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OPTIMIZATION OF THE MEDIUM FOR *LACTOBACILLUS ACIDOPHILUS* BY PLACKETT-BURMAN AND STEEPEST ASCENT EXPERIMENT*

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ABSTRACT

Background. *Lactobacillus acidophilus* not only improves the intestinal flora balance but also inhabits the growth of undesirable microorganisms in intestine, which is benefit to the health of humans and animals. Plackett-Burman and steepest ascent experiment are the rapid and concise ways of screening the main effective factors. This study is aimed to select the main influence factors and optimize the medium for *Lactobacillus acidophilus* by Plackett-Burman experiment and steepest ascent experiment.
Material and methods. The ideal carbon source was screened among glucose, maltose, lactose and whey powder, and the ideal nitrogen source was screened among casein hydrolysate, peptone, yeast extract powder, fish meal, carbamide, ammonium sulfate and sodium nitrate by single factor experiment. Plackett-Burman and steepest ascent experiment were applied to screen the main effective factors of *Lactobacillus acidophilus* among peptone, beef extract, yeast extract powder, glucose, K_2HPO_4, $C_6H_{14}O_7N_2$, CH_3COONa, $MgSO_4$ and Tween-80.
Result. The results indicated that glucose ($p = 0.01510$) as negative factor and K_2HPO_4 ($p = 0.02017$) as positive effect were the significant growth factors of *Lactobacillus acidophilus*, CH_3COONa ($p = 0.09273$) as positive effect was an important factor, and the optimized medium was as follows: glucose – 21 g/L, K_2HPO_4 – 3.5 g/L, CH_3COONa – 6.5 g/L, peptone – 10 g/L, beef extract – 8 g/L, yeast extract powder – 8 g/L, $C_6H_{14}O_7N_2$ – 2 g/L, $MgSO_4$ – 0.2 g/L and Tween-80 – 1 mL/L when the maximum viable count could achieve $2.72 \cdot 10^9$ cfu/mL.
Discussion. The experimental model is reliable and the experimental results are of good stability. Variance analysis is performed to determine the adequacy and significance of the linear model. Thus, Plackett-Burman and steepest ascent experiment improve the veracity of optimization the medium for *Lactobacillus acidophilus* compared with the previous research.

Key words: *Lactobacillus acidophilus*, Plackett-Burman experiment, steepest ascent experiment, optimization

INTRODUCTION

In recent years, with the awareness of the therapeutic effect on human health of consuming probiotic bacteria increasing (Gomes and Malcata, 1999), *Lactobacillus acidophilus* is a species of probiotic bacteria widely used as health foods and fermented milk (Fung et al., 2008). Dairy starter cultures (Surono and Hosono, 2002) are

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of industrial importance for fermented foods. As a major component of dairy starters *Lactobacillus acidophilus* has a "Generally Recognized as Safe (GRAS)" status in the USA and a "Qualified Presumption of Safety (QPS)" status in the European Union. Therefore, it is necessary to screen main influence factors of the medium in order to obtain an excellent starter with high viable counts.

The Plackett-Burman statistical method offers a design where variables are studied in n+1 experimental runs. This experimental design is an excellent screening method, because the required numbers of experimental runs are very few, leading to saving of time, chemicals, glassware and manpower (Carvalho et al., 1997; Srinivas et al., 1994). Moreover, the design is orthogonal in nature, implying that the effect of each variable worked out is pure in nature and not confounded with interaction among variables.

Response surface methodology (RSM) is a popular statistical method (Neelesh et al., 2014a) which contains a variety of statistical and mathematical techniques. This method is used by modelling and analysing the relationships between several independent variables and response variable(s) (Martins et al., 2013; Neelesh et al., 2014b; Piyushkumar et al., 2007). Thus, it has been widely used.

As far as can be ascertained, the present literature mainly contains the application of Plackett-Burman design and the optimization of fermentation medium (Zhang et al., 2013). The aim of the study was to screen the optimum medium for *Lactobacillus acidophilus* by Plackett-Burman and steepest ascent experiment. The optimized medium will improve the number of viable cells in MRS broth and prolong the growth cycle effectively, which provides the technical foundation for further producing probiotic bacteria powder.

