Lait (1994) 74, 207-215

Original article

Interrelationship of sugar metabolism (glucose, galactose, lactose) by *Leuconostoc mesenteroides* subsp *mesenteroides*

DQ Huang, H Prévost, C Diviès *

Laboratoire de Microbiologie, ENSBANA, 1, Esplanade Erasme, 21000 Dijon, France

(Received 2 August 1993; accepted 17 March 1994)

Summary — Growth of *Leuconostoc mesenteroides* subsp *mesenteroides* strain 19D on either glucose, lactose and galactose alone or in combination was studied. Growth on galactose was slow and addition of lactose or glucose to cells growing on galactose increased the growth rate and decreased galactose utilization. β-Galactosidase was synthesized in the presence of glucose, but the enzyme level was 2–3-fold higher in lactose grown cells. Galactokinase was strongly repressed by glucose but induced by galactose and lactose. Galactose did not accumulate in the media, suggesting that lactose transport was not *via* a lactose-galactose antiport. The fermentation balance of glucose, lactose and galactose generally agreed with the theoretical values, but in the presence of galactose and lactose, small quantities of formic acid and more acetate instead of ethanol were produced. Lactose was used rapidly alone, but slowly in the presence of galactose. In this case, a slight accumulation of glucose was observed, resulting in partial decrease of galactokinase activity and suggesting the repression of galactose metabolism.

***Leuconostoc* / lactose / galactose / β-galactosidase / galactokinase**

Résumé — Interrelation entre les métabolismes des sucres (glucose, lactose, galactose) par *Leuconostoc mesenteroides* subsp *mesenteroides*. *On a réalisé l'étude de la croissance et l'analyse enzymatique des cellules de* Leuconostoc mesenteroides *subsp* mesenteroides *(souche 19D) en milieu MRS additionné d'un des sucres glucose, lactose, galactose ou de leurs combinaisons 2 à 2. Il apparaît que la β-galactosidase est synthétisée en présence du glucose mais plus faiblement qu'en présence du lactose. La galactokinase réprimée par le glucose est induite par le lactose et le galactose. Au cours de l'utilisation du lactose, le galactose n'est pas accumulé, ce qui exclut un système de transport de type antiport lactose-galactose. L'hétérofermentation du lactose et galactose est caractérisée par une production supérieure d'acétate en comparaison à l'hétérofermentation du glucose. Le galactose ralentit l'utilisation du lactose, ce qui conduit à une accumulation du glucose, donc à la répression de la galactokinase.*

Leuconostoc / *lactose / galactose / β-galactosidase / galactokinase*

* Correspondence and reprints

INTRODUCTION

Leuconostoc are heterofermentative lactic acid bacteria which are often found in aromatic starters used to manufacture butter, fermented milks and some blue cheeses. These bacteria are also involved in a number of vegetable fermentations (Garvie, 1986). Despite industrial interest in *Leuconostoc*, there is a paucity of data concerning the metabolism of the sugars associated with milk, *ie* lactose, galactose, glucose. Recently published work mainly concerns the production of flavour compounds (*eg* diacetyl, acetate) from citrate and pyruvate (Cogan, 1987; Schmitt and Diviès, 1991; Starrenburg and Hugenholtz, 1991; Hugenholtz and Starrenburg, 1992).

In contrast to *Leuconostoc*, much work has been carried out on lactose metabolism in *Lactococcus* (McKay *et al*, 1969, 1970), *Lactobacillus* (O'Leary and Woychick, 1976), and *Streptococcus salivarius* subsp *thermophilus* (Tinson *et al*, 1982). The systems of lactose (galactoside) transport in these microorganisms include ATPase (*Leuc lactis*); ion-linked transport and exchange mechanisms (*S thermophilus* and *Lactobacillus delbrueckii* subsp *bulgaricus*) and phosphoenolpyruvate dependent phosphotransferase (PEP-PTS) (lactococci) (Poolman, 1993). In the PEP-PTS system, lactose is phosphorylated during transport into the cell yielding lactose-6-phosphate, which in turn is hydrolyzed by p-β-gal to glucose and galactose-6-phosphate (Thompson *et al*, 1979). These intermediates are then metabolized *via* the Embden-Meyerhof-Parnas and D-tagatose-6-phosphate pathways respectively (Bissett and Anderson, 1974). However, a lactose-negative *Lactococcus lactis* (DR1251) strain, which is deficient in lactose PTS activity, appears to utilize galactose *via* the Leloir pathway (LeBlanc *et al*, 1979). In lactobacilli, lactose is transported by a permease system, and is hydrolyzed by β-gal to glucose and galactose. Glucose is further metabolized through the Embden-Meyerhof-Parnas pathway, whereas galactose is metabolized through the Leloir pathway (Bissett and Anderson, 1974).

