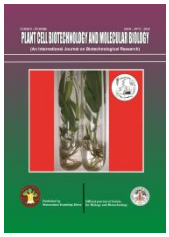

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Molecular Characterization of *Ralstonia mannitolilytica* Isolated from the Gut of *Brahmina coriacea* Grubs Using 16S Ribosomal RNA Gene Sequence Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Ralstonia mannitolilytica is an emerging aerobic gram-negative bacterium causing infection among immune compromised patients. It has been isolated from the soil, water, human body and plant surfaces but this is the first report of *Ralstonia mannitolilytica* isolated from the grubs of *Brahmina coriacea* in the world. The gut microbiota was isolated from different ecotypes of *B. coriacea*, collected from various parts of Himachal Pradesh, India. *Ralstonia mannitolilytica* strain GMG5 was found from the grubs of Seobagh population. *Ralstonia mannitolilytica* was able to degrade cellulose in the Carboxy Methyl Cellulose (CMC) media, where the cellulolytic index was recorded to be 0.33. The Cellulolytic bacterial isolates were identified using morphological, biochemical and 16S ribosomal RNA gene sequence analysis.

Keywords: Brahmina coriacea; Ralstonia mannitolilytica; gut microbiota; cellulolytic index; 16S ribosomal RNA.

1. INTRODUCTION

B. coriacea is a severe pest in both its larval and adult phases [1]. While grubs are considered the national pest of potatoes [2] and fruit/forest nurseries [3], adults severely harm a variety of forest and fruit trees. *B. coriacea* was observed to be feeding on apples and pears in the northwest Himalaya [4]. Formerly known as *Burkholderia pickettii*, *B. solanacearum*, *Pseudomonas solanacearum*, and *P. thomasii*, *Ralstonia* spp. is a Gram-negative, non-fermentative, aerobic rod-shaped species that contains *R. pickettii*, *R. insidiosa*, *R. solanacearum*, and *R. mannitolilytica* [5]. Tthis genus is frequently found in soil, water supplies, and plants [6]. It may even thrive in the presence of disinfectants and low nutrition environments.

Ralstonia species have the ability to form biofilms, which enable them to survive in the environment, elude the host's immune responses, and lead to invasive diseases. This is especially true for patients who are immune compromised, such as those who have solid organ or haematopoietic stem cell transplants, haematologic malignancies, patients in intensive care or who have Central Venous Catheters (CVCs), [6,7,8,9] patients with cystic fibrosis, and premature infants. This is especially true for these patients.

2. MATERIALS AND METHODS

The grubs of *B. coriacea* were collected from various locations of Himachal Pradesh, India (Fig. 1). The grubs were reared in the laboratory at Palampur and used for the microbial isolation. The bacterial isolation was done from 24 grubs of different locations on the nutrient agar media and categorized them on the basis of cellulolytic potential. The cellulose degrading bacteria were further identified by morphological, biochemical and molecular techniques.

Fig. 1. Third instar grub of *Brahmina coriacea*

3. RESULTS

The colony morphology of the isolate *Ralstonia mannitolilytica* strain GMG5 was circular, pulvinate, entire and white in colour. The rod shaped bacteria were arranged in chains, and showed negative results for gram's reaction. The isolate were able to utilize carbohydrates such as Maltose, Fructose, Dextrose, Galactose, Raffinose, Trehalose, Melibiose, Sucrose, L-arabinose, Mannose, Inulin, Glycerol, Salicin, Inositol, Sorbitol, Mannitol, Rhamnose, Cellobiose, Esculin hydrolysis and Malonate, whereas showed negative results for the Lactose, Xylose, Sodium Gluconate, Dulcitol, Adonitol, Arabitol, Erythritol, α-methyl-D-

