

Sequentially fermentation of traditional Chinese medicine by *Pichia kudriavzevii* and bifidobacteria for Skin Care Function

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Abstract

To explore the feasibility of the Skin Care Function of fermentative traditional Chinese medicine, *Pichia kudriavzevii* strains isolated from Strong-flavor baijiu grains were used to ferment food homologous traditional Chinese medicine with the assistance of bifidobacterial. We analyzed 16 *Pichia kudriavzevii* strains and 13 kinds of Chinese herbal medicines in the present study. Firstly, nine Chinese herbal medicines were selected according to the growth of bifidobacteria in the broth of 13 Chinese herbal medicines. The 9 *Pichia kudriavzevii* strains that could promote the growth of bifidobacteria were screened by sequential fermentation. The 9 *Pichia kudriavzevii* strains and bifidobacteria sequentially fermented the above nine kinds of traditional Chinese medicine broth according to different formulas. The broth's residual moisture rate, hydroxyl radical scavenging rate, and tyrosinase inhibition rate were detected to evaluate its skincare function. Better skin care function was found in the sequentially fermented broth of No.3 composed of *Rhodiola*, Raspberry, *Bletilla striata*, *Atractylodes* by YB07, and bifidobacterial, which displayed the moisture residual rate of 46.36%, the hydroxyl radical scavenging rate of 83.95% and the tyrosinase inhibition rate of 60.3%. Proteomic analysis by LC-MS showed that this broth contained 99 active peptides (< 1000 Da) in skin lotion. 24h skin patch and 30-day skin safety test preliminarily revealed the safety of the fermentation broth as a skin care product. This study provides a new perspective on food homologous traditional Chinese medicine for skin care products.

Introduction

With the rapid development of the skin care industry, people have put forward higher requirements for skin care products-natural, efficient, and environmental protection^[1]. Compared with synthetic skin care products, Chinese herbal medicine (CHM) has fewer side effects and higher market acceptance. It has become the mainstream of the cosmetics market^[2, 3]. There are more than 20 kinds of common Chinese herbal medicine raw materials (*Paeonia lactiflora*, *Atractylodes*, *Poria*, Witch hazel, Red pomegranate, *Rhodiola*, Almond, *Paeonia*, Ginseng, Huanglian, Aloe, Peppermint, *Astragalus*, Ginkgo, *Calendula*, Rose, Lingzhi, etc), its skincare principles can be mainly divided into three categories. Firstly, whitening is achieved by inhibiting the production of melanin by inhibiting tyrosinase activity, such as in *Salvia*, Motherwort, Safflower, etc^[4]; secondly, achieves anti-aging by eliminating free cell radicals, such as Ginseng, snow lotus, and *Rhodiola*, etc^[5]; thirdly, improve the skin's moisture absorption and moisturizing ability to achieve moisturizing, such as Yuzhu, *Astragalus*, Baiji, *Atractylodes*, etc^[6].

In addition to Chinese herbal medicine formulations, the processing or extraction process is also an essential factor affecting the skincare effects of CHM^[3, 7]. Fermentation is one of the processing methods of CHM. It mainly promotes the release of active ingredients through microbial enzymatic decomposition, transforms CHM ingredients to increase the content of active ingredients, or decomposes and transforms some ingredients with toxic and side effects.

At the same time, microorganisms can produce small molecular amino acids, polypeptides, nucleotides, vitamins, and other skin care activity substances, which have anti-aging, moisturizing, whitening, and

other effects through fermentation. Some of these particular metabolites also have unique skincare effects. For example, yeast products such as Pitera can promote skin metabolism^[8]. Therefore, microorganisms or their fermentation products have become an essential source of raw materials for natural active skin care products.

Currently, the microorganisms commonly used to produce skin care products are mainly yeast and lactic acid bacteria. Yeast such as *Pichia*, *Saccharomycopsis*, *Kluyveromyces*, *Schizosaccharomyces*, *Candida*, and other yeast species were used for skin care^[9, 10]. In addition, the most widely used lactic acid bacteria is *Bifidobacterium*^[11].

Chinese Strong-flavor baijiu cellar mud and fermented grains have continuously been fermented for many years. They contain many yeast resources with unique metabolisms or products^[12-14]. Among these yeasts, yeasts may produce skin care active substances, which can be used as a screening source for fermenting microorganisms in skin care products. The study found that single bacterial fermentation has the problem of incomplete types of secreted enzymes, and the synergy between the complex microorganisms can effectively avoid degradants' repression and feedback inhibition^[15]. However, there needs to be a report on the collaborative fermentation of traditional Chinese medicine by yeast and *Bifidobacterium* in skin care cosmetics. *Bifidobacteria* are strictly anaerobic bacteria with harsh culture conditions and prolonged growth. The growth of *bifidobacteria* requires the addition of appropriate growth-promoting factors^[16]. Adding common growth-promoting factors such as pig liver extract can cause the fermentation broth to have an unpleasant odor, increasing its market acceptance as a skin care raw material. In a previous study from our lab, yeast metabolites could be used as a liver extract substitute to help *bifidobacterial* growth and improve fermentation broth flavor.