Unlike lactococci, most strains of *S thermophilus* are unable to ferment galactose, either as the free sugar or that generated intracellularly by lactose hydrolysis (O'Leary and Woychick, 1976; Tinson *et al*, 1982). But for galactose-fermenting (Gal^+) strains, lactose and galactose can be transported by an ATP-dependent permease. The galactose formed intracellularly induces galactokinase (galK) and is further metabolized by enzymes of 1 the Leloir pathway (Hutkins *et al*, 1985a). GalK is repressed by glucose and lactose (Hutkins *et al*, 1985b). However, a high level of β-gal activity was detected in the presence of glucose and addition of lactose induced β-gal synthesis (Tinson *et al*, 1982). Recently, Poolman *et al* (1990) demonstrated that the galactose operon was located just upstream from the lactose operon in *S thermophilus*.

Since *Leuconostoc*, particularly *Leuc mesenteroides* subsp *mesenteroides*, are not used as acidifying starters, very little research has been devoted to lactose and galactose metabolism in these organisms. The objective of this study was to examine the utilization of sugars and the biosynthesis of β-gal and galK in *Leuc mesenteroides* subsp *mesenteroides* 19D, in order to enhance our understanding of the metabolic interrelationships of these sugars.

MATERIALS AND METHODS

Bacterial strains and culture media

Leuconostoc mesenteroides subsp *mesenteroides* 19D was obtained from Dr JJ Devoyod (INRA, 78350, Jouy-en-Josas, France), and was maintained by monthly transfer in MRS agar (De Man *et al*, 1960) and stored at 4°C. Stock cultures were maintained in skim milk supplemented

with 30% (v/v) glycerol in liquid nitrogen. Cells were grown in batch culture under static conditions at 30°C in MRS broth pH 6.8 (without regulation). Sugars were filter sterilized before addition to the sterile medium at a rate of 1% (w/v). Inocula were prepared from a single colony grown in MRS broth for 16 h at 30°C. The cells were harvested by centrifugation (4000 *g*, 4°C, 10 min) and washed twice with MRS broth without sugar. The cell biomass concentration was determined by measurement of the absorbance at 575 nm (Novaspec, 4049, LKB) and was converted to cell dry weight per unit volume using a calibration curve. The experimental growth rate (μ) was determined by the following formula: $\mu = dX / dt\ 1/X$; t was time (h), X was biomass (g/l).

Measurement of β-galactosidase

To assay β-gal *in situ*, bacterial cells were permeabilized by the method previously described by Miozzari *et al* (1978). Cells from 1 ml of culture were harvested by centrifugation (4000 *g*, 4°C, 10 min) and the supernatant stored at –20°C for biochemical analyses. The pellet was washed twice with 0.1 mol/l Tris-HCl (pH 7.2), resuspended in 1 ml of permeabilization buffer: 0.1 mol/l Tris-HCl (pH 7.2), containing 0.05% Triton X-100, and stored at –20°C for at least 15 h before use. β-Gal activity was estimated by measuring the rate of hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) (Citti *et al*, 1965). One unit of enzyme was equivalent to 1 nmol of *o*-nitrophenyl (ONP) liberated from ONPG per minute.

