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A strain of *Lactobacillus* isolated from Himalayan Ragi vinasse and its application in skincare

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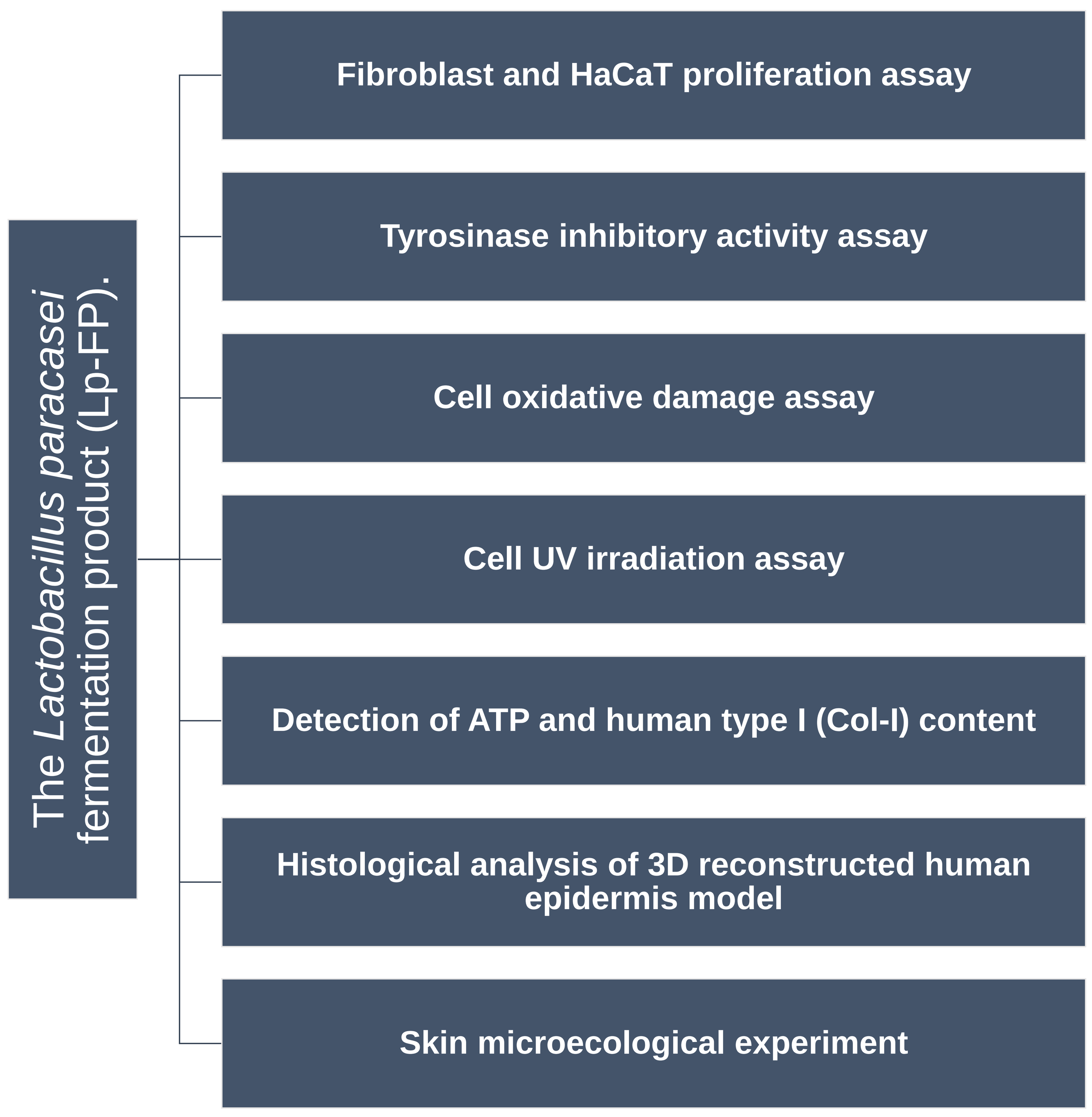
Introduction:

