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EFFECT OF NUTRIENTS ON THE GROWTH OF SELENIUM-ENRICHED *LACTICASEIBACILLUS RHAMNOSUS* L20

– Research paper –

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Abstract: Selenium-rich probiotics have the dual role of selenium and probiotics, and show great development potential in dairy products. Using *Lacticaseibacillus rhamnosus* L20 as the experimental strain, the effects of sodium selenite concentration and fermentation time on the growth and selenium conversion rate of *L. rhamnosus* L20, as well as the effects of carbon and nitrogen sources, prebiotics and inorganic salts on its growth were studied. The results showed that the appropriate sodium selenite concentration and fermentation time were 20 μg/mL and 16 h, and its selenium conversion rate and viable count were 88.83% and 2.34×10^8 CFU/mL, respectively. Carbon sources (glucose and lactose), organic nitrogen sources (soy peptone and beef extract), inorganic nitrogen sources (urea and Triammonium citrate), prebiotics (galactooligosaccharides and inulin) and inorganic salts (dipotassium hydrogen phosphate and sodium acetate) were beneficial to the growth of *L. rhamnosus* L20, and its dry weight was greater than 1.95 g/L, which provides a reference for further optimization of the proliferation culture medium of *L. rhamnosus* L20.

Keywords: Selenium-enriched probiotics, *Lacticaseibacillus rhamnosus*, Carbon and nitrogen source, Prebiotics

INTRODUCTION

In the mid-20th century, selenium was identified as a necessary trace element by WHO, and was listed as a dietary nutritional element by the China Nutrition Society in the 1880s. Selenium is considered an essential trace element because of its ability to improve immune function and its close relationship with many bodily functions. However, if the selenium intake exceeds 400 μg/d, it may cause poisoning. The World Health Organization recommends that the minimum dietary requirement for selenium be 40 μg/d. Although the content of selenium in human body is very small, it plays an irreplaceable role in human health by other elements, providing the necessary conditions for life to exist.

Selenium can be categorized into inorganic selenium, organic selenium and bio-nano selenium according to its chemical form and source. Inorganic selenium is mainly derived from selenium-containing ores, which are poorly absorbed and toxic in the human gut. Organic selenium is formed by combining with amino acids through a biotransformation pathway, which is more suitable as a source of selenium for the human body because of its higher absorption rate in the body and lower toxicity.

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Traditional synthetic methods for organic selenium include physical synthesis and chemical synthesis. The operation processes of the two kinds of synthesis are more complex, and the production cost is high, making large-scale production difficult. By contrast, the microbial preparation of organic selenium is a promising method, which efficiently converts inorganic selenium into organic selenium through microorganisms (Overschelde, Guisbiers, & Snyders, 2013), and can rapidly meet the needs of human beings, especially in selenium-deficient regions. Microbial transformation, which mainly utilizes the tolerance of natural or modified edible fungi to selenium ions and their growth and transformation characteristics. The conversion of inorganic selenium into the organic state is a hot topic of current research (Mangiapane, Pession, & Pessione, 2014; Kieliszek, Blazejak, GientkaI, 2015).

Probiotics are living microorganisms (Pereira, PiazentinI, & Deoliveira, 2022; Mohr, Pugh, & O'sullivan, 2022) that can confer health benefits to the host when administered in adequate amounts, and can alter the composition of the host's flora, produce more than basic nutritional value in the body, and exert health-promoting effects. A previous study (Yin, Shao, & Liao, 2021) has reported that probiotics can biotransform inorganic selenium into

organic selenium and synergistically exert their probiotic effects in the intestinal tract. The biotransformation of selenium in probiotics was achieved through anaerobic respiration and the dissimilatory reduction of selenate. A variety of microorganisms conduct respiratory metabolism in an anaerobic environment, and can act as electron receptors for selenate and selenite, converting them into SeNPs or selenoproteins (Janine, 2006). Microbial biosynthesis of organic selenium is primarily focused on selenium-rich yeast and selenium-rich lactic acid bacteria. At present, yeast is generally used as the preferred carrier for the enrichment and transformation of inorganic selenium by microorganisms (Zhu, Li, & Wang, 2020). However, studies have shown that when yeast enriched and converted selenium, selenomethionine (SeMet) is mainly produced, which binds non-specifically to protein. A variety of studies have found that some LAB strains can utilize the added selenium in the culture process and accumulate seleno-amino acids or nano-selenium in the body, and selenium-enriched lactic acid bacteria can be used to develop nutritional health-care product additives or serve as leavening agents to improve the flavor characteristics of fermented products (Wang, Zhao, & Li, 2021; Zhao, Zheng, & Zhu, 2021). Selenium-enriched *Lactobacillus casei* can also alleviate intestinal barrier dysfunction in animals by regulating endoplasmic reticulum stress and intestinal flora (Song, Qiao, & Chang, 2022).