MATERIAL AND METHOD

Materials

Glucose as the optimum carbon source was purchased from Tianjin FuChen chemical reagents factory (Tianjin, China). Both peptone and beef extract as the optimum nitrogen source came from BeiJing AoBoxing bio-tech Co. Ltd. K_2HPO_4 selected as the significant growth factor and purchased from Tianjin TianDa chemical reagents factory. CH_3COONa as an important factor was from Tianjin HongYan chemical reagent factory (Tianjin, China).

Bacterial strains

The probiotic lactic acid bacteria strain, namely *Lactobacillus acidophilus* (Chen et al., 2012) was obtained from College of Life Science and Engineering, Shaanxi University of Science and Technology, Xi'an. MRS agar medium was selected as colony counting culture medium, which was purchased from Qingdao Hope Biol-Technology Co. Ltd. (Qingdao, China).

Activation of bacteria and cultural methods. Inoculate *Lactobacillus acidophilus* in MRS broth (Hopebio, Qingdao, China) at 37°C for 24 h. The methods were used the methods of the toluidine blue staining and the microscopic examination to ensure no other harmful bacteria. Activate bacterial strains three successive times in anaerobic condition. MRS broth was sterilized at 115°C for 20 min (pH 6.2–6.4).

Determination of viable bacterial counts and pH evaluation. The activated bacterial strains with 0.9% NaCl were diluted to suitable concentration. 1 mL of the appropriate dilutability into MRS agar medium was taken, and cultured at 37°C for 48 h. The number of colony between 30 and 300 was selected, and then the viable count per milliliter (cfu/mL) was calculated.

The pH of the culture was evaluated by a pH-meter (pHS-3C, Shanghai Precision Scientific Instrument Co., Ltd, Shanghai, China).

RESULTS AND DISCUSSION

The effect of carbon sources on the growth for *Lactobacillus acidophilus*

The effect of carbon sources on the growth of *Lactobacillus acidophilus* is showed in Figure 1.

From Figure 1, we could find that the effect of different carbon sources on growth of *Lactobacillus acidophilus* had significant difference ($p < 0.05$). Whey powder and glucose were used better for *Lactobacillus acidophilus*, and whey powder had the best effect, less acid production and the number of colonies could rise to $1.8 \cdot 10^9$ cfu/mL.

It could be drawn from the above-mentioned analysis that whey powder was the ideal carbon source for