At present, with the improvement of consumer awareness, pursuits of green, nature, safety, and health are becoming more prevalent. The interest in new and better cosmetic ingredients which are originated from natural sources is also increasing [1].The more environmentally friendly and natural fermented cosmetics have received widespread attention. Microbes, especially probiotics, can produce a variety of active ingredients after fermentation, and its fermentation products have unique advantages in antioxidant, moisturizing, whitening, etc [2]. Therefore, it is extensive for the application of natural fermented products in cosmetics.
Ragi is a grain grown for food in the Himalayan region [3]. It is also used as substrate for traditional fermented alcoholic beverage because of high calories, vitamin content, as well as beneficial lactic acid bacteria and yeast [4]. The *Lactobacillus paracasei* is widely utilized as probiotics and also have a long history of safe human consumption [5,6]. Researches revealed that it could inhibit the growth of harmful bacteria, meanwhile, its exopolysaccharides with good antioxidant activity which can be used as health food additives [7,8] . This article will explore a strain of *Lactobacillus paracasei* was isolated from the ragi vinasse, and the potential of its fermented products in skin care efficacy.

Materials & Methods:

1. Strain separation and sample preparation
The strain was isolated from the ragi vinasse inTibet-China belongs to the Himalaya regionand was identified as *Lactobacillus paracasei* after culture and purification. The strain was inoculated in MRS to obtained seed liquid. After anaerobic incubation for 48 h at 37 °C, bacterial cells were removed by centrifugation (5,000 rpm, 30 min, 4 °C), and the supernatant was passed through a 0.22μm filtration to obtain Lactobacillus paracasei fermentation product (Lp-FP). The solid content of Lp-FP was 17mg/mL determined by loss in mass on drying. The Lp-FP samples used in this study were stored at -20 °C for the succeeding tests.
2. The skin care efficacy evaluation experiment
The following skin care efficacy evaluation experiment was performed on the fermentation product of *Lactobacillus paracasei* (Lp-FP).



Results & Discussion:

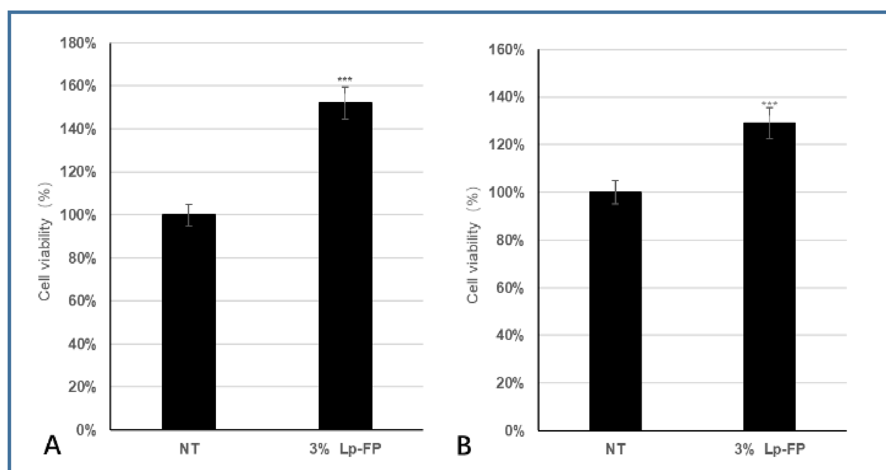
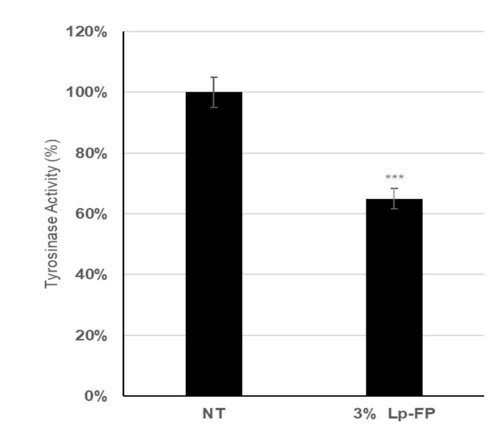


Fig.1 Proliferation effects of Lp-FP on Hacat (A) and FB (B) cells. NT= The group without Lp-FP treatment, *** p<0.001.

