

Review

Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria

J Cerning

Station de recherches laitières, INRA, CRJ, Domaine de Vilvert,
78352 Jouy-en-Josas cedex, France

Summary — The exopolysaccharide (EPS)-producing gram-positive bacteria which have been studied extensively in the last 10 years are the dairy lactic acid bacteria used in the manufacture of fermented milks such as *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp *bulgaricus* and *Lactococcus lactis* subsp *cremoris*. The role of exopolysaccharides in the manufacture of fermented milks and in particular yoghurt is well established; that is, they are essential for proper consistency and texture. A number of strains of these bacteria are capable of producing heteropolysaccharides composed of linear or branched repeating units varying in size from disaccharides to heptasaccharides. The final exopolysaccharide of high molecular weight (1 to 2×10^6) is formed by polymerisation of some hundreds to several thousands of these repeating units. Dairy propionibacteria are also capable of producing exopolysaccharides, but this area of research has received comparatively little attention. Nevertheless, similarities appear when the few results obtained with propionibacteria are compared with those concerning dairy lactic acid bacteria. Fermentation conditions (temperature and incubation time) and medium composition (carbon and nitrogen sources) affect the polymer yield and the sugar composition of the polymer. The most frequent identified monosaccharides in polysaccharides formed by propionibacteria are glucose, galactose and mannose. Small amounts of fucose and rhamnose have also been found. A striking difference exists in the molecular weight of polysaccharides produced by lactic acid bacteria and propionibacteria (*ie*, values for exopolysaccharides from the latter are in the range of 200 to 5 000). The polymer-producing ability is an extremely unstable property; it seems to be linked to the presence of plasmids of varying size in mesophilic lactic acid bacteria, whereas most of the exopolysaccharide-producing strains of thermophilic lactic acid bacteria do not harbour plasmids. Propionibacteria harbour plasmids, but their functions are not clearly established; therefore, the question of whether the EPS-producing trait of propionibacteria is plasmid encoded cannot be answered yet.

lactic acid bacteria / propionibacteria / exopolysaccharide

Résumé — Production de polysaccharides exocellulaires par des bactéries lactiques et les bactéries propioniques. Les bactéries Gram⁺ productrices d'exopolysaccharides les plus étudiées sont les bactéries lactiques utilisées dans la fabrication de divers laits fermentés, telles que *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp *bulgaricus* et *Lactococcus lactis* ssp *cremoris*. Le rôle des polysaccharides exocellulaires dans la fabrication des laits fermentés, et en particulier du

yaourt, n'est plus à démontrer. Il est en effet reconnu que leur présence est indispensable pour obtenir une texture convenable du produit. Un certain nombre de souches de ces bactéries produisent des hétéropolysaccharides constitués d'un enchaînement linéaire ou ramifié d'un petit nombre de sucres (1 à 7) appelé unité répétitive. Ces unités, par enchaînement linéaire ou ramifié d'une centaine à plusieurs milliers, forment le polysaccharide de poids moléculaire souvent très élevé (1 à 2×10^6). Les bactéries propioniques sont également capables de produire des EPS, mais les recherches dans ce domaine sont moins avancées. Toutefois, la comparaison des connaissances sur les bactéries lactiques d'intérêt laitier et les bactéries propioniques permet de dégager un certain nombre de similitudes. En effet, les conditions de culture (température, durée d'incubation) et la composition du milieu (source d'azote et de carbone) ont une influence considérable sur la production des EPS et leur composition en monosaccharides. Les monosaccharides les plus fréquemment identifiés dans les polysaccharides exocellulaires produits par les bactéries propioniques sont le glucose, le galactose, le mannose et parfois le rhamnose ou le fucose. En revanche, les poids moléculaires de l'ordre de 200 à 5 000 sont nettement inférieurs à ceux des exopolysaccharides produits par les bactéries lactiques. La capacité des bactéries lactiques de produire des polymères est une propriété extrêmement instable. Elle est portée par des plasmides de taille variable dans le cas des bactéries lactiques mésophiles, alors que celle des bactéries lactiques thermophiles semble sous contrôle chromosomique. Il est connu que les bactéries propioniques contiennent des plasmides (Rehberger et Glatz, 1990), mais en absence de travaux dans ce domaine, il n'est pas possible pour l'instant de lier la capacité de produire des EPS de ces bactéries à la présence d'un ou plusieurs plasmides.