The selenium-enriched probiotics have the double effects of selenium and probiotics (Nido, Shituleni, & Mengistu, 2016), and as a micro-ecological preparation, improve the bioavailability, improve the health condition of animals, improve the milk quality and supplement the selenium content in dairy animals, thereby contributing to improved production, but also combine the effects of the probiotics on improving the intestinal micro-ecological environment and enhancing immunity, and the unique double functions enable the selenium-enriched probiotics to play an irreplaceable role in dairy production.

Compared with selenium-enriched yeast, fewer studies have focused on selenium-enriched lactic acid bacteria as probiotics, and few studies on the effect of the composition of culture medium such as carbon source, nitrogen source and inorganic salt on the growth of selenium-enriched probiotics. In our previous study, we screened, obtained and identified a high selenium-enriched probiotic, *L. rhamnosus* L20 from 23 probiotic *Lactobacillus*. This study focused on the effects of sodium selenite, carbon source, nitrogen source, prebiotics and inorganic salt on the growth of *L. rhamnosus* L20, which would provide a reference for the subsequent optimization of the culture medium of *L. rhamnosus* L20.

MATERIALS AND METHODS

Microorganisms and Reagents

L. rhamnosus L20 was deposited in the School of Food Science and Engineering, Shaanxi University of Science and Technology.

Sodium selenite (Na_2SeO_3) was purchased from Shandong Siya Reagent Company, Shanghai, China. Other chemicals are purchased from local commercial sources and are of analytical grade quality.

Activation of cultures

The frozen and preserved probiotic lyophilized powder was inoculated into an MRS broth culture medium for activation, placed in a constant temperature incubator for culture at 37 °C for 24h, and transferred to the MRS broth culture medium for culture again at 37 °C for 24h after microscopic examination for no miscellaneous bacteria, and the activated bacteria were continuously activated for three generations, and stored at 4 °C for subsequent use.

Determination of the appropriate amount of selenium

Inorganic selenium sodium selenite inhibits the growth of probiotics, and a large amount of red nano-selenium will be produced under the environment of high concentration of inorganic selenium. At present, nano-selenium can only be used in health food and feed. The purpose of this study is to obtain high-activity probiotics containing organic selenium. The MRS broth medium with the sodium selenite concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg/mL was inoculated with selenium-enriched probiotics L20 with the inoculation amount of 1% and cultured at 37 °C for 24h to determine the viable count and conversion rate for optimizing the sodium selenite concentration.

Determination of selenium-enriched probiotic growth curve

The selenium-enriched probiotics were inoculated in the optimized medium with the inoculation amount of 1% and cultured at 37 ℃ for 32 h, and the viable count, dry weight and pH value were measured by sampling every 2h, with three parallel experiments conducted, and the growth curve of the selenium-enriched probiotics was drawn.

Screening of basic culture components

Because the components of the MRS culture medium are more complex, the cost is high. In this study, we

selected a simple and low-cost basal medium to reduce the impact on the test due to the complex composition. The growth of bacteria could not be separated from carbon source and nitrogen source. With MRS broth medium composition as reference, 2% glucose as the carbon source and 1% peptone as the nitrogen source. On this basis, the effects of components (beef extract, yeast extract, sodium acetate, ammonium citrate, magnesium sulfate, manganese sulfate, and dipotassium hydrogen phosphate) in MRS broth on the growth of selenium-enriched probiotics were investigated. The components capable of promoting the growth of the selenium-enriched probiotics are selected to form a basic culture medium of the selenium-enriched probiotics together with a carbon source and a nitrogen source.

Effects of carbon sources, nitrogen sources, prebiotics and salts on the growth of *L. rhamnosus* L20

Through the basal medium determined above, three parallel groups were set up to investigate the effects of carbon sources (glucose, lactose, maltodextrin, solublestarch and sucrose), organic nitrogen sources (yeast extract, peptone, soy peptone, beef extract, casein peptone and tryptone), inorganic nitrogen sources (ammonium sulfate, ammonium nitrate, potassium nitrate, urea, ammonium citrate dibasic and ammonium citrate), prebiotics (fructo oligosaccharide, galactooligosaccharides, inulin, Stachyose, isomaltooligosaccharide) and salts (dipotassium hydrogen phosphate, potassium dihydrogen phosphate, dibasic sodium phosphate, manganese sulphate, magnesium sulfate, sodium chloride, potassium chloride, sodium acetate) on the growth of selenium-rich probiotics were 2% carbon source, 1% organic nitrogen source, 0.5% inorganic nitrogen source, 0.5% prebiotic and 0.5% salt. The optimum medium composition of strain L20 was screened out.

