APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1995, p. 2840–2844 Vol. 61, No. 8
0099-2240/95/$04.00+0

Production of a Novel Extracellular Polysaccharide by *Lactobacillus sake* 0-1 and Characterization of the Polysaccharide

DICK J. C. VAN DEN BERG,[1] GERARD W. ROBIJN,[2] ANNETTE C. JANSSEN,[3] MARCO L. F. GIUSEPPIN,[3] ROB VREEKER,[3] JOHANNIS P. KAMERLING,[2] JOHANNES F. G. VLIEGENTHART,[2] AAT M. LEDEBOER,[3]* AND C. THEO VERRIPS[3]

Department of Molecular Cell Biology[1] and Department of Bio-Organic Chemistry,[2] State University of Utrecht, 3584 CH Utrecht, and Department of Gene Technology & Fermentation, Unilever Research Laboratorium Vlaardingen, 3133 AT Vlaardingen,[3] The Netherlands

Received 11 January 1995/Accepted 17 May 1995

A novel exopolysaccharide (EPS) produced by *Lactobacillus sake* 0-1 (CBS 532.92) has been isolated and characterized. When the strain was grown on glucose, the produced EPS contained glucose and rhamnose in a molar ratio of 3:2 and the average molecular mass distribution (M_m) was determined at 6×10^6 Da. At a concentration of 1%, the 0-1 EPS had better viscosifying properties than xanthan gum when measured over a range of shear rates from 0 to 300 s^{-1}, while shear-thinning properties were comparable. Rheological data and anion-exchange chromatography suggested the presence of a negatively charged group in the EPS. Physiological parameters for optimal production of EPS were determined in batch fermentation experiments. Maximum EPS production was 1.40 g · $liter^{-1}$, which was obtained when *L. sake* 0-1 had been grown anaerobically at 20°C and pH 5.8. When cultured at lower temperatures, the EPS production per gram of biomass increased from 600 mg at 20°C to 700 mg at 10°C but the growth rate in the exponential phase decreased from 0.16 to 0.03 g · $liter^{-1}$ · h^{-1}. EPS production started in the early growth phase and stopped when the culture reached the stationary phase. Growing the 0-1 strain on different energy sources such as glucose, galactose, mannose, fructose, lactose, and sucrose did not alter the composition of the EPS produced.

Many bacteria are known to produce exopolysaccharides (EPSs) that either are excreted in the growth medium or remain attached to the bacterial cell wall, thus forming capsular EPS (17). Over the last 20 years, extensive studies on EPS production have focused mainly on *Xanthomonas campestris*, *Rhizobium* spp., *Klebsiella* spp., *Pseudomonas* spp., *Acetobacter* spp., and *Escherichia coli* (17–19, 23), whereas relatively little attention has been paid to EPS production by lactic acid bacteria (LAB). However, recently a growing interest has been observed in EPS-producing LAB, especially because LAB are food grade and of major importance for the food industry. Polysaccharides can serve as viscosifying, stabilizing, emulsifying, gelling, or water-binding agents in a variety of food products and LAB might serve as an excellent source of food grade polysaccharides.

There exists a large variety of EPSs produced by an equally large number of LAB (1). Most of these strains were isolated from dairy products, but fermented meat also served as a source for EPS-producing LAB strains (12). Some EPS-producing LAB strains have been investigated in more detail, e.g., *Lactococcus lactis* isolated from various Scandinavian "ropy" fermented-milk products like Viili (10, 11, 14), *Lactobacillus kefiranofaciens* isolated from kefir grains (21), *Lactobacillus delbrueckii* subsp. *bulgaricus* isolated from various yogurts (2, 7, 13), and *Streptococcus thermophilus*, also isolated from yogurt (3, 6). Structural analysis combined with rheological studies revealed that there is considerable variation among the different EPSs. The monosaccharides most frequently occurring in the various EPSs are glucose and galactose, but rhamnose, fructose, mannose, galactosamine, and other sugars are also found. The production of intracellularly synthesized EPS by different LAB varies roughly from 50 to 350 mg · $liter^{-1}$ when the bacteria are grown under nonoptimized culture conditions (1).

