J Ind Microbiol Biotechnol (2003) 30: 661–668
DOI 10.1007/s10295-003-0095-4

ORIGINAL PAPER

A. D. Welman · I. S. Maddox

Fermentation performance of an exopolysaccharide-producing strain of *Lactobacillus delbrueckii* subsp. *bulgaricus*

Received: 14 April 2003 / Accepted: 13 September 2003 / Published online: 14 November 2003

Abstract The formation of exopolysaccharide (EPS) and extracellular metabolites was studied in a strain of *Lactobacillus delbrueckii* subsp. *bulgaricus* (NCFB 2483), grown under batch culture conditions in a semi-defined medium incorporating lactose and casein hydrolysate. Performance parameters were derived from the fermentation data, and kinetic models were applied in order to describe the production of EPS, extracellular metabolites, and biomass produced. Lactose was split intracellularly, with the resultant galactose being exported from the cell, and the glucose being metabolised further to EPS and lactic acid. Production of EPS, lactate, and galactose was closely growth-associated and followed a pattern of primary kinetics. A marginally lower galactose level relative to the modelled levels throughout most of the time course of the fermentation suggests that not all galactose is exported from the cell, and that a low level of flux to other metabolites, such as EPS, might exist.

Keywords Exopolysaccharide · *Lactobacillus delbrueckii* subsp. *bulgaricus* · Fermentation modelling

Introduction

The production of exopolysaccharides (EPS) by lactic acid bacteria (LAB) represents an attractive alternative to the microbial EPS that currently occupy the biopolymer market, viz. xanthan from *Xanthomonas campestris*, the gellans from *Sphingomonas paucimobilis* [26], acetan from *Acetobacter xylinum* [28], and dextran from *Leuconostoc mesenteroides* [7]. The reason for this is that many of the current biopolymers are produced by microbes that do not have GRAS (generally recognised as safe) status, hence limiting their use in foods. EPS from LAB currently play a role in the manufacture of fermented milk products such as yoghurt, imparting an improved rheology, texture and mouthfeel. In addition, these biopolymers have been strongly suggested to elicit health benefits [8]. The production of these polymers is, however, limited by the productive capacity of LAB, which are chiefly anaerobic organisms.

Some strains of *L. delbrueckii* subsp. *bulgaricus* serve as typical examples of LAB that can produce excess EPS. To date, the compositional structures of EPS from *L. delbrueckii* subsp. *bulgaricus* determined have varied; however, all have been found to be heteropolysaccharides, consisting of repeating units of monomers such as glucose, galactose, and rhamnose [2,12,13].

In this study, the fermentation performance of *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 was assessed in simple batch culture. The formation of EPS and other extracellular metabolites was monitored throughout the time-course of the fermentation, and performance characteristics were derived from the data. An attempt was made to simulate the production of these metabolites through the application of various models previously used to describe metabolite production in LAB. The development of these models is useful, as it enables prediction of EPS and metabolite formation through the measurement of a minimum number of parameters, e.g. biomass and lactose consumption.

A. D. Welman (✉) · I. S. Maddox
Institute of Technology and Engineering, Massey University, Palmerston North, New Zealand
E-mail: Alan.Welman@fonterra.com
Tel.: +64-6-3504649
Fax: +64-6-3504658

A. D. Welman
Fonterra Research Centre, Fonterra Cooperative Group Ltd., Private Bag 11029, Palmerston North, New Zealand

Materials and methods

Fermentation medium

The medium used was that described by Kimmel and Roberts [16], modified by the replacement of glucose with lactose as the sole sugar source (KM-l). This medium was based on the MRS medium

of de Man et al. [4], which has found wide utility in general studies on LAB.

The medium consisted of (in g l^{-1}): lactose 20, yeast nitrogen base (without amino acids and ammonia) 5, bacto casitone 20, sorbitan monooleate (Tween 80) 1, dipotassium phosphate (K_2HPO_4) 2, magnesium sulphate ($MgSO_4{\cdot}7H_2O$) 0.1, manganese sulphate ($MnSO_4{\cdot}4H_2O$) 0.05, ammonium citrate 2, sodium acetate 5. In all instances, the medium was prepared in separate, double-strength aliquots of lactose and the remainder of the nutrients as described above. After steam-sterilisation, the aliquots were allowed to cool, and pooled, achieving the desired concentration of medium constituents.

Bacterial strains and preparation of a working cell bank

The strain of *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 (NCIMB 702483) was obtained from the National Collection of Industrial and Marine Bacteria (NCIMB), Aberdeen, Scotland. The culture was grown at 37°C from a freeze-dried powder to late log-phase in medium 284 (NCIMB), modified by the deletion of glucose and incorporation of 20% glycerol, and adjusted to pH 6.2. The broth supernatant was removed by sterile centrifugation at 12,000 *g* for 15 min. The pellet was reconstituted with modified medium 284, as described above and preserved in 1 ml aliquots at −80°C.

