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Short Communication

Molecular characterization of a proteolytic bacterium in *Panchagavya*: An organic fertilizer mixture

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ABSTRACT

Fermented product of combination of five major substances obtained from cow, viz., urine, milk, ghee, curd, and dung, is known as *Panchagavya*. Its pro-agricultural and medicinal value has been traditionally known to Indian farmers from Vedic period. In this study, the proteolytic properties of *Panchagavya* were investigated using Skim Milk Agar (SMA) form, a commercially available *Panchagavya* product. Proteolytic bacteria, SNCK-3, was successfully isolated. Further identification using 16s rDNA sequencing revealed that SNCK-3 belonged to *Acinetobacter* spp., which is a species of biofertilizer group. This observation justified the pro-agricultural role of *Panchagavya*. The present study represents primary data and it is essential to develop a new area of research for exposing the invisible or dormant Vedic biotechnological concepts, like *Panchagavya*.

1. Introduction

'*Panchagavya*' (Sanskrit, for a blend of '*five products from cow*') is a traditional product prepared in India, by fermenting dung, urine, milk, curd and clarified butter (*ghee*) from cow [1,2]. Various ingredients are added to *Panchagavya*, other than the above combination, according to the locally practiced knowledge. For example, *Panchagavya* is prepared by mixing nine products, including cow dung, cow urine, milk, curd, butter, banana, tender coconut, sugarcane and water. *Panchagavya* is used in cowpathy and as a medicine for the treatment of several disorders, viz., allergies, colds, cough, asthma, skin infections etc. [3].

These contain macro- and micro-nutrients, amino acids, and growth promoting substances, like indole acetic acid, gibberellins and beneficial micro-organisms. *Panchagavya* has been reported to possess pro-agricultural activity (bio-control, biofertilizer, growth enhancer etc.), pharmacological value, growth stimulating activity, probiotic and antimicrobial potential. Beneficial effects of their biodynamic preparations on various crops have been reported. Biodynamic sprays of these preparations have increased the yield of cereals and vegetables significantly [1]. In Ayurveda, *Panchagavya* is used for purification of many herbal drugs and also as an important medicine. It is used in the treatment of different types of cancers and immune suppressive diseases. Deepika et al [4] reported the antimicrobial activity of *Panchagavya* against different microbial pathogens.

Presence of naturally occurring beneficial micro-organisms, predominantly bacteria, yeast, actinomycetes, and certain fungi have been reported in these biodynamic preparations. Research reports related to the isolation and characterizations of beneficial attributes of the bacteria present in biodynamic preparations are few [5]. And there are very few reports on the definitive identification of the beneficial bacteria in such biodynamic preparations, using molecular methods. Therefore, in this study, an attempt was made to isolate and perform molecular characterization of beneficial proteolytic bacteria from *Panchagavya*.

2. Materials and methods

2.1. Isolation and identification of proteolytic bacteria from *Panchagavya*

Commercially available *Panchagavya* preparation was procured from Green valley agency, Bangalore, and proteolytic bacteria were isolated and identified. *Panchagavya* sample was streaked on Skim Milk Agar (SMA) plates. The plates were incubated at room temperature for 24 h. A clear zone of inhibition on the SMA,

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indicated the presence of protease producing bacteria. Based on the zone of clearance, the bacterial colonies were further streaked on a fresh SMA plate to obtain pure culture.

2.2. Molecular identification (16s rDNA) of proteolytic bacteria from Panchagavya

Genomic DNA was isolated from overnight grown cultures in Minimal Salt Broth medium, by cetyltrimethyl ammonium bromide (CTAB) method [6]. PCR was performed using 16s rDNA universal primers (8F: 5′- AGA GTT TGA TCM TGG CTC AG -3′ and 519B2 R5′- ACG GCT ACC TTG TTA CGA CTT -3′), following standard procedures [7]. The amplified PCR products were sequenced using DNA Analyzer 3730 (Applied Biosystems, CA, USA). The resulting partial 16s rRNA gene sequences were searched in BLAST (Basic Local Alignment Search Tool) and closely related sequences were retrieved from the NCBI (National Center for Biotechnology Information) database. The unknown SNCK-3 sequence and retrieved sequences were aligned using ClustalW alignment tool in MEGA5 software. The evolutionary distance was inferred using the Neighbor-Joining method in MEGA5 [8].

3. Results

Proteolytic bacteria hydrolyze casein and produce soluble nitrogenous compounds, which are indicated as a clear zone, surrounding the respective colonies on SMA, which is a standard procedure [9,10]. One of the proteolytic bacterial colonies of SNK-3 (2 mm radius with regular margin, mucus, bulged, smooth and opaque proteolytic colony) from SMA was selected for the 16s rDNA based molecular identification. The 16s rDNA gene based analysis in the phylogenetic tree showed that SNCK-3 isolate was clustered along with *Acinetobacter* spp. (Fig. 1). The mean pairwise genetic distance (Kimura 2 parameter or K2P = 0.006%) between the isolate SNCK-3 and *Acinetobacter* spp. was smaller (Accession numbers KT025913.1, HQ199218.1 – *Acinetobacter calcoaceticus* and *Acinetobacter baylyi*) than the other species, such as, *Pseudomonas aeruginosa* (K2P = 0.189%) and *Comamonas kerstersii* (K2P = 0.256%). The isolate SNCK-3 was commonly clustered with all the *Acinetobacter* spp., which could not be identified upto species level. Sequence of *Acinetobacter* spp. SNCK-3 strain was submitted in the database of the National Center for Biotechnology Information (gene bank accession number KT588299).