To find a novel EPS with properties important for application in the food industry, an extensive screening of 600 LAB strains, which has revealed 30 EPS-producing LAB strains, has been carried out (22). Since the viscosity produced by *Lactobacillus sake*, which was isolated from a naturally fermented Belgium salami sausage, was the highest, one of these strains, called *L. sake* 0-1, was selected for further research. In this paper, we present data concerning sugar composition, molecular mass, and rheological characteristics of the purified 0-1 EPS, as well as growth characteristics and EPS production by *L. sake* 0-1 as a function of temperature and pH.

MATERIALS AND METHODS

Organism. *L. sake* 0-1 (deposited as CBS 532.92) was isolated from a naturally fermented Belgium salami sausage (22).

Media. *L. sake* 0-1 was routinely maintained in MRS broth (5). Stock cultures were made by mixing a pure culture that had been grown overnight with an equal volume of a 10% glycerol solution and storing the mixture at −80°C. These stock cultures were used as starting material for all the experiments. The semidefined medium (SDM) that was used for culturing *L. sake* 0-1 and for EPS production had the following composition (in grams · $liter^{-1}$): Na_2HPO_4, 10; KH_2PO_4, 12; ammonium citrate, 0.6; $MnSO_4$, 0.05; NZ-case Plus (Quest Chemicals), 20; glucose, 20; and yeast nitrogen base (Difco), 6.7. The pH of the medium after sterilization was 6.5. Glucose was autoclaved separately, while the yeast nitrogen base was dissolved in 100 ml of H_2O and filtered through 0.22-μm-pore-size Millex-GS filter units (Millipore). When cultures were grown under pH-controlled conditions, the buffering capacity of the medium was reduced to 1.0 g of Na_2HPO_4 and 1.2 g of KH_2PO_4 to allow a fast free acidification, due to lactate production, until the pH set point of each fermentation was reached.

Culture conditions. *L. sake* 0-1 stock culture was inoculated (1%, vol/vol) into 10 ml of fresh SDM. After incubation for 16 h (nonshaken, nonaerated) at 30°C,

* Corresponding author. Mailing address: Unilever Research Laboratorium, P.O. Box 114, 3130 AC Vlaardingen, The Netherlands. Phone: 3110-4605239. Fax: 3110-4605637.

this preculture was used for inoculating larger volumes of SDM. For pH-controlled batch fermentation experiments, a 2.5-liter-working-volume fermentation vessel with a six-blade disk turbine impeller (i.e., Rushton type) with a condenser for the outlet gas to reduce the evaporation of water was used. After a pH drop due to lactate production, the fermentation pH was maintained at its set point by titration with 4 M NaOH. Agitation was maintained at 200 rpm, and N_2 bubbling was controlled at 0.5 liter · min^{-1} to obtain anaerobic conditions.

Cell growth and determination of cell dry weight. Cell growth was monitored by measuring the optical density at 610 nm (OD_{610}) at constant time intervals during a fermentation experiment. A correlation between OD_{610} and biomass produced was determined by measuring the cell dry weight of the culture at different stages of the growth curve. To remove EPS from the bacterial cells, a sample of 20 ml was hydraulically sheared by forcing the viscous culture broth through a 20-ml syringe. The sample was centrifuged and washed three times in 10 mM phosphate buffer (pH 7.0). The pellet was dried at 80°C for 16 h and subsequently weighed.

Isolation and partial purification of the EPS. *L. sake* 0-1 was cultured in SDM for 2 days (nonshaken, nonaerated) at 20°C. To precipitate proteins, trichloroacetic acid was added to a final concentration of 4% and the culture was stirred for 2 h. Cells and precipitated proteins were removed by centrifuging for 30 min at 27,000 × *g*. The clear supernatant was collected, and the EPS was precipitated by adding 2 volumes of cold ethanol. The EPS was redissolved in ≈1/10 of the original volume of distilled H_2O and dialyzed for 2 to 3 days against distilled H_2O (changed twice each day). To remove any remaining undissolved material, the EPS solution was centrifuged and the clear supernatant was again precipitated with 2 volumes of ethanol. The resulting precipitate was dissolved in water and subsequently subjected to a fractionated precipitation at 30, 40, 50, 60, and 70% acetone, with intermediate centrifugation. The precipitated material collected from the 40% acetone fraction was further purified by gel filtration on a column (150 by 2.2 cm) of Sephacryl S-500 (Pharmacia) and eluted with 50 mM NH_4HCO_3, using refractive index monitoring (Bischoff 8100 RI detector). This material was lyophilized and stored at 4°C. To control the uniformity of the isolated EPS, a small amount of the purified polysaccharide (250 μg) was fractionated on a Superose-6 gel filtration column (30 by 1 cm; Pharmacia) and eluted with 50 mM NH_4HCO_3, using a Pharmacia fast protein liquid chromatography system with refractive index and UV monitoring (single-path monitor UV-1/280 nm, Pharmacia).

