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Lysinibacillus spp. were identified as the predominant bacteria on the surface of the dried Indian bay leaf purchased from the local farmers' market in the city of Visakhapatnam, India.

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Keywords: Indian bay leaf, 16S rRNA gene, Sanger sequencing, spore forming bacteria, dry spices, *Lysinibacillus* species.

Rising concerns about food-borne illnesses is an ongoing medical issue in India. Existence of multiple bacterial species in the moist street foods that are served unhygienically is commonly expected. However, dry spices such as the Indian bay leaf (IBL) are used in the preparation of street foods such as the biryani. Most of the spices are sold as dry products in the markets and we reasoned whether there would be any presence of dry-spore forming bacteria on these products. In this study we took the surface swabs of IBL purchased from the local farmers' market and were able to grow bacterial colonies in the laboratory on LB agar plates. These bacterial colonies were further cultured in LB broth and were genotyped by sequencing their 16S rRNA gene. Our results confirm the presence of *Lysinibacillus spp.* identified based on their 16S rRNA gene sequence. The biotransformation capabilities of these bacteria will be studied in future.

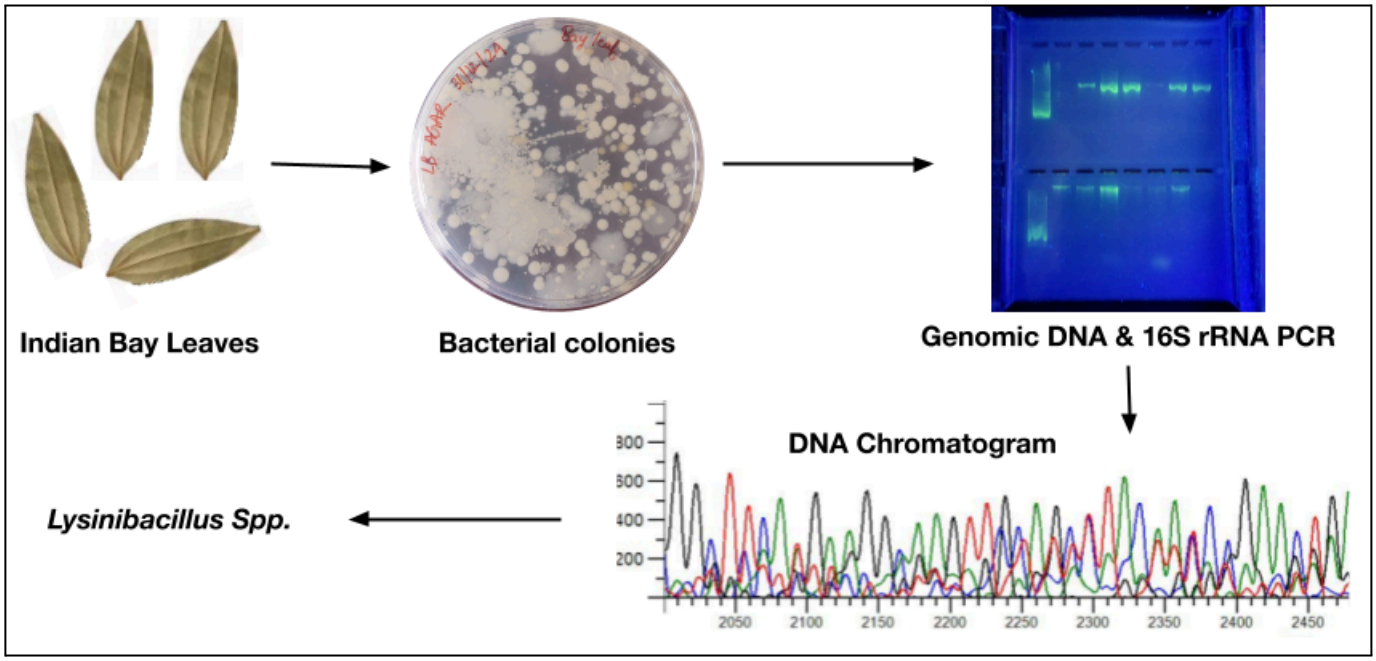

Figure 1. Overall process of bacterial species identification present on the Indian Bay Leaves.