Culture conditions

Aliquots (1 ml) of the culture strain were inoculated into 225 volumes of medium in 250 ml shake bottles (Duran; Schott, Mainz, Germany). The cultures were incubated without pH control in an orbital incubator shaker at a slow rotational speed (100 rpm), which was sufficient to keep the culture homogenous, and at a temperature of 37°C for 24 h. Trial experiments demonstrated that lactose consumption and metabolite production had ceased after 24 h. Six replicate cultures were used.

Sampling

Sample aliquots (15 ml) were withdrawn aseptically at 4-h intervals for the determination of biomass, microscopic cell counts, and sugar conversion to extracellular metabolites.

Analytical methods

Growth was monitored by absorbance measurement at 650 nm (A_{650}). Biomass was determined from a standard curve relating A_{650} to washed cell dry weights. Microscopic cell counts were measured using an improved Neubauer counting chamber (Weber Scientific, Teddington, UK).

EPS was subjected to crude isolation prior to analysis. Aliquots (100 µl) of whole broth underwent a 2-fold precipitation with chilled ethanol (2.9 ml distilled H_2O/7.0 ml 99.7% ethanol) for 24 h periods at 4°C. The precipitate was recovered by centrifugation (35,850 *g*, 40 min, 4°C) and resuspended in 1.0 ml distilled water. The total sugar concentration in the resuspended sample was measured according to the method of Dubois et al. [9], with dextran as the standard.

The concentrations of lactose, galactose and lactic acid were determined in duplicate by HPLC (Waters Alliance 2690 Separations module). The HPLC system was coupled with a refractive index detector (Waters 2410) and UV spectrophotometer (Waters 2487). The compounds were detected using a single column (Aminex HPX-87H, 300×7.8 mm, Bio-Rad, Richmond, Calif.) using 0.028 M H_2SO_4 as eluent, according to the method described in Ross and Chapital [25]; the column temperature was maintained at 40°C, and the flow rate at 0.6 ml min^{-1}. Cell-free supernatant fractions were diluted with distilled and filtered water (MilliQ) prior to analysis. Instrument, data accession, and processing methods were controlled using a Millenium software system. External standards were used for lactose, galactose, and lactate determination (Sigma, St. Louis, Mo.).

Results

EPS production was growth-associated, with a maximum value (0.16 g l^{-1}) measured at 20 h elapsed time (Fig. 1a). Lactate and galactose formation followed the growth trend (Fig. 1a, b), with maxima measured at 24 h. Acidification of the broth corresponded to lactate formation with pH values achieving minimum values from 20 h onwards. Microscopic cell counts and cell dry weights over the 24 h fermentation period are illustrated in Fig. 1b. A plot correlating values of the two variables obtained over the 24 h period yielded a RSQ value of 0.9998.

In order to generate a reasonable kinetic model describing the behaviour of biomass (X), extracellular products (P), and lactose utilisation (S), in *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 under the applied fermentation conditions, a variety of models were applied.

Biomass production

The logistic equation has been applied to describe the growth of microorganisms [1, 29, 17]:

$$\frac{dX}{dt} = \mu X\left(1 - \frac{X}{X_{max}}\right) \quad (1)$$

where X is the biomass concentration, X_{max} is the maximum biomass concentration, t is the elapsed time, and μ is the initial maximum specific growth rate. The above equation may be integrated and rearranged to give:

$$X(t) = \frac{X_o e^{\mu t}}{1.0 - (X_0/X_{max})(1.0 - e^{\mu t})} \quad (2)$$

A plot of $\ln[X/(1.0-X]$ against time yielded μ (the slope) (0.5 h^{-1}) and X_o, the initial minimum viable inoculum size (0.001 g l^{-1}) from the y intercept ($=-\ln[(X_{max}/X_o)-1.0]$, where $X = X(t)/X_{max}$. The value X_{max} was 0.83 g l^{-1} (Table 1). A simulation of biomass formation in *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 was then undertaken (Fig. 2a). The yield of biomass produced per lactose utilised ($Y_{x/s}$), and the volumetric rate of biomass production (r_x), corresponding to 20 h and 24 h of elapsed fermentation time, are listed in Table 1.

EPS production

For product formation, Luedeking and Piret [19] developed a model for lactic acid production in *L. delbrueckii*, viz.

