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Qualitative and quantitative analysis of SARS CoV-2 spike protein receptor binding domain expression in *Lactobacillus* with potential applications in edible vaccines.

Priyanka Boddepalli[1, 2], Chandini Kolluru[1, 2], Monalisa Chowdari[1, 2], Manikanta Sodasani[1], Madhuri Vissapragada[1], Bindiya Panchagnula[2], Sudhakarrao Pola[2], S. P. Dhanunjay Ponamgi[2] and Ravikiran S. Yedidi[1, 2, *]

[1]Department of Intramural research core, The Center for Advanced-Applied Biological Sciences & Entrepreneurship (TCABS-E), Visakhapatnam 530003, A.P. India; [2]Department of Biotechnology, Andhra University, Visakhapatnam 530003, A. P. India. (*Correspondence to R.S.Y.: tcabse.india@gmail.com).

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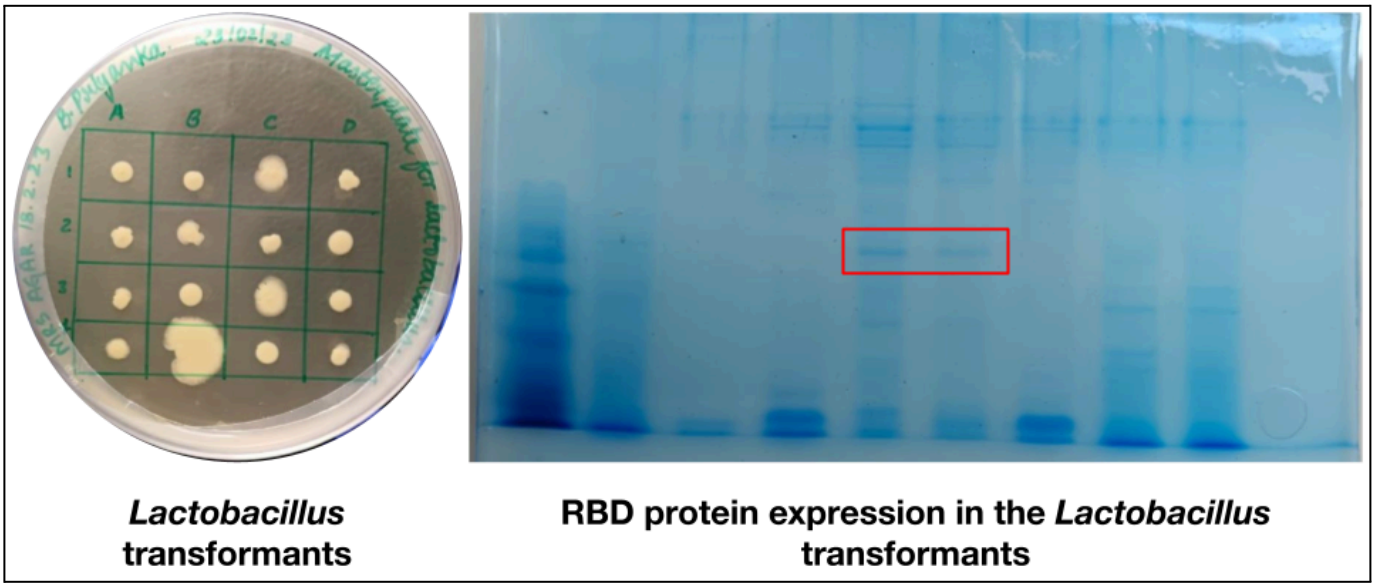
COVID-19 pandemic has claimed too many human lives in recent years due to the unavailability of vaccines in time. Many different strategies have been explored for COVID-19 vaccines such as the mRNA vaccine etc. However, irrespective of the vaccine strategy, side effects have been complained about in abundance so far. In this study we tested the feasibility of preparing an edible probiotic vaccine for COVID-19 using *Lactobacillus* bacteria that are transformed with a plasmid containing the SARS CoV-2 spike protein receptor binding domain (RBD). The expression levels of RBD in the Yogurt prepared by using the transformants was tested qualitatively and quantitatively using SDS-PAGE and ImagJ software, respectively. Our results suggest that RBD is expressed in selected transformants in measurable quantities thus offering a promising new approach to edible probiotic yogurt vaccines in the future.



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COVID-19 vaccines were designed using different strategies and were used during the pandemic [1]. However, none of them were edible and safe with negligible side effects [2]. Edible vaccines are often not only safe for consumption but also provide an alternative to painful oily injections for vaccine shots with a clinician's supervision [3].

Edible vaccines were designed for consumption in several ways in the past such as plant-based, etc. but a probiotic-based edible vaccine is still rarely seen across the globe [4, 5]. Probiotic bacteria such as *Lactobacillus* can be used for probiotic edible vaccines [6].

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Figure 1. Colonies of *Lactobacillus* isolated from the natural homemade yogurt. These colonies were obtained by streaking the yogurt on an MRS agar plate without any antibiotics followed by overnight incubation at 37 ℃.

