The first report of opportunistic human pathogenic bacteria isolated from Brahmina coriacea (Scarabaeidae: Coleoptera) in north-western Himalayas

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Abstract

Staphylococcus haemolyticus is an opportunistic human pathogenic bacterium, which constitutes the major part of human skin microflora. This bacterium showed resistance to most of the antibiotics, spread widely in hospitals and cause various infections in human beings. This bacterium has been reported from infected humans, animals and some insects, whereas, this is the first report of Staphylococcus haemolyticus from the scarabaeids in the world. The gut microbiota of white grubs helps in the digestion and assimilation of food such as cellulose, hemicellulose and pectin degradation by producing various enzymes. We have isolated 11 cellulolytic bacteria from the gut of Brahmina coriacea (Hope) grubs, which were collected from different locations of north-western Himalayas. S. haemolyticus was only reported from the grubs of Nauni, Solan region of Himachal Pradesh, India and identified by using 16S rRNA gene sequencing analysis. S. haemolyticus was able to degrade the cellulose in Carboxy Methyl cellulose (CMC) media. This bacterium can be used in industries for the management of agro-wastes, in pulp and paper industry and in biofuel production.

The first report of opportunistic human pathogenic bacteria*Staphylococcus haemolyticus* isolated from*Brahmina coriacea* (Scarabaeidae: Coleoptera) in north-western Himalayas

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Abbreviation Used:

CMC: Carboxy Methyl cellulose

FG: Foregut

MG: Midgut

HG: Hindgut

NCBI: National Center for Biotechnology Information

MEGA: Molecular Evolutionary Genetics Analysis

Abstract

Staphylococcus haemolyticus is an opportunistic human pathogenic bacterium, which constitutes the major part of human skin microflora. This bacterium showed resistance to most of the antibiotics, spread widely in hospitals and cause various infections in human beings. This bacterium has been reported from infected humans, animals and some insects, whereas, this is the first report of *Staphylococcus haemolyticus* from the scarabaeids in the world. The gut microbiota of white grubs helps in the digestion and assimilation of food such as cellulose, hemicellulose and pectin degradation by producing various enzymes. We have isolated 11 cellulolytic bacteria from the gut of*Brahmina coriacea* (Hope) grubs, which were collected from different locations of north-western Himalayas. *S. haemolyticus*was only reported from the grubs of Nauni, Solan region of Himachal Pradesh, India and identified by using 16S rRNA gene sequencing analysis. *S. haemolyticus* was able to degrade the cellulose in Carboxy Methyl cellulose (CMC) media. This bacterium can be used in industries for the management of agro-wastes, in pulp and paper industry and in biofuel production.

Keywords: *Staphylococcus haemolyticus* , Opportunistic, Scarabaeids, *Brahmina coriacea* , 16S rRNA, Agro-waste

Introduction

The white grubs are the larval stage of scarabs which live in the soil (Gardner, 1935). The scarab beetles are the common leaf chaffer, whereas grubs are among the most damaging soil pests (Chandel et al., 2021). The scarabaeids are polyphagous pest both in adult and larval stage and cause heavy damage on vegetables, lawn, field crops, their nurseries, fruit and forest trees (Chandel and Kashyap, 1997).

The species *Brahmina coriacea* is restricted to Himalyan region of India, Tibet and China (Mishra and Chandel, 2003). In Himachal Pradesh,*B. coriacea* is distributed in the mid and high hills of Mandi, Kullu, Chamba, Kinnaur, Solan, and Sirmaur districts. In Uttarakhand,*B. coriacea* is distributed throughout tropical, subtropical, and temperate zones (Singh et al*.,* 2003). According to Chandel et al. , (2015), Shimla hills are highly favourable for multiplication of *B. coriacea,* and tuber damage often exceeds 50 per cent in endemic areas of Shimla hills (Chandel et al*.,* 2021).

The evolutionary success showed the microbial activity of gut symbionts in white grub (Skowronek et al., 2020). The gut microbiota of white grubs performs wide services to the host insect such as digestion and assimilation of food (Msango Soko et al., 2020). The gut of white grubs possessed cellulolytic bacteria which helps them in consuming the cellulose by producing the cellulolytic enzyme (Inoue et al., 2005). Danu et al., (2023) isolated and identified the cellulose degrading bacterial isolates from the guts of different white grub species (*Anomala bengalensis* , *B. coriacea* , *Holotrichia longipennis* and *Holotrichia setticollis*) by using 16S rRNA sequencing. They identified four cellulolytic bacterial isolates from the gut of *B. coriacea* which are *Bacillus stratosphericus* ,*Bacillus licheniformis* , *Bacillus subtilis* and*Bacillus pumilus* .

