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Research Article

Isolation and identification of bacterial endomicrobiome of *Syzygium cumini* from Faridabad, India

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Abstract
Endomicrobiome is the diverse and dynamic microbial flora that resides in plant tissues, without harming and developing detrimental effects. *Syzygium cumini*, the black plum or jamun plant, is used as traditional medicine. This is a medicinal plant used against metabolic disorders like diabetes, hypertension, and obesity, etc. The present study aimed at isolating bacterial endomicrobiome from leaf and stem parts of *S. cumini* locally grown garden situated at 28°20′32″N 77°19′32″E, Faridabad, India. Bacterial endomicrobiome were isolated using nutrient agar plates and identified. The sterilized root and leaf explants were inoculated on the nutrient agar plate and incubated for 24 hours. Morphological, cultural, and staining features were noted for initial identification, and 16S rRNA sequencing to verify the strain. A total of 14 bacterial endomicrobiome isolates were recovered from stem and leaf samples of *S. cumini*. The leaf sample demonstrated a higher number of bacterial endomicrobiome isolates than the stem of *S. cumini*. All the obtained isolates were gram-positive, mostly endospore-forming. Morphologically, small rods, creamish, off-white, smooth with irregular margins in appearance while NS5 Bacillus sp. developed slow growing, with brownish colonies on agar medium. Bacillus genera were found as the dominating endomicrobiome of *S. cumini*. Molecular characterization confirmed that the endomicrobiome of *S. cumini* was dominated by the genus Bacillus, and *Bacillus subtilis* was found to be the dominant bacterial species in leaf samples. In contrast, *Bacillus safensis* was identified as the major bacterial endomicrobiome of *S. cumini* stems. The obtained sequences were deposited to NCBI under the accession no. *Bacillus* sp. JL2 PQ475951 and *Bacillus* sp. NS5 PQ495957. It is concluded that the bacterial endomicrobiome is attributed to the medicinal properties of *S. cumini*.

Keywords: Endomicrobiome, *Syzygium cumini*, 16S rRNA, *Bacillus sp*. JL2, *Bacillus sp.* NS5.

INTRODUCTION

Endomicrobiome is the diverse and dynamic microbial flora that resides in plant tissues, without harming and developing detrimental effects. Ideally, nonpathogenic to the host plant (Kandel *et al.,* 2017; Compant *et al.,* 2010). Bacterial endomicrobiome harbors a category of metabolite. Exploration and understanding of endomicrobiome and their metabolites are unleashing a path to applications in agriculture, pharmaceuticals, and healthcare applications. They are used as biocontrol agents, biofertilizers, and in mitigation of toxic effluents from soil and water, thus playing an important role in environmental management (Singh *et al*., 2022; Rizvi *et al*., 2022; Santoyo *et al*., 2016).

Syzygium cumini, the black plum or jamun plant, belongs to the family Myrtaceae, used as a traditional medicine for ages. The seeds known as “Maghz-e-

Jamun or Tukhm-e-Jamun" have antihyperlipidemia properties and are therefore used against hyperglycemia (Rizvi *et al.,* 2022). The medicinal properties of *S. cumini* are reported against metabolic disorders like Diabetes, hypertension, obesity, astringent, hemostatic and urinary incontinence, ulcer prevention, allergy, etc. The antidiabetic potentiality of *S. cumini* is reported in both *in vivo* and *in vitro* models (Rather *et al*., 2019; Qamar *et al*., 2022). Endomicrobiome may contribute to the medicinal properties of *S. cumini*. Cultivation and identification of bacterial endomicrobiome is crucial. 16S rRNA Gene sequencing is one of the most promising methods for identifying bacterial isolates. The method is ideally used for bacterial identification. The 16S rRNA gene coding is a 1500 base pairs long conserved nucleotide sequence which is present in bacterial species and interspersed with variable regions. These variable regions are specific to genera and species. The sequence provides a platform for bacteria identification (Dan *et al*., 2020). Till date cultivation and applications to *S. cumini* bacterial endomicrobiome are limited; therefore, the present study was designed to study the bacterial endomicrobiome of *S. cumini*. The study aimed at its isolation and identification through culture, staining and 16S rRNA characterization.