Determination of selenium

The determination principle of Chen Yongbo et al. was used (Chen, Xue, & Ma, 2010) under acidic conditions, tetravalent selenium reacts with 3,3'-diaminobenzidine to produce a yellow color skatole complex. According to the principle of similarity and compatibility, the skoelenocephalin complex can be extracted by organic solvents such as toluene or xylene in neutral solution, and its extract has the maximum absorbance at 420 nm. Then according to the spectrophotometric method for the degree of color development, the content of inorganic selenium contained can be determined. Finally, the conversion rate of selenium is measured according to the formula.

$$\text{Selenium conversion rate (\%)} = (A/B) \times 100 \quad [1]$$

Where A is total selenium content, and B is residual inorganic selenium content.

Statistical analysis of data

Statistical analysis was performed using SPSS to determine if the experimental data were significant. All trials were repeated three times.

RESULTS AND DISCUSSION

Determination of selenium standard curve

The content of inorganic selenium was determined by spectrophotometry. The feasibility of this method was judged by the recovery rate of 100% ±5%. According to the 3,3'-diaminobenzidine spectrophotometric colorimetry, a standard curve for selenium was established, as shown in Figure 1.

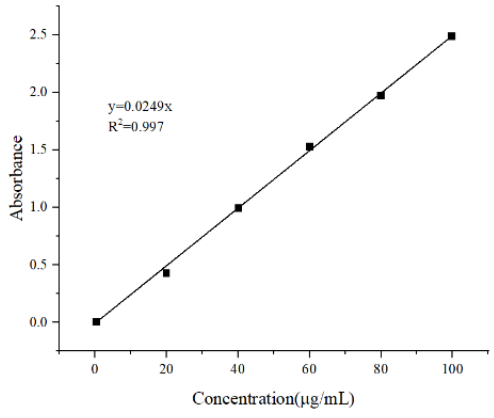


Figure 1 Standard curve of selenium

According to the standard curve, the linear regression equation was y=0.0249x with a regression coefficient (R^2) of 0.997 for selenium concentration in the range of 0-100 μg/mL, indicating that the linear relationship between absorbance and selenium concentration was good. The linear range was suitable for the determination of inorganic selenium content.

Effect of sodium selenite addition on the growth and selenium enrichment of *L. rhamnosus* L20

Sodium selenite was added to the prepared MRS broth medium according to different concentration gradients of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μg/mL, and then inoculated with the same inoculation amount of *L. rhamnosus* L20 for culture for 24 h, and the number of viable bacteria and conversion rate were measured, and the results are shown in Figure 2 and Figure 3.

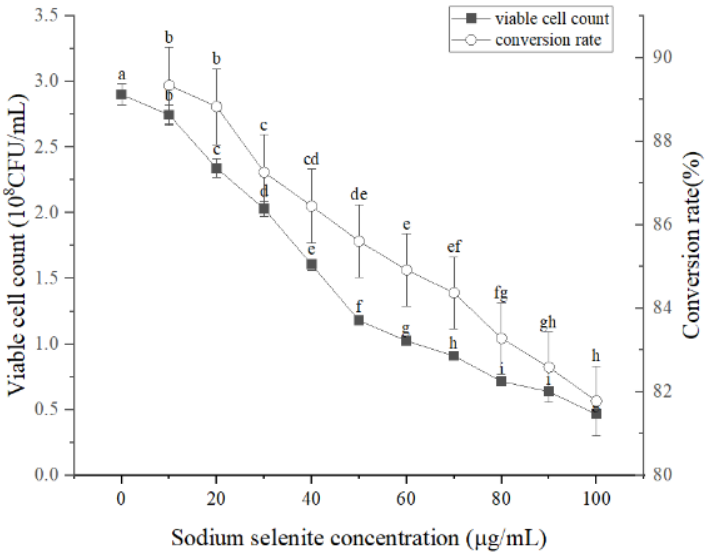


Figure 2 Effects of sodium selenite concentrations on the growth and Se-enriching ability of *L. rhamnosus* L20

