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Enhancement of Exopolysaccharide Production by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 with a Simplified Defined Medium

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The aim of this work was to investigate the medium requirements for growth and production of exopolysaccharides by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772. The strain was grown in batch cultures on a chemically defined medium, and the technique of single omission of medium components was applied to determine the nutritional requirements. The omission of aspartic acid, glutamic acid, or glycine affected growth only slightly, and the omission of glutamine, asparagine, or threonine resulted in a stronger reduction of the growth. All the other amino acids were essential. Multiple omissions of amino acids caused an almost complete loss of growth. *L. delbrueckii* subsp. *bulgaricus* required only riboflavin, calcium pantothenate, and nicotinic acid as individual vitamins. Surprisingly, when only these vitamins were present in the medium and other vitamins were not, less growth was observed than in the complete medium but the amount of exopolysaccharide produced was significantly greater. These observations were studied in more detail with a simplified defined medium in which *L. delbrueckii* subsp. *bulgaricus* was able to grow and produce exopolysaccharides. Although the final optical density in the simplified medium was lower, the production of exopolysaccharides was about twofold higher than in the complete medium.

The thermophilic lactic acid bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* are important in the dairy industry, since they are used in the fermentation of milk to form yogurt. Several strains which are capable of forming exopolysaccharides (EPS), which give a higher viscosity and a thicker texture to the product, have been isolated and analyzed (1–3, 9, 16, 24, 26). Since the addition of stabilizers of animal or plant origin to natural yogurts is prohibited in France and The Netherlands and since there is a growing popularity for food products without additions, the utilization of EPS-producing lactic acid bacteria has gained more popularity.

For optimal growth, lactic acid bacteria require very complex media like milk, whey ultrafiltrate, or complex synthetic media such as MRS broth (6) and M17 broth (25). However, for the investigation of the physiological background of EPS production in lactic acid bacteria, a chemically defined medium is required, and the exact nutritional requirements of the lactic acid bacteria remain unknown when complex media are used. A chemically defined medium containing a carbohydrate source, mineral salts, amino acids, vitamins, and nucleic acid bases is therefore more suitable to investigate the influence of nutrients on the growth, the metabolic pathways, and the synthesis of EPS in these bacteria. Recently, a simple synthetic growth medium for some lactic acid bacteria was formulated. These bacteria belong to the genera *Lactococcus* (5, 13), *Streptococcus* (21), and *Leuconostoc* (8). However, for *L. delbrueckii* subsp. *bulgaricus*, the minimal requirements are still unknown and the effect of medium composition other than the carbohydrate source on EPS production has not yet been elucidated. A synthetic culture medium in which several lactobacilli were able to grow has been formulated (15), but the growth requirements of *L. delbrueckii* subsp. *bulgaricus* were only partially investigated.

The use of chemically defined media is particularly important when the quantitative and qualitative production of EPS by lactic acid bacteria and the regulation of EPS synthesis are being studied. Previously, we used a chemically defined medium which contained a carbohydrate source, mineral salts, amino acids, nucleic acid bases and vitamins (10). In the present work we have simplified and optimized this medium for growth and EPS production by using the technique of the omission of a single medium component, which is generally used to investigate the requirements for medium components (22). By using this technique, we formulated a simplified chemically defined medium in which *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 was able to produce increased levels of EPS. Previously, it has been possible to study only the effect of the carbohydrate source. The strain metabolized only four carbohydrates, glucose, lactose, fructose, and mannose, and the production of EPS was dependent on the carbohydrate source. The strain produced considerably larger amounts of EPS when grown on glucose or lactose than when grown on fructose to equal cell densities. Compared with growth on the other carbohydrate sources, growth on mannose led to much lower levels of growth and EPS production (10, 11).

A defined medium is also very useful in the investigation of the composition of the EPS produced. It was found that complex media like MRS strongly interfere with the isolation procedure of macromolecules like EPS (9) and that the exact amount and composition of the EPS produced are unclear when these complex media are used. In the chemically defined

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medium used in our previous work, *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 grown on glucose produced two EPS fractions concurrently and in almost equal amounts; these two fractions differed in molecular weight and composition of the EPS repeating unit. The high-molecular-weight fraction (molecular weight, 1.7×10^6) contained glucose, galactose, and rhamnose at a ratio of 1:5:1, and the low-molecular-weight fraction (molecular weight, 4×10^4) was composed of glucose, galactose, and rhamnose at a ratio of 1:11:0.4. When fructose was used as the sole carbohydrate source, a low-molecular-weight EPS fraction was the major product (12). It is possible that not only the carbohydrate source but also other medium components affect the composition of the EPS.