EPS quantification. Samples taken from growing cultures were centrifuged for 20 min at 35,000 × *g*. To remove any monosaccharides, 3 ml of the clear supernatant was dialyzed for 3 days against distilled H_2O. EPS was then quantified colorometrically by adding 1 ml of anthrone reagent (0.2 g of anthrone, 10 ml of H_2O, 90 ml of H_2SO_4) to 0.2 ml of the dialyzed supernatant. The reaction mixture was incubated for 15 min at 90°C and cooled to room temperature, and the OD_{620} was measured. The EPS concentration was calculated from a calibration curve of a 1-g · $liter^{-1}$ 99% pure 0-1 EPS standard solution.

HPLC analysis. During the fermentation experiments glucose, lactate, acetate, ethanol, and formate levels were measured at regular time intervals. High pressure liquid chromatography (HPLC) samples were prepared by diluting the supernatant of the centrifuged culture samples fivefold with H_2O. A 20-μl portion was injected into an Aminex HPX-87H column (300 by 7.8 mm). As the mobile phase, a 0.0025 M H_2SO_4 solution was used at a fixed flow rate of 1 ml · min^{-1}.

Rheology. Flow curves with increasing shear rates of up to 300 s^{-1} were measured at 25°C with a Haake Rotovisco RV100 (system CV100, sensor system ZA-30). Measurements at low shear rates, i.e., from 0 to 10 s^{-1}, were carried out at 25°C with a Carrimed CSL rheometer.

Anion-exchange chromatography. A 2-ml portion of a 0.5-mg · ml^{-1} EPS solution was transferred to a 5-ml column containing Dowex resin (AG 1-X2, 100 to 200 mesh, acetate form; Bio-Rad). The column was eluted with 5 volumes of water, yielding fraction 1, and with 5 volumes of a 1 M NH_4HCO_3 solution, yielding fraction 2. The fractions were lyophilized, and the sugar composition was determined.

Sugar composition analysis. The sugar composition of the isolated EPS was determined by methanolysis, followed by N-reacetylation and trimethylsilylation as described by Gruter et al. (8). The resulting trimethylsilylated (N-reacetylated) methyl glycosides were analyzed on an SE-30 fused-silica capillary column (25 m by 0.32 mm; Pierce) with a Varian 3700 gas chromatograph (temperature program, 130 to 220°C at 4°C · min^{-1}).

Average molecular mass determination. The average molecular mass (M_m) of the purified 0-1 EPS was determined by a method that combined gel permeation chromatography with capillary viscometry, low-angle light scattering, and differential refraction analysis as described by Tinland et al. (20).

RESULTS

Physical and compositional analysis. For studying the nature of the EPS, a pure isolate, free of proteins and other contaminating compounds, was required. Starting with a 20-liter *L. sake* 0-1 culture in SDM, we obtained 1.5 g of pure EPS material that was used for a physical characterization, with the emphasis on rheological behavior.