MATERIALS AND METHODS

Sample collection

The healthy leaves and stem sample of *S. cumini* were collected from a locally grown garden, Goverdhan Kothi, near Government school and Canara Bank, situated at 28°20′32″N 77°19′32″E, Faridabad, India. Sample was taken carefully; tissue continuity was ensured and transferred to the laboratory. Immediately after, bacterial endomicrobiome isolation was conducted.

Surface sterilization

The collected plant samples were thoroughly washed with running tap water to remove excess soil particles and debris. The surfaces of these sample leaves were then sterilized by the method described by Marchut-Mikołajczyk *et al*., (2023) with slight modification. For surface sterilization, these leaves were first dipped into ethanol (70% C_2H_5OH) for 1 minute, and then transferred into sodium hypochlorite (0.5% NaOCl) for 5 minutes and finally into ethanol (70% C_2H_5OH) again for 30 seconds, then five times thoroughly rinsed with sterile double distilled water.

Isolation of bacterial endomicrobiome

To isolate bacterial endomicrobiome surface sterilized leaves and stem were cut into 1cm size pieces with the help of sterilized blades. These leaves and stem pieces were then placed on nutrient agar plates. Then, the plates were incubated at 37ºC for 24 hours. After incubation pure cultures were obtained. All the practicals were performed in triplicate.

Identification of bacterial endomicrobiome

After isolation, the bacterial endomicrobiome was examined for identification, and cultural characteristics and staining patterns were recorded for the preliminary analysis. The final identification was confirmed by 16S rRNA sequence analysis.

Cultural characteristics

After incubation, cultural characteristics: size, shape, color, texture, elevation, and margin of obtained bacterial endomicrobiome were observed and recorded.

Staining

For further identification, Gram staining and endospore staining were performed by following the methods described by Aneja (2003) and Cappuccino and Sherman (2008). The staining pattern to Gram's staining, endospore staining, and cell arrangement of obtained bacterial endomicrobiome was observed and recorded.

Molecular characterization

Molecular identification of obtained bacterial strains was performed at Bioserve Biotechnologies (India) Pvt. Ltd, Hyderabad, India. The microbial culture was processed, and genomic DNA was isolated using a Bacterial DNA isolation kit. Purity and concentration were confirmed by Denovix DS-11 spectrophotometer. The 16S rDNA region was amplified with Bacterial 16S rDNA PCR kit Fast (800, TaKaRa Bio; 10F/800R)). The amplification of 800bp amplicon of the obtained bacterial endomicrobiome strains and the positive control (*Escherichia coli*) was obtained using 16S rDNA Primer Mix and TaKaRa Taq™ HS Fast Detect Premix. No amplicon was obtained in the non-template control (NTC). The purification of amplicon was assessed via column purification and bi-directional cycle sequencing was carried out with forward primer and reverse primers using BDT V3.1 Cycle sequencing kit on ABI 3730 Genetic Analyzer as per the sequencing reaction protocol mentioned. Gene Tool software was used to generate a consensus sequence. BLAST analysis was carried out on the NCBI Genbank database. The first ten sequences in the database that showed the highest similarity were selected based on the maximum identity score, and the phylogenetic tree was prepared (Tamura *et al.,* 2021; Nei & Kumar, 2000; Saitou and Nei, 1987).

RESULTS AND DISCUSSION

A total 14 different isolates of bacterial endomicrobiome were isolated from stem and leaf samples of *S. cumini*. Comparatively, the number of bacterial endomicrobi-

ome isolates recovered from the leaf sample was higher than those isolated from the stem sample (Table 1, 2).

Phenotypic characterization

The cultural and staining characteristics of the obtained bacterial endomicrobiome were recorded. The obtained isolates were preliminarily characterized by colony appearance, opacity, texture, elevation, margin, and their microscopic appearance after Gram and endospore staining (Table 1, 2). The recovered bacterial endomicrobiome has developed smooth, creamy, off white colonies with irregular margins, opaque and thin rods. All were Gram-positive, and most of them were endospore-forming (Fig. 1). The obtained isolates were fast-growing, developing lawns within 24h of incubation. Exceptionally, the stem bacterial isolate NS5 was developing brownish, smooth, milky slow-growing colonies. All the isolates were named after the source of origin like, NS1, NS2, NS3, NS4 and NS5 for stem and JL1, JL2, JL3, JL4, JL5, JL6, JL7, JL8 and JL9 for leaf. Indrawati *et al*. (2021) reported similar findings with gram-positive, rod-shaped, milky or yellowish smooth bacterial isolates with *S. cumini*. Similarly, many authors have reported similar morphological findings with different plants (Marchut-Mikołajczyk *et al*., 2023; Singh *et al*., 2022; Chitranshi *et al*. 2022; Priti *et al*., 2020).