S. haemolyticus is a major species of Coagulase-negative Staphylococci (CoNS) and a component of the skin microflora (Saida et al., 2009). This species is the second most common and significant species of CoNS among isolates from clinical infections, accounting for 10–20% of clinical CoNS infections (Renaud et al., 1991). *S. haemolyticus* has been linked to a number of clinical infections (Szczuka et al., 2015), including meningitis, bacteremia, eye, skin, peritonitis, urinary tract, and male genital infections (Schuenck et al., 2008; Do Ferreira et al., 2011). Additionally, strains of *S. haemolyticus* were isolated from both dogs and their owners, raising the possibility of zoonotic transmission (Ruzauskas et al., 2014).

S. haemolyticus is known for its ability to form biofilms, which are essential for the spread of infection (Eltwisy et al., 2020). Additionally, *S. haemolyticus* generates a number of invasive enzymes and toxins that alter host immune responses and cause damage to host cells, aiding in the bacterial pathogenesis process (Eltwisy et al., 2020). A newly discovered pathogen that causes nosocomial infections is *S. haemolyticus* . It is unclear what exactly contributes to *S. hemolyticus* survival and spread in hospitals (Cavanagh et al.,

2014). The abundance of insertion sequences and resistance to multiple antibiotics are features of the *S. haemolyticus* genome that cause hospital infections (Hosseinkhani et al., 2016; Ahmed et al., 2015).

The aim of present investigation was to isolate and identify the cellulose degrading bacteria from the gut of most notorious and polyphagus white grub, *B. corieacea* and estimated their cellulolytic index for utilize in future studies for decomposing of organic matter and utilization in biofuel production in industries.

Materials and methods

2.1 Collection and Identification of *B. coriacea* grubs

The actively feeding third instar grubs were collected during October to November at different locations of north-western Himalayas. The *B. coriacea* grubs were identified by examining their raster pattern (Thakur et al., 2022).

2.2 Dissection and isolation of gut microbiota from grubs of*B. coriacea*

The entire dissection procedure was aseptically performed under aseptic conditions in laminar flow hood. After dissection, different segments of the gut as foregut (FG), midgut (MG) and hindgut (HG) were separated carefully and transferred to 1.5 mL eppendorf tubes. Isolation of gut microbiota were done by using serial dilution agar plating method. The bacteria were isolated on nutrient agar media.

2.3 Morphological characterization

Morphological characterization was done by using Bergey's Manual of Systematic Bacteriology (Boone et al., 2001). The individual colonies were assessed in terms of shape, margin, elevation and pigmentation. Isolated colonies were subjected to gram staining, and colony characteristics as bacterial shape, gram reaction and bacterial arrangements were analyzed with a phase contrast microscope.

2.4 Screening and characterization of cellulose degrading bacteria

Cellulose degrading ability of the gut bacteria was tested by overnight placing the bacterial culture at the center of CMC agar plate, and incubating the CMC agar plates at 37^{o}C (Treather and Wood, 1982), and the degradation of CMC was seen as a clear halo around the bacterial colony. The cellulolytic index of the isolates was measured by the following formula (Ferbiyanto et al., 2015)

$$Cellulolytic\ index = \frac{\text{Diameter of clear zone (mm)} - Diameter\ of\ bacterial\ colony\ (mm)}{Diameter\ of\ bacterial\ colony\ (mm)}$$

2.5 Biochemical characterization

Isolates were characterized for their carbohydrate metabolizing abilities using a KB009 HiCarbohydrate kit (HiMedia laboratories Pvt. Ltd.). Similarly, the isolates were characterized for different enzymatic activities *viz.* cellulolytic activity, starch hydrolysis and urease activity.

2.6 16S rRNA gene sequencing analyses

The bacterial isolates were molecularly analysed by Sanger dideoxy sequencing of the obtained PCR products, and identified by comparing them with sequences already available in NCBI database.

2.7 Phylogenetic analysis

All the generated sequences were compared with the sequences available in GeneBank using the BLASTn programme (http://www.ncbi.nlm.nih.gov). The phylogenetic analysis was done using Molecular Evolutionary Genetics Analysis (MEGA X) software (Kumar et al., 2018).