In this work, the effect of the omission of medium components on the growth of and EPS production by *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 was investigated and a simplified defined medium, in which the strain produced a considerably larger amount of EPS, was formulated.

MATERIALS AND METHODS

Bacterial strain and culture medium. *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 was obtained from the National Collection of Food Bacteria (Reading, United Kingdom). Cultures were stored at −80°C in MRS medium (6) plus 15% glycerol until required and were reactivated in MRS medium at 37°C and initial pH 6.0 for 16 h. Growth experiments were performed in a chemically defined medium in which growth of and EPS production by the strain were shown previously (10); this medium contained 7.4 mM KH_2PO_4, 14.1 mM Na_2HPO_4, 4.3 mM citric acid, 82.3 mM sodium acetate, 18.7 mM NH_4Cl, 0.8 mM $MgSO_4 \cdot 7H_2O$, 0.3 mM $MnCl_2 \cdot 4H_2O$, 1 ml of Tween 80 per liter, 0.07 mM adenine, 0.07 mM guanine, 0.09 mM uracil, 0.07 mM xanthine, 1.1 mM L-alanine, 0.5 mM L-arginine, 0.7 mM L-asparagine, 0.8 mM L-aspartic acid, 2.5 mM L-cysteine, 0.7 mM L-glutamine, 0.7 mM L-glutamic acid, 1.3 mM glycine, 0.6 mM L-histidine, 0.8 mM L-isoleucine, 0.8 mM L-leucine, 0.6 mM L-lysine hydrochloride, 0.7 mM L-methionine, 0.6 mM L-phenylalanine, 0.9 mM L-proline, 1.0 mM L-serine, 0.8 mM L-threonine, 0.5 mM L-tryptophan, 0.6 mM L-tyrosine, 0.9 mM L-valine, 1 ml of a trace element solution (81.1 mM HCl, 0.8 mM $CoCl_2 \cdot 6H_2O$, 0.01 mM $CuCl_2 \cdot 2H_2O$, 7.5 mM $FeCl_2 \cdot 4H_2O$, 0.1 mM H_3BO_3, 0.2 mM $Na_2MoO_4 \cdot 2H_2O$, 0.1 mM $NiCl_2$, 0.5 mM $ZnCl_2$) per liter, and 1 ml of a vitamin solution (0.7 mM *p*-aminobenzoic acid, 0.08 mM biotin, 0.1 mM folic acid, 0.24 mM lipoic acid, 1.6 mM nicotinic acid, 0.4 mM calcium pantothenate, 2.1 mM pyridoxamine, 0.3 mM pyridoxine, 0.27 mM riboflavin, 0.6 mM thiamine, 0.07 mM vitamin B_{12}) per liter. Glucose was used as the carbohydrate source at an initial concentration of 111 mM unless otherwise stated. The defined medium was sterilized by being passed through a 0.2-μm-pore-size sterile filter (Schleicher & Schuell, Dassel, Germany, or Gelman Sciences, Ann Arbor, Mich.).

Growth, EPS isolation, and EPS characterization. Batch cultivations were performed in unshaken, nitrogen-flushed sealed bottles (volume, 115 ml) containing 50 ml of defined medium at 37°C and initial pH 6.0, unless otherwise stated. Growth was monitored after 48 h by measurement of the optical density at 600 nm (OD_{600}). EPS were isolated as described previously (10), the total carbohydrate content of the isolated EPS was measured by the phenol-sulfuric acid method of Dubois et al. (7), and the sugar composition of the EPS was determined after hydrolysis by high-performance liquid chromatography (10). The molecular weight of the EPS was measured by high-performance size exclusion chromatography as described previously (12).

Growth experiments to evaluate nutritional requirements. To identify the nutritional requirements of *L. delbrueckii* subsp. *bulgaricus* NCFB 2772, the strain was grown in batch cultures with defined medium and the technique of omission of one of the medium components was used. Before being used for growth experiments, cells grown in defined medium were centrifuged (15,000 × *g* for 10 min at 4°C), washed twice in 20 mM phosphate buffer (pH 6.0), resuspended in the same buffer, and finally added to medium from which one or more of the medium components had been omitted. In these test media, the strain was subcultured three times in succession by adding 0.5 ml of a culture incubated for 48 h to 50 ml of fresh medium. After this, the OD_{600} of the third subcultures were compared with the OD_{600} of a positive control without nutrient omissions. These experiments were performed in triplicate.