In the present study, we optimized the fermentation of raw materials through yeast screening and CHM formulation based on yeast-*bifidobacteria* synergistic fermentation of CHM. We evaluated the antioxidant, whitening, and moisturizing properties of the fermentation broth, and then the skin care properties of the fermentation broth were improved. Therefore, this fermented formula is expected to develop into a new yeast-plant binary skin care product.

Material And Methods

Chinese Herb, yeast strains, and inoculation cultures

Purslane, Chuanxiong, Astragalus, Ginkgo, Safflower, Atractylodes, Rhodiola, Raspberry, Honeysuckle, *Sophora japonica*, *Bletilla striata*, *Poria* and *Pueraria* were purchased from the local farmers' markets (Yibin City, Sichuan Province, China). Yeasts (*Pichia kudriavzevii*) were isolated from a famous Strong-flavor baijiu enterprise cellar. The *Bifidobacterium* capsule was purchased from the Lizhu pharmaceutical factory (Zhuhai, Guangdong, China). The strains were maintained at 4°C on YPD agar (containing 20g/L peptone, 20g/L glucose monohydrate, and 10 g/L yeast extract, which regulates the pH to 5.0 using lactic acid). All other reagents were of analytical grade.

The screen of fermentation materials

Preparation of CHM broth and bean sprout juice

100 g of Chinese herbal medicines or bean sprouts were soaked in 1000 g of distilled water for 30 min, decocted for 30 min, then filtered and maintained at 4°C.

The growth evaluation of bifidobacterium in CHM

The activated bifidobacteria were inoculated into the 200 mL broth mixed with 180mL CHM broth, 2 g sucrose, and 20 mL bean-sprout juice and cultured at 37°C for 48 hours. The fermentation broth was diluted 10 times, and the OD_{600nm} was detected by UV-Spectrophotometer (Unico UV-2600A) with the blank of non-inoculated fermentation broth.

The growth evaluation of *Pichia kudriavzevii* strains in CHM

Adding sterile glucose solution (20 g/L) and CHM extract to the test tube controlled the volume (mL) ratio of the fermentation broth of 9:1. The activated yeast strains were inoculated into the fermentation broth and cultured in a 28°C incubator for 2 days. A control test (without activated yeast) was set in the experiment. The fermentation broth was diluted three times, and the OD value was detected at a wavelength of 600 nm by UV-Spectrophotometer (Unico UV-2600A). The well-grown yeast was selected as the follow-up experimental object according to the measured OD value.

Sequentially fermentation in CHM by *Pichia kudriavzevii* strains and Bifidobacterium

The strains screened above were mixed and cultured with their respective suitable CHM fermentation broths, cultured at 28°C, 40 rpm for 2 days, and then centrifuged at 3000 rpm for 5 min. The supernatant was removed and inoculated with bifidobacteria, then cultured at 37°C for 48 hours. The fermentation broth was diluted ten times, and the OD_{600nm} was detected by a UV-Spectrophotometer (Unico UV-2600A). The score of CHM was evaluated by measuring the moisturizing function (40%), antioxidant capacity (30%), and tyrosinase inhibition rate (30%) of the fermentation broth shown in Supplementary Table 1.

The moisture retention of fermentation broth was detected according to the light industry standard of the People's Republic of China QB/T 4256-2011 "Guideline for the evaluation of cosmetics moisturizing efficacy". Scavenging against superoxide anion radicals was used by Chang-Chai Ng and Chuang-Yi Wang^[17]. Sample pretreatment: The fermentation broth was centrifuged at 4800 r/min for 20 min, and the supernatant was taken. And then, 1 mL of the supernatant was added with 1 mL H₂O₂ (8.8 mmol/L), 1 mL FeSO₄ solution (0.9 mmol/L), and 1.0 mL salicylic acid (9 mmol/L). After 60 min reaction under 37°C, the absorbance of each sample was measured at 510 nm, and the hydroxyl radical scavenging capacity was calculated based on the absorbance of a control sample as a standard. Tyrosinase inhibitory activity was determined using the slightly modified method described by Hye-Young Shin and Sung-Min Kim^[18]. Sample pretreatment: The fermentation broth was centrifuged at 4800 r/min for 20

min, and the supernatant was taken. Phosphate buffer (2 mL, 0.1 M, pH 6.8), sample extract (1 mL), and tyrosinase solution (1,500 U/mL in phosphate buffer) were mixed and incubated at 37°C for 10 min. Then, 0.5 mL of 5 mM L-DOPA was added to the mixture and incubated at 37°C for 10 min. The absorbance of each sample was measured at 475 nm, and the inhibitory activity was calculated based on the absorbance of a control sample.

Evaluation of Safety and Skin Care Effect

The safety of fermentation broth was evaluated on the skin. The fermentation broth was centrifuged at 4800 r/min for 20 min, and the supernatant was filtered with 0.22µm membrane for determination. The examination method was referred to China State Food and Drug Administration GGTG-2015-13053 "Safety and Technical Standards for Cosmetics-Human Skin Patch Test". 30 volunteers aged 18-60 who met the experimental requirements were selected as the subjects, and the human skin patch test was carried out using the repetitive open smear test. The flexed side of the forearm was used as the test site, with an area of 3×3 cm². Moreover, the test site should be kept dry and avoid contact with other external preparations. Apply about 0.050±0.005 g(mL)/time of the test substance to the test site evenly, twice a day, for 7 consecutive days, and observe the skin reaction simultaneously. The skin reaction was observed according to the skin reaction evaluation criteria of the repetitive open smear test in Table 1, and the results were recorded. A skin smear test evaluated the application's effect on improving adverse skin conditions. These volunteers aged 20-30 with skin discomforts such as sensitivity, blemish-prone, oil-prone, or dry were selected and randomly distributed 30 lotions and 30 controls (distilled water with 0.01% caramel coloring added). Observations and experiments were carried out regarding the human skin patch test.