Galactokinase assay

Cells were grown in MRS broth containing filter sterilized carbohydrate solutions of either glucose (55.6 mmol/l), galactose (55.6 mmol/l) or lactose (27.8 mmol/l). Cells from 100 ml of medium were harvested and washed twice with 50 mmol/l potassium phosphate buffer (pH 7.2), suspended in 10 ml of the same buffer, sonicated for 15 min (Vibra Cell Sonics Materials, Danbury, CT, USA) and before centrifugation (6000 *g*, 4°C, 15 min). The supernatant was stored at 4°C for assay. All assays were performed within 5 h of cell disruption. GalK activity was assayed by the method of Ballard (1966). The assay mixture contained 0.3 mol/l triethanolamine-HCl (pH 7.8), 1 mol/l KCl, 50 mmol/l ATP, 60 mmol/l $MgCl_2$, 20 mmol/l PEP, 10 mmol/l NADH, 150 mmol/l L-cysteine, 0.5 mol/l NaF, and 20 mmol/l D-galactose in a final volume of 1.4 ml, with non-limiting amounts of pyruvate kinase (10 U) and lactate dehydrogenase (10 U). The galactose was added last to allow correction for NADH oxidase and myokinase activity. The reduction of NADH was measured at 340 nm at 30°C. GalK activity was expressed as nmol of NADH reduced per min, per mg of protein. Protein concentrations were determined by the method of Lowry *et al* (1951) with bovine serum albumin as standard.

Analytical methods

The substrates (glucose, lactose, galactose) and products (D-lactate, ethanol, acetate, formate) were determined enzymatically using Boehringer Mannheim kits.

RESULTS

Sugar utilization by growing cells

As indicated in figure 1, the growth of *Leuc mesenteroides* subsp *mesenteroides* 19D was slow on galactose. When glucose (fig 1a) was added to this growth medium, growth was increased. The addition of glucose immediately resulted in exponential growth ($\mu = 0.26\ h^{-1}$). Growth after the addition of lactose was slightly increased (fig 1b), but without exponential growth. The addition of glucose or lactose caused a small slowing down in galactose consumption, which still continued to be used by cells.

The growth of *Leuc mesenteroides* subsp *mesenteroides* 19D on lactose was exponential (fig 2a). The growth rate was $0.15\ h^{-1}$. When glucose was added to the medium containing lactose, it was increased to $0.20\ h^{-1}$. Glucose was used very rapidly and retarded lactose utilization. Growth on glucose was also exponential ($\mu = 0.21\ h^{-1}$) (fig 2b). When lactose was added to this

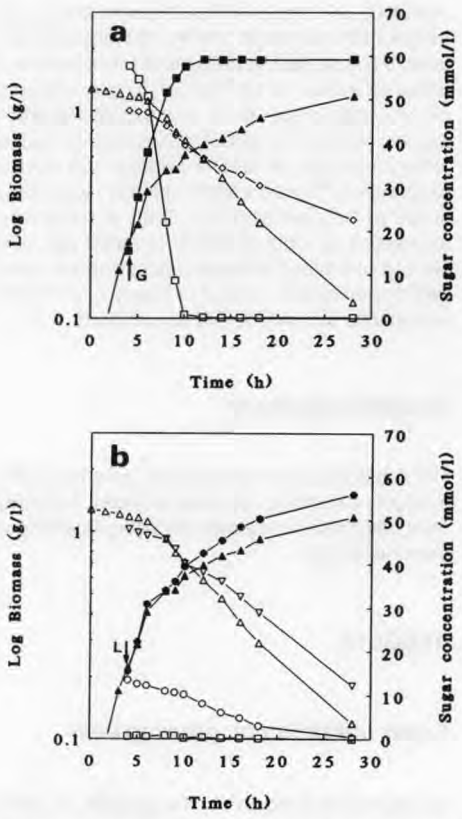

Fig 1. Utilization of glucose or lactose by *Leuconostoc mesenteroides* subsp *mesenteroides* 19D grown at 30°C in the presence of galactose. At the time indicated by the arrows, the culture was divided and 55.6 mmol/l glucose (**G**) or 27.8 mmol/l lactose (**L**) was added to one half medium. **a**. Growth on galactose (▲) and galactose + glucose (■); concentration of galactose in the growth medium in the absence (Δ) and presence (◊) of glucose; concentration of glucose (□) in the growth medium. **b**. Growth on galactose (▲) and galactose + lactose (●); concentration of galactose in the growth medium in the absence (Δ) and presence (∇) of lactose; concentration of lactose (O) and glucose (□) in the growth medium.