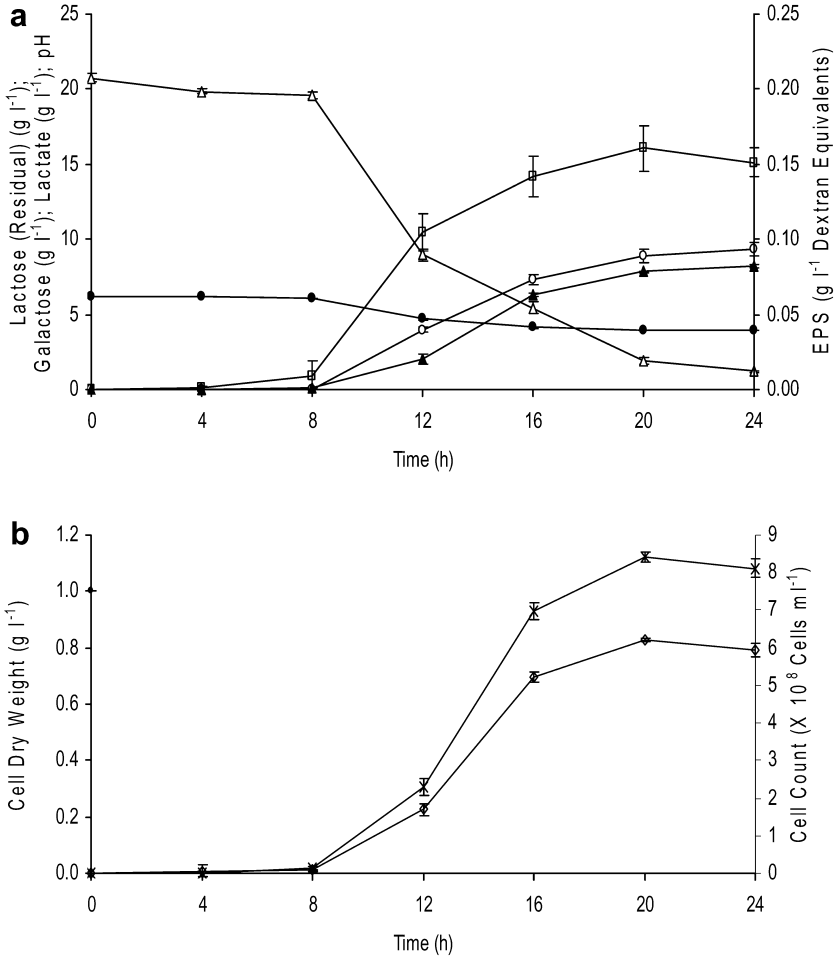

Fig. 1a, b Time-course of growth, lactose consumed and extracellular metabolite production associated with *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2483 grown in KM-l medium at 37°C. **a** △ Residual lactose, ○ galactose, ● pH, ▲ lactate, □ EPS; **b** ◇ biomass (cell dry weight) titre, × microscopic cell count. All values are the mean of six experiments

Table 1 Experimentally derived parameters related to biomass, yield of biomass on lactose consumed, maximum growth rate, and rate of biomass production in *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2483 after 20 h growth in KM-l medium at 37°C. X_{max} Maximum biomass,X' maximum biomass prior to exopolysaccharide (EPS) degradation, X_o initial minimum inoculum biomass, $Y_{X/S}$ yield of biomass on lactose consumed, r_X volumetric rate of biomass production, *CDW* cell dry weight, μ_{max} maximum specific growth rate

$X_{\max}$ (g CDW l^{-1})	X' (g CDW l^{-1})	X_o (g CDW l^{-1})	$Y_{X/S}$ [g CDW (g lactose consumed)$^{-1}$]	$\mu_{\max}$ (h^{-1})	r_X (g CDW l^{-1} h^{-1})
0.83	0.83	0.001	0.04	0.5	0.04

$$\frac{dP}{dt} = nX + m\frac{dX}{dt} \quad (3)$$

The equation is split into a non-growth associated term nX, and a growth-associated term mdX/dt describing product formation. Integration of the equation describes the way in which product evolves with time [16]:

$$P(t) = P_o + mX_o\{e^{\mu t}[1.0 - (\bar{X}_o).(1.0 - e^{\mu t})] - 1.0\} + n(X_{\max}/\mu)\ln[1.0 - \bar{X}_o.(1.0 - e^{\mu t})] \quad (4)$$

in which P_o is the product titre at time $t = 0$. The factor n is calculated from:

$$n = \frac{(dP/dt)_{stat}}{X_{\max}} \quad (5)$$

Product formation in *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 is growth-associated (Fig. 1a), and the calculation of n is thus not included in the model simulation. The factor m (mg P mg X^{-1}) was obtained from the slope of a plot of the integrated form of the Luedeking-Piret equation against $X - X_o$. In the case of EPS production, m was calculated to be 203 mg EPS (dextran equivalents) (g biomass)$^{-1}$.