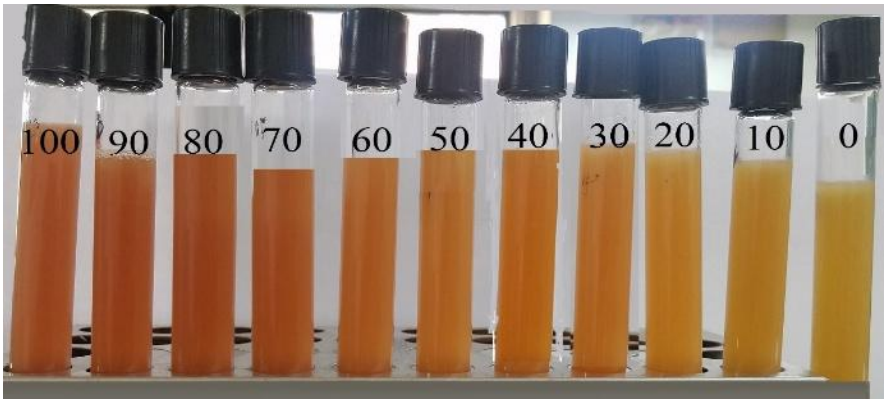


Figure 3 Variation of the color of MRS broth with different sodium selenite concentrations fermented by *L. rhamnosus* L20

In this experiment, ANOVA was used to investigate the effects of different sodium selenite concentrations on the number of viable bacteria and transformation rate of *L. rhamnosus* L20. The descriptive statistics showed that *L. rhamnosus* L20 had the highest number of viable bacteria at a sodium selenite concentration of 0 μg/mL (M=2.942, SD=0.573), and the conversion rate of *L. rhamnosus L20* reached the maximum at a sodium selenite concentration of 10 μg/mL (M=89.797, SD=6.456); the results of the analysis of variance (ANOVA) showed significant differences differences in the effects of different sodium selenite concentrations on the viable count and transformation rate of *L. rhamnosus* L20 ($p<0.05$).

As can be seen from Figure 3, sodium selenite concentration at 0-20 μg/mL, the viable count of *L. rhamnosus* L20 decreasesed slowly with an increase in sodium selenite concentration. The range of 20-50 μg/mL is where the concentration of sodium selenite is found, the number of L20 viable bacteria decreased rapidly. When the concentration of sodium selenite reached 50 μg/mL, the rate of decline of L20 viable bacteria slowed down again. The concentration of sodium selenite was found to be 0-20 μg/mL, the number of viable bacteria of *L. rhamnosus* L20 varied in a small range, which was suitable for the growth of strains L20. Sodium selenite, when present at a concentration of 10 μg/mL, the transformation rate of *L. rhamnosus* L20 was 89.34%. Concurrently, the increasing concentration of sodium selenite resulted in a gradual decrease in the conversion rate of L20. Notably, at a concentration of 100 μg/mL, the conversion rate of L20 was recorded at 81.78%, signifying a substantial decline in the reaction rate.

In Figure 3, from right to left, the concentrations of sodium selenite were 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg/mL in sequence. From the results, it could be seen that the color of the *L. rhamnosus* L20 culture solution became red and gradually deeper with the increasing sodium selenite concentration, suggesting that red nano-selenium was produced in the culture solution and its content gradually increased. The color of the medium was more intense at 30 μg/mL sodium selenite than at 20 μg/mL sodium selenite. When the concentration of sodium selenite was 0, 10 or 20 μg/mL, there was no significant difference in the color of the culture solution.

The purpose of this study was to prepare probiotics rich in organic selenium, and the optimal sodium selenite concentration for *L. rhamnosus* L20 was 20 μg/mL. However, as reported by Xia SK et al. (Xia, Chen, & Liang, 2007), the optimal amount of sodium selenite in *L. delbrueckii* was 16 μg/mL. This difference is due not only to differences in strain characteristics but also to the presence of other metal ions that enhance the strain's tolerance to inorganic selenium (Calomme, Vanden Berghe, 2010).

Effect of culture time on the growth and selenium enrichment of *L. rhamnosus* L20

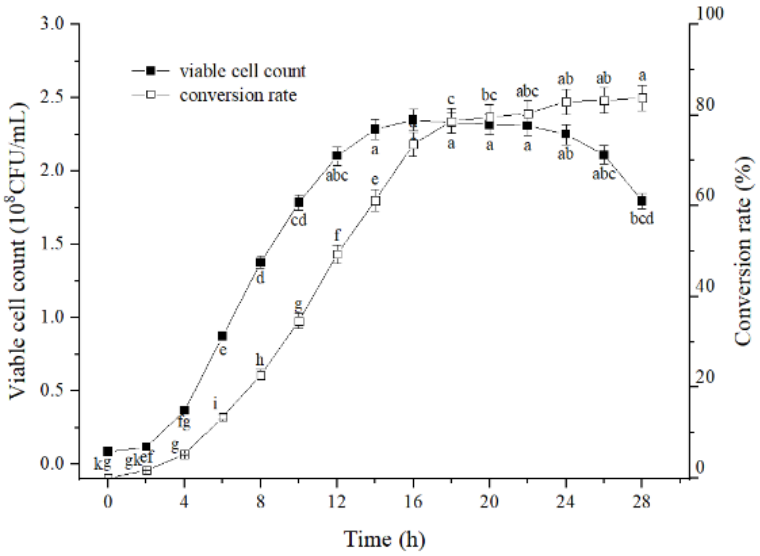
L. rhamnosus L20 was inoculated with 1% inoculation in MRS broth medium with sodium selenite concentration of 20 μg/mL, cultured at 37°C, and samples were taken every two hours to measure the number of viable bacteria and conversion rate of *L. rhamnosus*, and the results are shown in Figure 4. In this experiment, one-way analysis of variance (ANOVA) was used to investigate the effects of incubation time on the viable count and transformation rate of *L. rhamnosus* L20. The descriptive statistics showed that *L. rhamnosus* L20 had the highest number of viable bacteria when the incubation time was 16h (M=2.383, SD=0.051); the results of ANOVA showed that there was a significant difference between the effects of incubation time on the number of viable bacteria and the transformation rate of *L. rhamnosus* L20 ($p<0.05$).