bactérie lactique / bactérie propionique / exopolysaccharide

INTRODUCTION

Bacteria synthesize a number of polysaccharides which are defined by their location relative to the cell. Some are intracellularly located in the cytosol and used as carbon sources, others are cell wall constituents such as peptidoglycan and teichoic acids and a third group is located outside the cell wall. The latter group can take the form of an adherent, often covalently bound, cohesive layer forming a morphological entity termed *capsule* or *capsular polysaccharide* (CPS). Alternatively, the polymer can consist of a slime polysaccharide with little or no cell association or attachment to the cell surface. In some cases, both capsular and unattached polysaccharides are produced by the same organism and distinguishing between the 2 forms can be difficult. Depending on their structural relationship to the bacterial cell, these polymers have been variously named *slime*, *capsular* or *microcapsular polysaccharides*. The term *exopolysaccharides* (EPS) as proposed by

Sutherland (1972) provides a general name for all these forms of bacterial polysaccharides found outside the cell wall and will be used in this article. In contrast to leuconostocs and oral streptococci, which produce homopolysaccharides, composed of a single sugar (dextrans, α -glucans, fructans), dairy lactic acid bacteria and propionibacteria produce heteropolysaccharides, composed of repeating units varying in size from disaccharides to heptasaccharides.

The aim of this overview is to summarize information available on EPS produced by dairy lactic acid bacteria and dairy propionibacteria. The EPS-producing dairy lactic acid bacteria, which play a beneficial role in the rheological behaviour and the texture of fermented milks, have been studied more extensively in the past 10 years than EPS-producing dairy propionibacteria. However, the comparison of current knowledge in the area of research discussed here is an attempt to reach a common concept in EPS production by the 2 species.

PRODUCTION

EPS production of dairy starters has been assessed previously and continues to be estimated by visual observations or viscosity measurements of fermented milks. Even though viscosity measurements used as an indication for EPS production in liquid media are difficult to interpret, they are at present the only means available to test ropy bacteria easily and rapidly. Recently, methods have been developed for quantifying more precisely the EPS (Cerning *et al.*, 1986, 1988; Garcia-Garibay and Marshall, 1991; Marshall *et al.*, 1995) which are based on the isolation of EPS by ethanol precipitation. Most are tedious and time-consuming and require equipment for precise sugar analysis which is not always available in microbiology laboratories. Furthermore, dairy lactic acid bacteria and propionibacteria are very low EPS producers compared to other microorganisms as, for example, leuconostocs; that is, the yields of EPS are in the range of milligrams rather than grams. This makes the analytical approach and the isolation of EPS from complex fermentation medium particularly difficult.

The quantities of EPS produced in milk by different species and strains vary considerably; the amount of EPS reportedly range from 50 to 350 mg/l for *S thermophilus* (Cerning *et al.*, 1988; Doco *et al.*, 1990), from 60 to 150 mg/l for *Lb bulgaricus* (Cerning *et al.*, 1986; Garcia-Garibay and Marshall, 1991) and from 80 to 600 mg/l for *Lc lactis* subsp. *cremoris* (Cerning *et al.*, 1992). Much lower yields (25 mg/l) have been reported recently for a strain of *Lc lactis* subsp. *cremoris* (Marshall *et al.*, 1995). EPS production from *Lb casei*, which is relatively low in skim milk (50 to 60 mg/l), is stimulated by the addition of glucose or sucrose with values reaching almost 200 mg EPS/l. At present, quantitative results on EPS production by propionibacteria are not available. Data reported by Racine *et al.* (1991) (15 g/l) are in fact values for the dry weight of a crude

precipitate which was not dialysed. Consequently, the crude preparations include whey powder, remaining sugars and minerals, which all precipitate with ethanol and should have been eliminated before EPS analysis.