EPS production in *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 closely follows the trend of biomass for-

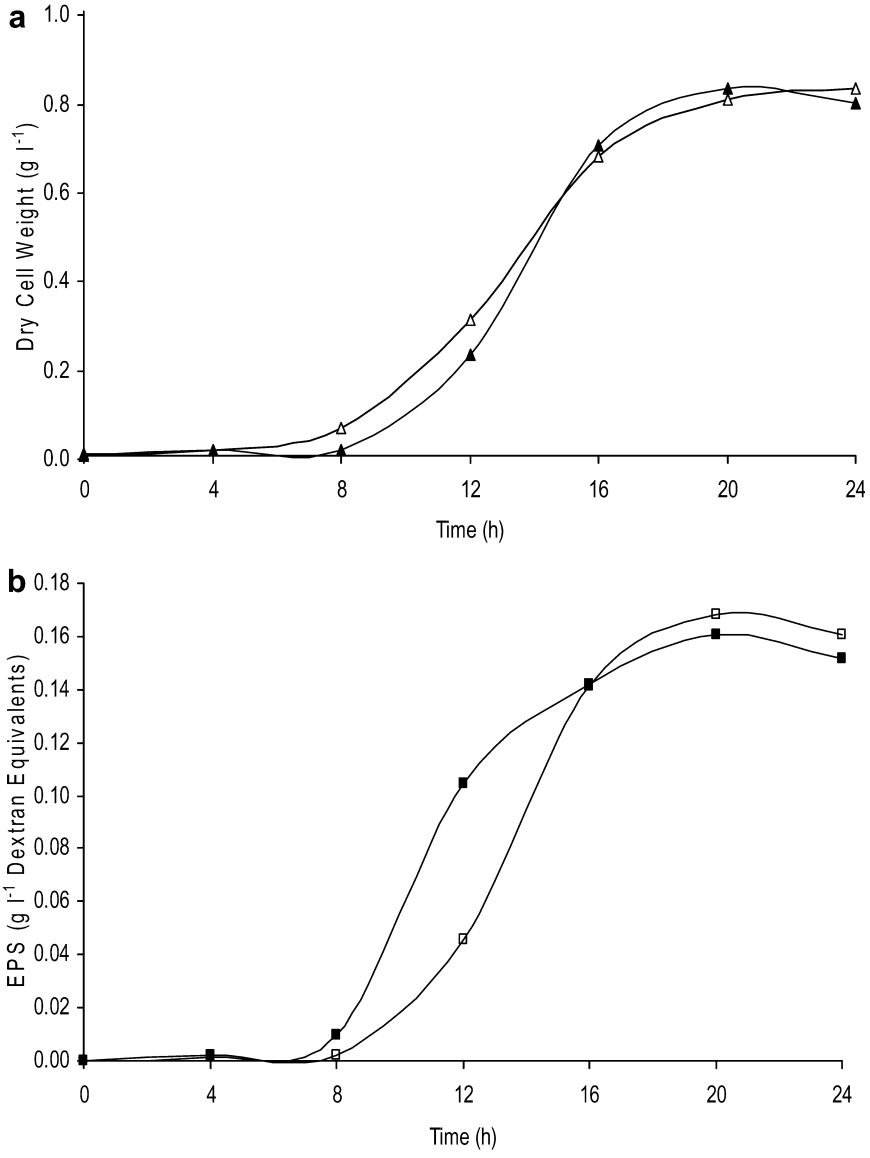

Fig. 2a, b Simulation of growth and EPS formation in *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 grown in KM-l medium at 37°C. **a** ▲ Actual biomass data, △ simulated biomass data, **b** ■ actual EPS titre, □ simulated EPS titre, over a period of 20 h

mation (growth-associated), reaching a measured maximum at 20 h (Fig. 1a, b). However, a low level of lactose consumption continues until the end of the growth period (Fig. 1a).

A simulation of EPS formation by *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 using the Luedeking-Piret equation is shown in Fig. 2b. The simulation was not attempted beyond 20 h fermentation, as the EPS titre decreased after this time, possibly due to degradation of the polymer.

Galactose production

L. delbrueckii subsp. *bulgaricus* strains, which are galactose-negative, import lactose by a lactose/galactose antiport transport system [5], as has been found in *Streptococcus thermophilus* LY03 [14].

Using sets of data from the 24 h fermentation profiles of *L. delbrueckii* subsp. *bulgaricus* NCFB 2483, a linear relationship can be shown to exist between galactose produced and lactose consumed (Fig. 3). During growth and maintenance of the bacteria, galactose efflux may be described by the equation [6]:

$$\frac{\mathrm{dGal}}{\mathrm{d}t} = \frac{-1}{Y_{\mathrm{S/gal}}} \frac{\mathrm{d}S}{\mathrm{d}t} \tag{6}$$

where $Y_{\mathrm{S/gal}}$ represents the yield coefficient for galactose [a value of 2 g lactose consumed (g galactose produced)$^{-1}$, was used]. A simulation of galactose efflux over a 24 h fermentation period using the above relationship is shown in Fig. 4a.