Utilisation du glucose ou du lactose par Leuconostoc mesenteroides *var* mesenteroides *19D cultivée à 30°C en présence du galactose. Comme il est indiqué par les flèches, la culture est divisée et 55,6 mmol/l de glucose (**G**) ou 27,8 mmol/l de lactose (**L**) sont ajoutées au milieu. **a.** Croissance sur galactose (▲) et galactose + glucose (■) ; concentration en galactose en absence (Δ) ou en présence (◊) de lactose; concentration en glucose (□). **b.** Croissance sur galactose (▲) et galactose + lactose (●) ; concentration en galactose en absence (Δ) ou en présence (∇) de lactose ; concentration en lactose (O) et en glucose (□).*

growth medium, the growth rate was almost the same ($\mu = 0.22\ h^{-1}$). Lactose was used slowly until the glucose had disappeared, after which it was used more rapidly; there was no effect on glucose utilization.

Product formation from carbohydrate

Fermentation of 1 mol of glucose by *Leuconostoc* produces 1 mol of D-lactate, 1 mol of ethanol (mainly) + acetate and 1 mol of CO_2. For lactose, these figures are doubled (Garvie, 1986). The yields of these compounds from lactose, glucose, and galactose were as predicted (table I). The use of galactose and lactose as the sole sources of carbohydrate led to a greater accumulation of acetate per hexose. In this manner, the ratio of ethanol to acetate was smaller than that from glucose.

Enzymes implicated in lactose hydrolysis

In a previous study, we demonstrated that β-gal activity was about 100 times greater than that of p-β-gal in *Leuc mesenteroides* subsp *mesenteroides* 19D, and was doubled when the cells were grown on lactose (Huang *et al*, 1994a, in press). GalK activity was only detected in cells grown on galactose and lactose; none was present in glucose grown cells (Huang *et al*, 1994b, in press). These results show that β-gal is mainly responsible for lactose hydrolysis in *Leuc mesenteroides* subsp *mesenteroides* and that galK is probably a key enzyme in

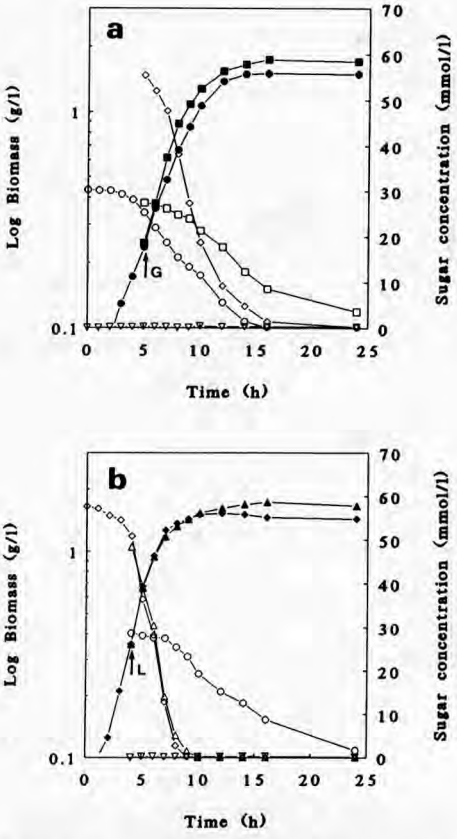

Fig 2. Preferential fermentation of lactose or glucose by *Leuconostoc mesenteroides* subsp *mesenteroides* 19D. The culture was grown on lactose **(a)** and glucose **(b)**. At the time indicated by the arrows, the culture was divided and 55.6 mmol/l glucose (**G**) or 13.9 mmol/l lactose (**L**) was added to the respective media. **a.** Growth on lactose (●) and lactose + glucose (■); concentration of lactose in the absence (O) or presence (□) of glucose; concentration of glucose (◊) and galactose (▽). **b.** Growth on glucose (♦) and glucose + lactose (▲); concentration of glucose in the absence (◊) or presence (△) of lactose; concentration of lactose (O) and galactose (▽) in the growth media.