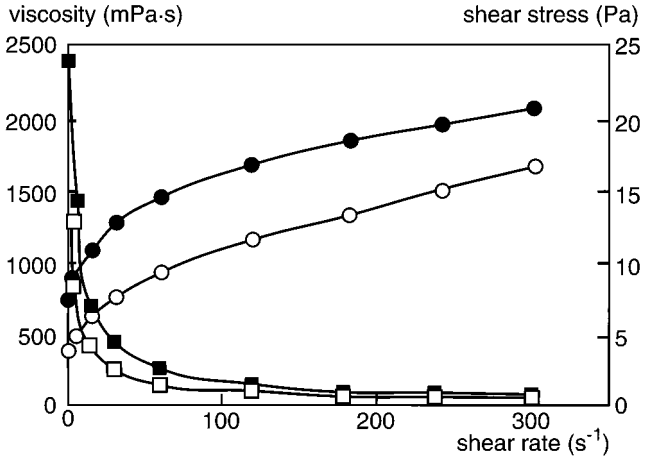

FIG. 1. Flow curves of a 1% (wt/vol) aqueous solution of purified 0-1 EPS and xanthan gum. The shear stress, τ (in pascals), was measured as a function of the shear rate, γ (in reciprocal seconds). The calculated viscosities (in millipascal seconds) are also indicated in the graph. Symbols: □, viscosity of xanthan; ■, viscosity of 0-1 EPS; ○, shear stress of xanthan; ●, shear stress of 0-1 EPS.

Rheological characteristics of both the 0-1 EPS and a commercially available xanthan gum were compared. Flow curves were made for a 1% xanthan gum and a 1% 0-1 EPS solution. Figure 1 shows the shear stress (τ) and the apparent viscosity (η) as functions of the shear rate (γ). The viscosity of the *L. sake* 0-1 EPS was higher throughout a range of increasing shear rates from 0 to 300 s^{-1}, while shear-thinning properties of the new EPS were as good as those of xanthan gum. Good shear-thinning properties, i.e., a drop in viscosity with increasing shear rate, are important for food applications when considering processing costs and mouthfeel of food products. Since viscosity is highly dependent on the average molecular mass distribution (M_m), we were interested in the sizes of the respective polymers. Measurements were carried out as described above. The average M_m distribution of the 0-1 EPS was determined as 6×10^6 Da, which is in the same range as that of the xantham gum that was used as a reference and of which the average M_m distribution was determined to be between 4×10^6 and 9×10^6 Da.

Analysis of the sugar composition revealed the presence of glucose and rhamnose, occurring in a molar ratio of 3:2. At present, there are strong indications that the EPS is negatively charged. Purifying the isolated EPS over a Dowex anion-exchange chromatography column resulted in almost complete loss of the EPS. Eluting the column with water, yielding fraction 1, or with a 1 M NH_4HCO_3 solution, yielding fraction 2, was not sufficient to remove the EPS from the column material. After lyophilization of fractions 1 and 2, a sugar analysis revealed that only 2% of the EPS was recovered in fraction 2, leaving 98% of the EPS attached to the column. Furthermore, a study of the effects of the addition of salt on the rheological behavior of the 0-1 EPS indicated the presence of charge. Viscosity was measured as a function of shear rate, and results of 1% (wt/wt) 0-1 EPS solutions of neutral pH in pure water and in different concentrations of NaCl were compared (Fig. 2). Increasing the concentration of NaCl from 0 to 0.2 and 0.5% resulted in a decreased viscosity over the whole range of shear rates measured. This observation, that the addition of ions drastically affects the viscosity of the polymer solution, strongly suggests the presence of a charged group in the EPS.

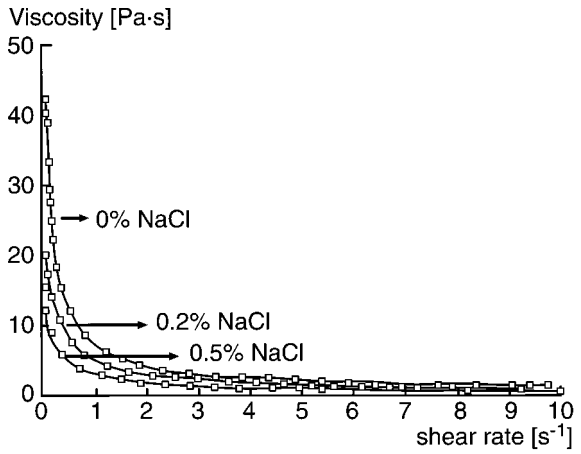

FIG. 2. Influence of NaCl on the viscosity of a 1% (wt/vol) solution of purified 0-1 EPS. Viscosity (in pascal seconds) was measured as a function of shear rate, which ranges from 0 to 10 s^{-1}, using a Carrimed CSL rheometer.