Proteomics analysis

The fermentation broth was centrifuged at 4800 r/min for 20 min, and the supernatant was filtered with 0.22µm membrane for determination. The mixture of peptides of sample in 0.1% formic acid was analyzed using LC/MS (Thermo Scientific), and the examination method was referred to Renata M. Santos and Fabio C.S (Santos R et al., 2017). It was loaded in Accucore C18 (2.1×150 mm, 1.5 mm). The gradient used was 98% of solvent A (98% H₂O, 2% ACN, 0.1% formic acid) until 32% of solvent B (98% H₂O, 2% ACN, 0.1% formic acid) for 75 min; 32% to 90% of solvent B for 5 min; and 90% of solvent B for 5 minutes). The full scan and MS/MS acquisition was made in a positive mode applying a Data Dependent Acquisition. The operating parameters of the mass spectrometer were: spray voltage at 3.8 kV, 45 arbitrary units of sheath gas flow rate and 10 arbitrary units of aux gas flow rate and 275°C in the heated capillary.

Statistical analysis

Experiment results in this study were reported as means ± standard deviation of three replicates. Analysis of variance and significant difference tests were performed using SPSS (version 20.0). Mean differences

were considered significant when $P < 0.05$ and extremely significant $P < 0.01$. Analysis of heatmap were performed using GraphPad Prism 9.1.2.

Results

Screen of CHM for Bifidobacterium

Extracts of Bifidobacterium and many CHM have been used widely in skin care products. Firstly we selected food homologous Chinese herbal medicines that could ensure the growth of bifidobacterial by detecting the OD600nm increase in 13 kinds of CHM fermentation broth [20]. It found that bifidobacterial grow significantly differently in 13 kinds of CHM broth, and the CX showed the best effect on promoting the growth of bifidobacterial, then were the FP, and the OD600nm of the broth in which bifidobacterial increased to more than 0.5 in 9 kinds of CHM containing the HQ, the JYH, the BJ, the HJT, the FPZ, the HH, the Hh, the CX and the BZ. Subsequently, these nine kinds of CHM were used to screen the yeast strains that could grow in them.

The growth of yeast strains in nine kind of CHM

The growth of 16 *Pichia kudriavzevii* strains in 9 kinds of CHM broth was evaluated according to the OD560nm of the broth, as reported previously^[21]. The results (see Fig.2) showed that these yeast strains were more suitable for specific traditional Chinese medicine. the CX, BJ, HJT, Hh, FPZ, HQ, JYH, HH, and BZ support the growth of 6, 10, 9, 10, 9, 12, 7, 10, 11, and 15 yeast strains well respectively. Most of these yeast strains could grow well in the broth of Raspberry, safflower, and *Rhodiola rosea*, and the adaptability of yeast to CHM showed the difference in strain level, such as YB01 could grow in nine kinds of CHM, and the FPZ and the Hh showed good growth (OD600 nm ≥ 0.8) of the broth. Subsequently, the CHM which support 9 yeast strains well were mixed in different formula to culture these strains to produce skin care products.

Combination of Chinese herbal medicine for the fermentation of yeast

Compound CHM could possess the probable advantages of enhanced efficacy and reduced toxicity, expanding their safety and efficacy^[22]. CHM that support the growth of some yeast strains shown in Fig 2 were combined to culture this strain based on the deletion method, and the skin-care activity of this different fermented broth was evaluated depending on the moisture retention, tyrosinase inhibition rate, and hydroxyl radical scavenging ability. The specific results are shown in Supplementary Table 1. The results showed that the yeast strains grew well in the combined broth of different CHM, and 15 groups of CHM were prepared for 9 yeast strains that showed good skin care efficacy (Table 2). For example, the highest moisturizing rate was 89.33% (No.14), the highest tyrosine clearance of 93.67% (No.15) and the best hydroxyl radical scavenging was 85.68% (No.5). Subsequently, the corresponding bifidobacterium was added for further skincare activity evaluation.

Mixed fermentation of yeast and bifidobacterium in compound CHM

Subsequent cultures of bifidobacterial after yeasts could improve the survival of bifidobacteria during fermentation^[23, 24]. Chinese herbal medicines that contain complex chemical compounds not only possess the probable advantages of enhanced efficacy but also have improved complexity and influence on microbial growth^[22].