The EPS-producing trait in thermophilic and mesophilic lactic acid bacteria is unstable. This instability has been attributed to loss of plasmids in mesophilic lactic acid bacteria (Vedamuthu and Neville, 1986; Wright and Tynkkynen, 1987; Vescovo *et al.*, 1989; Kojic *et al.*, 1992; Cerning *et al.*, 1994). For thermophilic bacteria such as *S thermophilus* and *Lb bulgaricus*, this may not be the explanation as these strains apparently do not harbour plasmids. Propionibacteria are known to contain plasmids (Rehberger and Glatz, 1990), but their functions have not been clearly established. Therefore, the question whether the EPS-producing ability of propionibacteria is plasmid-encoded cannot be answered yet.

Decline in viscosity and EPS yield upon prolonged incubation has been tentatively explained as a result of EPS hydrolysis by extracellular glycohydrolases in *S thermophilus* (Cerning *et al.*, 1988) and *Lactococcus lactis* subsp. *cremoris* (Macura and Townsley, 1984), but this has not been proved experimentally. Similar observations are described for *Propionibacterium acidipropionici* fermented in whey-based medium (Racine *et al.*, 1991).

Effects of fermentation conditions on EPS production

Synthesis and secretion of EPS occur during different growth phases and the type of polymer is influenced by growth conditions such as temperature and incubation time. The yield of EPS produced by lactic acid bacteria and propionibacteria is not a direct function of growth. Indeed, EPS production has often been found to be greater at lower temperatures. Schellhaass (1983), Teggtatz

(1990) and Mozzi *et al* (1995) demonstrated an increased EPS production from *S thermophilus* and *Lb bulgaricus* at incubation temperatures of 32 or 37°C instead of 42°C. EPS production from mesophilic lactic acid bacteria is almost 50% higher when the organisms are grown at 25°C instead of 30°C (Cerning *et al*, 1992). For some strains of *Lc lactis* subsp *cremoris*, temperatures as low as 18°C have been chosen to enhance EPS synthesis (Kontusaari and Forsen, 1988). *P acidi propionici* produced more EPS at 25°C, a temperature slightly lower than the optimum growth temperature (Racine *et al*, 1991). Among the many species of propionibacteria tested for EPS production by Skogen *et al* (1974), some showed an increase in viscosity of over 100% when grown at 15°C rather than at 21°C (*P zeae*) or 21°C instead of 32°C (*P arabinosum*). These results are consistent with the proposed mechanism, namely that slowly growing cells exhibit much slower cell wall polymers synthesis, making more lipid carrier available for EPS synthesis (Sutherland, 1972, 1982).

Adjustment of the pH to 6.0 of the whey-based fermentation medium for growth of *S thermophilus*, *Lb bulgaricus* and *Lc lactis* supposedly promotes EPS formation as relative viscosities increase (Schellhaass, 1983). Skogen *et al* (1974) found that various initial pH levels of the medium (yeast extract-sodium lactate) had a considerable effect on absolute viscosities obtained with *P zeae* P74, the highest viscosity being obtained with an initial pH of 8. The optimum pH value for EPS synthesis by *P acidi propionici* in whey-based medium is close to 6.0 (Racine *et al*, 1991).

Effect of medium composition on EPS production

Many of the investigations on EPS formation by starter cultures used in the manufacture

of yoghurt and fermented milks (*S thermophilus*, *Lb bulgaricus*, *Lc lactis* subsp *cremoris*) have been carried out with milk (*ie*, lactose being the fermentable sugar). Attempts have been made to produce EPS on milk-derived medium such as milk ultrafiltrate or whey concentrates. Recently, chemically defined medium have been used successfully for EPS production from *Lc lactis* subsp *cremoris* and *Lb casei* (Gruter *et al*, 1993; Cerning *et al*, 1994; Marshall *et al*, 1995). Compared to milk or whey, synthetic media allow faster and easier isolation of EPS and facilitate the study of the influence of individual components of the medium (*eg*, influence of sugars on EPS production).

