Short Communication

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

D.S. Sayi, Surya Mohan, K. Vinod Kumar

*Sree Narayana College Kollam, Department of Botany and Biotechnology, Kollam, Kerala, India*

**Article info**

Article History:
Received 2 November 2016
Received in revised form 30 March 2017
Accepted 13 April 2017
Available online 6 December 2017

Keywords:
Acinetobacter spp.
Identification
Proteolytic
Panchagavya

**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*.

* © 2017 Transdisciplinary University, Bangalore and World Ayurveda Foundation. Publishing Services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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,
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’- ACC 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 up to 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).](image-url)
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.

Sources of funding

Department of Botany and Biotechnology, Sree Narayana College Kollam provided the fund for carrying M.Sc biotechnology dissertation work.

Conflict of interest

None.

Acknowledgement

Authors would like to thank Department of Biotechnology, Sree Narayana College Kollam for providing the facility for carrying out this work.

References

[1] Somasundaram E, Amanullah MM, Vaiyapuri K, Thirukkanumar K, Sathyamoorthi K. Influence of organic sources of nutrients on the yield and economics of crops under maize based cropping system. J Appl Sci Res 2007;3:1774–7.
[2] Thamaraj K, Ganesh P, Suresh Kumar R, Anandan A, Kolanjinathan K. A critical review on Panchagavya—a boon plant growth. Inter J Pharm Biol Arch 2011;2:1611–4.
[3] Palival R, Sahni YP, Singh SK, Sen S. Effect of Panchagavya on central actions in albino cats. Pharm Sci Monit 2013;4:3940–6.
[4] Deepika M, Nashima K, Rajeswari S. Antimicrobial activity of Panchagavya against urinary track infection. Int J Curr Pharm Res 2006;8:68–70.
[5] Nagaraj N, Sreenivasa MN. Influence of bacteria isolated from Panchagavya on seed germination and seed vigour in wheat. Karnataka J Agric Sci 2009;22:230–1.
[6] Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, et al. Current protocols in molecular biology. New York, NY: John Wiley & Sons, Inc.; 1999.
[7] Lane DJ, Pace Olsen CJ, Stahl DA, Sogin ML, Pace NR, et al. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci USA 1985;82:6955–9.
[8] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011;28:2731–9.
[9] Downes FP, Ito KE. Compendium of methods for the microbiological examination of foods. 4th ed. Washington, D.C: APHA; 2001.
[10] Wehr HM, Frank JH. Standard methods for the microbiological examination of dairy products. 17th ed. Washington, D.C: APHA Inc.; 2004.
[11] Rohkabakhsh-Zamin F, Sachdev D, Kazemi-Pour N, Engineer A, Pardesi KR, Zinjarde S, et al. Characterization of plant-growth-promoting traits of *Acinetobacter* species isolated from rhizosphere of *Pennisetum glaucum*. J Microbiol Biotechnol 2011;21:556–66.
[12] Yadav BK, Lourdraj CA. Effect of organic manures and Panchagavya spray on yield attributes and economics of rice (Oryza sativa). Crop Res 2006;31:1–5.
[13] Perumal K, Praveena K, Stalin V, Janarthanam B. Assessment of selected organic manures as plant growth hormones and their impact on the growth attributes of *Alium cepa* Lin. Curr Sci 2006;8:46–51.
[14] Natarajan K, Panchagavya—a manual. Mapusa, Goa, India: Other Indian Press; 2002. p. 313.
[15] Kumar MS, Bharath M, Josmin Laali Nisha LL, Basavaraju H. Field efficacy of Panchagavya on insect pests recorded during the study in *Tectona grandis*. Int J Res Agri Fores 2015;2:1–8.
[16] Shekarappa SM, Balakal RA. Evaluation of plant products in combination with cow urine and Panchagavya against sorghum shoot fly, *Atherigona soccata* Rondani. Karnataka J Agric Sci 2009;22:618–20.