As can be seen from Figure 4, the number of viable bacteria and culture time of *L. rhamnosus* showed a logarithmic growth trend, 0-2 h was the delay period of the growth of *L. rhamnosus*, L20 grew slowly, 2-16 h was the logarithmic growth phase of L20, during which the viable count of L20 increased rapidly, the number of viable bacteria reached the maximum value of 2.34×10^8 CFU/mL at 16 h, and then entered the growth stability phase at 16-24 h, and the L20 strain entered the decay phase after 24 h. With the increase of the number of viable bacteria of L20 strains, the transformation rate of inorganic selenium also increased, and the growth trend of the transformation rate gradually slowed when the viable counts stabilized at 16 h. There is a certain lag between the conversion rate and the number of viable bacteria, which is because it takes a certain amount of time for the bacteria to transform inorganic selenium. Without changing other culture conditions, the optimal culture time of strain L20 was about 16 h. Through the above results, the optimal culture time for strain *L. rhamnosus* L20 was approximately 16 h without changing other culture conditions. According to Sun Jianchun et al.(Sun, Wu, & Zou, 2022), the conversion rate was 50.34% and the selenium-rich content was as high as 5 658.256 μg/g when the culture time of *L. rhamnosus* plus selenium was 18 h. This may be due to the fact that *L. rhamnosus* was cultured at a temperature of 38°C and sodium selenite was added at a concentration of 12 μg/mL, which increased the amount of the culture.

Screening of basal culture components of *L. rhamnosus* L20

Taking the composition of MRS broth medium as a reference, 2% glucose was selected as the carbon source and 1% peptone as the nitrogen source to ensure the growth of *L. rhamnosus* L20.

The activated *L. rhamnosus* L20 was inoculated at a 1% inoculum, incubated at 37°C for 16 h, and the pH, OD_{600} value, and dry weight of the culture medium were measured. The results are shown in Figure 5.



. Figure 4. Growth curve and conversion rate of *L. rhamnosus* L20

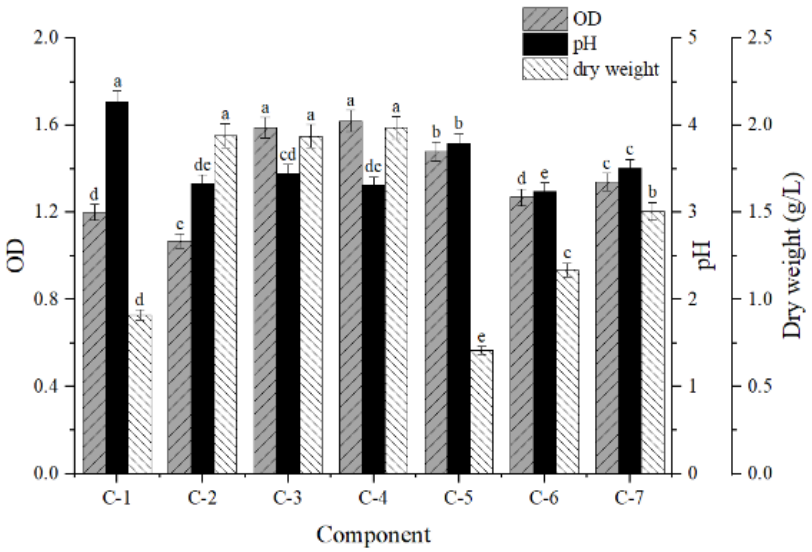


Figure 5. Screening of the basic medium components of *L. rhamnosus* L20. (C-1-7 respectively represent: sodium acetate, magnesium sulfate, beef extract, yeast extract, ammonium citrate, manganese sulfate, dipotassium hydrogen phosphate)

One-way analysis of variance (ANOVA) was used to investigate the effects of different basal medium compositions on the pH, OD_{600} value and dry weight of *L. rhamnosus* L20. Descriptive statistics showed that the OD_{600} value was highest when the medium contained C-4 (M=1.640, SD=0.020), the dry weight of the biomass was also highest (M=1.980, SD=0.014), and the pH value was lowest (M=3.351, SD=0.051); the results of the analysis of variance (ANOVA) showed that different basal mediums had an effect on the pH value of *L. rhamnosus* L20, OD_{600} values and the dry weight of the bacteria were significantly different ($p < 0.05$).