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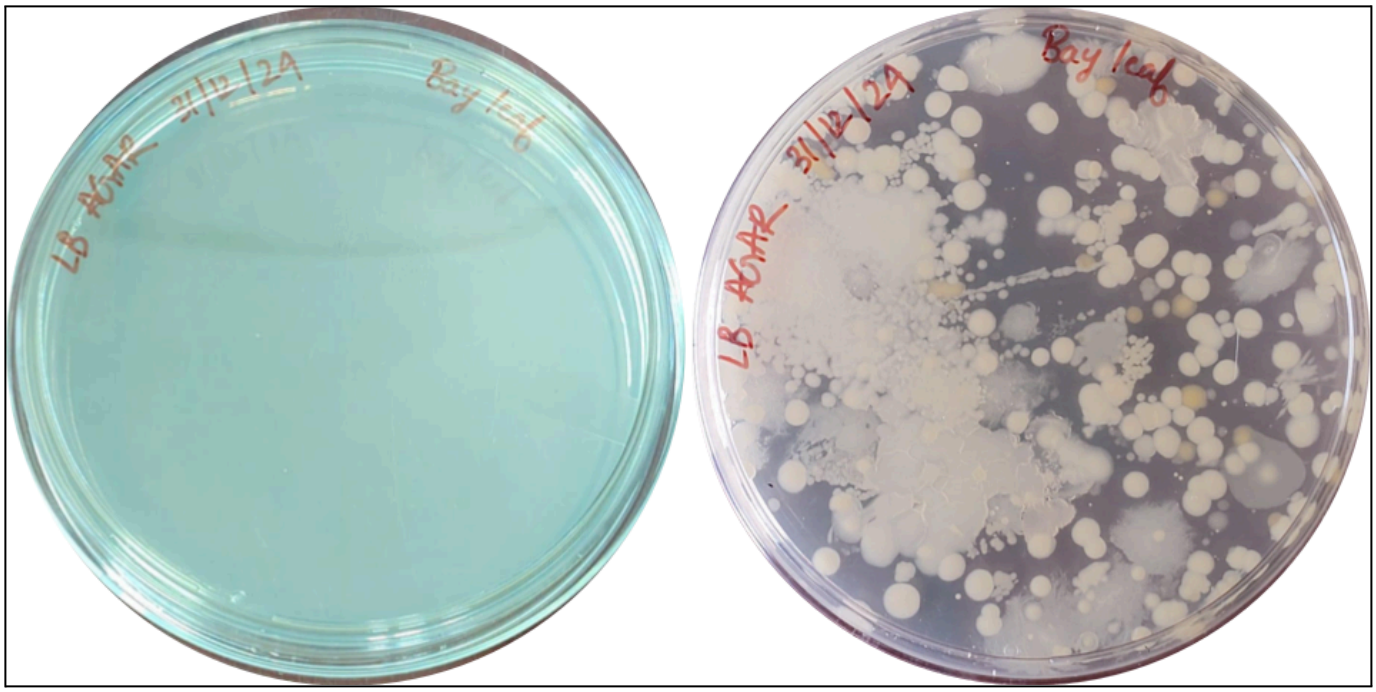

Figure 2. LB-Agar plates streaked with the swabs exposed to the surface of the Indian Bay Leaf before (left panel) and after (right panel) incubation at 37 ℃ overnight.

Bacterial growth is commonly seen in moist street foods due to unhygienic ways of storing and serving them by the street vendors [1, 2]. However, we reasoned whether there will be any bacteria on the dry spices that are used in the preparation of street food such as biryani, etc. We chose the Indian Bay Leaf (IBL) (*Cinnamomum tamala*) that is most commonly used as a spice for flavoring biryani, curries, etc. Based on the dry characteristic of the IBL spice, we did not expect that any bacteria would survive due to lack of moisture. But we parallelly reasoned that spore-forming bacteria may however leave behind their spores that can sustain through dry conditions for prolonged periods of time [3].

In this study we purchased the IBL from a local farmers' market in order to analyze the presence of any spore-forming bacteria using the overall process displayed in Figure 1. The leaves were packed in bags during the transportation to the laboratory to avoid any further cross-contamination possibilities on the way. The leaves were unpacked in the sterile environment in the laminar air flow chamber. Autoclaved earbuds were used to wipe the surface of the IBL which were then used to streak sterile and autoclaved LB agar plates. A negative control plate that was not streaked was also incubated along with the streaked plate overnight at 37 ℃. Followed by the overnight incubation, the plates were observed for colony formation. As shown in Figure 2, the plate that was streaked with the swab from IBL displayed multiple colonies of more or less the same morphology apparently. The bacterial colonies were analyzed separately to know their identity and the analyses will be published elsewhere. In this study, a mix of all colonies was taken as inoculum to grow an overnight culture in 5 ml sterile LB broth.