Production of EPS. To find optimal culture conditions for EPS production, the influence of temperature and pH was investigated. Table 1 shows the influence of temperature on maximum cell growth and EPS production. From these results, it is clear that there is an inverse relation between the EPS production per gram of biomass and the growth temperature. The lower the temperature, the higher the EPS production levels per gram of biomass produced. However, at low temperatures, growth is relatively slow and also the growth yield, expressed as drw_{max} (maximum dry weight) in Table 1, is low compared with that of cultures at 20, 25, or 30°C. Therefore, the optimal temperature for EPS production is 20°C rather than a low temperature of 15 or 10°C. At 20°C, the growth rate in the exponential phase is 0.16 $g \cdot liter^{-1} \cdot h^{-1}$, drw_{max} = 2.31 g, and the EPS production is 1,375 $mg \cdot liter^{-1}$.

Figure 3 shows the results of the fermentations at different pHs. The data clearly show the dependence of growth and EPS production on the pH. At pH 5.8, EPS production was highest at 1,375 $mg \cdot liter^{-1}$, whereas at pH 5.5 the amount of EPS released in the growth medium was decreased to 875 $mg \cdot liter^{-1}$ and at pH 6.2 production stopped at 1,100 $mg \cdot liter^{-1}$. On the other hand, growth was stimulated more at pH 6.2, indicating that the conversion from sugar to EPS is more efficient at pH 5.8 whereas sugar is more efficiently converted to biomass at pH 6.2 (Fig. 3C and D). Both growth and EPS production decreased dramatically when the pH dropped from 5.5 to 5.0. Figure 3A shows that at a concentration of 5 g of lactate $\cdot$ $liter^{-1}$, growth stops completely and no glucose is converted to EPS. At higher pHs, however, all the glucose is converted to biomass, lactate, and EPS. EPS production starts almost simultaneously with growth and reaches a maximum rate when the culture is in its exponential growth phase. The maximum amount of glucose converted to EPS is relatively small, i.e., only 6.9% at pH 5.8. Under the conditions used, we could not detect acetate, ethanol, or formate, showing that the metabolism is purely homofermentative with lactate as the only detectable end product.

Growth of *L. sake* 0-1 on different energy sources such as glucose, galactose, mannose, fructose, lactose, and sucrose did not change the sugar composition of the EPS produced.

TABLE 1. Influence of temperature on growth and EPS production[a]

Temp (°C)	*dX/dt* (g/liter/h)[b]	drw_{max} (g/liter)	EPS concn[c]	
			mg/liter	mg/g (dry wt)
10[d]	0.03	1.35	950	700
15	0.07	1.80	1,200	670
20	0.16	2.31	1,375	595
25	0.18	2.12	975	460
30	0.22	1.97	550	280

[a] Cultures were grown anaerobically in a 2.5-liter-working-volume fermentation vessel at pH 5.8 as described in the text. All experiments were carried out in duplicate, and the results were within an error range of 10%.
[b] *dX/dt* is a measure for the growth rate of the cells.
[c] EPS was quantified by the anthrone reaction as described in the text.
[d] At 10°C, the experiment was stopped after 3 days; the concentration of glucose was still 5 g $liter^{-1}$.

DISCUSSION

A viscometric analysis of a novel EPS produced by *L. sake* 0-1, isolated from sausage, has been presented, and the influence of several fermentation parameters on production has been determined. Compositional analysis showed that the EPS consists of glucose and rhamnose in a molar ratio of 3:2. An attempt to purify the EPS over a Dowex column resulted in a 98% loss of the polysaccharide because of a strong binding to the anion-exchange material. This indicates the presence of a negative charge linked to the EPS. The effect of NaCl added to a 1% EPS solution (Fig. 2) supports this conclusion. A detailed structural analysis of the repeating unit of the isolated EPS has been carried out by Robijn et al. (16). Their results, including nuclear magnetic resonance spectroscopy analysis of partially hydrolyzed EPS fragments, also clearly demonstrate that the isolated material from an *L. sake* 0-1 culture contained only one EPS. When dissolved in pure water, the conformation of the negatively charged polysaccharide is such that the hydrodynamic volume and thus the intrinsic viscosity (η) is relatively large. This is mainly due to repulsion effects of the intramolecular negative charges. By adding small amounts of NaCl, shielding of the charged groups in the EPS will result in a decrease of the repulsion effects, which then leads to a smaller hydrodynamic volume and thus a lower intrinsic viscosity.