Contradictory results have been reported regarding the effect of stimulating factors on EPS production. Enhanced EPS production and growth were obtained when hydrolysed casein was added to skim milk cultures of *Lb bulgaricus* (Garcia-Garibay and Marshall, 1991). According to Schellhaass (1983), neither growth nor EPS production was specifically linked to the presence of casein or whey proteins in the medium for thermophilic and mesophilic lactic acid bacteria. However, others found that casein stimulates EPS production but not growth of *Lb bulgaricus* (Cerning *et al*, 1986). It has also been reported that *Lb bulgaricus* is able to produce the same amount of EPS in milk and milk ultrafiltrate, but that *S thermophilus* cannot (Cerning *et al*, 1990). On the other hand, supplementation of milk ultrafiltrate with glucose or sucrose stimulates EPS production by *Lb casei* and modifies the composition of the EPS (Cerning *et al*, 1992). Experiments with a defined medium for growth of 2 strains of *Lc lactis* subsp *cremoris* showed that the organisms produce the same EPS in comparable amounts in this medium compared to milk (Gruter *et al*, 1992; Marshall *et al*, 1995).

Fermentation experiments for production and analysis of EPS of propionibacteria have been carried out either with depro-

teinized whey-based medium supplemented with ammonium chloride and lactose (Racine *et al*, 1991) or yeast extract broth supplemented with lactate, glucose or sucrose (Skogen *et al*, 1974). A high carbon:nitrogen ratio (60 g lactose/l) enhanced EPS production of *P. acidipropionici*. This is in agreement with results obtained with other bacteria (Sutherland, 1972, 1982). A great number of carbon sources have been tested by Skogen *et al* (1974) and a considerable increase in viscosity was obtained with *P. zeae* P74 grown on glucose, maltose and raffinose. When the Skogen group's paper was published, very little was known about EPS production by dairy lactic acid bacteria and propionibacteria. At present, it is generally accepted that the 2 species are able to produce EPS in media containing various carbon sources (Cerning *et al*, 1992, 1994). Not only the nature of the carbon source and sometimes the combination of sugars, but also their concentration have a stimulating effect on EPS synthesis.

PROPERTIES

Isolation and purification

As mentioned earlier, isolation and subsequent purification of EPS is difficult and tedious, in particular when EPS-producing microorganisms such as thermophilic and mesophilic lactic acid bacteria are grown in a coagulated milk system. However, even isolation of EPS from lesser complex medium such as whey, milk ultrafiltrate or chemically defined media must be considered with caution. EPS is always found together with and surrounding the microorganisms that have produced it and from which they can hardly be entirely separated. Furthermore, it is mixed with different carbohydrates and proteins or peptides from

the medium itself. Such contaminations are inherent to the isolation procedure and ethanol precipitation. Moreover, when a medium contains yeast extract, isolated EPS are always heavily contaminated with the high molecular weight fraction of mannanes which are not eliminated by dialysis. This concept is often overlooked and may account for some of the discrepancies in the literature.

Purification of EPS from dairy lactic acid bacteria has been successfully achieved by DEAE cellulose chromatography (Oda *et al*, 1983; Cerning *et al*, 1986; Doco *et al*, 1990; Nakajima *et al*, 1990; Marshall *et al*, 1995) and this technique should be convenient as well for purification of EPS from propionibacteria. Contaminating proteins or peptides can also be removed by gel filtration chromatography, the principal problem here may be the occasionally high viscosities of the EPS solutions.

TCA precipitation of contaminating material such as proteins or peptides from milk cultures has been proposed by Garcia-Garibay and Marshall (1991). This method has the advantage of being faster than the previously mentioned techniques. However, a great proportion of the EPS will co-precipitate in TCA and it is important to wash the precipitate at least once if not twice for complete EPS recovery.