Simplified defined medium for growth and EPS production. Based on the results of the analyses of the nutritional requirements of *L. delbrueckii* subsp. *bulgaricus* NCFB 2772, a simplified medium containing all the components necessary for growth and EPS production by the strain was synthesized. Growth and EPS production were investigated in a pH-controlled batch culture, using a glass fermentor (Applikon, Schiedam, The Netherlands) with a working volume of 1.5 liters at 40°C, pH 6.1 ± 0.1, 50 rpm, and on N_2 atmosphere. Glucose was used as the carbohydrate source at an initial concentration of 166 mM. Growth and EPS production by *L. delbrueckii* subsp. *bulgaricus* in the simplified medium were compared with growth and EPS production under the same conditions in the complete medium.

TABLE 1. Nutrient requirements of *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 in defined medium investigated by omission of a single medium component

Omitted medium component	OD_{600}[a]	Omitted medium component	OD_{600}[a]
None	2.0 ± 0.18	L-Isoleucine	0.3 ± 0.10
Phosphate	0.0 ± 0.02	L-Leucine	0.0 ± 0.01
Citric acid	1.3 ± 0.21	L-Lysine	0.0 ± 0.02
Sodium acetate	0.1 ± 0.03	L-Methionine	0.3 ± 0.07
NH_4Cl	1.8 ± 0.18	L-Phenylalanine	0.3 ± 0.08
$MgSO_4$	0.0 ± 0.01	L-Proline	0.0 ± 0.00
$MnCl_2$	0.2 ± 0.04	L-Serine	0.0 ± 0.01
Tween 80	0.7 ± 0.02	L-Threonine	0.8 ± 0.06
Trace elements	1.9 ± 0.26	L-Tryptophan	0.2 ± 0.03
		L-Tyrosine	0.3 ± 0.05
Adenine	0.9 ± 0.09	L-Valine	0.0 ± 0.00
Guanine	2.1 ± 0.21		
Uracil	0.0 ± 0.02	*p*-Aminobenzoic acid	2.1 ± 0.22
Xanthine	2.1 ± 0.17	Biotin	2.2 ± 0.12
		Folic acid	1.9 ± 0.15
L-Alanine	0.0 ± 0.00	Lipoic acid	1.9 ± 0.22
L-Arginine	0.0 ± 0.02	Nicotinic acid	0.0 ± 0.00
L-Asparagine	0.9 ± 0.04	Calcium pantothenate	0.4 ± 0.10
L-Aspartic acid	1.5 ± 0.17	Pyridoxamine	2.2 ± 0.06
L-Cysteine	0.4 ± 0.08	Pyridoxine	2.3 ± 0.15
L-Glutamic acid	1.6 ± 0.17	Riboflavin	0.6 ± 0.10
L-Glutamine	1.3 ± 0.11	Thiamine	2.3 ± 0.24
Glycine	1.6 ± 0.10	Vitamin B_{12}	1.6 ± 0.12
L-Histidine	0.1 ± 0.02		

[a] OD measurements were performed after 48 h of incubation. Values are the means ± standard deviations of triplicate measurements.

RESULTS

Gas requirement. The growth of *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 was not significantly affected by the composition of the gas phase. Growth and EPS production under a nitrogen atmosphere, an N_2-CO_2 atmosphere with different ratios, and air were comparable. Growth was lower in the presence of 100% O_2 in shaken sealed bottles (volume, 115 ml) with 25 ml of medium in a horizontally shaking water bath at 37°C, resulting in a final OD_{600} of 0.9; however, glucose fermentation pattern and EPS production related to growth were not affected.

Nutritional requirements for growth. It appeared that most amino acids were essential for the growth of *L. delbrueckii* subsp. *bulgaricus* NCFB 2772. The single omission of aspartic acid, glutamic acid, or glycine affected growth only slightly, whereas the omission of asparagine, glutamine, or threonine resulted in a stronger reduction of growth (Table 1). No growth was observed when any of the other amino acids was omitted from the growth medium. The strain did not grow when only the essential amino acids were present. When a growth medium without aspartic acid, glutamic acid, and glycine was used, almost no growth was observed after 48 h but the OD_{600} of the culture increased to 0.9 after 96 h. Omissions of single or multiple amino acids did not affect the amount of EPS relative to the cell density or the sugar composition of the EPS (data not shown). The same was observed when guanine or xanthine was omitted from the growth medium. On the other hand, the omission of uracil prevented growth completely and the omission of adenine resulted in a reduction of the OD_{600} and EPS production by approximately 50%.