The OD_{600nm} value at the end of fermentation could reflect the growth of bifidobacterium in the fermentation broth. The growth of different yeast strains and bifidobacteria in different fermentation broths is shown in Table 1. The results showed that yeast strains grew differently in 15 compound CHM, the growth of No.1-YB14 and No.5-YB12 was inhibited, and the remaining 8 kinds of yeasts all had their suitable compound CHM. Four yeast strains exist in fermentation formulas that were suitable for the growth of subsequent culture, namely No.3 YB07, No.7 YB15, No.8 YB15, No.12 YB11, and No. 14 YB11, No.15 YB16. 7 compound CHM that is fermented by specific yeast strains could promote the growth of bifidobacterial, this may be due to the growth factors produced by the corresponding yeast strains and benefit the bifidobacteria, while the secondary metabolic of yeast are usually unstable^[25] and may and be affected by the formula of CHM, so are the growth factors produced by yeast.

Moisture retention of fermentation broth

The residual water content of subsequent mixed fermentation broth by bifidobacterium and yeast in different compound CHM is shown in Fig. 4. The moisturizing performance of all CHM was improved after fermentation by yeast and bifidobacterial. This may be related to the fermentation metabolites of bifidobacterials such as equol that an improvement of skin moisturizing^[26]. Among them, No. 8 has the significantly highest moisture residual rate (49.93%±0.035), and the moisturizing effect was the best. The three groups of formulas of yeast strain YB07 and bifidobacterial had low moisturizing properties, which were 42.85% (No. 2), 46.36% (No. 3), and 47.37% (No. 4), respectively. This may be related to the raspberry which reduces the moisturizing performance of fermentation broth^[27], and the raspberry may inhibit the growth of microbes by producing polyphenols with antibacterial activity^[28], this further weakens the moisturizing properties of the fermentation broth due to the metabolites or cellular substance of microbes.

Scavenging against superoxide anion radical

The IIS pathway is the first confirmed pathway to regulate aging^[29], Some of the antiaging effects of traditional Chinese medicine are involved in this pathway^[30], so hydroxyl radical scavenging of fermentation could reflect the antioxidant capacity of fermentation broth. Fig. 5 showed that only 5 of the 15 compound CHM showed certain hydroxyl radical scavenging ability, while the hydroxyl radical scavenging ability of subsequent fermentation by bifidobacterium after yeast in 8 kinds of compound CHM increased by 50%-95.8% of the unfermented blank, especially the hydroxyl radical clearance rate of No. 9 compound CHM increased the most to the 95.82% of the blank dramatically, only the hydroxyl radical clearance rate of No. 15 decreased, it indicated that the growth of yeast strains and bifidobacterium improved the hydroxyl radical clearance rate of fermented broth. It may be due to the

polysaccharides from yeast, bifidobacterium, and medicinal plants that are reported to possess antioxidant activities^[31, 32].

Antioxidant activity analysis

The tyrosinase inhibition rate at the end of fermentation could reflect the whitening ability in fermentation broth, the products of bifidobacteria decrease the melanogenesis process of melanoma cells by inhibiting tyrosinase activity, which may improve whitening ability^[33], and the growth of bifidobacteria can also affect the production of tyrosinase to varying degrees^[11]. Fig. 6 showed that the tyrosinase inhibition rate of 15 kinds of fermentation broth varied from 0% to 36.56% according to the compound CHM, the compound CHM of No. 7 and No. 8 showed higher tyrosinase inhibition rate, and it changed after the fermentation by yeast and bifidobacterial. The tyrosinase inhibition rate increased to 60.30%, 44.47%, 56.8%, and 71.07% in the fermentation broth by the strains of YB07 (No.3), YB15 (No. 7), YB11 (No. 14) and YB16 (No.15) respectively, especially in the broth of the No. 15, the inoculation of yeast strain YB16 and bifidobacteria improved the tyrosinase inhibition rate by 67.29% when compared with the blank, while it decreased to 0.27%, 5.27%, 20.14% and 0% in the fermentation broth by the strains of YB14 (No. 1), YB07 (No. 2), YB07 (No. 4) and YB15 (No. 8) respectively, indicating that the tyrosinase has been activated owing to the fermentation of yeast and bifidobacteria.

In summary, the *Rhodiola*, Raspberry, *Bletilla striata*, and *Atractylodes* were suitable for the strain of YB07(No.3), *Bletilla striata*, Safflower, *Sophora japonica* and Chuanxiong were suitable for the strain of YB05(No.7), and Honeysuckle, raspberry, *Sophora japonica*, *Atractylodes macrocephala* and *Bletilla striata* were suitable for the strain of YB11(No.14), respectively.

Preliminary evaluation of Safety and Skin care effect

24h skin patch test and 30-day skin safety test were carried out for the broth of No. 3 (YB07, chuanxiong, safflower, *Bletilla striata*, astragalus, raspberry and *atractylodes*), No. 7 (YB15, *Bletilla striata*, Safflower, *Sophora japonica* and Chuanxiong), and No. 14 (YB11, Honeysuckle, raspberry, *Sophora japonica*, *Atractylodes macrocephala* and *Bletilla striata*) and the results were shown in Table 3, the fermentation broth has obvious skin soothing effect compared with the control group ($P < 0.05$), 83% of people gave positive evaluation to the broth of No. 3 in the aspect of skin care effect and no discomfort reaction was reported.