Utilisation préférentielle du glucose ou du lactose par Leuconostoc mesenteroides *var* mesenteroides *19D. Comme il est indiqué par les flèches, la culture est divisée et 55,6 mmol/l de glucose (**G**) ou 13,9 mmol/l de lactose (**L**) sont ajoutées au milieu. **a.** Croissance sur lactose (●) et lactose + glucose (■) ; concentration en lactose en absence (O) ou en présence (□) de glucose ; concentration en glucose (◊) et en galactose (▽). **b.** Croissance sur glucose (♦) et glucose + lactose (▲) ; concentration en glucose en absence (◊) ou en présence (△) de lactose; concentration en lactose (O) et en galactose (▽).*

Table I. Fermentation balances of *Leuconostoc mesenteroides* subsp *mesenteroides* strain 19D grown in MRS at 30°C with different substrates.
Bilans de fermentation de Leuconostoc mesenteroides *var* mesenteroides *cultivé à 30°C sur milieu MRS en présence de différents sucres.*

Substrates [a]	*Yields (mmol/mmol)*				
	D-Lactate	*Ethanol*	*Acetate*	*Formate*	*EtOH/Acetate* [b]
Glucose	0.89	0.84	0.045	0.004	18.6
Galactose	0.98	0.67	0.193	0.049	3.5
Lactose	1.81	1.73	0.200	0.039	8.6

[a] Cells were grown in MRS medium without citrate and acetate, containing glucose or galactose (55.6 mmol/l) or lactose (27.8 mmol/l). Fermentation products were determined in the culture supernatant of the stationary phase.
[b] EtOH/acetate: ratio between the yields of ethanol and acetate.
[a] Les cellules sont cultivées sur milieu MRS modifié (sans citrate, sans acétate) contenant 55,6 mmol/l de glucose, de galactose ou 27,8 mmol/l de lactose. Les produits de fermentation sont déterminés sur le surnageant de la culture en phase stationnaire. [b] EtOH/Acétate: rapport entre les rendements respectifs en éthanol et en acétate.

the utilization of galactose. Therefore, further studies were focused on these two enzymes.

In the presence of glucose, the rates of lactose and galactose utilization were strongly reduced (figs 1, 2). The effect of glucose (β-gal) and galactose (galK) was studied (figs 3, 4). A high level of β-gal activity was observed for strain 19D grown on lactose. When glucose was added to cells grown on lactose, a significant decrease in β-gal activity was observed, suggesting repression of β-gal synthesis. When lactose was added to cells growing on glucose,

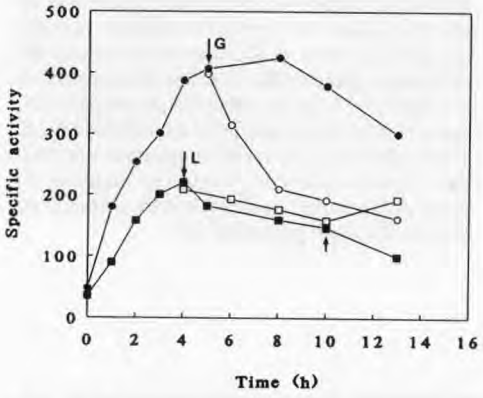

Fig 3. β-Galactosidase activity of *Leuconostoc mesenteroides* subsp *mesenteroides* strain 19D growing on glucose (■) and lactose (●). At the points indicated by the bigger arrows, 55.6 mmol/l glucose were added to lactose grown cells (**G**) and 13.9 mmol/l lactose to glucose grown cells (**L**). Activity in the presence of glucose (■); glucose + lactose (□); lactose (●); lactose + glucose (O). The smaller arrows indicate the time at which glucose was exhausted in the medium.