As shown in Fig.1, 3% Lp-FP could promote the proliferation on HaCaT cells and FB Cells to 152% and 129%, respectively. Lp-FP can promote the proliferation of human skin cells, and maintaining a healthy skin condition, and having anti-aging potential.



As shown in Fig.2, the results of B16 tyrosinase assay showed that Lp-FP effectively inhibited tyrosinase activity, which was reduced to 35% at a concentration of 3%.

Fig.2 Tyrosinase activity was determined by L-DOPA oxidation assay in B16. NT= The group without Lp-FP treatment, *** p<0.001.

As shown in Fig.3A and 3B, under the treatment of hydrogen peroxide, Lp-FP can increase the viability of HaCat and FB cells by 17.60% and 24.56%, respectively. Moreover, as shown in Figure 3C and 3D, under UVA and UVB radiation, HaCat cell viability can be increased by 9.07% and 30.60, respectively. Lp-FP exhibits a protective effect against damage caused by hydrogen peroxide and ultraviolet rays.

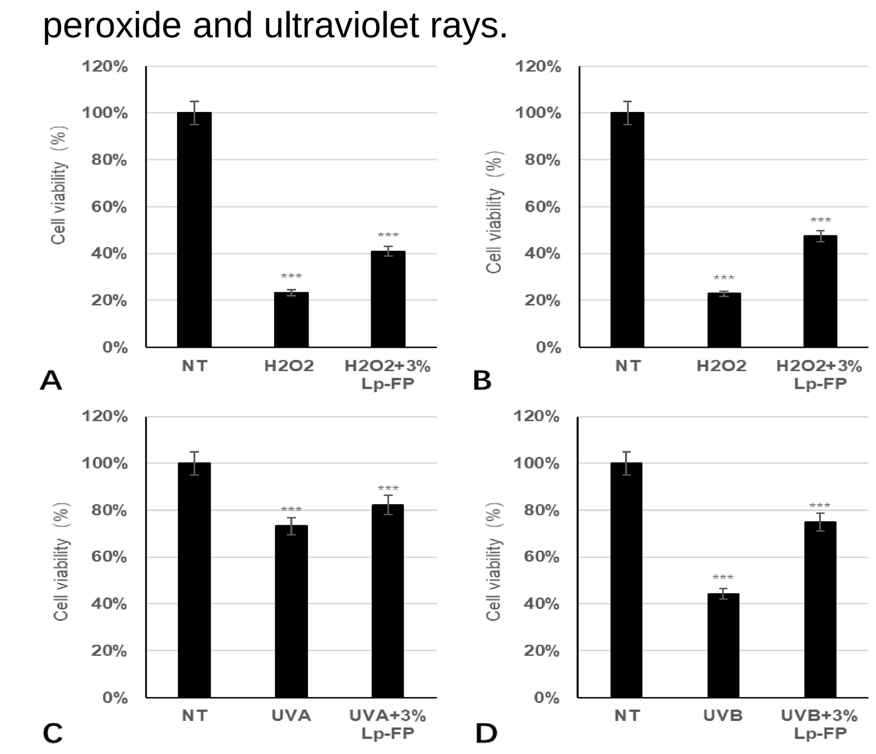


Fig.3 The cell viability after UV irradiation and H2O2 oxidation damage. A/B: Hacat (A) and FB (B) cell viability after H2O2 oxidation damage. C/D: Hacat cell viability after UVA (365nm, 10J/cm2) and UVB (312nm, 40mJ/cm2) irradiation damage. NT= The group without Lp-FP treatment, *** p<0.001.