Proteomics analysis

Many multifunctional bioactive peptides can simultaneously affect beneficially multiple physiological pathways in the skin^[34], especially those with molecular weight between 500-1000Da obtained more attention for they can overcome the skin barrier^[35] and have better skin care effects. A total of 99 active peptides with molecular weight between 500-1000 Da were detected in the broth of No.3, including 52 from *Pichia* and 49 from *bifidobacterium* shown in Supplementary Table. The 10 important peptides with a relative abundance of over 1% are shown in Table 4. For example, UDP-glucose 4-epimerase could

induce the conformational change of gale, activate the reduction of NAD^+ , produce NADH, and promote cell productivity^[36]. α -L-arabinofuranosidase is the main glycosidase (a lignocellulose-degrading enzyme) that causes the degradation of arabinose residues in cell wall polysaccharides, which can improve the efficiency of fermenting sugars in plant biomass^[37]. Peroxisome membrane proteins are located on the peroxisome monolayer membrane, contain antioxidant systems and oxidative systems, and affect the oxidation-antioxidant properties of cells. Mannose-1-phosphate guanosyltransferase is a key enzyme in mannose synthesis, and the increase in mannose content helps to enhance the antioxidant activity of polysaccharides^[38]. Mitogen-activated protein kinase (MAPK) signaling pathway is an important pathway in the inflammatory transduction network^[39]. The chorismate enzyme is involved in the shikimic acid pathway, which can convert shikimic acid into chorismic acid, which in turn inhibits the conversion into tyrosine, and has a whitening effect^[40].

Discussion

The growth conditions of bifidobacteria are complex and require certain growth factors^[16]. In contrast, the pig liver extract that usually supplies growth-promoting factors may cause an unpleasant odor to the fermentation broth, reducing its market acceptance as a skin care raw material. Although *Bifidobacterium* can hardly survive in CHM broth, In our study, sequential fermentation of *Bifidobacterium* after *Pichia kudriavzevii* was proved feasible. This may be due to the metabolites of *Pichia kudriavzevii* yeasts that promote bifidobacterial growth. Or the conversion of the inhibitory substances from CHM by *Pichia kudriavzevii* strains.

Based on the skincare activity of CHM, fermentation of CHM not only promote the release of active ingredients through microbial enzymatic decomposition^[7], such as lactic acid^[41], bioactive peptides, which possess the status of Generally Recognized as Safe (GRAS) food substance^[34]. It also forms abundant cell lysates of yeast and bifidobacteria that proved to have skincare effects. This reminds the simultaneous utilization of plant and active microbial substances in studying full-effect skin care products. Considering the almost infinite combination of microorganisms and CHM, several prospective skin care products could emerge, while there is still a long way to go before that, for example, in the research on the action mechanism of such skin care products

Declarations

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Tables

Table 1 Skin Reaction Evaluation Criteria for Repeated Open Smear Test on Skin

degree of response	Rating level	Clinical manifestation of skin reaction
-	0	Negative reaction
±	1	Weak erythema, dry skin, wrinkled skin
+	2	Erythema, edema, papule, wind mass, scaling and fissure
++	3	Obvious erythema, edema and blister
+++	4	Severe erythema, edema, bullae, erosions, hyperpigmentation or hypopigmentation, acneiform changes

Table 2 Fermentation formula with remarkable skin care function

Groups	Fermentation Recipe Bacteria, TCM	Residual moisture %	Tyrosinase inhibition %	Hydroxyl radical scavenging %
No.1	YB14 HJT, Hh, BZ, CX	73.06±1.32a	84.75±0.39d	69.87±0.11a
No.2	YB07 HJT, FPZ, JYH, BJ, BZ	84.99±1.28e	44.68±0.57a	124.80±0.46a
No.3	YB07 HJT, FPZ, BJ, BZ	87.38±0.12b	51.50±0.14b	92.17±0.78a
No.4	YB07 HJT, FPZ, JYH, BJ	82.29±1.13a	63.33±0.13a	82.66±0.01a
No.5	YB12 HJT, FPZ, BZ, HH	87.42±0.15fg	106.33±0.82f	95.68±0.45a
No.6	YB15 BJ, Hh, HH, FPZ, CX	81.24±0.75d	65.33±0.1c	77.97±0.08a
No.7	YB15 BJ, Hh, HH, CX	78.93±0.23c	143.67±0.16h	38.44±0.20a
No.8	YB15 BJ, Hh, PFZ, CX	88.65±0.38gh	160.00±9.01i	35.21±0.27a
No.9	YB10 HJT, JYH, BZ, CX	88.82±0.36gh	138.00±0.17g	82.29±0.07a
No.10	YB03 CX, BJ, JYH, HH, BZ	82.24±1.34d	274.33±0.11k	59.40±0.06a
No.11	YB13 BJ, Hh, FPZ,BZ	87.06±0.13f	138.00±0.02g	80.56±0.32a
No.12	YB11 Hh, FPZ, JYH, BZ, BJ	88.20±0.27fgh	89.00±0.21de	254.64±0.54a
No.13	YB11 Hh, FPZ, HH, BZ, BJ	88.08±0.64fgh	171.33±0.1j	223.97±0.26b
No.14	YB11 JYH, FPZ, HH, BZ, BJ	89.33±0.07h	275.33±0.47k	204.32±0.09a
No.15	YB16 BJ, Hh, FPZ, JYH	85.33±0.79e	93.67±0.71e	56.37±0.33a