Activité de la β-galactosidase chez la souche de Leuconostoc mesenteroides *var* mesenteroides *19D cultivée en présence de glucose (■) et de lactose (●). Comme il est indiqué par les grandes flèches, 55,6 mmol/l de glucose sont ajoutées aux cellules cultivées sur lactose et 13,9 mmol/l de lactose sont ajoutées aux cellules cultivées sur glucose. Activité sur milieu glucose (■); glucose + lactose (□); lactose (●); lactose + glucose (O). Les petites flèches indiquent le moment où le glucose est épuisé.*

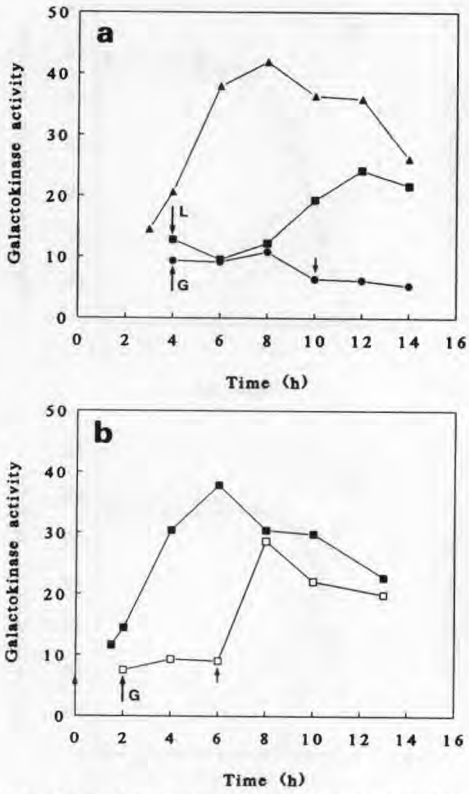

Fig 4. Effect of glucose and lactose on galactokinase activity in *Leuconostoc mesenteroides* subsp *mesenteroides* strain 19D growing on galactose. At the points indicated by the bigger arrows, 55.6 mmol/l glucose (**G**) and 27.8 mmol/l lactose (**L**) **(a)** or 22.2 mmol/l of glucose **(b)** were added to the medium. The smaller arrows indicated the points of glucose exhaustion of the medium. **a.** Galactokinase activity on galactose (▲); galactose + lactose (■); galactose + glucose (●). **b.** Galactokinase activity on lactose (■); lactose + glucose (□).

Effet du glucose et du lactose sur l'activité de la galactokinase chez la souche de Leuconostoc mesenteroides *var* mesenteroides *19D cultivée en présence de galactose. Comme indiqué par les grandes flèches, 55,6 mmol/l de glucose (G) et 27,8 mmol/l de lactose (L)* ***(a)*** *ou 22,2 mmol/l de glucose* ***(b)*** *sont ajoutées au milieu de culture. Les petites flèches indiquent le moment où le glucose est épuisé (figs 4a, 4b).* ***a.*** *Activité de la galactokinase sur milieu galactose (▲); galactose + lactose (■); galactose + glucose (●).* ***b.*** *Activité de la galactokinase sur milieu lactose (■); lactose + glucose (□).*

there was no difference in β-gal activity. However, induction of β-gal activity by lactose was observed only following complete exhaustion of glucose in the medium (fig 3).

When cells were grown on galactose, high galK activity was observed, and following addition of lactose and glucose, significant decreases in enzymatic activity occurred, indicating repression of galK synthesis (fig 4a). The addition of lactose also caused a decrease in galK activity. This sugar is only used slightly in the presence of galactose and induces a momentary accumulation of glucose (0.82 mmol/l) (fig 1b), which probably leads to a decrease of galactokinase activity. As soon as the glucose produced from lactose was exhausted, catabolite repression was lifted, and galK activity increased (fig 4a). Figure 4b shows that galK activity was induced by lactose, since when glucose was added to the cells grown on lactose, a significant decrease of galK activity was observed, which was further increased after glucose exhaustion.