Molecular characterization and phylogenetic analysis

Out of 14 pure bacterial isolates, only two showed distinct morphology. The both bacterial endomicrobiome isolates, namely, JL2 and NS5. The recovered isolates were successfully amplified and sequenced using 16S rRNA primers. Obtained data of 16S rRNA gene sequence, BLAST analysis, and similarity indices were depicted in Table 3. Both the recovered bacterial endomicrobiome of the leaf and stem belonged to the Bacillus genera. The neighbor joining phylogenetic tree was constructed (Fig. 2).

BLAST and Phylogenetic analysis reflected that the isolate *Bacillus* sp. JL2, bacterial endomicrobiome, and *Schizygum cumini* leaf showed a 100% match identity with *Bacillus* sp. (in firmicutes) strain FRL13108 and isolated *Bacillus* sp. NS5, bacterial endomicrobiome, *S. cumini* stem, had 100% similarity with *Bacillus safensis* strain LBRN-2 (Table 3). Obtained sequences of both isolates were deposited to the GenBank, NCBI Bankit under the following accession numbers *Bacillus s*p. JL2 accession no. PQ475951 and *Bacillus* sp. NS5 PQ495957. 16S rRNA sequencing, BLAST, and phylogenetic analysis are among the best methods for the identification of bacterial endomicrobiome or endophytes. Many authors have identified bacterial endomicrobiome with 16S rRNA sequencing methods (Emitaro *et al*., 2024; Bartholomew Saanu, 2024; Raimi *et al*., 2023; Ambikapathy *et al*., 2022; Rekha *et al*., 2015). It is evident that the endomicrobiome of *S. cumini* is dominated by different strains of Bacillus genera. Molecular characterization confirmed that the *Bacillus subtilis* was found to be the dominant bacterial species in leaf samples whereas the *Bacillus safensis* was obtained as the major bacterial endomicrobiome of *S. cumini* stem. Limited data is identified with bacterial endomicrobiome (Indrawati *et al*., 2021) of *S. cumini,*

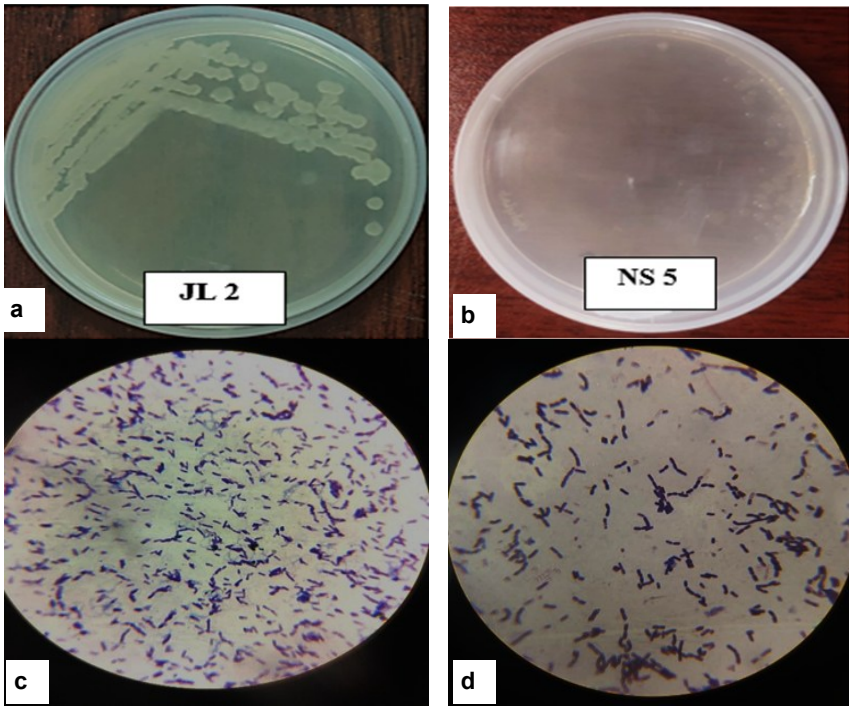

Fig. 1: *Obtained Bacterial* endomicrobiome *of Syzygium cumini: (a), (b) growth on agar plate (after 24 h incubation); (c), (d) microscopic image under 100X magnification.*