Physicochemical characteristics and sugar composition

The EPS have apparent molecular weights that range from 5×10^5 (*Lb. bulgaricus*) (Cerning *et al*, 1986), 1.7×10^6 (*Lc. lactis* subsp. *cremoris*) (Nakajima *et al*, 1990), 1.6×10^6 (*Lb. helveticus*) (Yamamoto *et al*, 1994) to 1×10^6 (*S. thermophilus*) (Doco *et al*, 1990) (table I). EPS containing 2 fractions of different molecular weights (2×10^6 and 3.5×10^3) have been obtained from *S. thermophilus* (Cerning, 1990) and from *Lc. lactis*

Table I. Heteropolysaccharides from lactic acid bacteria and propionibacteria.
Hétéropolysaccharides produits par les bactéries lactiques et les bactéries propioniques.

<i>Species/subspecies</i>	<i>Component sugars</i>	<i>Mol weight</i>	<i>References</i>
<i>S thermophilus</i>	Galactose, glucose, N-acetyl-glucosamine	1×10^6	Doco <i>et al</i> , 1990
<i>Lb delbrueckii</i> subsp <i>bulgaricus</i>	Galactose, glucose rhamnose	5×10^5	Cerning <i>et al</i> , 1986; Gruter <i>et al</i> , 1993
<i>Lb helveticus</i>	Glucose, galactose, rhamnose, N-acetyl-glucosamine	1.6×10^6	Yamamoto <i>et al</i> , 1994
<i>Lb paracasei</i> subsp <i>paracasei</i>	Glucose, galactose rhamnose		Cerning <i>et al</i> , 1992, 1994
<i>Lb helveticus</i> var <i>jugurti</i>	Glucose, galactose	2×10^6	Oda <i>et al</i> , 1983
<i>Lc lactis</i> subsp <i>cremoris</i>	Galactose, glucose rhamnose, phosphate	1.7×10^6	Nakajima <i>et al</i> , 1990
<i>Lc lactis</i> subsp <i>cremoris</i>	Galactose		Gruter <i>et al</i> , 1992
<i>Lc lactis</i> subsp <i>cremoris</i>	Galactose, glucose N-acetyl-glucosamine	1×10^6	Marshall <i>et al</i> , 1995
<i>Lc lactis</i> subsp <i>cremoris</i>	Glucose, galactose, rhamnose, N-acetyl-glucosamine	1×10^4	Marshall <i>et al</i> , 1995
<i>P freudenreichii</i> subsp <i>shermanii</i>	Glucose, galactose methylpentose	5×10^3	Crow, 1988
<i>P zeae</i> 74	Galactose, glucose, mannose		Skogen <i>et al</i> , 1974
<i>P acidi propionici</i>	Glucose, galactose, mannose, rhamnose, fucose	6×10^3	Racine <i>et al</i> , 1991

subsp *cremoris* ($>1 \times 10^6$ and 1×10^4) (Marshall *et al*, 1995).

A striking difference exists in the molecular weights of EPS from lactic acid bacteria and those reported for EPS from propionibacteria. The EPS isolated from a fermentation medium of *P freudenreichii* subsp *shermanii* contained 3 fractions with molecular weights of 200 to 350, 350 to 450 and 5×10^3 (Crow, 1988), while that from *P acidi propionici* had a molecular weight of 5.8×10^3 (Racine *et al*, 1991). EPS of such small molecular weights are not likely to play a role in the rheological behaviour and viscosity of a medium. The EPS had been isolated after prolonged incubation (80 to 90 h), thus it cannot be excluded that some degradation of the EPS occurred; these results need more confirmation.

Some of the EPS exhibit remarkable thickening properties: the intrinsic viscosity of EPS from *S thermophilus* is 1.75 dl/g (Doco *et al*, 1990); from *Lb casei*, 1.13 dl/g; and from *Lb bulgaricus*, 4.7 dl/g. Similar information on EPS from propionibacteria is not available.