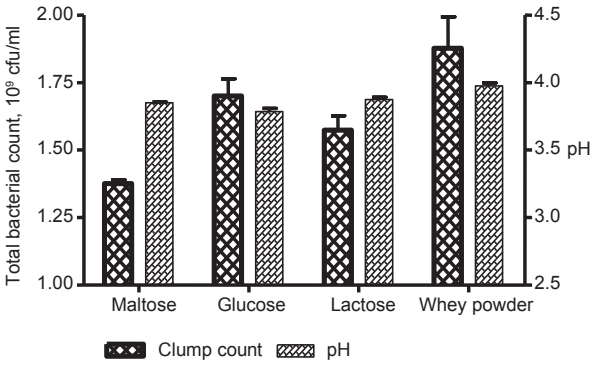

Fig. 1. Effect of carbon source on the growth of *Lactobacillus acidophilus*

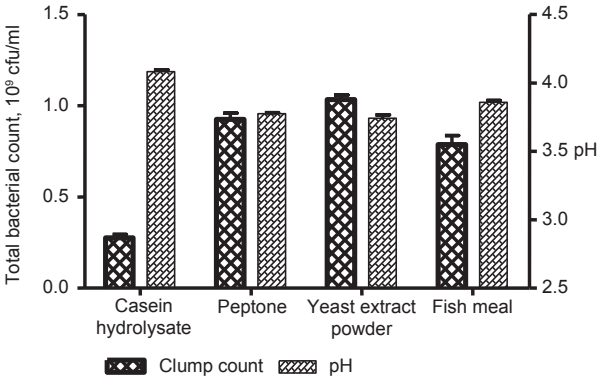

Fig. 2. Effect of organic nitrogen on the growth of *Lactobacillus acidophilus*

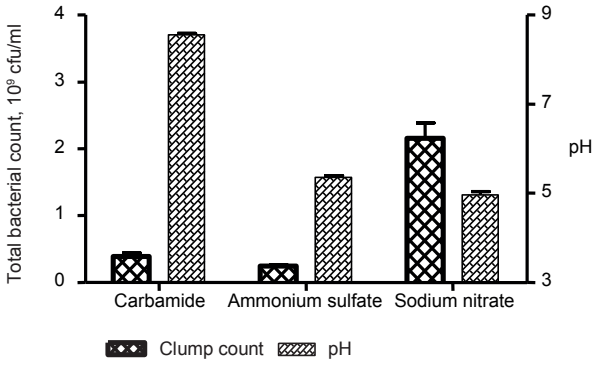

Fig. 3. Effect of inorganic nitrogen on the growth of *Lactobacillus acidophilus*

Lactobacillus acidophilus, but it easily to produced precipitation and would be inconvenient for subsequent preparation of bacterial powder, so glucose was used as the carbon source instead.

The effect of nitrogen sources on the growth for *Lactobacillus acidophilus*

The effect of nitrogen sources on the growth of *Lactobacillus acidophilus* and the pH value of culture medium were studied. The results are shown in Figure 2 and 3.

Figure 2 and 3 respectively show that the differences of different nitrogen sources were significant ($p < 0.001$) on the growth and acid content metabolized. Organic nitrogen source could promote better growth of *Lactobacillus acidophilus* than inorganic nitrogen source, because it contained protein, free amino acid, peptides, glucide, fat and growth factor. Yeast extract and peptone were ideal nitrogen source of *Lactobacillus acidophilus*, it could be found that complex nitrogen source had better effect than single nitrogen source when compared with the basic medium, and that the colony number of single yeast extract was $1.1 \cdot 10^9$ cfu/mL, while the colony number of the complex nitrogen source in MRS was $1.7 \cdot 10^9$ cfu/mL.

It could be drawn from the above-mentioned analysis that single nitrogen source had not better effect than complex nitrogen source, so the compound of yeast extract and peptone were used as complex nitrogen source.

Screening of significant growth factors of medium for *Lactobacillus acidophilus*

Considering the ideal carbon source, nitrogen source and the culture medium compositions of MRS broth from Figure 3, nine kinds of factors were studied. The factors levels coding is shown in Table 1.

The design and results of tests are shown in Table 2. The response value *Y* represented viable count in the liquid fermentation, and the unit was 10^9 cfu/mL.

Analysis of variance was shown in Table 3 in which the effect of each factor on the growth of *Lactobacillus acidophilus*: glucose (X_4) > K_2HPO_4 (X_5) > CH_3COONa (X_7) > yeast extract (X_3) = $MgSO_4$ (X_8) > peptone (X_1) > beef extract (X_2) = $C_6H_{14}O_7N_2$ (X_6) = Tween-80 (X_9). The factor whose reliability was more than 95% ($0.01 < p < 0.05$) was defined as a remarkable factor and the factor whose reliability was more

Table 1. Factors levels coding table of Plackett-Burman

Variables	Factors	Lower level g/L	Higher level g/L
X_1	peptone	10	15
X_2	beef extract	8	12
X_3	yeast extract powder	4	6
X_4	glucose	20	30
X_5	K_2HPO_4	2	3
X_6	$C_6H_{14}O_7N_2$	2	3
X_7	CH_3COONa	5	7.5
X_8	$MgSO_4$	0.2	0.3
X_9	Tween-80	1	1.5