As shown in figure 5, among the seven components, the pH value of the culture medium under the action of yeast extract is the lowest. In terms of OD value, the OD_{600} value of yeast extract was the highest among the seven components, which was 1.62, and the dry weight of yeast extract was also the highest among the seven components, indicating that its promoting effect on L20 was higher than that of the other six components, and the dry weight of yeast extract was 1.9849g. It can be seen that the growth promotion effect of yeast extract on *L. rhamnosus* L20 is better than that of other components.

At this point, the base medium composition can be determined as: 2% glucose, 1% peptone and 0.5% yeast dip. In the study of Xu Xilin et al (Xu, Zhao, & Zheng, 2022), the optimum base medium composition of Lactobacillus rhamnosus was 2% glucose, 3.5% peptone and 3.5% yeast dip, the reason for the difference in the optimum additions is not only the difference in the characteristics of the strains, but also may be related to the purity and source of the different medium components.

Effects of various nutrients on the growth of *L. rhamnosus* L20

On the basis of basal medium, the effects of 2% carbon sources (glucose, lactose, maltodextrin, solublestarch and sucrose) and 0.5% inorganic nitrogen sources (ammonium sulfate, ammonium nitrate, potassium nitrate, urea, ammonium citrate dibasic and ammonium citrate), 0.5% prebiotics (Fructooligosaccharides, Galactooligosaccharides, Inulin, Stachyose and Isomaltooligosaccharide), 0.5% inorganic salts (dipotassium hydrogen phosphate, potassium dihydrogen phosphate, dibasic sodium phosphate, manganese sulphate, magnesium sulfate, sodium chloride, potassium chloride and sodium acetate), and 1% organic nitrogen sources (yeast extract, peptone, soy peptone, beef extract, casein peptone and tryptone) on the growth of *L. rhamnosus* L20 ware investigated. Each experimental group was incubated at 37°C for 16 h with basal medium as the control group, and the results are shown in Figure 6.

ANOVA confirmed significant differences in the effects of different nutrients on the pH, OD_{600}, and dry weight of L. rhamnosus L20 ($p<0.05$).

As can be seen from Figure 6 (a), glucose and lactose were the carbon sources, the most significant effect on the growth of *L. rhamnosus* L20 was promoted. When glucose was used as the only carbon source, the OD value of the fermentation broth was 1.62, the pH was 3.31, and the dry weight of the bacteria was 1.99 g/L. When lactose was used as the only carbon source, the OD value of the fermentation broth was 1.56, the pH was 3.28, and the dry weight of the bacteria was 2.07 g/L.

