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Culture Conditions Optimization of Tibetan Kefir Grains by Response Surface Methodology

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Abstract

Tibetan kefir grains are naturally-existing yogurt starter and have some unique benefits. In this study, response surface analysis was used to optimize their biomass production. Results showed that optimal culture conditions were skim milk concentration, 41.6%; temperature 30.05℃; inoculation amount, 1.86%; time, 20h and shaker rotating speed, 0r/min, at which the growth rate was 14.33%, 39.4% more than initial. In addition skim milk concentration, temperature and inoculation amount were proved to be significant factors on biomass production.

Keywords: Tibetan kefir grains; response surface analysis; biomass; growth rate

1. Introduction

Kefir grains are a kind of yogurt starter which are white to yellow-white, gelatinous and variable in size (Fig. 1) and consist of a complex microbial symbiotic mixture of lactic acid bacteria, yeasts and few acetic acid bacteria which stick to a polysaccharide-protein matrix [1]. After successive fermentations, they can break up to new generation grains which have the same characteristics as old ones. Mainly there are two origins of them, one is Caucasus [2] and the other is Tibet [3].

Fig. 1. Tibetan kefir grain

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Recently, the increased interest in kefir grains has developed because their unique fermentation performance [4], the kefiran they produced [5] and various of biological activities such as anti-tumor [6], anti-microbiology [7] and immune modulation [8]. Some of the studies were to improve their production. Guzel-Seydim, et al [9] investigated the effect of different growth conditions on biomass increase in kefir grains and found that the highest biomass increase (392%) was found after a week. Gorsek and Tramsek [10] established a continuous two-stage bioreactor system for kefir grains production. Bekatorou, et al [11] found that either fructose or glucose and sucrose could give largest biomass production in a 24h experiment. In this study, we investigated the biomass of Tibetan kefir grains cultured in different conditions by response surface analysis methodology (RSM).

2. Material and Methods

2.1. Cultivation of Tibetan kefir grains

The samples of Tibetan kefir grains were obtained from private households in Lhasa. The grains were preserved in sterilized milk (5%, w/v) from Mengniu Dairy at 4℃, and activated at 28 ℃, for 24 h, 3 times [12]. The biomass of the grains was measured every 24 h after washed by sterile water and blotted by filter paper. Biomass growth rate was got according to the formula below, which was used to evaluate effects of culture conditions. All results were carried out 3 times for means.

$$v = \frac{X_{n+1} - X_n}{X_n} \times 100\% \quad (1)$$

v, growth rate; Xn, biomass weight after n days (g); Xn+1, biomass weight after (n+1) days (g)

2.2. Single factor experiments

Single factor changed separately when other variables unchanged and Tibetan kefir grains were cultured at different changed conditions to evaluate the effects. Initial culture conditions [5] were used as control: 20% (w/v) of skim milk, 5% (w/v) of inoculation amount, 0 r/min of shaker rotating speed, cultured at 28℃ for 24h. Facilitation of different culture conditions was evaluated and they were skim milk concentration (10-50%), inoculation amount (1-5%), shaker rotating speed (0-200r/min), temperature (25-37℃), time (8-24h).

2.3. Response surface analysis methodology (RSM)

Fractional Factorial Design (FFD) and Central Composite Design (CCD) were used for RSM [13] according to the results of single factor experiments by Design-Expert software v. 6.0 (Stat-Ease, Inc.). In FFD, 3 levels and 5 factors were chose as showed in Table 1. CCD was done according to FFD experiments and ANOVA. Levels and factors chose in CCD were showed in Table 2. When the optimal level was got, additional test was done to verify the results.