Table 3 The experimental results of fermentation liquid improving the basic condition of skin

Sample	Improve skin condition now (the number of people)	No effect (the number of people)	Discomfort (the number of people)	Not sure if skin condition improved (the number of people)
experience group No. 3	25	1	0	4
experience group No. 7	20	3	0	2
experience group No. 14	18	4	0	3
control group	3	18	0	10

Table 4 The most important of 10 peptides in fermentation broth of No.3

Retention time	Accessions	Marked as	Description
2.40	A3LU10	Pichia	DNA mismatch repair protein MSH3
3.37	A3LR68	Pichia	Autophagy protein 5
18.52	E8MF10	Bifidobacterium	UDP-glucose 4-epimerase
21.29	Q841V6	Bifidobacterium	Intracellular exo- α -(1 $\rightarrow$ 5)-L-arabinofuranosidase
22.12	Q9HG09	Pichia	Peroxisomal membrane protein PEX14
25.86	Q9P8N0	Pichia	Mannose-1-phosphate guanylttransferase
26.15	B7GQH1	Bifidobacterium	Protein translocase subunit SecA
28.85	Q8G3I2	Bifidobacterium	Isoleucine-tRNA ligase
29.04	A3LN91	Pichia	Mitogen-activated protein kinase HOG1
46.29	B7GS97	Bifidobacterium	Chorismate synthase

Figures

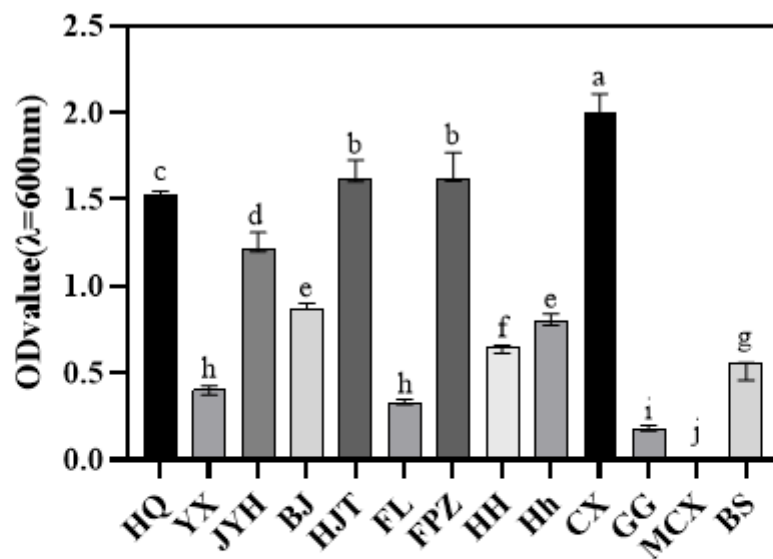


Fig.1 Effects of Different Traditional Chinese Medicines on the Growth of Bifidobacterium

HQ: Astragalus, YX: Ginkgo, JYH: Honeysuckle, BJ: Bletilla striata, HJT: Rhodiola, FL: Poria, FPZ: Raspberry, HH: Sophora japonica, Hh: Safflower, CX: Chuanxiong, GG: Pueraria, MCX: Purslane, BZ: Atractylodes. The same is below.

Figure 1

See image above for figure legend.

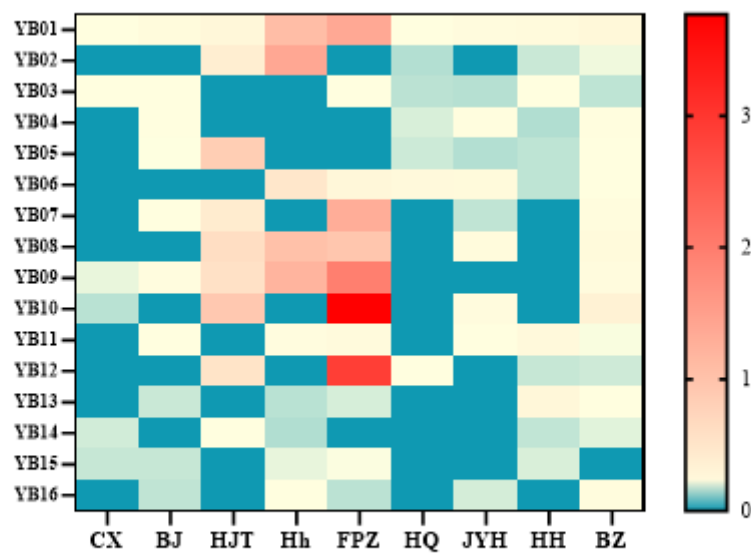


Fig.2 The growth of yeast in 9 kinds of CHM

Figure 2

See image above for figure legend.