In this study, we isolated the *Lactobacillus spp.* from natural homemade yogurt and transformed them to check whether it is possible to express the antigenic proteins such as the SARS CoV-2 spike protein receptor binding domain (RBD) in this bacteria through transformation and analyzed the protein expression both qualitatively (SDS-PAGE) and quantitatively (pixel quantification using ImageJ software) [7]. *Lactobacillus* bacteria were isolated from the natural homemade yogurt by streaking on a freshly prepared and autoclaved De Man–Rogosa–Sharpe (MRS) agar plate without any antibiotics. After incubation at 37 ℃ overnight, the colonies were obtained as shown in Figure 1. Bacterial minicultures were prepared using MRS liquid broth without antibiotics. These cultures were further used to prepare competent cells using the standard $CaCl_2$ method. The freshly prepared competent cells were then transformed with a plasmid containing RBD coding gene along with the ampicillin-resistance gene (Amp^r). This plasmid was prepared previously [8]. Transformation was performed using the standard Heat-shock method in which the competent cells were briefly incubated at 42 ℃ in the presence of the plasmid and were quenched on ice [9]. The transformants were then plated on MRS agar plates containing 50 µg/ml ampicillin as a selection marker. A master plate shown in Figure 2 was prepared to screen the transformants for RBD expression using SDS-PAGE. RBD bands were further quantified using ImageJ software [10].

Freshly prepared competent cells always yielded multiple colonies of transformants compared to frozen and thawed ones that showed relatively lesser transformation efficiency. Multiple transformant colonies were obtained from which random colonies were selected and plated on the master plate shown in Figure 2. A total of 16 colonies were plated in a grid drawn on the master plate for further evaluation for the RBD expression. Whole cell lysates were prepared from the minicultures of these selected colonies from the master plate for RBD expression analysis using SDS-PAGE. Among the lysates of 16 colonies, 2 colonies showed expression of RBD protein in reasonably good quantities that were sufficient for the pixel quantification method for estimating the RBD expression. These two colonies were further propagated in the presence of ampicillin to retain the plasmid. The data from pixel quantification of the 2 bands from the SDS-PAGE (Figure 2) was found to be 6,500 units compared to the background which was found to be <1,000 units as described previously [10]. These results suggest that the 2 chosen colonies expressed reasonable amounts of the RBD protein and that they can be used further for the preparation of future yogurt-based vaccines for COVID-19. The glycerol stocks of the two colonies selected were used for the expression of RBD protein at least two more times to test the consistency of RBD expression and were found to be efficiently consistent. Additionally, we tested new batches of *Lactobacillus* bacteria that were isolated from additional samples of natural homemade yogurt and were transformed with the RBD plasmid to test the reproducibility by different people in the laboratory. The results in this study were reproducible irrespective of the person who conducted the experiment and various batches of yogurt samples that were used to isolate the *Lactobacillus* bacteria.

In conclusion we have shown in this study that viral antigenic proteins such as the RBD in the current study can be conveniently expressed in the common probiotic bacteria such as *Lactobacillus* that can be used further to prepare probiotic edible vaccines with almost no side effects. Such probiotic vaccines due to their edible nature would be easily administered without painful oily injections, especially to the kids of all ages. The RBD expression in *Lactobacillus* will be further optimized in future to produce more amounts of RBD to meet the vaccine criteria that can elicit a successful immune response upon consumption. Animal studies are yet to be performed to test the antigenicity of RBD produced from the yogurt to test whether it can produce measurable antibody titers before starting the human trials. Based on these current studies, an edible probiotic vaccine will be prepared in the future for COVID-19 followed by other infectious diseases. In addition to the regular yogurt taste, various flavors such as vanilla, strawberry, chocolate, etc. will be tested to improve the palatability of the yogurt vaccines.

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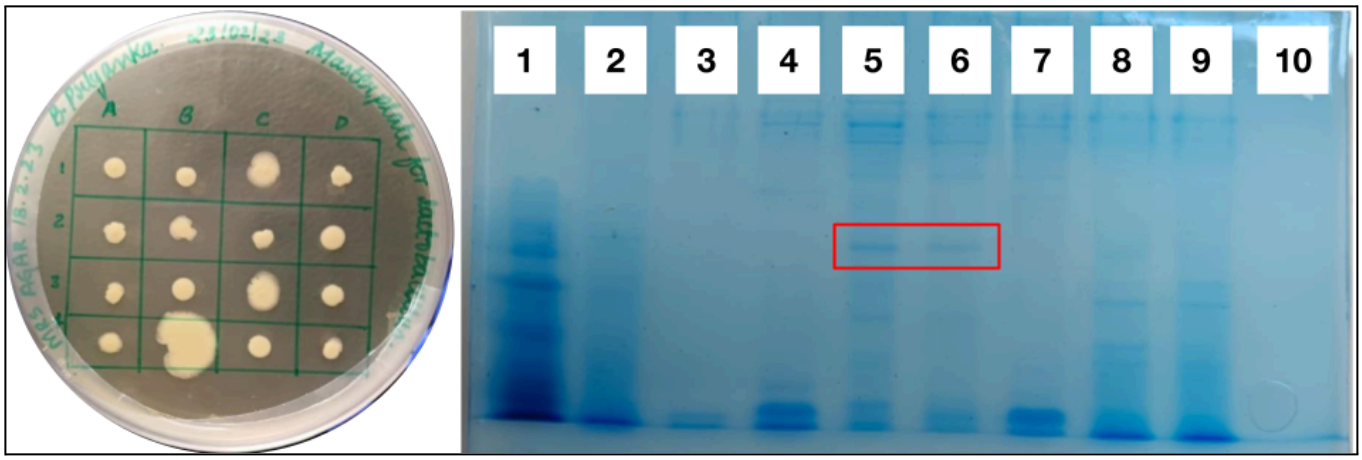