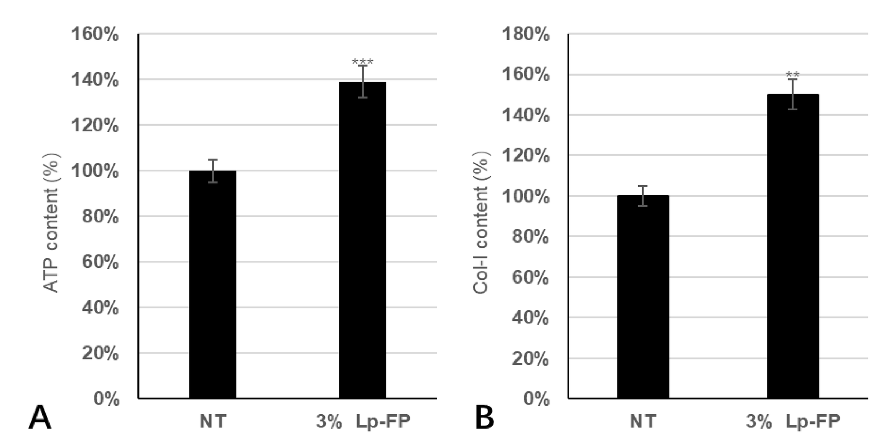


Fig.4 The ATP content in Hacat cells (A) and COl-I secretion in FB cells (B). NT= The group without Lp-FP treatment, ** p<0.01, *** p<0.001.

As shown in Fig.4, Lp-FP increased the ATP content of HaCat cells by 35% compared to the untreated group. And it also increased the Col-I secretion of FB cells by 50%. It shows that Lp-FP can significantly improve skin vitality and anti-aging effects.

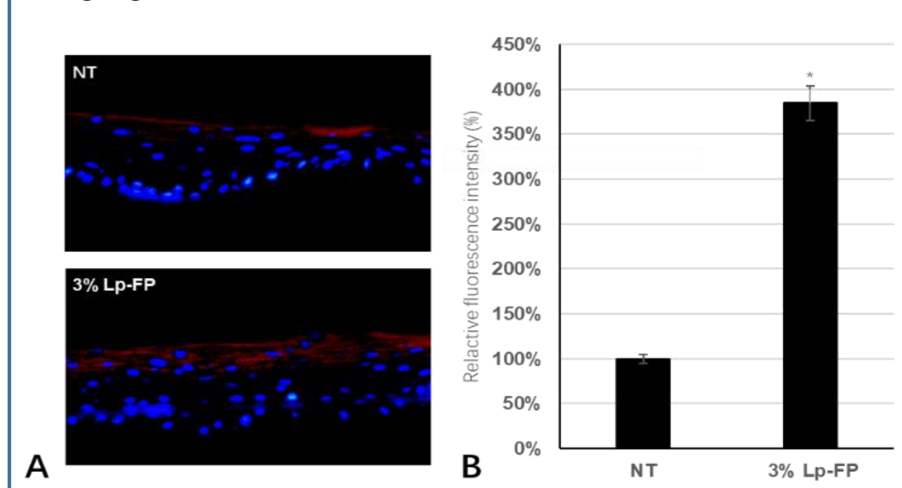


Fig. 5 Immuno-fluorescent staining of Fillaggrin (A) and relative fluorescence intensity of protein Fillaggrin (B). NT= The group without Lp-FP treatment, * p<0.05.

The Lp-FP were applied onto the reconstructed skin, visual histological evaluation from Figure A shows that Fillaggrin is significantly up-regulated. It can be seen from Figure B that Lp-FP can significantly enhance the content of filaggrin. The results of the 3D RHE model further indicate that Lp-FP is beneficial to the skin, strengthens the skin barrier, and maintains the skin stability.