Table 2. Experimental design and results of Plackett-Burman

RUN	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	Y, 10^9 cfu/mL
1	1	–1	1	–1	–1	–1	1	1	1	1.75
2	1	1	–1	1	–1	–1	–1	1	1	1.66
3	–1	1	1	–1	1	–1	–1	–1	1	1.79
4	1	–1	1	1	–1	1	–1	–1	–1	1.69
5	1	1	–1	1	1	–1	1	–1	–1	1.74
6	1	1	1	–1	1	1	–1	1	–1	1.76
7	–1	1	1	1	–1	1	1	–1	1	1.71
8	–1	–1	1	1	1	–1	1	1	–1	1.74
9	–1	–1	–1	1	1	1	–1	1	1	1.72
10	1	–1	–1	–1	1	1	1	–1	1	1.78
11	–1	1	–1	–1	–1	1	1	1	–1	1.74
12	–1	–1	–1	–1	–1	–1	–1	–1	–1	1.73

Table 3. ANOVA of Plackett-Burman

Source	df	*SS*	*MS*	*F*	*p*	Significance
X_1	1	0.000208	0.000208	1.923077	0.29986	
X_2	1	8.33E-06	8.33E-06	0.076923	0.80755	
X_3	1	0.000408	0.000408	3.769231	0.19171	
X_4	1	0.007008	0.007008	64.69231	0.015108	*
X_5	1	0.005208	0.005208	48.07692	0.020173	*
X_6	1	8.33E-06	8.33E-06	0.076923	0.80755	
X_7	1	0.001008	0.001008	9.307692	0.092735	
X_8	1	0.000408	0.000408	3.769231	0.19171	
X_9	1	8.33E-06	8.33E-06	0.076923	0.80755	

*** $p < 0.0001$ – highly significant, ** $p < 0.001$ – very significant, * $p < 0.05$ – significant.

than 90% ($0.05 < p < 0.1$) was important factor in general statistics.

It was shown that glucose (X_4) and K_2HPO_4 (X_5) were remarkable factors, and CH_3COONa (X_7) was important factor on growth of *Lactobacillus acidophilus* in Table 3. Similarly, from Figure 4, we could find that the factors of X_4, X_5 and X_7 had greater effect on the growth of *Lactobacillus acidophilus*, it also indicated positive or negative effects and the influence intensity of each factor on the response values.

We screened two significant factors of glucose (X_4), K_2HPO_4 (X_5) and one important factor of CH_3COONa (X_7) by the Plackett-Burman experiment design and then did further research and analysis. The other factors were determined as: the X_1 to –1 level, X_2 to –1 level, X_3 to 1 level, X_6 to –1 level, X_8 to –1 level and X_9 to

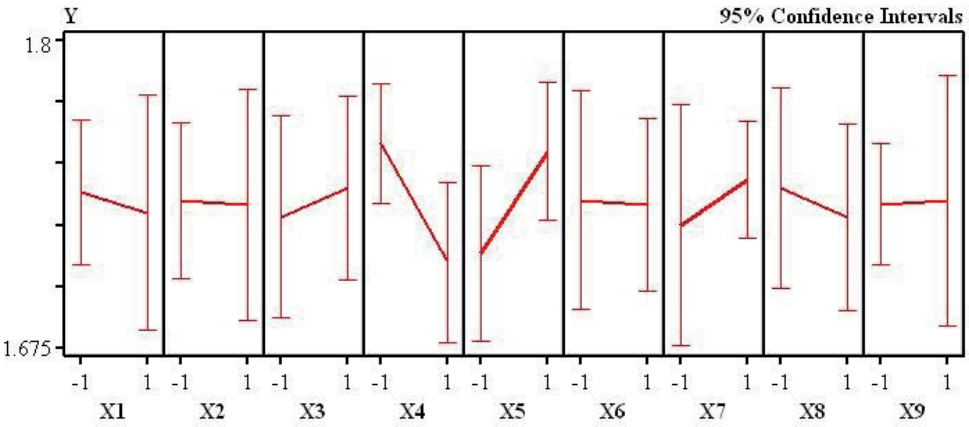

Fig. 4. 95% confidence interval of growth factors

Table 4. Design and results of the path of steepest ascent experiment

Number of steps	X_4	X_5	X_7	Number of colony 10^9 cfu/mL
1	30	2	5	1.67
2	27	2.5	5.5	1.86
3	24	3	6	2.41
4	21	3.5	6.5	2.72
5	18	4	7	1.45

–1 level, namely, peptone 10 g/L, beef extract 8 g/L, yeast extract powder 8 g/L, citric acid hydrogen ammonia 2 g/L, magnesium sulfate 0.2 g/L and Tween-80 1 ml/L according to Figure 4.