Figure 2. Master plate showing colonies of *Lactobacillus* transformants and SDS-PAGE analysis of RBD expression. The master plate has a grid in which the transformed colonies were grown in the presence of 50 µg/ml ampicillin. These colonies were further tested for the RBD protein expression. The SDS-PAGE gel shown on the right side contains protein ladder in the first lane (left most lane) followed by the whole cell lysates from the transformants from the master plate. Evidently, colonies 4 and 5 in lanes 5 and 6, respectively show the RBD expression highlighted by red box.

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

Author contributions: The first author, P.B.1 did most of the wet lab work. C.K. and M.C. repeated all the wet lab work for consistency. M.S. and M.V. supervised P.B.1, C.K. and M.C. in the wet lab work and data analysis. P.B. and S.P co-supervised the first author P.B.1 D.P. co-supervise C.K. and M.C. R.S.Y. is the principal investigator who designed the project, trained P.B.1, C.K., M.C., M.S. and M.V., secured funding and material for the project, provided the laboratory space and facilities needed. R.S.Y. wrote, edited and finalized the manuscript.

References

1. Science Brief: COVID-19 Vaccines and Vaccination. In: National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases. *CDC COVID-19 Science Briefs*. Atlanta (GA): Centers for Disease Control and Prevention (US); September 15, 2021.
2. Sohrab SS. An edible vaccine development for coronavirus disease 2019: the concept. *Clin Exp Vaccine Res*. 2020; 9(2): 164-168. doi:10.7774/cevr.2020.9.2.164.
3. Paradia PK, Bhavale R, Agnihotri T, Jain A. A Review on Edible Vaccines and Biopharmaceutical Products from Plants. *Curr Pharm Biotechnol*. 2023;24(4):495-509. doi:10.2174/138 920102366622080315103 9.
4. Gunasekaran B, Gothandam KM. A review on edible vaccines and their prospects. *Braz J Med Biol Res*. 2020;53(2):e8749. Published 2020 Jan 24. doi:10.1590/1414-431X20198749.
5. Tomás, C.C., Oliveira, E., Sousa, D. *et al.* Proceedings of the 3rd IPLeiria's International Health Congress. *BMC Health Serv Res* 16 (Suppl 3), 200 (2016). https://doi.org/10.1186/s12913- 016-1423-5.
6. Rosales-Mendoza S, Angulo C, Meza B. Food-Grade Organisms as Vaccine Biofactories and Oral Delivery Vehicles. *Trends Biotechnol*. 2016;34(2):124-136.
7. Posimsetti, M., Sanapala, S. S., Vissapragada, M. and Yedidi, R. S. (2022). Design of a pixel-based quantification method for in vitro colorimetric enzyme assay to evaluate enzyme inhibition in a dose-dependent manner. TCABSE-J, Vol. 1, Issue 4:22-27. Epub: Oct5th , 2022.
8. Vissapragada, M., Gollu, S., Addala, S., Sodasani, M., Aggunna, M., Mukala, N., Pola, S., Panchagnula, B. and Yedidi, R.S. (2023). PCR amplification of wild type receptor binding domain coding gene of SARS CoV-2 spike protein for cloning into a bacterial expression vector. TCABSE-J, Vol. 1, Issue 6:18-23. Epub: Aug 20 th , 2023.
9. Bellana, P., Eddumala, N., Pinipe, J., Marada, K., Mahapatro, R., Addala, S., Sodasani, M. and Yedidi, R.S. (2024). In vitro horizontal transfer of ampicillin-resistance gene between bacterial species with implications in antibiotic-resistance problem in human microbiome. TCABSE-J, Vol. 1, Issue 8:1-5. Epub: Jul 20 th , 2024.
10. Vissapragada M, Addala S, Aggunna M, Sodasani M, Grandhi AVKS, Yedidi RS. Leveraging the potential of bacterial lateral gene transfer in boosting the efficacy of an edible probiotic prototype yogurt vaccine for COVID-19. *Biochem Biophys Res Commun*. 2024;734:150622. doi:10.1016/j.bbrc.2024.150622.