The omission of ammonium chloride from the growth me-

TABLE 2. Effect of individual and multiple omission of nonessential vitamins on the growth of and EPS production by *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 in defined medium

Omission	OD_{600}[a,b]	EPS production (mg/liter)[b]	Specific EPS production (mg of EPS/mg of cell dry weight)[c]
None	2.0 ± 0.18	43.3 ± 1.0	28.9
p-Aminobenzoic acid	2.1 ± 0.22	35.2 ± 3.4	22.3
Biotin	2.2 ± 0.12	39.3 ± 2.6	23.8
Folic acid	1.9 ± 0.15	40.0 ± 3.9	28.1
Lipoic acid	1.9 ± 0.22	32.9 ± 2.6	23.1
Pyridoxamine	2.2 ± 0.06	34.4 ± 2.7	20.8
Pyridoxine	2.3 ± 0.15	55.4 ± 3.4	32.1
Thiamine	2.3 ± 0.24	39.1 ± 2.9	22.7
Vitamin B_{12}	1.6 ± 0.12	40.0 ± 3.5	33.3
All nonessential vitamins[d]	1.1 ± 0.10	41.1 ± 3.7	49.8

[a] OD measurements were performed after 48 h of incubation.
[b] Values are the means ± standard deviations of at least triplicate measurements.
[c] The specific EPS production was calculated from a standard curve of OD_{600} against cell dry weight (10).
[d] All nonessential vitamins: *p*-aminobenzoic acid, biotin, folic acid, lipoic acid, pyridoxamine, pyridoxine, thiamine, and vitamin B_{12}.

dium had only a small effect on the growth of *L. delbrueckii* subsp. *bulgaricus*. The strain grew well when the trace elements ($FeCl_2$, $CoCl_2$, $ZnCl_2$, H_3BO_3, Na_2MoO_4, $NiCl_2$, and $CuCl_2$) were omitted individually or all at once. Sodium acetate, $MgSO_4$, and $MnCl_2$ appeared to be essential for growth. The omission of Tween 80 or citric acid decreased growth. Addition of $CaCl_2$ (10 μM) to the growth medium had no effect. In all these omission experiments, growth and EPS production were proportional and no changes in the EPS sugar composition were observed. The addition of 20 mM morpholinepropanesulfonic acid (MOPS) buffer together with a 10-fold-lower level of phosphate did not affect the growth and EPS production of the strain significantly. When the vitamins were omitted individually from the growth medium, only riboflavin, calcium pantothenate, and nicotinic acid appeared to be essential for growth whereas a slightly lower OD_{600} was found when vitamin B_{12} was omitted.

Enhanced EPS production by multiple-vitamin omission. A single omission of the nonessential vitamins did not change the specific EPS production, but when *L. delbrueckii* subsp. *bulgaricus* was grown in a medium containing no vitamins except riboflavin, calcium pantothenate, and nicotinic acid, the OD_{600} of the cultures was much lower after 48 h whereas the specific EPS production increased significantly (Table 2). Omission of single vitamins did not change the monomeric sugar composition of the EPS. Omission of multiple vitamins resulted in EPS with a slightly lower content of galactose monomer.

In view of the nutritional requirements, a simplified medium was formulated for growth and EPS production by *L. delbrueckii* subsp. *bulgaricus* NCFB 2772. Compared to the complete growth medium, the simplified medium contained no NH_4Cl, trace elements, guanine, xanthine, or vitamins except for riboflavin, calcium pantothenate, and nicotinic acid. Since the omission of multiple amino acids strongly reduced the growth of the strain, all the amino acids were included. A pH-controlled batch culture experiment was performed to compare growth and EPS production by strain NCFB 2772 in the complete medium and the simplified medium. In the simplified medium, strain NCFB 2772 was able to grow with a maximal growth rate of 0.18 h^{-1} and a final OD_{600} of 1.4 and produced 250 mg of EPS per liter when grown in a pH-controlled batch culture. On the other hand, in the complete medium the growth rate was 0.23 h^{-1} and the final OD_{600} was 2.3, while the EPS production was only 130 mg/liter (Fig. 1). It was observed that in both the complete medium and the simplified medium, EPS production continued after growth had ceased, but beyond the stationary growth phase significantly more EPS was produced in the simplified medium than in the complete medium. In the simplified medium, the EPS produced had a monomeric sugar composition of glucose, galactose, and rhamnose in a ratio of 1:5.7:0.8, whereas in the complete medium this ratio was 1:6.3:0.8. Molecular weight analysis of the isolated EPS showed the presence of two fractions with molecular weights of 1.4×10^6 and 7.0×10^4. In both the EPS produced in the complete medium and the EPS produced in the simplified medium, the high-molecular-weight fraction was present at a twofold higher concentration than was the low-molecular-weight fraction.