EPS from *S thermophilus* CNRZ 404 contains neutral sugars (*ie*, galactose and glucose, glucosamine) along with low levels of pentose sugars and mannose (Cerning *et al*, 1988; Doco *et al*, 1990). EPS from *Lb bulgaricus* contains galactose, glucose and rhamnose (Cerning *et al*, 1986; Gruter *et al*, 1993). Recently, 2 EPS concurrently produced by the same strain of *Lc lactis* subsp *cremoris* have been identified. One has a high molecular weight ($< 1 \times 10^6$) and contains galactose, glucose and glucosamine; the second is smaller (1×10^4), is a charged polymer with a high phosphate content and the sugar constituents are galactose, glucose, glucosamine and rhamnose (Marshall *et al*, 1995). A high phosphate content of EPS produced by another strain of *Lc lactis* subsp *cremoris* has been reported by Nakajima *et al* (1990).

The structure of the repeating units of EPS produced by several lactic acid bacteria including *S thermophilus* (Doco *et al*, 1990), *Lb bulgaricus* (Gruter *et al*, 1993), *Lc lactis* subsp *cremoris* (Gruter *et al*, 1992; Marshall *et al*, 1995) and *Lb helveticus* (Yamamoto *et al*, 1994), have been elucidated.

Little literature has been devoted to the composition of EPS produced by propionibacteria. However, as mentioned previously, when the few obtained results are compared with those from dairy lactic acid bacteria, similarities appear. The EPS produced by *P freudenreichii* subsp *shermanii* contained high proportions of methylpentoses along with lower amounts of glucose and galactose (Crow, 1988). *P zeae* P74 produced an EPS composed of large amounts of mannose and lesser quantities of glucose and galactose (Skogen *et al*, 1974). The EPS was produced on a yeast extract containing medium, therefore one cannot rule out the possibility of mannose overestimation. The EPS isolated from *P acidi propionici* grown on whey-based medium was composed of a water-soluble and water-insoluble fraction (Racine *et al*, 1991). The soluble fraction was composed of 22% rhamnose, 10% mannose and 34% of both galactose and glucose. The water-insoluble fraction of the EPS was composed of 7% fucose, 22% mannose, 40% galactose and 31% glucose. Further investigations are needed to conclude whether 2 distinct EPS were produced here. Fractions of different solubilities have also been reported for EPS from *S thermophilus* (Cerning *et al*, 1988).

Functions of EPS

The functions of EPS from propionibacteria have not been studied to a large enough extent, while those of EPS from thermophilic and mesophilic dairy starter are now well recognized. In Scandinavian countries,

slime-forming strains of *Lc lactis* subsp *cremoris* are used in the manufacture of fermented milks such as "Viili" or "Lonfil", where the production of EPS is essential for proper consistency (Kontusaari and Forsen, 1988). EPS producing starter cultures such as *S thermophilus* and *Lb bulgaricus* are largely used in yoghurt manufacture, particularly in France and the Netherlands where the addition of texture-promoting additives is not allowed. It is obvious that EPS are necessary to increase viscosity and improve the texture of fermented milks, but the magnitude of increase varies because of differences in culture strain, incubation conditions total solid content and viscosity measurements. Moreover, viscosity may not be only affected by the amount of EPS released, but also by an EPS with slightly different structure, resulting in different rheological characteristics of the medium. The complex relationship between viscosity and EPS production have been described earlier (Schellhaass and Morris, 1985; Teggatz and Morris, 1990) and it is well established that in ropy cultures EPS not only interacts with the caseins but is also attached to the bacterial surface, the EPS and bacteria interaction being disrupted with increasing shear stress. It is therefore evident that the role of slime-producing cultures is particularly important in stirred yoghurt. Furthermore, it has been claimed that EPS isolated from lactic acid bacteria cultures may have anti-tumor activity (Oda *et al*, 1983; Doco *et al*, 1990).