DISCUSSION

When cells of *Leuc mesenteroides* subsp *mesenteroides* 19D were cultivated on glucose as the sole carbohydrate source, β-gal was synthesized, but the activity increased 2–3-fold in the medium containing lactose. Moreover, a decrease of enzyme level was observed when glucose was added to cells grown on lactose. These results are in agreement with those obtained for *S thermophilus* (Tinson *et al*, 1982), and are different from those obtained for *E coli* by Loomis and Magasanik (1967). Furthermore, in the latter organism, the effect of glucose was more marked, and β-gal was not induced while the glucose present in medium was not used. *Leuc mesenteroides* subsp *mesenteroides* 19D possessed weak p-β-gal activity. It seems that a lactose permease system (without translocation) is involved in lactose transport. This is different from lactococci, which have a lactose PEP-PTS system (McKay *et al*, 1970). In *Leuc mesenteroides* subsp *mesenteroides*, galK activity was highest when the cells were grown on lactose and galactose as sole substrate; this result differs from that observed in a *S thermophilus* Gal$^+$ strain, in which galK was induced by galactose, but repressed by lactose (Thomas and Crow, 1984; Hutkins *et al*, 1985b), indicating that galK is subject to an induction-repression mechanism (Hutkins *et al*, 1985b). In *Leuconostoc*, galactose did not accumulate in the medium, suggesting that the mechanism of lactose transport could not be a type of lactose-galactose antiport (Hutkins and Ponne, 1991), but may be a lactose-H$^+$ symport (Poolman, 1993).

The fermentation balances of *Leuc mesenteroides* subsp *mesenteroides* 19D grown on different sugars support the operation of the heterofermentative pathway (Garvie, 1986). Fermentation of galactose and lactose lead to slight formation of formate, and increased acetate production instead of ethanol. This may be explained by activity of pyruvate formate lyase, by which pyruvate is broken down into formic acid and acetyl-CoA (Kandler, 1983). The formation of NAD(P)H necessary for the production of ethanol is limited in the presence of galactose. This suggests the possibility of a partial utilization of the D-tagatose-6-phosphate pathway (Bissett and Anderson, 1974) during galactose or lactose fermentation by *Leuconostoc*. This pathway does not produce NAD(P)H by glucose-6-phosphate dehydrogenase, which would explain the reduced amounts of ethanol formed. An increased yield of lactate was obtained from galactose fermentation, confirming this hypothesis. The fact that there is only a small utilization of galactose or lactose by *Leuconostoc via* the D-tagatose pathway, means the yield of D-lactate may not be increased significantly, thus galactose is fer-

mented predominantly *via* the Leloir pathway in *Leuconostoc.*

The interrelationships between the simultaneous utilization of glucose, lactose, galactose have been studied in *S thermophilus* (Tinson *et al*, 1982; Thomas and Crow, 1984). The inability of *S thermophilus* to use galactose is linked to the lack of galK activity. The presence of galK activity in *Leuconostoc* explains the utilization of galactose. GalK was induced by galactose and lactose, but strongly repressed by glucose. It should be noted, however, that galK activity was partially repressed by lactose, when this was added to cells grown previously on galactose. This was probably due to a small accumulation of glucose (0.82 mmol/l), which repressed galK activity. Recently, we have demonstrated that the lactose phenotype of strain 19D is plasmid-linked and that the β-gal gene is localized on a plasmid of 47.3 kb. Both strains of Lac^+ and its mutant Lac^- show the same growth rate as when they were grown on MRS galactose (55.6 mmol/l) (Huang *et al*, 1994b) indicating that the lactose plasmid does not affect galactose metabolism. These results differ from those observed in *Lactococcus lactis* (LeBlanc *et al*, 1979). It is important to consider the interrelationships between sugar metabolism and the genetic aspects in exploiting the potential of *Leuconostoc* to grow with other lactic acid bacteria.