Lactate production

L. delbrueckii subsp. *bulgaricus* NCFB 2483 is homofermentative as no organic acids apart from lactate were detected in fermentation samples. Lactic acid formation can similarly be related to lactose utilisation by the yield

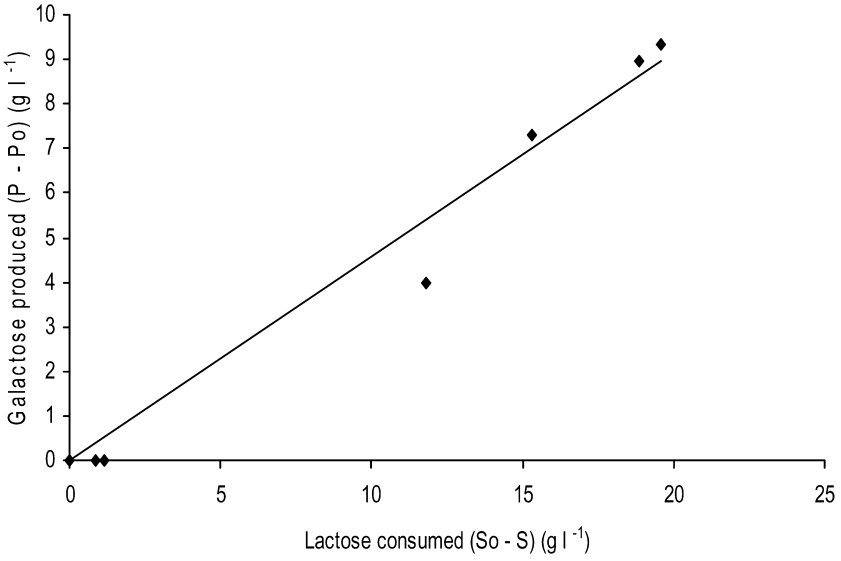

Fig. 3 Relationship between galactose efflux and lactose consumed in *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 grown in KM-l medium at 37°C

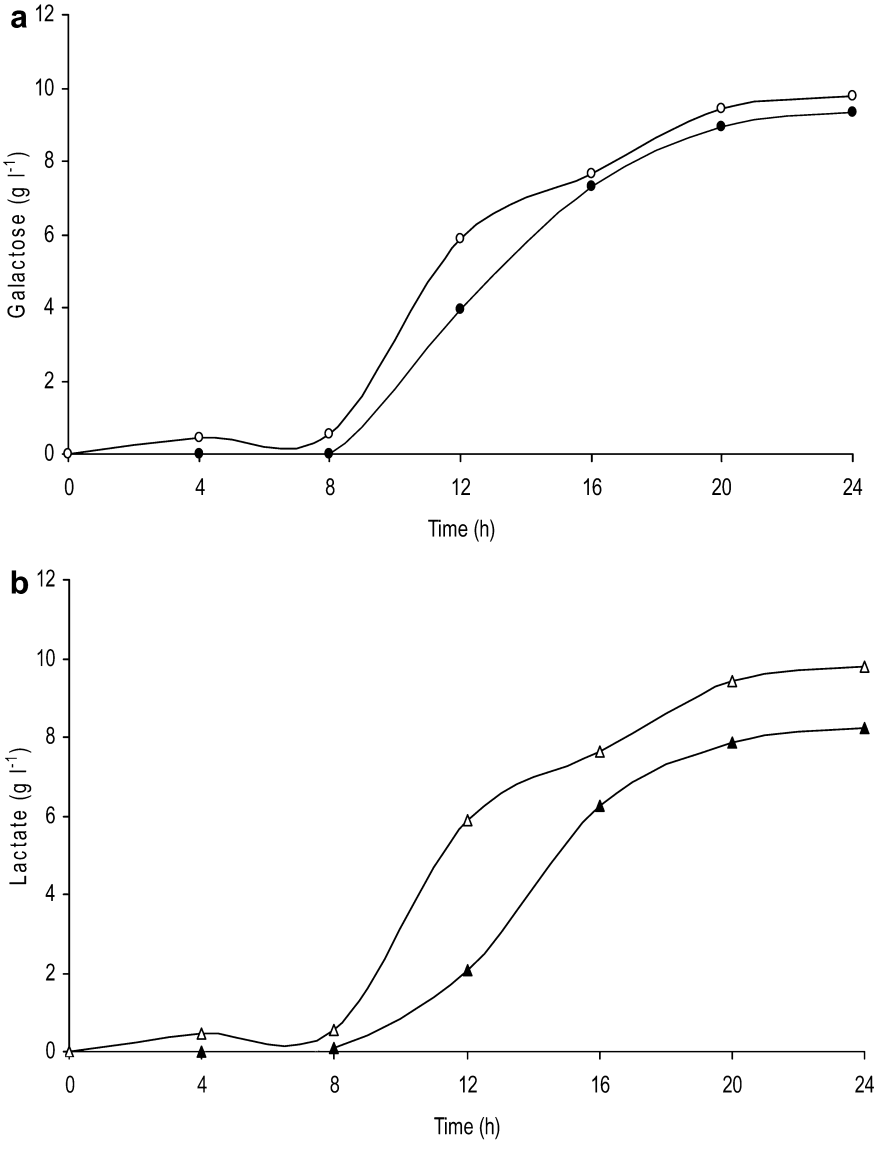

Fig. 4a, b Simulation of galactose and lactate formation in *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 grown in KM-l medium at 37°C. **a** ● Actual galactose titre, ○ simulated galactose titre; **b** ▲ actual lactate titre, △ simulated lactate titre