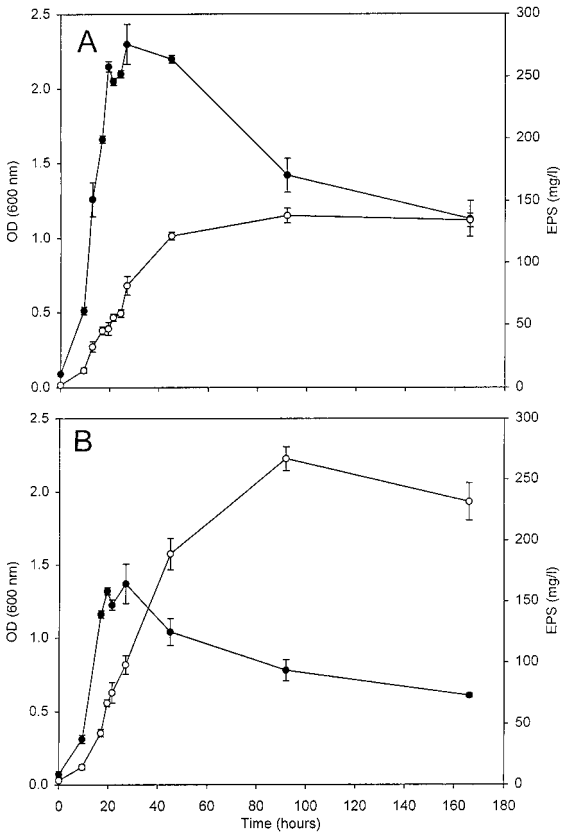

FIG. 1. Growth of and EPS production by *L. delbrueckii* subsp. *bulgaricus* in pH-controlled batch cultures at 40°C and pH 6.0 ± 0.1 in complete defined medium (A) and simplified medium (B). Each value represents the mean of duplicate measurements and varied from the mean by not more than 10%. ●, OD_{600}; ○, EPS production.

DISCUSSION

L. delbrueckii subsp. *bulgaricus* NCFB 2772 produces EPS when grown in a defined medium supplemented with a carbohydrate source. The amount of the EPS produced was thought to be affected only by the growth temperature or the carbohydrate source, and it was believed that EPS production was strictly coupled to growth (10, 11). In this work, the nutritional requirements of the strain were investigated to determine

whether other nutrients can affect the growth of and EPS production by the strain.

Use of the technique of single-component omission gave a clear indication of the nutrient requirement for the growth of *L. delbrueckii* subsp. *bulgaricus* NCFB 2772. Nevertheless, the strain did not grow if all the components which were individually not required were omitted. For instance, a single omission of either aspartic acid, glutamic acid, or glycine resulted in good growth of the culture, whereas growth was poor when these three amino acids were all absent. Since nitrogen metabolism in this strain is not well understood yet, it is difficult to predict the combined effect of amino acids in the growth medium. Compared to other lactic acid bacteria such as *Lactobacillus plantarum* (23), *Lactococcus lactis* (13), and *Streptococcus thermophilus* (21), *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 required more amino acids. In contrast to the findings of Ledesma et al. (15), who proposed that a requirement for glutamic acid, valine, and leucine is a taxonomic criterion for the genus *Lactobacillus*, *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 grew well in the absence of glutamic acid. The omission of single or multiple amino acids had no effect on the production of EPS relative to cell growth. Regarding the vitamin requirements, it appeared that only nicotinic acid, calcium pantothenate, and riboflavin were essential for growth. Ledesma et al. (15) found that *L. delbrueckii* subsp. *bulgaricus* ATCC 9224 required nicotinic acid and calcium pantothenate for growth, since these vitamins are involved in coenzyme biosynthesis by lactobacilli. Riboflavin, a component of flavin coenzymes, appears to be essential for the growth of lactic acid bacteria (5).

