# Study on the skin anti-aging and barrier repair effects of Himalayan soil yeast culture broth

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## Introduction:

Tibet is rich in biological resources, its ecological environment is diverse, and its geographical environment is complex. The bacterial strain bank has a very high prospect for development and utilization [1]. The yeast strains contain many natural anti-oxidant and anti-aging active ingredients with low toxic and side effects [2]. With multiple functions, they are increasingly used in the field of skin care [3]. Budding yeast Saccharomyces cerevisiae is an important industrial microorganism, which is widely used in food, medicine, light industry, energy and other fields [4]. In this study, soil yeast was isolated and screened from Himalayan soil, and the skin care efficacy of its culture broth was explored.

Laminin 332 plays a vital role in cell adhesion, growth, migration and differentiation [5]. Tyrosinase activity is an important indicator for detecting the whitening effect of skin cells [6]. Senescent cells usually become larger, and the expression of β-galactosidase with high enzymatic activity at pH 6.0 is a potential cause of biological aging [7].

Filaggrin is a key protein required for the formation of the stratum corneum barrier. It maintains skin moisture by binding water and is called natural moisturizing factor (NMF) [8]. Involucrin is an important part of the skin barrier. Skin barrier disruption can change the expression of integument protein [9]. Various major cross-linked proteins, which are regulated by involucrin, bind to the intermediate filament keratin to form cornified envelopes (CE) [10]. Claudin-1, also called tight junction protein, is an important transmembrane protein in tight junctions, and it forms the skin barrier together with keratinocytes [11].

## Materials & Methods:

**Optimal concentration screening**

Normal human skin cells, including dermal fibroblasts and epidermal keratinocytes, were extracted from healthy foreskin biopsies. After serial subcultures, fibroblasts and keratinocytes were cultivated with growth medium supplemented with SC02 culture broth. The MTT method was used to test the cell survival rate of SC02 culture broth with a concentration of 0.5%, 1%, 2%, 5% and the NT group without SC02 culture broth on FB and KC. The DNA and ATP content test of SC02 culture broth on FB refers to the steps of DNA assay kit and ATP assay kit.

**Laminin 332 content, tyrosinase activity of B16 and SA-β-gal positive rate test**

Test the Laminin 332 content of KC without SC02 culture broth and with the optimal concentration of SC02 culture broth. The expression of laminin 332 in keratinocytes monolayer based on cell-based ELISA assay. Read the plates with Multimode Plate Reader. Choose the top-read mode. Excitation filter 485nm/Emission filter 535nm. Test the tyrosinase activity of B16 without SC02 culture broth and with SC02 culture broth at the optimum concentration. Refer to the operation steps of tyrosinase activity kit for details. Test the SA-β-gal positive rate of FB without SC02 culture broth and with SC02 culture broth at the optimum concentration. SA-β-gal test of SC02 culture broth on FB refers to the steps of senescence β-galactosidase staining kit. The percentage of SA-β-gal positive cells were quantified with the Cell Counter plugin of ImageJ 1.52f software.

**Construction of 3D epidermal model and immunohistochemistry**

The P1 generation KC was used to construct the epidermal model. Set the untreated one as the NT group, and set the 2% SC02 culture broth global culture as the sample group. HE staining, Ki67 staining of IHC and Filaggrin, Involucrin and staining of IF were performed respectively. To explore the effect of SC02 culture broth on the 3D skin model. A Scope A1 microscope was used to photograph the staining results for statistical analysis.

## Results & Discussion:

- **Efficacy test of SC02 culture broth on human skin cells**

Different concentrations of SC02 culture broth showed a certain proliferation effect on FB and KC compared with the untreated group, also increase the DNA and ATP content of FB. And promoted the content of laminin in KC, increased the stability of the basement membrane. 2% of the SC02 culture broth can significantly inhibit the tyrosinase activity of B16. 2% of SC02 culture broth significantly reduces the activity of SA-β-gal and delaying cell senescence on FB.

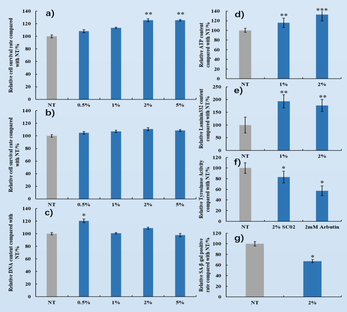

Figure 1 2D efficacy test of SC02 culture broth

- **Efficacy test of SC02 culture broth treatment on 3D recombinant human epidermal model**

The structure of the epidermal model of the 2% SC02 culture broth treatment group (Figure 2b) was more regular, and it has a more complete basal layer and stratum corneum than the control group (Figure 2a), showing that it has a better function of improving the skin barrier.

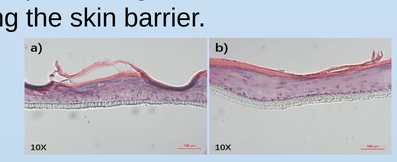

Figure 2 HE staining of 3D recombinant human epidermal model

The results of IHC staining on the epidermal model in Figure 3b show that the basal layer of the epidermal model using SC02 culture broth has obvious expression, and the expression level was significantly higher than that of the control group (Figure 3a), indicating that the epidermal model using SC02 culture broth has stronger proliferation activity.