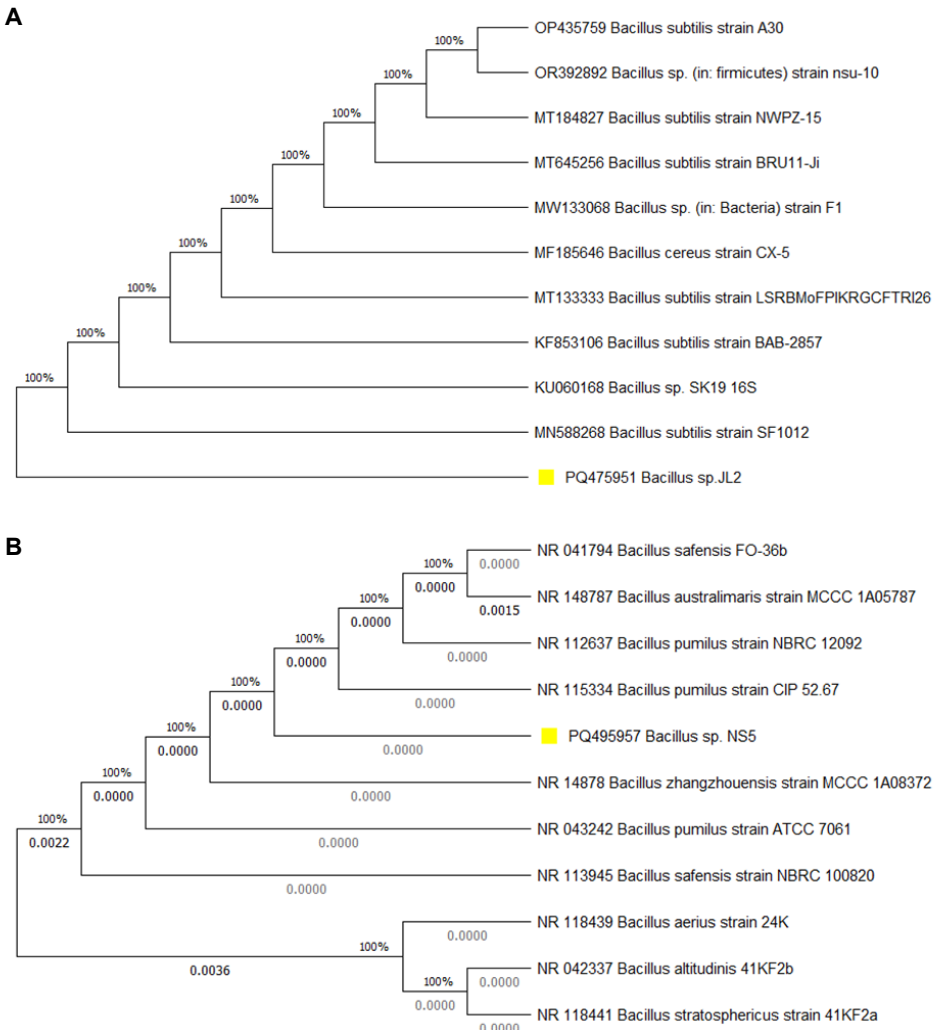

Fig. 2. *Evolutionary analysis by neighbor-joining phylogenetic tree of (a) Bacillus sp. JL2 and (b) Bacillus sp. NS5, bacterial endomicrobiome of S. cumini leaf and stem.* *Boot strap value ≥ 50%; * Yellow box indicates the query sequence.