The results analysis of steepest ascent experiment

The results were as shown in Table 4 which showed that optimum value area was located in step 4. Glucose (X_4) as the negative effect should be reduced; K_2HPO_4 (X_5) and CH_3COONa (X_7) as positive effect should be increased. According to the effect of three factors, changed direction and a set of experimental runs were designed.

CONCLUSIONS

This study result showed that glucose (X_4) and K_2HPO_4 (X_5) were remarkable factors, and CH_3COONa (X_7) was important factor on growth of *Lactobacillus acidophilus*. The viable count would increase with the X_5 and X_7, and decrease with X_4. Effect of each factor on the growth of *Lactobacillus acidophilus*: glucose (X_4) > K_2HPO_4 (X_5) > CH_3COONa (X_7) > yeast extract (X_3) = $MgSO_4$ (X_8) > peptone (X_1) > beef extract (X_2) = $C_6H_{14}O_7N_2$ (X_6) = Tween-80 (X_9). The optimized medium component was as follows: glucose – 21 g/L, K_2HPO_4 – 3.5 g/L, CH_3COONa – 6.5 g/L, peptone – 10 g/L, beef extract – 8 g/L, yeast extract powder – 8 g/L, $C_6H_{14}O_7N_2$ – 2 g/L, $MgSO_4$ – 0.2 g/L and Tween-80 – 1 mL/L when the maximum viable count could achieve $2.72 \cdot 10^9$ cfu/mL.

REFERENCES

Ariga, O., Toyofuku, H., Minegishi, I., Hattori, T., Sano, Y., Nagura, M. (1997). Efficient production of recombinant enzymes using PVA encapsulated bacteria. J. Ferm. Bioeng., 84, 553–557.

Bai, J. L., Mo, S. P., Zheng, W. L. (2007). *Lactic acid* bacteria enrichment medium nutrition factor optimization industry. J. Food Ferm. Ind., 33(2), 79–81.

Bukharin, O. V., Sgibnev, A. V. (2013). Effect of metabolites of H_2O_2-producing lactobacilli on functional activity of lysozyme. Zhur. Mikrob. Epidem. Iimmunobiol., 4, 60–64.

Carvalho, C. M. L., Serralheiro, M. L. M., Cabral, J. M. S., Airebarros, M. R. (1997). Application of factorial design to the study of transesterification reactions using cutinase in AOT-reversed micelles. Enz. Microb. Techn., 27, 117–123.

Chen, H., Li, C. N., Shu, G. W., Wang, C. F. (2012). Screening of nitrogen sources in the medium for Streptococcus

thermophilususing Plackett-Burman design. Adv. Mat. Res., 531, 532–535.

Colombo, M., Oliveira, A. E. Z., Carvalho, A. F., Nero, L. A. (2014). Regulation of exopolysaccharide production by *Lactobacillus lactic* subsp. Food Microbiol., 39, 89–95.

Fung, W. Y., Woo, Y. P., Liong, M. T. (2008). Optimization of growth of Lactobacillus acidophilus FTCC 0291 and evaluation of growth characteristics in soy whey medium: a response surface methodology approach. J. Agric. Food Chem., 56, 7910–7918.

Gomes, A. M. P., Malcata, F. X. (1999). Dobacterium spp. and Lactobacillus acidophilus: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trends Food Sci. Tech., 10, 139–157.

Gran, H. M., Gadaga, H. T., Narvhus, J. A. (2003). Utilization of various starter cultures in the production of Amasi, a Zimbabwean naturally fermented raw milk product. Int. J. Food Microbiol., 88, 19–28.