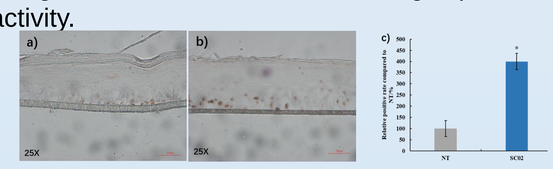

Figure 3 IHC staining of Ki67 protein of 3D recombinant human epidermal model

The expression of filaggrin, involucrin and claudin-1 in the SC02 culture broth group was significantly higher than that in the control group, and the stratum corneum was thicker and firmer. It can be concluded that SC02 culture broth can improve the stratum corneum barrier, making the skin epidermal cell connection structure more complete and the skin tougher.

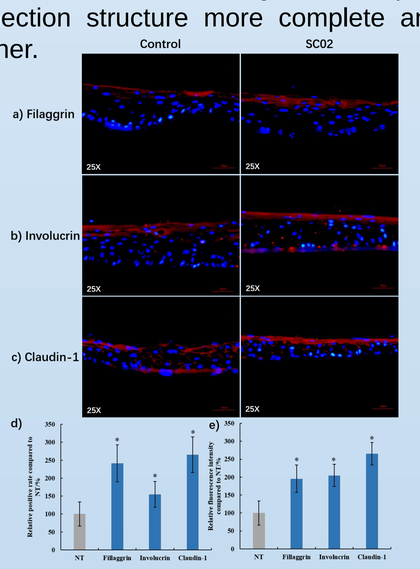

Figure 4 IF staining of Filaggrin, Involucrin and Claudin-1 protein in epidermal model

## Conclusions:

Culture broth of the budding yeast strain SC02 culture broth can promote the cell viability of FB and KC to 125.6% and 110.8%, respectively, at a concentration of 2%. When supplemented at a concentration of 2%, the contents of DNA and ATP of FB are also relatively high. Compared with the untreated group, 2% of SC02 culture broth treated group has lower relative SA-β-gal positive rate (67.4%) and stronger anti-aging ability. The whitening effect was associated with inhibition of tyrosinase activity (83.1%). A significant increase in the relative content of laminin 332 (176.8%) and a certain barrier repair ability were observed in the treated group. From the HE staining results of the epidermal model, it is found that the treated group can thicken the stratum corneum. The relative expression of Ki67 in the epidermal model treated with the treatment was 400%, much higher than that of the untreated group, which could promote the proliferation of basal cells. At the same time, the positive rate and fluorescence intensity of Filaggrin, Involucrin and Claudin-1 of the epidermal model were significantly higher than those of the untreated group, which could strengthen the skin barrier.

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## References:

1.Hu, W., Zhang, Q., Li, D., Cheng, G., Mu, J., Wu, Q., et al. (2014). Diversity and community structure of fungi through a permafrost core profile from the Qinghai-Tibet Plateau of China. J. Basic Microbiol. 54, 1331–1341.
2.Sun P, Wang B W, Jiang X X, et al. Effect of Selenium Yeast on Tissue Selenium Content and Anti-oxidation Function of Fatty-liver Geese[J]. Journal of Shenyang Agricultural University, 2009, 40(5): 575-579.
3.Gioti A, Nystedt B, Li W, et al. Genomic Insights into the Atopic Eczema-Associated Skin Commensal Yeast Malassezia sympodialis [J]. Mbio, 2013, 4(1):00572-12.
4.Cherry J M, Hong E L, Amundsen C, et al. Saccharomyces Genome Database: the genomics resource of budding yeast[J]. Nucleic Acids Research, 2012, 40(Database issue):700-705.
5.Huang, Chen. Laminin332 mediates proliferation, apoptosis, invasion, migration and epithelialtomesenchymal transition in pancreatic ductal adenocarcinoma[J]. Molecular Medicine Reports, 2021. 23(1):1-1.
6.Kim J, Yun E, Yu S, et al. Different Levels of Skin Whitening Activity among 3,6-Anhydro-l-galactose, Agarooligosaccharides, and Neoagarooligosaccharides [J]. Marine Drugs, 2017. 15, 321.
7.Gruber H. Senescence in Cells of the Aging and Degenerating Intervertebral Disc: Immunolocalization of Senescence-Associated β-Galactosidase in Human and Sand Rat Discs [J]. Spine, 2007.
8.Sanja, Kezic, Patrick, et al. Loss-of-Function Mutations in the Filaggrin Gene Lead to Reduced Level of Natural Moisturizing Factor in the Stratum Corneum[J]. Journal of Investigative Dermatology, 2008. 128, 2117–2119.
9.H Törmä, et al. Skin barrier disruption by sodium lauryl sulfate-exposure alters the expressions of involucrin, transglutaminase 1, profilaggrin, and kallikreins during the repair phase in human skin in vivo [J]. Journal of Investigative Dermatology, 2008, 128(5):1212-1219.
10.Theerawatanasirikul S, Suriyaphol G, Thanawongnuwech R, et al. Histologic morphology and involucrin, filaggrin, and keratin expression in normal canine skin from dogs of different breeds and coat types[J]. Journal of Veterinary Science, 2012, 13(2) : 163-170.
11.Robert, Gruber, Christian, et al. Diverse Regulation of Claudin-1 and Claudin-4 in Atopic Dermatitis[J]. The American Journal of Pathology, 2015, 185(10):2777-2789.