The bacterial cells were harvested by centrifugation and the pellet was further used for the extraction of bacterial genomic DNA as described previously [4]. The genomic DNA was then checked qualitatively on an agarose gel and was further used as a template for the amplification of the 16S rRNA gene. Polymerase chain reaction-based amplification of the bacterial 16S rRNA gene was performed. The final PCR product was outsourced for Sanger sequencing in order to identify the bacterial species. The chromatograms were carefully analyzed for base calling and the final DNA sequence was used to search online in the NCBI-BLAST server. As shown in Figure 3, a total of 105 hits were obtained in the NCBI-BLAST search out of which, 98 hits were *Bacillus spp.* and 91 hits were *Lysinibacillus spp*. With >86 % of the identified hits dominated by the *Lysinibacillus spp*., we confirm that the IBL were infested with either *Lysinibacillus spp*. or their spores to survive the dry conditions. It has been widely known that the *Lysinibacillus spp*. are a common environmental contaminant that may accidentally enter the food chain and cause disease to humans [5-7].

Taxonomy	Number of hits	Number of Organisms
⊟Bacteria	105	32
. ⊟Bacillales	98	26
. . ⊟Bacillaceae	96	24
. . . ⊟Lysinibacillus	91	21
. . . . Lysinibacillus macroides	5	1
. . . . Lysinibacillus sphaericus	15	1
. . . . Lysinibacillus pakistanensis	8	1
. . . . Lysinibacillus xylanilyticus	20	1
. . . . ⊞unclassified Lysinibacillus	37	15
. . . . Lysinibacillus boronitolerans	1	1
. . . . Lysinibacillus fusiformis	5	1
. . . ⊞unclassified Bacillus (in: firmicutes)	5	3
. . Staphylococcus sp. MF3	1	1
. . uncultured Planococcaceae bacterium	1	1
. ⊞unclassified Bacteria	6	5
. uncultured bacterium	1	1

Figure 3. Screenshot of the bacterial species identified by the NCBI-BLAST search.

With this information we further evaluated the biotransformation capabilities of *Lysinibacillus spp*. bacteria to evaluate whether the metabolites from the IBL can be biotransformed into useful and/or harmful substances by these bacteria in the human gut microbiome. The biotransformation data are published as a full research article in this issue of TCABSE-J [8].

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Author contributions: B.S. designed and performed all the wet lab experiments. M.V.S.A., L.A., V.M.A., M.K., H.T.M. and A.A. assisted B.S. in various aspects of the project. R.S.Y. is the principal investigator who designed the project, trained all students, secured required material for the project, provided the laboratory space and facilities needed. R.S.Y. wrote, edited and finalized the manuscript.

Conflict of interest: The authors declare no conflict of interest in this study.

References

1. Chumber, S. K., Kaushik, K., & Savy, S. (2007). Bacteriological analysis of street foods in Pune. *Indian journal of public health*, *51*(2), 114–116.
2. Sharma, I., & Mazumdar, J. A. (2014). Assessment of bacteriological quality of ready to eat food vended in streets of Silchar city, Assam, India. *Indian journal of medical microbiology*, *32*(2), 169–171.
3. Mathot, A. G., Postollec, F., & Leguerinel, I. (2021). Bacterial spores in spices and dried herbs: The risks for processed food. *Comprehensive reviews in food science and food safety*, *20*(1), 840–862.
4. Sundaram, B., Akula, L., Akella, M.V.S., Mir, H.T., Shaik Azeez, Lebesa, M.N., Allu, J., Agathambadi, K., Sodasani, M, Aggunna, M., Addala, S., Vissapragada, M. and Yedidi, R.S. (2024). Exploring the street food bacterial diversity in the city of destiny using the bacterial 16S rRNA genotyping. TCABSE-J, Vol. 1, Issue 7:21-25. Epub: July 6 th , 2024.
5. Torres, J. L., & Faulhaber, J. R. (2023). Triple-valve endocarditis due to *Lysinibacillus sphaericus* infection. *IDCases*, *33*, e01856.
6. Wenzler, E., Kamboj, K., & Balada-Llasat, J. M. (2015). Severe Sepsis Secondary to Persistent Lysinibacillus sphaericus, Lysinibacillus fusiformis and Paenibacillus amylolyticus Bacteremia. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases*, *35*, 93–95.
7. Kinoshita, T., Sahara, S., Amano, T., Ito, M., Sakakibara, T., Takimoto, N., Osada, Y., & Oka, K. (2023). First Case Report of Peritoneal Dialysis-associated Peritonitis Caused by Lysinibacillus sphaericus. *Internal medicine (Tokyo, Japan)*, *62*(19), 2919–2922.
8. Sundaram, B., Akella, M.V.S., Akula, L., Addala V.M., Kinjarapu, M., Mir, H.T., Ambidi, A. and Yedidi, R.S. (2025). Identification of highly toxic metabolites produced by the bacterial biotransformation in the Indian bay leaf methanolic extracts using GC-MS: a new perspective for food-borne illnesses. *TCABSE-J*, Vol. 1, Issue 9:24-29. Epub: Mar 30th, 2025.