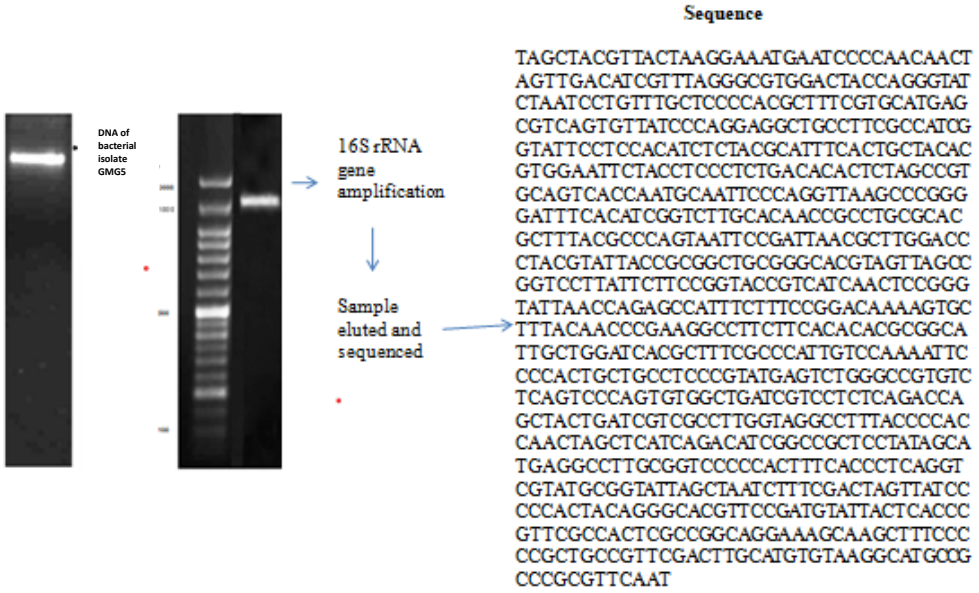

Fig. 2. Molecular identification of bacterial isolate GMG5 based on 16S rRNA amplification (Lane M, DNA marker; Lane1, GMG5)

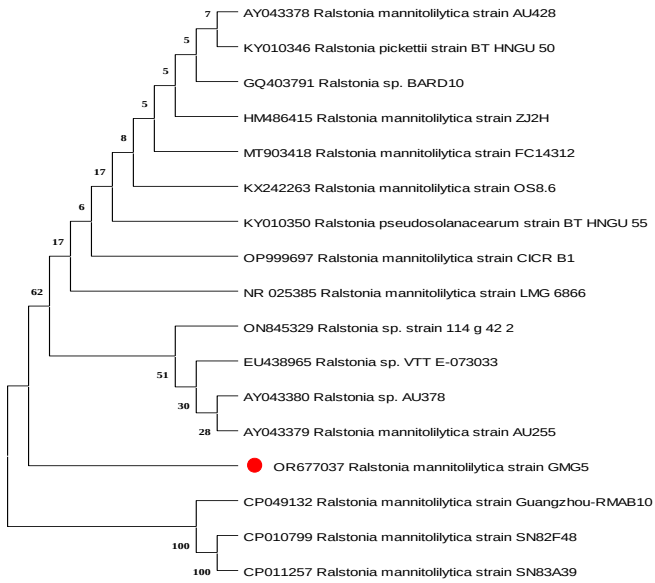

Fig. 3. Phylogenetic tree, showing distant relationship of *Ralstonia mannitolilytica* strain GMG5 with other strains

glucoside, Melezitose, α-methyl-D-mannoside, Xylitol, ONPG, D-arabinose, citrate and Sorbose carbohydrates. *Ralstonia mannitolilytica* showed negative results for starch hydrolysis and urease activity. The bacterial strains were identified by sequencing of the obtained PCR product (Fig. 2), and by compare it with the available sequences in the GenBank. The *Ralstonia mannitolilytica* strain GMG5 (OR677037) showed 99.40 per cent similarity with the NCBI accession of *Ralstonia mannitolilytica* strain Guangzhou-RMAB10.

Phylogenetic tree was constructed between *Ralstonia mannitolilytica* strain GMG5 and its closest strains in the GenBank, NCBI using neighbor-joining method of 16S rRNA gene sequences (Fig. 3). The figure showed

evolutionary relationship of different strains of *Ralstonia mannitolilytica*. The bacterial strain GMG5 depicted high relatedness with the closest strains at boot-strap value ranged from 75 to 100 % at 1000 replicates.

4. DISCUSSION

Ralstonia spp. has emerged as an opportunistic pathogen in nosocomial settings [5,6,7,10,8, 11,12,13,14,15,16]. While *Ralstonia* spp. can cause invasive disease, most reports have shown a good prognosis and low mortality. For instance, during an outbreak in an oncology day ward, 22 patients with CVC developed bacteremia, which was suspected to be caused by flushing the CVCs with contaminated saline; however, symptoms improved in call cases after the removal of their CVCs [7]. Another report from a hemodialysis unit at a tertiary hospital recorded 16 cases of bacteremia outbreaks, with a high probability of dialysis water being the source. *R. mannitolilytica* has been found to modulate biofilm in plastic water pipes, and contaminated water supplies such as respiratory gas humidification devices and multi-dose saline bottles could be sources of *R. mannitolilytica* infection [7,11,17]. *R. mannitolilytica* could not be easily eradicated using disinfection protocols due to its ability to form biofilm structures that provide increased protection against external stress, such as disinfectants, and the ability to slow bacterial growth within the biofilm [18]. In this study, we have isolated *R. mannitolilytica* strain GMG5 from the gut of *Brahmina coriacea* grubs, which can be used as a cellulose degrading bacteria for the degradation of the agricultural waste and in bio-fuel industry.

4.1 Compliance with Ethical Standards

We believe that our findings could be of interest to the readers.We certify that this is an original work and the same is not submitted or published earlier elsewhere. We declare that there are no conflicts of interests associated to this manuscript. In addition, the manuscript meets all applicable standards with regard to the ethics of experimentation and research, and there is no duplicate publication, fraud, plagiarism, or concerns about animal or human experimentation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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