Rheological properties were comparable to those of xanthan gum, and viscosity was even higher under all shear rates tested. The average M_m distribution was determined as 6×10^6 Da for the 0-1 EPS, whereas it was between 4×10^6 and 9×10^6 Da for xanthan gum. As the M_m of the two polymers is of the same order of magnitude, these results indicate that the high viscosity of the 0-1 EPS is an intrinsic property and is not due to large differences in M_m. We therefore consider this novel EPS to be a serious option for application in a variety of food products.

It is shown that the growth temperature of the *L. sake* 0-1 is an important parameter that can dramatically influence the amount of EPS produced. From Table 1, it is clear that at relatively low temperatures of 10 to 20°C, EPS production is more than doubled when compared with production at an optimal growth temperature of 30°C. This indicates that EPS production is enhanced at lower growth rates of the EPS-producing strain. Similar results have been reported for slime-forming *Lactococcus lactis* (4) and *Lactobacillus casei* (9). This effect has been explained (1) by the fact that at suboptimal growth temperatures, more undecaprenol (C_{55}) lipid carrier is available for the synthesis of the repeating unit of the EPS. Undecaprenol plays a key role in the biosynthesis of EPS but also in that of peptidoglycan, lipopolysaccharide, and teichoic acid. It may therefore be expected that at higher growth rates, more undecaprenol will be used for the production of cell wall