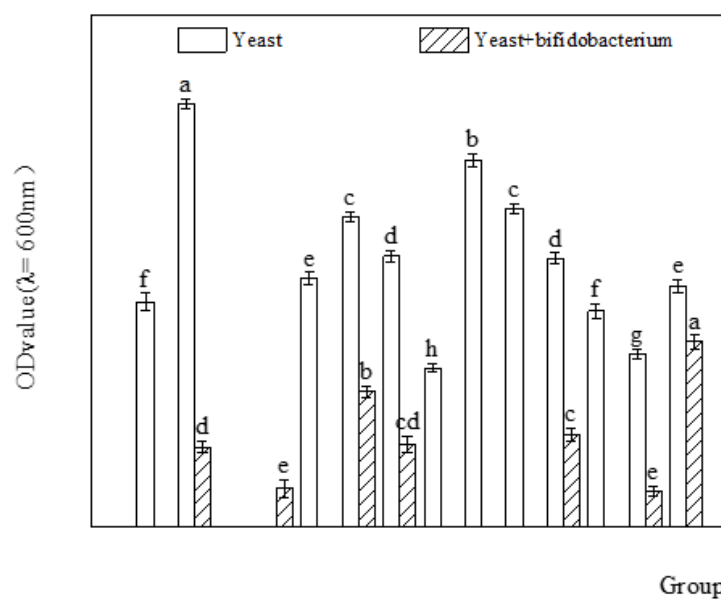


Fig. 3 Effects of different compound CHM on growth of yeast and Bifidobacterium

Figure 3

See image above for figure legend.

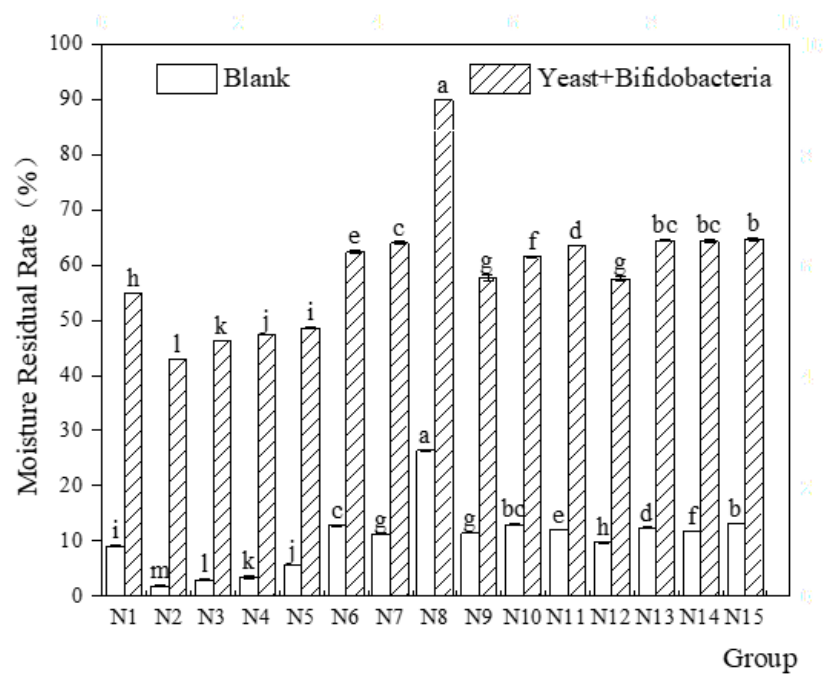


Fig. 4 Influence of different fermentation formulas on the residual water content of fermentation broth

Figure 4

See image above for figure legend.

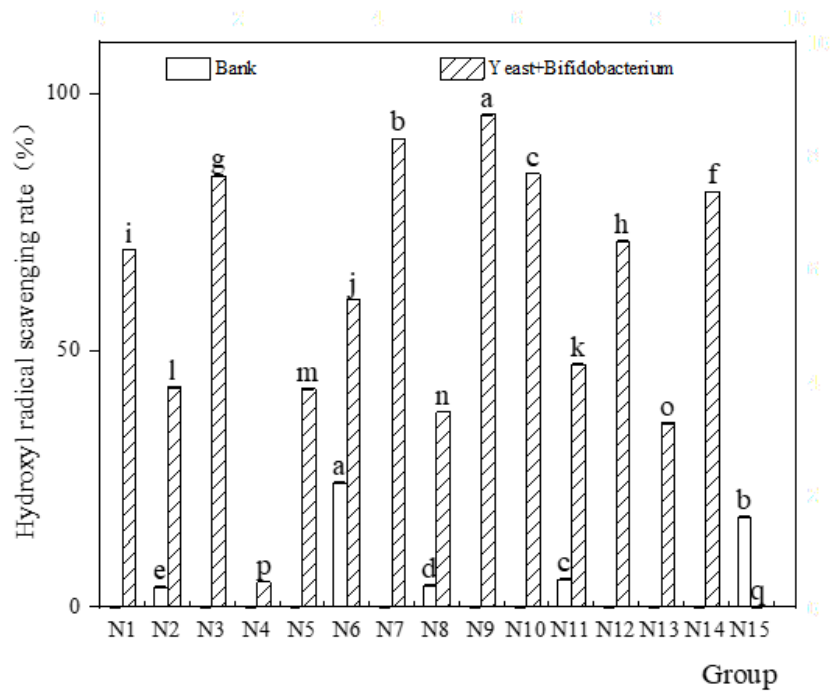


Fig.5 Effects of different fermentation formulas on hydroxyl radical scavenging

Figure 5

See image above for figure legend.