REFERENCES

Ballard FJ (1966) Purification and properties of galactokinase from pig liver. *Biochem J* 98, 347-352

Bissett DL, Anderson RL (1974) Lactose and D-galactose metabolism in group N streptococci: presence of enzymes for both the D-galactose 1-phosphate and D-tagatose 6-phosphate pathways. *J Bacteriol* 117, 318-320

Citti JE, Sandine WE, Elliker PR (1965) β-Galactosidase of *Streptococcus lactis. J Bacteriol* 89, 937-942

Cogan TM (1987) Co-metabolism of citrate and glucose by *Leuconostoc* spp: effect on growth, substrates and products. *J Appl Bacteriol* 53, 551-558

De Man JC, Rogosa M, Sharpe ME (1960) A medium for the cultivation of lactobacilli. *J Appl Bacteriol* 23, 130-135

Garvie EI (1986) Gram positive cocci - Genus *Leuconostoc. In: Bergey's Manual of Systematic Bacteriology, vol II, 9th edn.* Williams and Wilkins Co, Baltimore. 1071-1075

Huang DQ, Prévost H, Diviès C (1994a) Principal characteristics of β-galactosidase from *Leuconostoc* spp. *Int Dairy J*, in press

Huang DQ, Prévost H, Kihal M, Diviès C (1994b) Instability of plasmid encoding for β-galactosidase in *Leuconostoc mesenteroides* subsp *mesenteroides. J Basic Microbiol*, in press

Hugenholtz J, Starrenburg MJC (1992) Diacetyl production by different strains of aromabacteria *Lactococcus lactis* subsp *diacetylactis* and *Leuconostoc* spp. *Appl Microbiol Biotechnol* 38, 17-22

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

Hutkins R, Morris HA, McKay LL (1985a) Galactose transport in *Streptococcus thermophilus. Appl Environ Microbiol* 50, 772-776

Hutkins R, Morris HA, McKay LL (1985b) Galactokinase activity in *Streptococcus thermophilus. Appl Environ Microbiol* 50, 777-780

Kandler O (1983) Carbohydrate metabolism in lactic acid bacteria. *Antonie van Leeuwenhoek* 49, 209-224

LeBlanc DJ, Crow VL, Lee LN, Garon CF (1979) Influence of the lactose plasmid on the metabolism of galactose by *Streptococcus lactis. J Bacteriol* 137, 878-884

Loomis WF, Magasanik B (1967) Glucose-lactose diauxie in *Escherichia coli. J Bacteriol* 93, 1397-1401

Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 193, 265-275

McKay LL, Walter LA, Elliker PR (1969) Involvement of phosphoenolpyruvate in lactose utilization by group N streptococci. *J Bacteriol* 99, 603-610

McKay LL, Miller III A, Sandine WE, Elliker PR (1970) Mechanism of lactose utilization by lactic acid streptococci: enzymatic and genetic analyses. *J Bacteriol* 102, 804-809

Miozzari GF, Niederberger P, Hutter R (1978) Permeabilization of microorganism by Triton X-100. *Anal Biochem* 90, 220-233

O'Leary VS, Woychick JH (1976) Utilization of lactose, glucose and galactose by a mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk treated with lactase enzyme. *Appl Environ Microbiol* 32, 89-94

Poolman B (1993) Energy transduction in lactic acid bacteria. *FEMS Microbiol Rev* 12, 125-148

Poolman B, Royer TJ, Mainzer SE, Schmidt BF (1990) Carbohydrate utilization in *Streptococcus thermophilus*: characterization of the genes for aldolase 1-epimerase (mutarotase) and UDP glucose 4-epimerase. *J Bacteriol* 172, 4037-4047

Schmitt P, Diviès C (1991) Co-metabolism of citrate and lactose by *Leuconostoc mesenteroides* subsp *cremoris*. *J Ferment Bioeng* 71, 1-3

Starrenburg MJC, Hugenholtz J (1991) Citrate fermentation by *Lactococcus* and *Leuconostoc* spp. *Appl Environ Microbiol* 57, 3535-3540

Thomas TD, Crow VL (1984) Selection of galactose-fermenting *Streptococcus thermophilus* in lactose-limited chemostat cultures. *Appl Environ Microbiol* 48, 186-191

Thompson J, Turner KW, Crow VL (1979) Galactose fermentation by *Streptococcus lactis* and *Streptococcus cremoris*: pathways, products and regulation. *J Bacteriol* 144, 672-682

Tinson W, Hillier AJ, Jago GR (1982) Metabolism of *Streptococcus thermophilus*: utilization of lactose, glucose and galactose. *Aust J Dairy Technol* 37, 8-13