Table 1. Cultural characteristics of obtained bacterial endomicrobiome of *Syzygium cumini*

Isolate	Characteristics				
Code	**Elevation**	**Shape**	**Color**	**Texture**	**Margin**
JL1	Flat	Lawn	Off white	Smooth	Entire
JL2	Flat	Lawn	Creamish	Smooth	Irregular
JL3	Flat	Lawn	Off white	Smooth	Entire
JL4	Flat	Lawn	Off white	Smooth	Entire
JL5	Flat	Lawn	Creamy white	Smooth	Irregular
JL6	Flat	Lawn	Off white	Smooth	Entire
JL7	Flat	Small	Off white	Smooth	Entire
JL8	Flat	Lawn	Off white	Smooth	Entire
JL9	Flat	Lawn	Off white	Smooth	Entire
NS1	Flat	Lawn	Off white	Smooth	Entire
NS2	Flat	Lawn	Off white	Smooth	Entire
NS3	Flat	Lawn	Off white	Smooth	Entire
NS4	Flat	Lawn	Off white	Smooth	Entire
NS5	Slightly raised	Lawn	Brownish	Smooth	Entire

*JL = Bacterial Endomicrobiome *S. cumini* Leaf Sample;*NS = Bacterial Endomicrobiome *S. cumini* stem sample

Table 2: Staining Characteristics of obtained bacterial endomicrobiome of *Syzygium cumini*

Isolate Code	Gram's Staining			Endospore Staining
	Shape		Cell Arrangement	
JL1	Positive	Small rods	Single	+
JL2	Positive	Small rods	Single	+
JL3	Positive	Small rods	Small Chain	+
JL4	Positive	Small rods	Single	+
JL5	Positive	Small rods	Single	+
JL6	Positive	Small rods	Single	+
JL7	Positive	Small rods	Single	+
JL8	Positive	Small rods	Small Chain	+
JL9	Positive	Small rods	Single	+
NS1	Positive	Small rods	Single	+
NS2	Positive	Small rods	Small Chain	+
NS3	Positive	Small rods	Single rods	+
NS4	Positive	Small rods	Single	+
NS5	Positive	Small rods	Single	+

*JL = Bacterial Endomicrobiome *S. cumini* Leaf Sample;*NS = Bacterial Endomicrobiome *S. cumini* stem sample.* + = Endospore present.

however, many authors have reported the fungal endomicrobiome of *S. cumini* (Samapti *et al*., 2022; Nurhaida andYenn *et al*. 2019). Indrawati *et al*. (2021) have reported that fruit endomicrobiome of *S. cumini* was dominated by *Bacillus* sp. The obtained data have supported the present research. Many authors have reported Bacillus as the dominating endomicrobiome with different plants (Emitaro *et al*., 2024; Singh *et al*., 2022; and Bolivar-Anillo *et al*., 2021).

Endomicrobime plays a crucial role, as it produces a variety of bioactive compounds of industrial interest. Compounds such as Pseudomycins, Ecomycins, Munumbicins, and Xiamycins demonstrate antibacterial, antimycotic, and antiplasmodial activities that are useful in the production of antibiotics (Hnamte *et al*., 2024; Digra and Nonzom, 2023; Christina *et al*., 2013), agriculture (Burragoni and Jeon, 2021; Ek-Ramos, 2019), plant stress management (Ameen *et al*., 2024; Fayha, 2024; Kaur and Karnwal, 2023; Chaudhary *et al*., 2022; Liu *et al*., 2022), The bacterial endophyte produced compounds with medicinal properties, including lobophorins, xiamycins, and sespenins that possess anti-inflammatory, anticancer, and anti-diarrheal properties (Zotchev, 2024; Chitranshi *et al*., 2022; Burragoni and Jeon, 2021; Luo *et al*., 2021; Wei *et al*., 2011; Ding *et al*., 2010; Jiang *et al*., 1999). Toxoflavin, derived from the Burkholderia gladioli a bacterial endophyte is reported as potential anticancer drug candidate (Zotchev, 2024; Li *et al*., 2019). The obtained bacterial endomicrobiome shared a wide spectrum of potential applications in different domains. Many authors have reported different applications and potentiality of *Bacillus* sp. and *Bacillus safensis* endophytes. Wu *et al*. (2019) have suggested diesel hydrocarbon bioremediation and plant growth-promoting capabilities of *Bacillus safensis*. Similarly, *Bacillus* sp. has been remarked for its antiviral activity against groundnut bud virus (Gayathri *et al*., 2024), protease production (Elaine Mankge *et al.,* 2024), disease suppression as biocontrol (Bolivar-Anillo *et al*., 2021), and plant growth promotion (Adeleke *et al*., 2021) in vegetable crops (Miljaković *et al*., 2020), and many more. The present result depicted the bacterial endomicrobiome of *S. cumini* plant, possibly a major contributor to its medicinal properties.