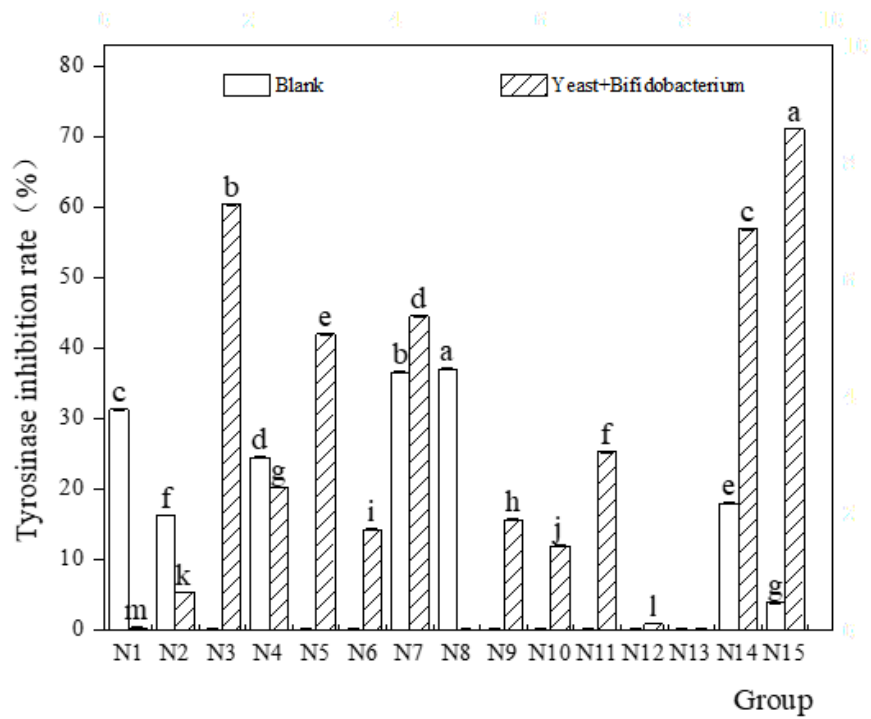


Fig. 6 Effects of different fermentation formulas on tyrosinase inhibition rate

Figure 6

See image above for figure legend.

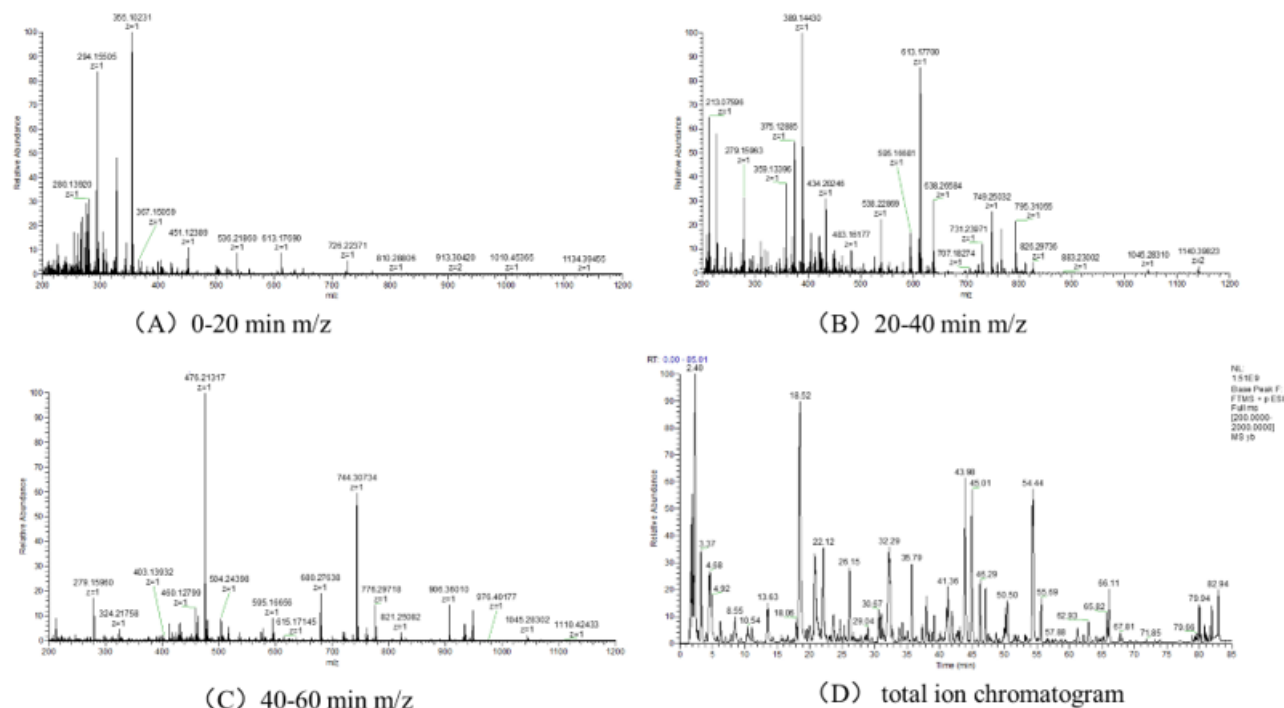


Fig. 7 Total ion chromatogram of fermentation liquid

Figure 7

See image above for figure legend.

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