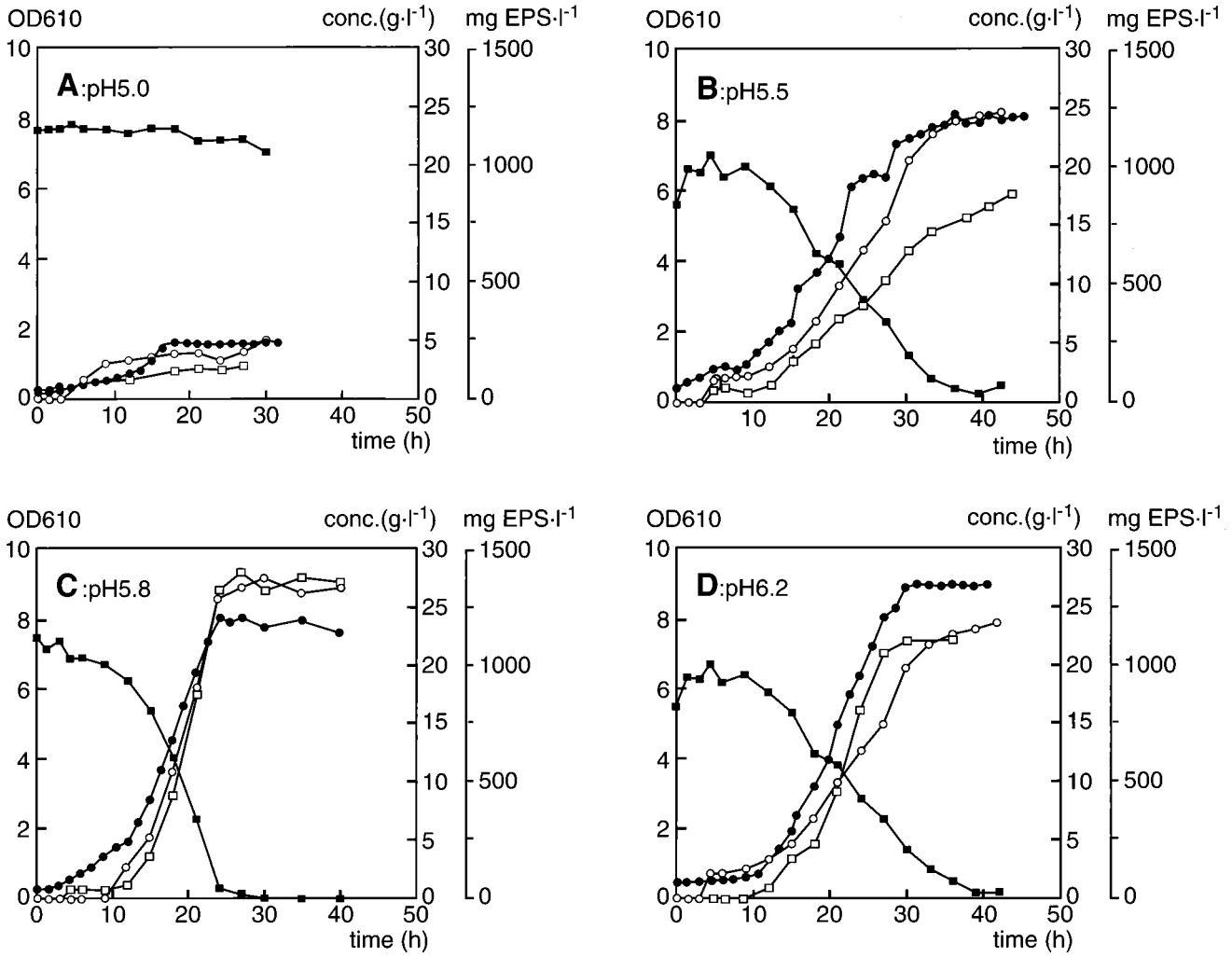

FIG. 3. Growth, EPS production, and glucose conversion at different pHs. Experiments were carried out in duplicate in a 2.5-liter-working-volume fermentation vessel at 20°C, and the results are within an error range of 10%. Anaerobic conditions were achieved by passing 0.2 liter of N_2 gas over the culture medium, and agitation was maintained at 200 rpm. Fermentations at four different pH values were investigated, pH 5.0 (A), pH 5.5 (B), pH 5.8 (C), and pH 6.2 (D), which were obtained by titrating the produced lactate with a 4 M NaOH solution. Growth was monitored by measuring the OD_{610}. EPS was quantified by the anthrone reaction as described in the text. Symbols: ●, OD_{610}; □, EPS production (milligrams per liter); ■, glucose conversion (grams per liter); ○, lactate concentration (grams per liter).

material than for the biosynthesis of EPS, resulting in decreased slime production. On the other hand, it is also possible that at optimal growth temperatures, the organism simply uses excess sugar in cell wall biosynthesis.

Considering the results of the study of physiological parameters influencing the growth of *L. sake* 0-1 and its subsequent EPS production, one may conclude that an optimal production per OD unit is obtained when cells are grown anaerobically at 20°C and the pH is controlled at 5.8. Under these conditions, 1,375 mg of EPS · $liter^{-1}$ is produced, which means that 6.9% of the initial 20 g of glucose per liter is effectively converted to EPS.

Fermentation experiments under conditions of stationary pH (Fig. 3) suggest that EPS production is linked to growth. From the results of the experiment at pH 5.0, it is clear that EPS production ends when growth stops, although most of the glucose is still present in the medium (Fig. 3A). Whether this means that we are dealing with a real dependence on growth or that this can be explained by inhibition of the metabolism by the presence of 0.5% lactate at pH 5.0 must be studied in more detail. The results presented in this study (Fig. 3B to D) suggest that the 0-1 EPS is not produced via an overflow metabolism as is the case for xanthan production by *Xanthomonas campestris* (19) and for the production of EPS by *Zoogloea ramigera* (15), where production continues after the stationary phase has been reached.

From an economics point of view, a 10-fold increase in 0-1 EPS production, to obtain 10 to 15 g · $liter^{-1}$, is required to use this EPS as a food additive. Since EPS production seems to be coupled to growth and since growth is presumably inhibited by the formation of lactate, we believe that there is scope for productivity improvement by reducing the concentration of lactate in the culture broth. Part of our research is therefore focused on this aspect. Moreover, the present EPS production level of 1.4 g · $liter^{-1}$ may be enough for possible applications in situ.

Future work will be focused on the effects of oxygen on the metabolism of *L. sake* 0-1 and its EPS production. Furthermore, the influence of the lactate that is produced during growth will be investigated.

ACKNOWLEDGMENTS

We thank Marguerite Rinaudo for determining the average molecular mass distributions (M_m) at her laboratory in the Centre de Recherches sur les Macromolécules Végétales, Grenoble Cedex, France.

This work was supported in part by the Commission of the European Communities under grant of the BRIDGE T-project Biotechnology of Lactic Acid Bacteria (contract BIOT CT-910263).