Results

3.1 Isolation of gut microbiota from grubs of *B. coriacea*

Total 11 cellulolytic bacteria were isolated from the grubs of *B. coriacea* . Most of the bacterial isolates were already reported, but*S. haemolyticus* was reported for the first time from the hindgut region of this grub. Figure 1 depicts dissection of grub, isolation of hindgut microflora and cellulase activity shown by *S. haemolyticus.*

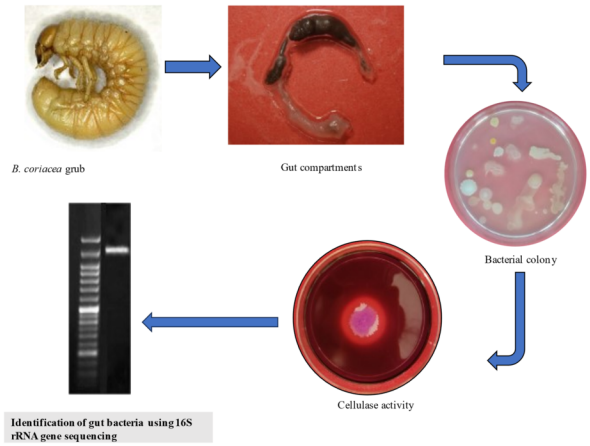

Fig.1 Schematic presentation of isolation and identification of*S. haemolyticus* from the hindgut of *B. coriacea*

3.2 Morphological characterization

The bacterial isolate *S. haemolyticus* showed positive gram's reaction, and they were singly arranged cocci bacteria. The colony morphology of the bacteria was recorded to be Circular, Convex, Entire and cream coloured.

3.3 Biochemical characterization

S. haemolyticus strain was able to utilize lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melibiose, sucrose, mannose, inulin and esculin hydrolysis, whereas L-arabinose, sodium gluconate, glycerol, salicin, dulcitol, inositol, sorbitol, mannitol, adonitol, arabitol, erythritol, α-methyl-D-glucoside, rhamnose, cellobiose, melezitose, α-methyl-D-mannoside, xylitol, ONPG, D-arabinose, citrate utilization, malonate utilization and sorbose were not utilized by this species.

This bacterial strain is able to utilize cellulose by producing cellulase enzyme, and the cellulolytic index was recorded to be 0.5.*S. haemolyticus* showed positive results to starch hydrolysis by producing amylase enzyme, which degrade starch and produce clear halo around the bacterial colony. This species showed negative results for urease activity.

3.4 16S rRNA gene sequencing analyses

The bacterial isolate was identified by using 16S rRNA gene sequencing method (Fig.2). The sequence has been submitted to the GenBank nucleotide sequence database under the accession no. OR677034. The bacterial isolate DHG6 (OR677034) had a similarity of 100 % with National Center for Biotechnological Information (NCBI) accession of*Staphylococcus haemolyticus* strain SCAID URN1-2019 (CP052055).

3.5 Phylogenetic tree analyses

Phylogenetic tree was constructed between *Staphylococcushaemolyticus* strain DHG6 and its closest strains in the GenBank using neighbour-joining method of 16S rRNA sequences (Fig.3). The dendrogram in figure

showed evolutionary relationship of different strains of *S. haemolyticus* . The bacterial strain DHG6 showed high relatedness with the closest strains at boot -strap value ranged from 75 to 100 % at 1000 replicates.

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Fig.3 Phylogenetic tree constructed by using 16S rRNA gene sequences, showing distant relationship of *S. haemolyticus* strain DHG6 with other strains

Discussion

Insect possessed diverse microbiota in their gut which provide physiological as well as ecological benefits to hosts (Jang and Kikuchi, 2020). In insects, gut symbionts help in the degradation of cellulose, hemicellulose, detoxification and also protect the host insect from pathogens by producing various antimicrobial compounds (Jang and Kikuchi, 2020). Lemke et al., (2003) initiated the comprehensive study of gut microflora in white grub species, *Pachnoda ephippiata* . They reported that white grubs possess diverse microbiota in the gut which involved in degradation of cellulose, microbial fermentation and proteolytic activities. Msango Soko et al., (2020) isolated culturable cellulolytic bacteria from the midgut of *A. dimidiata* , which was similar to our results. They reported *Pseudomonas aeruginosa* ,*Bacillus subtilis* , *Bacillus cereus* and *Bacillus toyonensis* from the gut. These results showed that the cellulolytic gut bacteria of white grubs play important role in the decomposition of cellulose rich food eaten by the white grubs (Cazemier et al., 2003; Huang et al., 2010; Msango Soko et al. 2020).

Danu et al., (2023) isolated and identified gut bacteria isolated from the *B. coriacea* grubs. They reported *Bacillus stratosphericus* , *Bacillus pumillus* , *Bacillus subtilis* and*Bacillus licheniformis* as the most potent cellulose degrading bacteria, which was similarly found in our investigation but *S. haemolyticus* was reported for the first time from scarabaieds as the potent cellulose degrading bacteria with its pathogenic nature.

In this investigation, *S. haemolyticus* was isolated from the hindgut of *B. coriacea* at Nauni, Solan in north-western Himalayas, these bacteria have potential for use in degradation of cellulose rich organic waste and also in the production of biofuel. Therefore, further investigations are planned to evaluate the efficiency of this cellulolytic bacterial isolate in decomposing the agricultural wastes.

The authors have declared no conflict of interest

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