Table 1. Range of values for FFD

Levels	Factor 1 (*%*): Skim milk concentration	Factor 2 (*℃*): Temperature	Factor 3 (*%*): Inoculation amount	Factor 4 (*h*): Time	Factor 5 (*r/min*): Shaker rotating speed
-1	30	28	1	16	50
0	40	30	2	20	100
1	50	32	3	24	150

Table 2. Range of values for CCD

Levels	Factor A (%): Skim milk concentration	Factor B (℃): Temperature	Factor C (%): Inoculation amount
-1.68	23.2	21.6	0.32
-1	30	25	1
0	40	30	2
1	50	35	3
1.68	56.8	38.4	3.68

3. Results and Discussion

3.1. Single factor experiments

Growth rate was 10.28%, when Tibetan kefir grains were cultured at initial conditions. As milk concentration went up, the growth rate increased as showed in Fig.2-A, but when it was 50%, the growth rate dropped. Because when milk concentration was too high, lactic acid bacteria, yeasts and acetic acid bacteria would change lactose to lactic acid rapidly and pH value went down sharply, which would block the biomass production, in like manner, temperature (Fig.2-B) and inoculation amount (Fig.2-C) were. Fig.2-D showed that when cultured for 20h or 24h, growth rates were similar, so 20h was chose. In shaker rotating speed test (Fig.2-E), growth rate was changeless at whichever speed, so we deduced that there was balance between facultative anaerobic bacteria and aerobic yeasts and their competition for oxygen was not intense. According to results above, the level of other factors, which gave the highest growth rate was chose as the center level (Table 1).

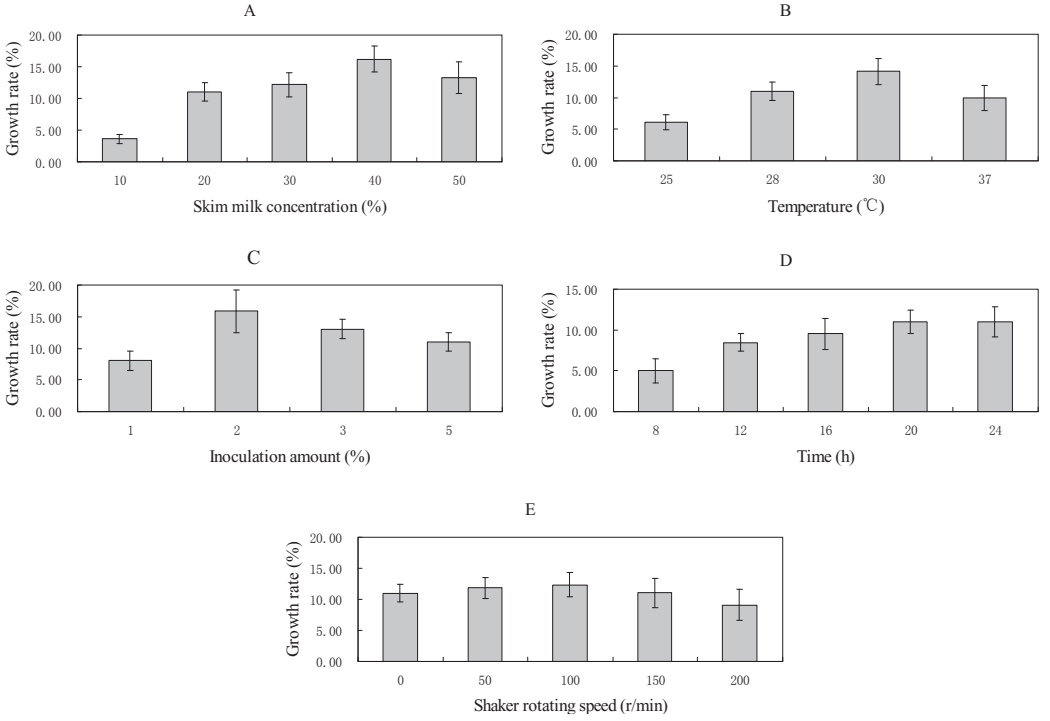

Fig. 2. Optimization of culture conditions

(A) Skim milk concentration; (B) Temperature; (C) Inoculation amount; (D) Time; (E) Shaker rotating speed

3.2. Response surface analysis methodology (RSM)

20 tests were given by Design-Expert software for FFD, and the responses (growth rate) were got as showed in Table 3. ANOVA showed that R^2 was 0.983 and curvature was significant (0.012), which indicated that optimization point was in tests. Factor 1 (skim milk concentration), factor 2 (temperature) and factor 3 (inoculation amount) had significant influence on responses ($P<0.05$), so they were used in CCD (Table 2) while factor 4 and factor 5 were kept at the optimal level as showed in Table 1. ANOVA of CCD showed that model terms were significant ($P<0.05$) and lack of fit was not significant (P=0.2162). Final equation in terms of coded factors was got (2), which gave Fig. 3.

