273.

Singh M.P., Bisht R.S. and Mishra P.N. (2003) Distribution of white grub fauna in Garhwal hills of western Himalayas. Indian Journal of Entomology 65(2): 217–221.

Soldera J., Nedel W.L., Cardoso P.R. and d'Azevedo P.A. (2013) Bacteremia due to *Staphylococcus cohnii* ssp. *urealyticus* caused by infected pressure ulcer: case report and review of the literature. Sao Paulo Medical Journal 131: 59–61. doi: 10.1590/S1516-31802013000100010.

Thakur A., Chandel R.S., Ramappa K. and Guleria N. (2022) Biodiversity and identification of potato white grubs in high hills of Himachal Pradesh. Potato Journal 49: 174–187.

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