At present, the functions of EPS from propionibacteria have not been investigated. EPS produced from 3 species of propionibacteria (Reddy *et al*, 1973) did not induce detectable precipitating antibody formation in rabbits and it was concluded that the EPS did not have immunologic properties. It was suggested that growth of propionibacteria in whey would be a means of upgrading the dairy by-product by lactose fermentation and a possibility of EPS production (Racine *et al*, 1991).

BIOSYNTHESIS AND ASSEMBLY

EPS are synthesized in different growth phases and under a variety of conditions depending on the organism studied. Two distinct mechanisms of synthesis are known for exopolysaccharides. Homopolysaccharides such as dextrans and levans produced by leuconostocs and oral streptococci are synthesized by an extracellular process involving enzymes which are either secreted from the bacteria or loosely associated with the cell surface. Heteropolysaccharides are synthesized by a more complex system in that they are produced at the cytoplasmic membrane utilizing precursors formed intracellularly. In contrast to exopolysaccharides and capsular polysaccharides from gram-negative bacteria (for reviews see Sutherland, 1982; Whitfield and Valvano, 1993), which have been extensively investigated, little is known, as yet, on the biosynthesis of EPS produced by dairy lactic acid bacteria and propionibacteria. It is, however, probable that the mechanism proposed for EPS and capsular polysaccharides from gram-negative bacteria can also be accepted for EPS from gram-positive bacteria, because the structure of the latter are based, as are the former, on the polymerization of repeating sugar units (Doco *et al*, 1990; Gruter *et al*, 1992, 1993; Nakajima *et al*, 1992; Yamamoto *et al*, 1994; Marshall *et al*, 1995).

The synthesis of bacterial EPS involves a larger number of enzymes which are not unique to EPS formation. Sugar nucleotides (nucleotide diphosphate sugars) play an important role in EPS synthesis: they are the activated form of the monosaccharides and provide the microbial cell with a means of interconversion of various monosaccharides through epimerization, dehydrogenation and decarboxylation reactions.

Furthermore, isoprenoid glycosyl carrier lipids are involved in EPS synthesis (Sutherland, 1982; Sutherland and Tait,

1992; Whitfield and Valvano, 1993). The lipid involved is undecaprenol phosphate, identical to the carrier lipid also involved in the synthesis of cell wall polymers such as lipopolysaccharides, peptidoglycan and teichoic acids. This explains why EPS production is reduced under conditions which stimulate increased lipopolysaccharide or teichoic acid production due to the competition for the same lipid carrier. In addition, EPS production by dairy lactic acid bacteria and propionibacteria has been found to be greater at lower growth temperatures (Racine *et al.*, 1991; Cerning *et al.*, 1992). If cells are growing more slowly, then cell wall polymer formation will be slower, thereby making more isoprenoid phosphate available for EPS synthesis.

CONCLUSION

Among the 3 essential groups of EPS produced by lactic acid bacteria, dextrans and mutans have been the subject of numerous investigations because they have a wide field of industrial and medical applications. Cell surface polysaccharides or capsules from gram-negative and gram-positive bacteria have been studied extensively because they play a critical role in antigenicity and because many encapsulated bacteria are pathogens. EPS produced by dairy lactic acid bacteria and propionibacteria have not been investigated in depth. However, over the past 10 years, there has been an increasing interest in this field, because *in situ* production of EPS in fermented milks increases viscosity, improves stability in these products and thus has a clear economic benefit. Dairy propionibacteria as well as lactic acid bacteria have the potential to ferment low-value substrates such as whey, which could be a means of upgrading this dairy by-product by lactose fermentation with concurrent production of a useful polymer for food and nonfood uses. There are

still major gaps in our knowledge such as what governs the amount and the structural organization of EPS in a given organism and how can the instability of the EPS-producing trait be explained? Particularly intriguing questions are, what is the precise role played by undecaprenol lipids, how are the EPS transported across the cytoplasmic membrane, periplasm or outer membrane and how is the biosynthesis regulated? Answers to these questions may be available in the near future, provided the scientific community continues the research effort in this field.

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