coefficient for lactic acid, viz $Y_{S/lactate}$, which represents the lactose consumed (g) per lactate produced (g). The equation [3] for lactate production is:

$$\frac{\mathrm{dLactate}}{\mathrm{d}t} = \frac{-1}{Y_{S/\mathrm{lactate}}} \frac{\mathrm{d}S}{\mathrm{d}t} \quad (7)$$

A simulation of lactate production using the above relationship is shown in Fig. 4b. The value of $Y_{S/lactate}$ used was 2 g lactose consumed (g lactate produced)$^{-1}$.

Fermentation performance

Parameters associated with fermentation performance were calculated from experimental data, as well as from the simulated results derived from the applied models for EPS, lactate and galactose formation (Table 2) (maximum product titre, yield of product on biomass, and volumetric and specific rates of product formation). Specific yields and rates of production were calculated at 20 h for EPS production, and at 24 h for lactate and galactose, as these times corresponded to the point at which the respective products were at a maximum during the time course of the fermentations.

Discussion

EPS production by *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 is growth-associated (Fig. 1a, b), corresponding with previous findings in *L. delbrueckii* subsp. *bulgaricus* [11], and is consistent with the nature of EPS production in thermophilic LAB in general [6]. The maximum titre of EPS produced (160 mg l^{-1}) was higher in comparison to the 140 mg l^{-1} (approximate) determined by Toba et al. [27] for *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 grown in skim milk, but using ultrafiltration to separate the polysaccharide prior to analysis by the phenol-sulphuric acid method. In the present study, the reduction in EPS titre after achieving a maximum level at 20 h fermentation time can most probably be ascribed to the action of glycohydrolases, as was found by Gassem et al. [10] and Petry et al. [24] in *L. delbrueckii* subsp. *bulgaricus*, and the cessation of biomass production. Biomass measurements correlated well with microscopic cell counts (Fig. 1b), reflecting the inhibitory effect on growth of lactic acid accumulation and nutrient limitation (Fig. 1a). Carbon flux after the 20 h period is therefore almost exclusively diverted to galactose and lactate formation (Fig. 1a), and cell maintenance.

Metabolite production in *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 followed a pattern of primary kinetics, characterised by biosynthesis of the metabolites almost simultaneously with growth and approaching a maximum rate near the end of this period (Fig. 1a, b). This pattern is described for *Lactobacillus bulgaricus* CRL 420 by Manca de Nadra et al. [20], for *L. delbrueckii* subsp. *bulgaricus* NCFB 2772 by Grobben et al. [11], and in *L. delbrueckii* subsp. *bulgaricus* RR by Kimmel and Roberts [16].

Use of the logistic equation provided a satisfactory model to describe the kinetics of biomass formation in *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 (Fig. 2a). This equation has been used successfully to model the individual patterns of growth in a mixed culture of *S. thermophilus* and *L. bulgaricus* in a medium consisting of lactose, peptone, and yeast extract [1], and for *S. thermophilus* LY03 by Degeest and De Vuyst [3]. This equation is well known, and has been used in prior studies to describe the growth of LAB [22, 23, 18].

The Luedeking-Piret equation, originally developed for the formation of lactic acid by *L. delbrueckii* [19] and used by Weiss and Ollis [29] to describe the kinetics of polysaccharide production by *Xanthomonas campestris* B-1459, was applied to simulate EPS production by *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 (Fig. 2b). The simulation was undertaken over the first 20 h of fermentation, as it was evident that the EPS became degraded after this time. The largest difference between the experimentally derived values for EPS production and the simulated values, was observed from 8 h into the fermentation onwards (Fig. 2b), during which period EPS production and biomass formation departed from the defined relationship. The model predicts higher EPS_{max} and $Y_{EPS/X}$ values than those obtained experimentally (Table 2). This could be ascribed to the inhibitory effects of lactic acid formation on biomass

Table 2 Experimentally-derived and simulated yield coefficients and rates of production of EPS, lactate, and galactose produced by *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 grown in KM-l medium at 37°C. EPS concentrations expressed as grams dextran equivalents per litre