REFERENCES

1. **Cerning, J.** 1990. Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiol. Rev. **87:**113–130.
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. J. Desmazeaud, and M. Landon.** 1988. Exocellular polysaccharide production by *Streptococcus thermophilus*. Biotechnol. Lett. **10:**255–260.
4. **Cerning, J., C. Bouillanne, M. Landon, and M. Desmazeaud.** 1992. Isolation and characterization of exopolysaccharides from slime-forming mesophilic lactic acid bacteria. J. Dairy Sci. **75:**692–699.
5. **De Man, J. C., M. Rogosa, and M. E. Sharpe.** 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. **23:**130–135.
6. **Doco, T., J. M. Wieruszeski, and B. Fournet.** 1990. Structure of an exocellular polysaccharide produced by *Streptococcus thermophilus*. Carbohydr. Res. **196:**1–9.
7. **Garcia-Garibay, M., and V. M. E. Marshall.** 1991. Polymer production by *Lactobacillus delbrueckii* ssp. *bulgaricus*. J. Appl. Bacteriol. **70:**325–328.
8. **Gruter, M., B. R. Leeflang, J. Kuiper, J. P. Kamerling, and J. F. G. Vliegenthart.** 1993. Structural characterization of the exopolysaccharide produced by *Lactobacillus delbrueckii* ssp. *bulgaricus* rr grown in skimmed milk. Carbohydr. Res. **239:**209–226.
9. **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.
10. **Kontusaari, S., and R. Forsén.** 1987. Finnish fermented milk "Viili": involvement of two cell surface proteins in production of slime by *Streptococcus lactis* ssp. *cremoris*. J. Dairy Sci. **71:**3197–3202.
11. **Macura, D., and P. M. Townsley.** 1984. Scandinavian ropy milk—identification and characterization of endogenous ropy lactic streptococci and their extracellular excretion. J. Dairy Sci. **67:**735–744.
12. **Mäkelä, P. M.** 1992. Fermented sausage as a contaminating source of ropy slime-producing lactic acid bacteria. J. Food Prot. **55:**48–51.
13. **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* CRL 420. Milchwissenschaft **40:**409–411.
14. **Nakajima, H., S. Toyoda, T. Toba, T. Itoh, T. Mukai, H. Kitazawa, and S. Adachi.** 1989. A novel phosphopolysaccharide from slime-forming *Lactococcus lactis* ssp. *cremoris* SBT 0495. J. Dairy Sci. **73:**1472–1477.
15. **Norberg, A. B., and S. O. Enfors.** 1982. Production of extracellular polysaccharide by *Zoogloea ramigera*. Appl. Environ. Microbiol. **44:**1231–1237.
16. **Robijn, G. W., D. J. C. van den Berg, H. Haas, J. P. Kamerling, and J. F. G. Vliegenthart.** Determination of the structure of the exopolysaccharide produced by *Lactobacillus sake* 0-1. Carbohydr. Res., in press.
17. **Sutherland, I. W.** 1972. Bacterial exopolysaccharides. Adv. Microb. Physiol. **8:**143–213.
18. **Sutherland, I. W.** 1982. Biosynthesis of microbial exopolysaccharides. Adv. Microb. Physiol. **33:**79–150.
19. **Sutherland, I. W.** 1990. Biotechnology of microbial exopolysaccharides. Camb. Stud. Biotechnol. **9:**1–151.
20. **Tinland, B., J. Mazet, and M. Rinaudo.** 1988. Characterization of water-soluble polymers by multidetection size-exclusion chromatography. Makromol. Chem. **9:**69–73.
21. **Toba, T., S. Abe, K. Arihara, and S. Adachi.** 1986. A medium for the isolation of capsular bacteria from kefir grains. Agric. Biol. Chem. **50:**2673–2674.
22. **Van den Berg, D. J. C., A. Smits, B. Pot, A. M. Ledeboer, K. Kersters, J. M. A. Verbakel, and C. T. Verrips.** 1993. Isolation, screening and identification of lactic acid bacteria from traditional food fermentation processes and culture collections. Food Biotechnol. **7:**189–205.
23. **Whitfield, C.** 1988. Bacterial extracellular polysaccharides. Can. J. Microbiol. **34:**415–420.