Multiple omission of all nonessential vitamins reduced the growth of the strain but, surprisingly and in contrast to the results obtained for the amino acids, affected the production of EPS strongly. For the first time, it was observed that EPS production in a lactic acid bacterium was affected by a growth factor other than the carbohydrate source, temperature, or pH and that the regulation of the EPS production beyond the stationary growth phase was influenced by the medium composition. Up to now, nothing was known about the relationship between the vitamin requirement and EPS production in lactic acid bacteria. Cerning et al. (4) and Kojic et al. (14) found that *L. casei* CG11 produces EPS when grown in a basal minimal medium (19) containing only folic acid, nicotinic acid, calcium pantothenate, pyridoxine, and riboflavin. The EPS production by *L. casei* CG11 continued beyond the stationary growth phase. *L. delbrueckii* subsp. *bulgaricus* CRL 420 also produced some EPS after growth had ceased (16), but this organism was grown in a complex medium, so that the influence of vitamins on the EPS production by this strain is unknown. We demonstrated that only the total amount of EPS produced was affected by the omission of multiple vitamins and that the ratio of the high-molecular-weight fraction and the low-molecular-weight fraction of the EPS was not affected, as was found when fructose instead of glucose was used as the carbohydrate source (12). This means that the omission of multiple vitamins affected the production of both the high-molecular-weight fraction and the low-molecular-weight fraction, in contrast to the carbohydrate source, whose effect was mainly on the production of the high-molecular-weight EPS fraction (12).

It was found that EPS production under controlled pH was significantly higher than in acidified batch cultures (Table 2; Fig. 1). This was also observed in our previous work (10), and it was found that *L. casei* also produced considerably more EPS when grown under constant pH than without pH control (20). Higher EPS production as a result of maintaining the pH of the culture medium at a constant value was also reported for *Xanthomonas campestris* (18) and *Pseudomonas* sp. strain EPS-5028 (17).

In conclusion, a simplified defined medium was formulated in which *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 grew less well than in the complete defined medium but produced about twice as much EPS and in which the EPS production continued strongly after cell growth had ceased. The single-omission technique appeared to be indirectly successful. By using this technique, the nutritional requirements were found and omission of multiple vitamins resulted in enhanced EPS production, although it is unclear which factor is responsible for this enhancement. More investigations on the physiological effects of vitamins on the production of EPS will be necessary for a better understanding of the mechanisms of EPS production.