EPS	EPS_{max} (g l^{-1})	$Y_{EPS/X}$ [g EPS (g CDW)$^{-1}$]	r_{EPS} (g EPS l^{-1} h^{-1})	$r_{EPS\ (specific)}$ [g EPS (g CDW)$^{-1}$ h^{-1}]
Experimental	0.16	0.19	0.01	0.01
Simulated	0.17	0.21	0.01	0.01
Lactate	$Lactate_{max}$ (g l^{-1})	$Y_{Lactate/X}$ [g lactate (g CDW)$^{-1}$]	$r_{lactate}$ (g lactate l^{-1} h^{-1})	$r_{lactate\ (specific)}$ [g lactate (g CDW)$^{-1}$ h^{-1}]
Experimental	8.23	10.39	0.34	0.43
Simulated	9.78	11.87	0.41	0.49
Galactose	$Galactose_{max}$ (g l^{-1})	$Y_{Galactose/X}$ [g galactose (g CDW)$^{-1}$]	$r_{galactose}$ (g galactose l^{-1} h^{-1})	$r_{galactose\ (specific)}$ [g galactose (g CDW)$^{-1}$ h^{-1}]
Experimental	9.34	11.80	0.39	0.49
Simulated	9.78	11.90	0.41	0.50

production and EPS-producing ability during the latter part of the fermentation. However, predicted and experimental volumetric and specific rates of EPS production were similar (Table 2).

A linear relationship between galactose efflux and lactose consumed is shown in Fig. 3. The yield coefficient for galactose, $Y_{S/gal}$ [theoretically, 2 g lactose consumed (g galactose produced)$^{-1}$] may be used to model galactose production (Fig. 4a). The simulated profile of galactose formation is similar to that obtained experimentally. This is consistent with the nature of the mechanism by which lactose is split by a β-galactosidase into equimolar quantities of glucose and galactose, with galactose being exported from the cell via an antiport system [15, 30]. The marginally lower galactose levels relative to the modelled levels throughout most of the time course of the fermentation suggest that not all galactose is exported from the cell, and that a low level of flux to other metabolites might exist. Deviation from the theoretical value of $Y_{S/gal}$ has been reported in *S. thermophilus* LY03 by Degeest and De Vuyst [3]. This was explained by the hypothesis that not all the galactose was exported from the cell, but that some was converted to lactic acid. It is hence not inconceivable that a similar partial utilisation of galactose might exist in *L. delbrueckii* subsp. *bulgaricus* NCFB 2483, although this remains to be proved. Marshall et al. [21] reported assimilation of galactose into EPS in galactose-negative strains of *L. delbrueckii* subsp. *bulgaricus*. Higher predicted specific yields and rates of galactose production relative to the experimental results (Table 2) lend credence to this proposition; however, nutrient limitation and inhibitory stress by lactate may also afford a reason for differences between the experimental and modelled results.

Simulated levels of lactic acid production (Fig. 4b) were consistently higher than the experimentally derived values. This could be ascribed to the fact that, whilst lactose is split on an equimolar basis to galactose, which is exported, and glucose, some of this glucose is used for building cell mass and for producing exopolysaccharide. The model hence does not take into account this additional carbon requirement, and assumes that the metabolic destination of all glucose carbon is lactate. The corresponding differences between experimentally derived and simulated specific yields and rates of lactate production (Table 2) support this observation; however, it is also likely that lactate accumulation exerted an inhibitory effect on cell metabolism.

In conclusion, *L. delbrueckii* subsp. *bulgaricus* NCFB 2483 follows a primary kinetic pattern with respect to production of EPS, lactate, and galactose. After growth ceases, EPS titres diminish, possibly as a consequence of degradation by glycohydrolases. Kinetic models applied to describe the formation of these metabolites suggest that whilst the bulk of galactose is exported unutilised via a lactose/galactose antiport, there exists the possibility that some galactose may be diverted for utilisation intracellularly for lactate and EPS production; however, this remains to be proved.

Acknowledgement This study was supported in part by the New Zealand Foundation for Research, Science, and Technology through the TBG program.