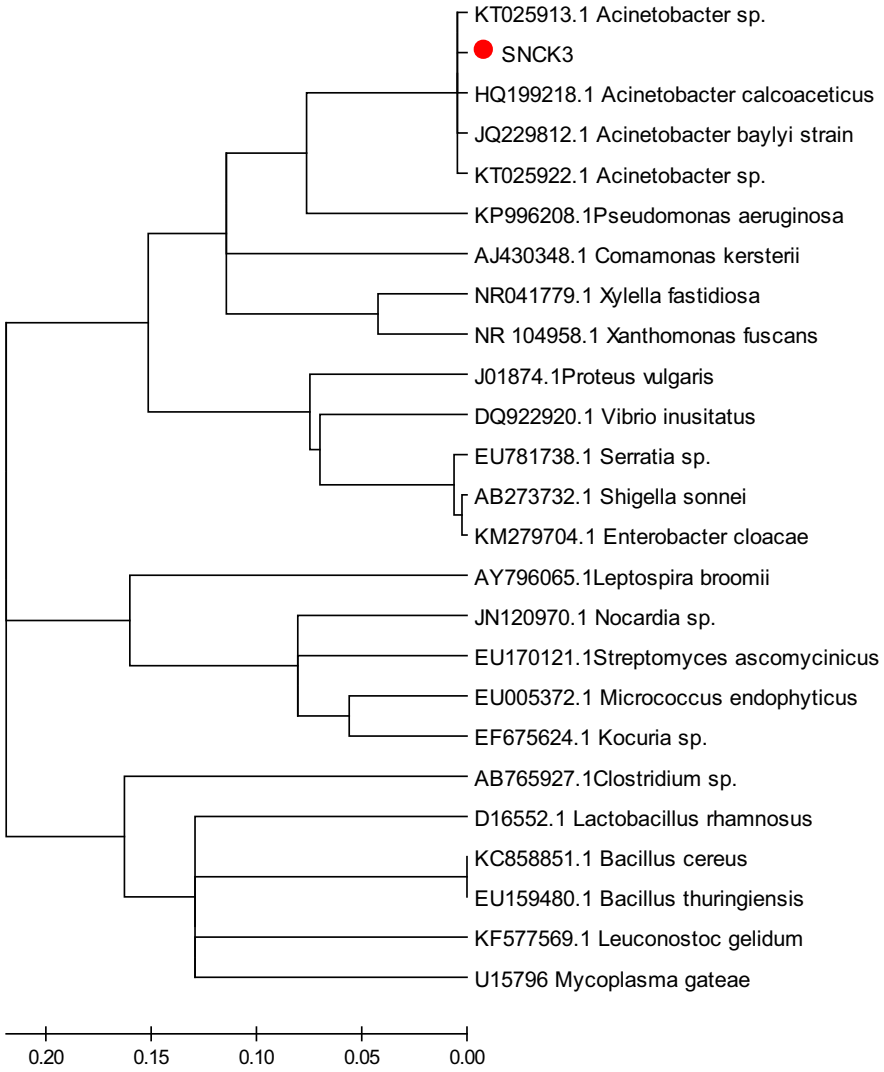

Fig. 1. The inferred phylogenetic tree showing the proteolytic bacterial isolate SNCK-3, including 24 reference strains. Evolutionary distances were determined with pairwise dissimilarities of the 16s rRNA gene sequences, and the dendrogram was generated using the neighbor-joining algorithm (MEGA5).

4. Discussion

Acinetobacter spp. is known for its plant-growth-promoting traits, viz. solubilized phosphates and zinc oxide, and for the production of siderophores for iron absorption [11]. It has been reported that *Acinetobacter* spp. significantly enhanced the shoot height, root length, etc. Thus, the current proteolytic strain of *Acinetobacter* spp. (SNCK-3) from *Panchagavya* can be considered as a biofertilizer and their beneficial properties need to be explored.

The strain (SNCK-3) is able to produce protease, and may directly or indirectly influence the pro-agricultural and medicinal properties of *Panchagavya*. *Panchagavya* is reported to enhance the biological efficiency of various crop plants and also the quality of fruits and vegetables. Microbiological studies reported that *Panchagavya* contains biofertilizers, like phosphobacteria, *Azospirillum*, *Pseudomonas, Azotobacter* and *Lactobacillus* [12]. Microbes can play a crucial role in the addition of growth regulatory substance, such as Indole Acetic Acid (IAA) and other growth nutrient factors to *Panchagavya*, which can cause a tremendous influence on the growth of crops [13].

The present study identified a proteolytic bacterium from *Panchagavya* and succeeded in the isolation and identification of a biofertilizer type of proteolytic *Acinetobacter* spp. *Panchagavya* induces resistance to pests and diseases, improves the quality of fruits and vegetables, and enhances the growth and vigor of crops [14]. *Panchagavya* has also been reported to possess insecticidal and anti-larval activity. Proteolytic properties of *Acinetobacter* spp. have been found to be crucial for the insecticidal activity of *Panchagavya* [15,16]. Till date, there are no studies on the activity of proteolytic bacteria from *Panchagavya*.

5. Conclusion

A proteolytic bacterium was isolated from *Panchagavya* and identified genotypically belonging to *Acinetobacter* spp, which are well known for their biofertilizer properties. The current microbiological analysis of *Panchagavya* generated a primary data in terms of its biofertilizer potential. However, a detailed study needs to be carried out to investigate the microbial biodiversity related to pro-agricultural and medicinal importance of *Panchagavya*. Through these studies, the biotechnological knowledge of *Panchagavya* in Vedic scripts can be explored.

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Conflict of interest

None.

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