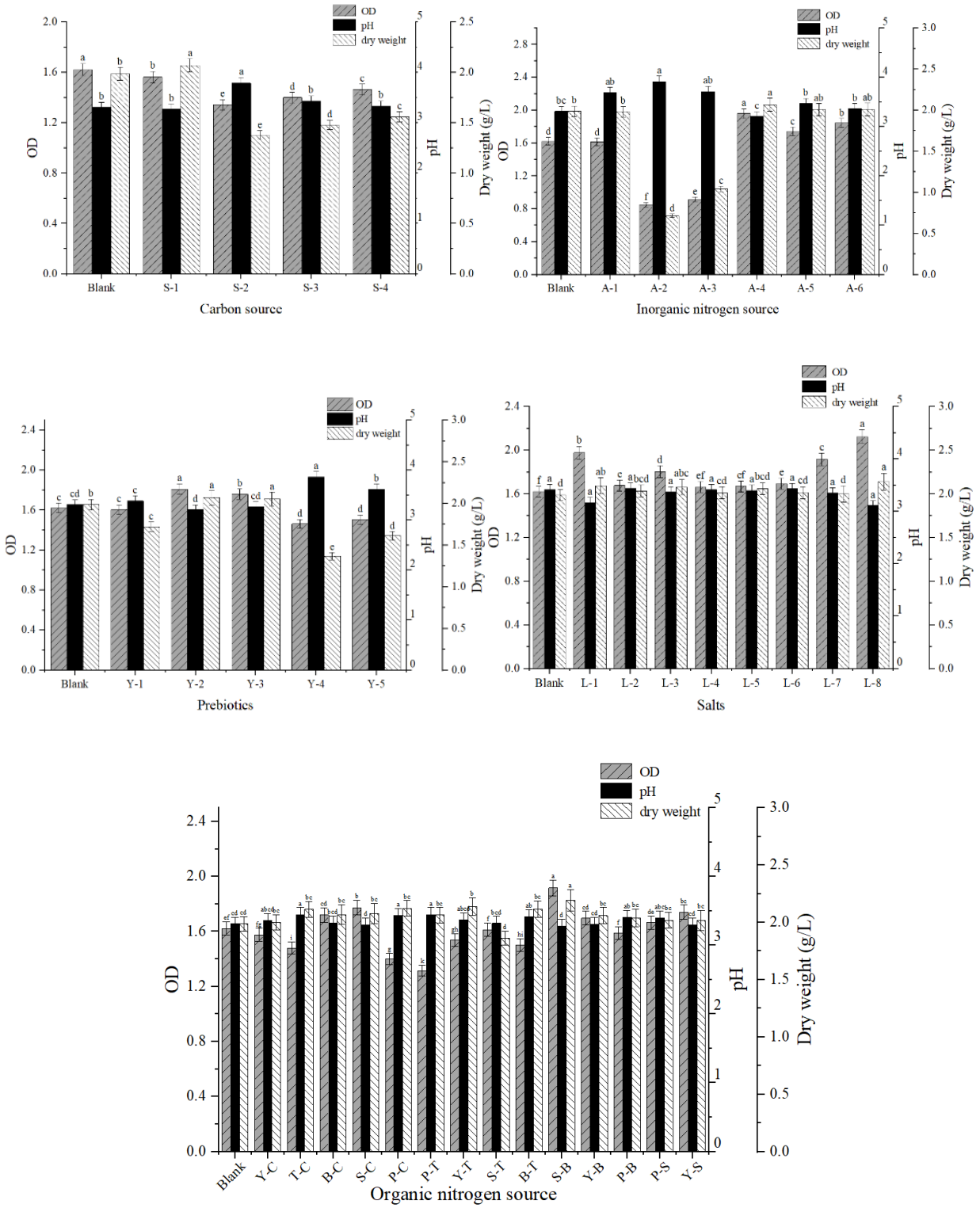


Figure 6. Effects of various nutrients on the growth of *L. rhamnosus* L20. a: carbon source, S1-4 respectively represent: lactose, maltodextrin, solublestarch, sucrose; b: inorganic nitrogen source, A1-6 respectively represent: ammonium sulfate, ammonium nitrate, potassium nitrate, urea, ammonium citrate dibasic, ammonium citrate; c: salts, L1-8 respectively represent: dipotassium hydrogen phosphate, potassium dihydrogen phosphate, dibasic sodium phosphate, manganese sulphate, magnesium sulfate, sodium chloride, potassium chloride, sodium acetate; d: prebiotics, Y1-5 respectively represent: fructo oligosaccharide, galactooligosaccharides, inulin, stachyose, isomaltooligosaccharide; e: organic nitrogen source, Y, P, S, B, C, T respectively represent: yeast extract, peptone, soy peptone, beef extract, casein peptone and tryptone

However, maltodextrin, soluble starch and sucrose were not as good as glucose and lactose in promoting the growth of strain L20, and glucose and lactose could be selected as carbon sources in subsequent experiments.

As can be seen from Figure 6 (b), urea and ammonium citrate have the most significant effect on the growth of *L. rhamnosus* L20 among the inorganic nitrogen sources. The OD value, pH value and dry weight of urea were 1.96, 3.21 and 2.07 g/L, respectively. The OD value, pH value and dry weight of ammonium citrate were 1.843, 3.37 and 2.0105 g/L, respectively. The OD value and dry weight of the cells were higher than those of other inorganic nitrogen sources, and the pH value was lower than that of other inorganic nitrogen sources. It was determined that urea and ammonium citrate were selected as inorganic nitrogen sources for follow-up tests.

The prebiotic galactooligosaccharides and inulin had the most significant effect on the growth of *L. rhamnosus* L20 (Figure 6 c). The OD value, pH value and dry weight of galacto-oligosaccharides were 1.81, 3.21 and 2.07 g/L, respectively. The OD value, pH value and dry weight of inulin were 1.76, 3.27 and 2.05 g/L, respectively. The OD value and dry weight of the bacteria were higher than those of other prebiotics, and the pH value was lower than that of other prebiotics. It was determined that galacto-oligosaccharides and inulin were selected as prebiotics in the follow-up experiments.