Conclusion

In the present study, 14 bacterial endomicrobiome isolates were recovered from the stem and leaf samples of *S. cumini*. The leaf sample (9) demonstrated a higher number of bacterial endomicrobiome isolates than the stem (5) of *S. cumini*. All the obtained isolates were gram-positive endospore-forming. Morphologically, small rods, mostly creamish, off white, smooth colonies with irregular margins. Exceptionally, the stem bacterial isolate NS5 was developing brownish, smooth, milky, slow-growing colonies. Molecular characterization confirmed that the endomicrobiome of *S. cumini* was dominated by Genus Bacillus, and *B. subtilis* was found as the dominant bacterial species in leaf samples, whereas the *B. safensis* was obtained as the major bacterial endomicrobiome of *S. cumini* stem. The obtained sequence was deposited to NCBI under accession no. *Bacillus* sp. JL2 PQ475951 and *Bacillus* sp. NS5 PQ495957. Both strains are renowned for their diverse applications. The medicinal properties of *S. cumini* may be sourced or supported with *S. cumini* plant bacterial endomicrobiome or their bioactive compounds. Further

Table 3. BLAST percent similarity index of Bacterial endomicrobiome recovered from (a) *Bacillus sp*. JL2 leaf and (b) *Bacillus sp*. NS5 stem of *Syzygium cumini*.

A

Description	Max Score	Total Score	Query Cover	E value	Percent Identity
Bacillus sp. (in firmicutes) strain FRL13108 16 ribosomal RNA gene	1264	1264	100%	0.0	100.00%
Bacillus subtilis strain AAUBC-BsB1 16S ribosomal RNA gene	1264	1264	100%	0.0	100.00%
Bacillus stercoris strain V6335 16S ribosomal RNA gene	1264	1264	100%	0.0	100.00%
Bacillus stercoris strain V4367 16S ribosomal RNA gene	1264	1264	100%	0.0	100.00%
Bacillus stercoris strain V3823 16S ribosomal RNA gene	1264	1264	100%	0.0	100.00%
Bacillus stercoris strain V3228 16S ribosomal RNA gene	1264	1264	100%	0.0	100.00%
Bacillus stercoris strain V3086 16S ribosomal RNA gene	1264	1264	100%	0.0	100.00%
Bacillus sp. (in firmicutes) strain C1 16S ribosomal RNA gene	1264	1264	100%	0.0	100.00%
Bacillus subtilis strain BS-2301 16S ribosomal RNA gene	1264	1264	100%	0.0	100.00%
Bacillus licheniformis strain PBN2 16S ribosomal RNA gene	1264	1264	100%	0.0	100.00%

B

Description	Max Score	Total Score	Query Cover	E value	Percent Identity
Bacillus pumilus strain LBUM494 chromosomes	1275	10158	100%	0.0	100.00%
Bacillus safensis subsp. Safensis strain ENBC1 16S ribosomal gene	1275	1275	100%	0.0	100.00%
Bacillus safensis strain LBRN-2 16S ribosomal RNA gene	1275	1275	100%	0.0	100.00%
Bacillus safensis strain D012 16S ribosomal RNA gene	1275	1275	100%	0.0	100.00%
Bacillus sp. (in. firmicutes) strain *pumilis* 16S ribosomal RNA gene	1275	1275	100%	0.0	100.00%
Bacillus safenisis strain MN1 16S ribosomal RNA gene	1275	1275	100%	0.0	100.00%
Bacillus safensis strain JJ1244 chromosome complete	1275	10185	100%	0.0	100.00%
Bacillus pumilis strain JJ950 chromosome complete	1275	10163	100%	0.0	100.00%
Bacillus safensis strain B1 16S ribosomal RNA gene	1275	1275	100%	0.0	100.00%
Bacillus safensis strain AR505 chromosome	1275	10169	100%	0.0	100.00%

research is needed to identify the bioactive compound for *S. cumini's* medicinal and plant growth-promoting properties.

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Conflict of interest

The authors declare that they have no conflict of interest.

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