$$Response1 = 13.77+0.37A+0.088B-0.45C-1.17A2-0.97B2-1.60C2+0.071AB+0.036AC+0.58BC \quad (2)$$

Solution was given in this model and it was A, 0.16, B, 0.01 and C, -0.14, at which, expected response could reach 13.83%. Additional test was done to verify expected response. As factor 4 and factor 5 were not significant, they were set at 20h and 0r/min separately for cost cutting. The growth rate of 14.33% was got at these conditions (skim milk concentration, 41.6%; temperature 30.05℃; inoculation amount, 1.86%; time, 20h and shaker rotating speed, 0r/min). Compared with initial growth rate (10.28%), it had been raised by 39.4%. In keeping with results of single factor experiment, both skim milk concentration and temperature should not be too high, while effects of time and shaker rotating speed were not significant on growth rate.

Table 3. Results of FFD

Runs	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Y/ Growth rate (%)
1	1	-1	1	-1	1	12.69
2	-1	1	1	1	-1	15.09
3	1	-1	1	-1	-1	11.29
4	1	1	1	1	1	12.55
5	0	0	0	0	0	12.26
6	0	0	0	0	0	13.56
7	1	1	-1	-1	-1	10.99
8	-1	1	-1	1	1	9.80
9	1	1	-1	1	-1	10.35
10	-1	-1	-1	1	-1	6.11
11	-1	1	1	-1	1	11.48
12	0	0	0	0	0	13.00
13	1	-1	1	1	-1	9.17
14	1	-1	-1	-1	-1	13.35
15	0	0	0	0	0	12.36
16	1	1	-1	-1	1	10.95
17	1	1	1	-1	-1	11.51
18	-1	-1	-1	-1	1	8.34
19	-1	-1	1	1	1	10.49
20	1	-1	-1	1	1	10.93

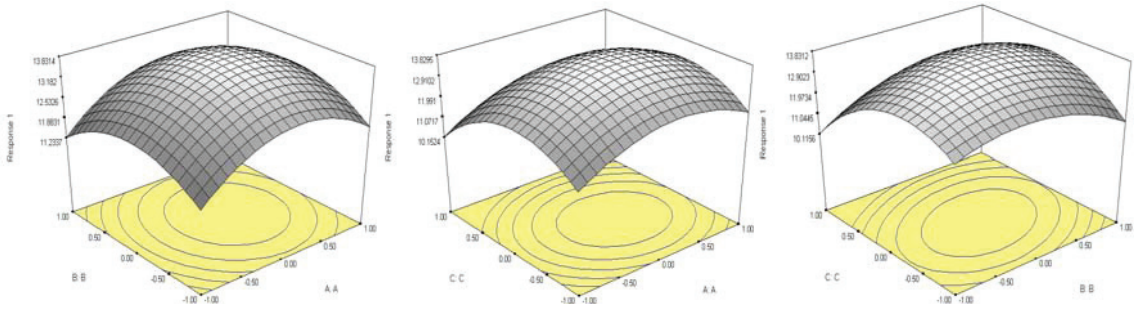

Fig. 3. Response surface plot of the 3 factors of CCD (A) Factor A&B; (B) Factor A&C; (C) Factor B&C

4. Conclusion

As a naturally-existing yogurt starter, Tibetan kefir grains have some unique benefits and have attracted increasing attentions. In this study, FFD and CCD are used for RSM to optimize biomass production of Tibetan kefir grains according to the results of single factor experiments. From the above results, it is obvious that various culture conditions effect biomass production a lot. The optimal culture conditions are skim milk concentration, 41.6%; temperature 30.05℃; inoculation amount, 1.86%; time, 20h and shaker rotating speed, 0r/min, at which the growth rate is 14.33%, 39.4% more than initial. In addition, skim milk concentration, temperature and inoculation amount are significant factors on biomass production.

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