As can be seen from Figure 6 (d), dipotassium hydrogen phosphate and sodium acetate have the most significant effect on the growth of *L. rhamnosus* L20. The OD value, pH value and dry weight of potassium phosphate were 1.977, 3.20 and 2.0928 g/L, respectively. The OD value, pH value and dry weight of sodium acetate were 2.1245, 3.11 and 2.1395 g/L, respectively. The OD value and dry weight of the bacteria were higher than those of other salts, and the pH value was lower than that of other salts. Follow-up tests were determined to select dipotassium hydrogen phosphate and sodium acetate.

In Fig. 6 (e), the nitrogen source combination of soy peptone and beef extract had the most significant effect on the growth of *L. rhamnosus* L20, and its OD value, pH value and dry weight were 1.9165, 3.28 and 2.1937 g/L, respectively.

In summary, by comparing the promotional effects of each nutrient on the dry weight, OD value and pH of *L. rhamnosus* L20, the suitable selenium-enriched medium for L20 was screened as nitrogen source of glucose and lactose, organic nitrogen source of soybean peptone and beef dip powder, inorganic nitrogen source of urea and trinitrammonium citrate, prebiotic of oligogalactose and inulin, and salts of dipotassium hydroxide phosphate and sodium acetate.

Hui Jing et al (Hu, Yu, Hu, 2016) reported the optimal medium for *L. rhamnosus* as: glucose 13.33 g, yeast powder 26.67 g, dipotassium hydrogen phosphate 2.00 g, sodium acetate 5.00 g, magnesium sulphate 0. 58 g, manganese sulphate 0.25 g, and citrate diamine 2.00 g. At this time, the highest OD value was measured, the density of bacteria was 0.343, and the wet weight of bacteria was 1.325g after 24h of incubation on this medium, and the total number of bacteria could reach 1.57×10^{12}/g. Wang Zhiding et al (Wang, Liu, Li, 2013) obtained a better medium for *L. rhamnosus* by one-way test and orthogonal analysis as 2.5% glucose, 1.5% yeast powder, 2.5% tryptone, 0.20% diammonium hydroxycitrate, 0.2% dipotassium hydrogen phosphate, and 0.5% anhydrous sodium acetate, and the maximum number of the bacterial organisms was reached at 16 h of incubation and could reach up to 9.4×10^{9} CFU/mL

Hu Bo et al (Hu, Chen, Nan, Liang, 2010) carried out bacterial enrichment culture study on *L. rhamnosus*, and optimized the medium as 65g/L glucose, 15g/L yeast powder, 28g/L beef meal, 30g/L soybean peptone, 43g/L potassium dihydrogen phosphate, 3g/L anhydrous sodium acetate, and 2g/L ammonium citrate, and the viable bacterial counts were measured to be increased from 10^{8}CFU/mL before optimization to 10^{10}CFU/mL before optimization. The optimal medium for *L. rhamnosus* was obtained from the test by the authors. The reason for the differences in the growth of nutrients on *L. rhamnosus* may be the differences in strains, the genetic background of *L. rhamnosus* from different sources differ in their ability to utilize nutrients and their needs. At the same time, the enzyme system in the bacterial cell has different metabolic ability to different carbon sources, and different nitrogen sources need to be transformed by different metabolic pathways, and their metabolic efficiency of carbon and nitrogen sources and other nutrients are different, thus affecting the optimal combination of culture medium. In addition, different environmental conditions will affect the enzyme activity, thus affecting the metabolism and utilization of nutrients; for different applications, the performance requirements for strains are different, which also affects the optimization direction of the medium. In conclusion, the differences in the composition and content of the optimal culture medium for *L. rhamnosus* are the result of a variety of factors, and in the process of practical application, it is necessary to optimize and adjust the medium according to the specific strains, cultivation conditions and purposes, in order to obtain the best cultivation effect.

CONCLUSION

Sodium selenite concentration and fermentation time had significant effects on the growth and selenium conversion rate of *L. rhamnosus* L20. The optimal sodium selenite concentration for conversion of inorganic selenium to organic selenium by *L. rhamnosus* L20 was 20 μg/mL, the selenium conversion rate was 88.83%, and the number of viable bacteria was 2.34×10^8 CFU/mL. The suitable nitrogen sources of selenium-rich medium for this strain were glucose and lactose, the organic nitrogen sources were soy peptone and beef extract, the inorganic nitrogen sources were urea and ammonium citrate, the prebiotics were galacto-oligosaccharides and inulin, and the salts were dipotassium hydrogen phosphate and sodium acetate. The results of this study provide a reference for the further optimization of *L. rhamnosus* L20 media.

ACKNOWLEDGMENTS

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