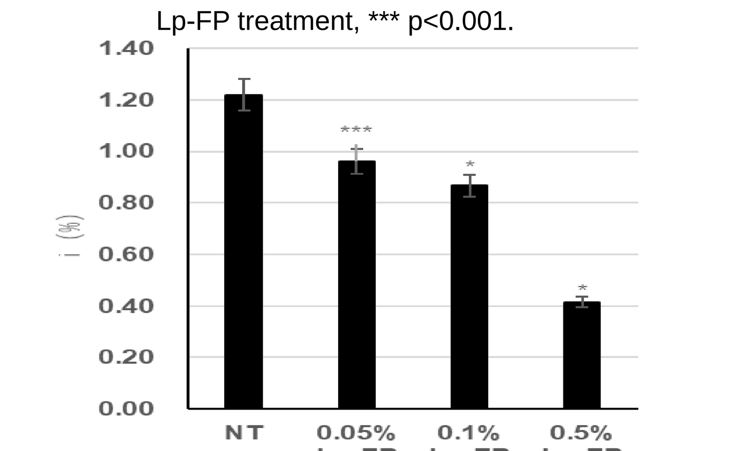


Fig. 6 Influences of Lp-FP on S. aureus and S. epidermidis. NT= The group without Lp-FP treatment, * p<0.05, *** p<0.001.

The effect of Lp-FP on the skin flora on the ratio of *S. a*/*S. e* is shown in Fig.6. This indicates that Lp-FP can significantly reduce the biomass ratio, and has the potential to adjust the ratio of the ratio of *S. a*/*S. e* in the microbial flora on the skin surface to a more healthy state.

Conclusions:

In this study, a series of assays have been conducted to verify the skin care efficacy of Lp-FP. The results show that *the Lactobacillus paracasei* fermentation product has unique advantages in anti-oxidation, skin whitening and other aspects. That can be used as a highly active and safe fermentation material on the cosmetic market, also providing a new possible way for cosmetic research and its market applications. In the future, the active ingredients and its skin care mechanism need to be further studied.

References:

1. Puebla-Barragan S, Reid G (2021) Probiotics in Cosmetic and Personal Care Products: Trends and Challenges. Molecules 26;26(5):1249.
2. Sivamaruthi B S, Kesika P, Chaiyasut C (2018) A review on anti-aging properties of probiotics. International Journal of Applied Pharmaceutics 10(5): 23-27.
3. Ray S, Bagyaraj D J, Thilagar G, et al (2016) Preparation of Chyang, an ethnic fermented beverage of the Himalayas, using different raw cereals. Journal of Ethnic Foods 3(4): 297-299.
4. Thapa S, Tamang J P (2004) Product characterization of kodo ko jaanr: fermented finger millet beverage of the Himalayas. Food Microbiology 21(5): 617-622.
5. Lee, H. Y. (2019) Improvement of skin barrier dysfunction by Scutellaria baicalensis GEOGI extracts through lactic acid fermentation. Journal of cosmetic dermatology, 18(1), 183-191.
6. Martinez, F. A. C., Balciunas, E. M., Salgado, J. M., Gonzalez, J. M. D., Converti, A., & de Souza Oliveira, R. P. (2013) Lactic acid properties, applications and production: a review. Trends in food science & technology, 30(1), 70-83.
7. Tsai, C. C., Chan, C. F., Huang, W. Y., Lin, J. S., Chan, P., Liu, H. Y., & Lin, Y. S. (2013) Applications of Lactobacillus rhamnosus spent culture supernatant in cosmetic antioxidation, whitening and moisture retention applications. Molecules, 18(11), 14161-14171.
8. Kim, N. Y., Kwon, H. S., & Lee, H. Y. (2017) Effect of inhibition on tyrosinase and melanogenesis of Agastache rugosa Kuntze by lactic acid bacteria fermentation. Journal of cosmetic dermatology, 16(3), 407-415.