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REFERENCES

1. **Cerning, J.** 1990. Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiol. Rev. **87:**113–150.
2. **Cerning, J., C. Bouillanne, M. J. Desmazeaud, and M. Landon.** 1986. Isolation and characterization of exocellular polysaccharide produced by *Lactobacillus bulgaricus*. Biotechnol. Lett. **8:**625–628.
3. **Cerning, J., C. Bouillanne, M. Landon, and M. J. Desmazeaud.** 1988. Exocellular polysaccharide production by *Streptococcus thermophilus*. Biotechnol. Lett. **10:**255–260.
4. **Cerning, J., C. M. G. C. Renard, J. F. Thibault, C. Bouillanne, M. Landon, M. Desmazeaud, and L. Topisirovic.** 1994. Carbon source requirement for exopolysaccharide production by *Lactobacillus casei* CG11 and partial structure analysis of the polymer. Appl. Environ. Microbiol. **60:**3914–3919.
5. **Cocaign-Bousquet, M., C. Garrigues, L. Novak, N. D. Lindley, and P. Loubiere.** 1995. Rational development of a simple synthetic medium for the sustained growth of *Lactococcus lactis*. J. Appl. Bacteriol. **79:**108–116.
6. **De Man, J. C., M. Rogosa, and M. E. Sharpe.** 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. **23:**130–135.
7. **Dubois, M., K. A. Gilles, J. K. Hamilton, and P. A. Rebers.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28:**350–356.
8. **Foucaud, C., A. Francois, and J. Richard.** 1997. Development of a chemically defined medium for the growth of *Leuconostoc mesenteroides*. Appl. Environ. Microbiol. **63:**301–304.
9. **Garcia-Garibay, M., and V. M. E. Marshall.** 1991. Polymer production by *Lactobacillus delbrueckii* ssp. *bulgaricus*. J. Appl. Bacteriol. **70:**325–328.
10. **Grobben, G. J., J. Sikkema, M. R. Smith, and J. A. M. de Bont.** 1995. Production of extracellular polysaccharides by *Lactobacillus delbrueckii* ssp. *bulgaricus* NCFB 2772 grown in a chemically defined medium. J. Appl. Bacteriol. **79:**103–107.
11. **Grobben, G. J., M. R. Smith, J. Sikkema, and J. A. M. de Bont.** 1996. Influence of fructose and glucose on the production of exopolysaccharides and the activities of enzymes involved in the sugar metabolism and the synthesis of sugar nucleotides in *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772. Appl. Microbiol. Biotechnol. **46:**279–284.
12. **Grobben, G. J., W. H. M. van Casteren, H. A. Schols, A. Oosterveld, G. Sala, M. R. Smith, J. Sikkema, and J. A. M. de Bont.** 1997. Analysis of exopolysaccharides produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 grown in continuous culture on glucose and fructose. Appl. Microbiol. Biotechnol. **48:**516–521.
13. **Jensen, P. R., and K. Hammer.** 1993. Minimal requirements for exponential growth of *Lactococcus lactis*. Appl. Environ. Microbiol. **59:**4363–4366.
14. **Kojic, M., M. Vujcic, A. Banina, P. Cocconcelli, J. Cerning, and L. Topisirovic.** 1992. Analysis of exopolysaccharide production by *Lactobacillus casei* CG11, isolated from cheese. Appl. Environ. Microbiol. **58:**4086–4088.
15. **Ledesma, O. V., A. P. de Ruiz Holgado, G. Oliver, G. S. de Giori, P. Raibaud, and J. V. Galpin.** 1977. A synthetic medium for comparative nutritional studies of lactobacilli. J. Appl. Bacteriol. **42:**123–133.
16. **Manca de Nadra, M. C., A. M. Strasser de Saad, A. A. Pesce de Ruiz Holgado, and G. Oliver.** 1985. Extracellular polysaccharide production by *Lactobacillus bulgaricus*. Milchwissenschaft **40:**409–411.
17. **Marqués, A. M., I. Estañol, J. M. Alsina, C. Fusté, D. Simon-Pujol, J. Guinea, and F. Congregado.** 1986. Production and rheological properties of the extracellular polysaccharide synthesized by *Pseudomonas* sp. strain EPS-5028. Appl. Environ. Microbiol. **52:**1221–1223.
18. **Moraine, R. A., and P. Rogovin.** 1973. Kinetics of the xanthan fermentation. Biotechnol. Bioeng. **15:**229–238.

19. **Morishita, T., Y. Deguchi, M. Yajima, T. Sakurai, and T. Yura.** 1981. Multiple nutritional requirements of lactobacilli. Genetic lesions affecting amino acid biosynthetic pathways. J. Bacteriol. **148:**64–71.
20. **Mozzi, F., G. S. de Giori, G. Oliver, and G. F. de Valdez.** 1996. Exopolysaccharide production by *Lactobacillus casei* under controlled pH. Biotechnol. Lett. **18:**435–439.
21. **Neviani, E., G. Giraffa, A. Brizzi, and D. Carminati.** 1995. Amino acid requirements and peptidase activities of *Streptococcus salivarius* subsp. *thermophilus*. J. Appl. Bacteriol. **79:**302–307.
22. **Reiter, B., and J. D. Oram.** 1962. Nutritional studies on cheese starters. I. Vitamin and amino acid requirements of single strain starters. J. Dairy Res. **29:**63–77.
23. **Ruiz-Barba, J. L., and R. Jiménez-Diaz.** 1994. Vitamin and amino acid requirements of *Lactobacillus plantarum* strains isolated from green olive fermentations. J. Appl. Bacteriol. **76:**350–355.
24. **Schellhaass, S. M.** 1983. Characterization of exocellular slime produced by bacterial starter cultures used in the manufacture of fermented dairy products. Ph.D. thesis. University of Minnesota, St. Paul.
25. **Terzaghi, B., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. **29:**807–813.
26. **Wacher-Rodarte, C., M. V. Calvan, A. Farres, F. Gallardo, V. M. E. Marshall, and M. Garcia-Garibay.** 1993. Yogurt production from reconstituted skim milk powders using different polymer and non-polymer forming starter cultures. J. Dairy Res. **60:**247–254.