Kirmizakis, P., Tsamoutsoglou, C., Kayan, B., Kalderis, D. (2014). Subcritical water treatment of landfill leachate: Application of response surface methodology. J. Environ. Manag., 146, 9–15.

Li, J. X., Luo, Y., Tian, P. F. (2010). Optimizing *lactobacillus casei* fermentation culture medium of progretive bacteriocins by the response surface method. J. Henan Univ. Techn., 31(3), 45–49.

Looijesteijn, P. J., Boels, I. C., Kleerebezem, M., Hugenholtz, J. (1999). Regulation of exopolysaccharide production by *Lactobacillus lactic* subsp. cremorisby the sugar source. Appl. Environ., 65, 5003–5008.

Martins, A. C., Bukman, L., Vargas, A. M. M., Barizão, É. O., Moraes, J. C. G., Visentainer J. V., Almeida, V. C. (2013). The antioxidant activity of teas measured by the FRAP method adapted to the FIA system: Optimizing the conditions using the response surface methodology. Food Chem., 138, 574–580.

Mundra, P., Desai, K., Lele, S. S. (2007). Application of response surface methodology to cell immobilization for the production of palatinose. Biores. Techn., 98, 2892–2896.

Naveena, B. J., Altaf, M., Bhadriah, K. G. (2005). Reddy Selection of medium components by Plackett–Burman design for production of L(+) *lactic acid* by *Lactobacillus amylophilus* GV6 in SSF using wheat bran. Biores. Techn., 96, 485–490.

Neelesh, S., Garima, S., Mahe, T., Himanshu, R., Onkar, N. S., Arvind, M. K. (2014). Cicer α-galactosidase immobilization onto functionalized graphene nanosheets using response surface method and its applications. Food Chem., 142, 430–438.

Singh, N., Srivastava, G., Raghubanshi, H., Srivastava, O. N., Kayastha, A. M. (2014). Cicer α-galactosidase immobilization onto functionalized graphene nanosheets using response surface method and its applications. Food Chem., 142, 430–438.

Park, J. K., Chang, H. N. (2000). Microencapsulation of the microbial cells. Biotechn. Adv., 18, 303–319.

Piyushkumar, M., Kiran, D., Lele, S. S. (2007). Application of response surface methodology to cell immobilization for the production of palatinose. Biores. Techn., 98, 2892–2896.

Surono, I., Hosono, A. (2002). Starter cultures. Nagano-Ken, Japan: Shinsyu University.

Srinivas, M. R. S., Naginchand, Lonsane, B. K. (1994). Use of Plackett-Burman design for rapid screening of several nitrogen sources, growth/product promoters, minerals and enzyme inducers for the production of alpha-galactosidase by *Aspergillus niger* MRSS 234 in solid state fermentation. Bioproc. Eng., 10, 139–144.

Tian, H. T., Jia, Y. M., ..., Ma, W. (2002). *Thermophilic streptococcus* growth promoting substance research and proliferation medium optimization selection. J. Food Sci., 23(5), 60–62.

Wang, Y. R., Liu, D. W., Zhang, M. H. (2010). The response surface method optimization of *Lactobacillus acidophilus* whey medium. China Brew., 10, 77–78.

Yi, H. X., Han, X., Du, M. etc. (2010). MRS culture medium components of *Lactobacillus* J23 effects of antibacterial peptide Bac-J23. China Brew., 31(5), 56–58.

Zhao, R. X., Sun, J. L., Li, M. J. (2005). *Lactobacillus acidophilus* assimilates cholesterol capacity in MRS medium. Chin. J. Microecol., 17(4), 254–255.

Zhang, S. W., Huang, J. F., Luo, L. X. (2013). Optimization of fermentation medium for protease production by *Bacillus subtilis*. China Brew., 251(2).

Zhuang, X. L., Zhang, H. X., Ma, G. R. (2000). The main factors that influence the milk peptide chain bacteria fermentation. Chem. Metal., 21(1), 93–97.

Zhang, Y., Leng, Y. F., Li, J. H. (2011). The response surface method optimization of *Lactobacillus acidophilus* proliferation medium. Chinese Brew., 134–137.

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