References

1. Berkman T, Bozoglu TF, Özilgen M (1990) Mixed culture growth kinetics of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Enzyme Microb Technol 12:138–140
2. Cerning J, Bouillanne C, Desmazeaud MJ, Landon M (1986) Isolation and characterisation of exocellular polysaccharide produced by *Lactobacillus bulgaricus*. Biotechnol Lett 8:625–628
3. Degeest B, de Vuyst L (1999) Indication that the nitrogen source influences both amount and size of exopolysaccharides produced by *Streptococcus thermophilus* LY03 and modelling of the bacterial growth and exopolysaccharide production in a complex medium. Appl Environ Microbiol 65:2863–2870
4. De Man JC, Rogosa M, Sharpe ME (1960) A medium for the cultivation of lactobacilli. J Appl Bacteriol 23:130–135
5. De Vos WM, Vaughan EE (1994) Genetics of lactose utilisation in lactic acid bacteria. FEMS Microbiol Rev 15:216–237
6. De Vuyst L, Vanderveken F, Van de Ven S, Degeest B (1998) Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in milk medium and evidence for their growth-associated biosynthesis. J Appl Microbiol 84:1059–1068
7. De Vuyst L, de Vin F, Vaningelgem F, Degeest B (2001) Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. Int Dairy J 11:687–707
8. Duboc P, Mollet B (2001) Applications of exopolysaccharides in the dairy industry. Int Dairy J 11:759–768
9. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356
10. Gassem MA, Schmidt KA, Frank JA (1997) Exopolysaccharide production from whey lactose by fermentation with *Lactobacillus delbrueckii* ssp. *bulgaricus*. J Food Sci 62:171–173
11. Grobben GJ, Sikkema J, Smith MR, de Bont JAM (1995) Production of extracellular polysaccharides by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 grown in a chemically defined medium. J Appl Bacteriol 79:102–107
12. Grobben GJ, Van Casteren WHM, Schols HA, Oosterveld A, Sala G, Smith MR, Sikkema J, de Bont JAM (1997) Analysis of the exopolysaccharides produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 grown in continuous culture on glucose and fructose. Appl Microbiol Biotechnol 48:516–521
13. Gruter M, Leeflang BR, Kuiper J, Kamerling JP, Vliegenthart JFG (1993) Structural characterisation of the exopolysaccharide produced by *Lactobacillus delbrueckii* subspecies *bulgaricus* RR grown in skimmed milk. Carbohydr Res 239:209–226
14. Hutkins RW, Ponne C (1991) Lactose uptake driven by galactose efflux in *Streptococcus thermophilus*: evidence for a galactose-lactose antiporter. Appl Environ Microbiol 57:941–944
15. Kandler O (1983) Carbohydrate metabolism in lactic acid bacteria. Antonie van Leeuwenhoek 49:209–224
16. Kimmel SA, Roberts RF (1998) Development of a growth medium suitable for exopolysaccharide production by *Lactobacillus delbrueckii* ssp. *bulgaricus* RR. Int J Food Microbiol 40:87–92
17. Klimek J, Ollis DF (1980) Extracellular microbial polysaccharides: kinetics of *Pseudomonas* sp., *Azotobacter vinelandii*, and *Aureobasidium pullulans* batch fermentations. Biotechnol Bioeng 22:2321–2342

18. Lejeune R, Callewaert R, Crabbe K, de Vuyst L (1998) Modelling the growth and bacteriocin production by *Lactobacillus amylovorus* DCE 471 in batch cultivation. J Appl Microbiol 84:159–168
19. Luedeking R, Piret EL (1959) A kinetic study of the lactic acid fermentation. Batch process at controlled pH. J. Biochem Microbiol Technol Eng 1:393–412
20. Manca de Nadra MC, Strasse de Sand AM, Pesce de Ruiz Hoglado AA, Oliver G (1985) Extracellular polysaccharide production by *Lactobacillus bulgaricus*. Milchwissenschaft 40:409–411
21. Marshall VM, Laws AP, Gu Y, Levander F, Radstrom P, De Vuyst L, Degeest B, Vaningelgem F, Dunn H, Elvin M (2001) Exopolysaccharide-producing strains of thermophilic lactic acid bacteria cluster into groups according to their EPS structure. Lett Appl Microbiol 32:433–437
22. Mercier P, Yerushalmi L, Rouleau D, Dochain D (1992) Kinetics of lactic acid fermentation on glucose and corn by *Lactobacillus amylophilus*. J Chem Technol Biotechnol 55:111–121
23. Parente E, Ricciardi A, Addario G (1994) Influence of pH on growth and bacteriocin production by *Lactococcus lactis* subsp. *lactis* 140 NWC during batch fermentation. Appl Microbiol Biotechnol 41:388–394
24. Petry S, Furlan S, Crepeau M-J, Cerning J, Desmazeaud M (2000) Factors affecting exocellular polysaccharide production by *Lactobacillus delbrueckii* subsp. *bulgaricus* grown in a chemically defined medium. Appl Environ Microbiol 66:3427–3431
25. Ross LF, Chapital DC (1987) Simultaneous determination of carbohydrates and products of carbohydrate metabolism in fermentation mixtures by HPLC. J Chromatogr Sci 25:112–117
26. Sutherland IW (1998) Novel and established applications of microbial polysaccharides. Trends Biotechnol 16:41–46
27. Toba T, Uemura H, Itoh T (1992) A new method for the quantitative determination of microbial extracellular polysaccharide production using a disposable ultrafilter membrane unit. Lett Appl Microbiol 14:30–32
28. Van Kranenburg R, Boels IC, Kleerebezem M, de Vos WM (1999) Genetics and engineering of microbial exopolysaccharides for food: approaches for the production of existing and novel polysaccharides. Curr Opin Biotechnol 10:498–504
29. Weiss RM, Ollis DF (1980) Extracellular microbial polysaccharides I. Substrate, biomass, and product kinetic equations for batch xanthan gum fermentation. Biotechnol Bioeng 22:859–873
30. Zourari A, Accolas JP, Desmazeaud MJ (1992) Metabolism and biochemical characteristics of yogurt bacteria. A review. Lait 72:1–34