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Physiological responses of *Lactococcus lactis* ML3 to alternating conditions of growth and starvation

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Abstract. *Lactococcus lactis* species can survive periods of carbohydrate starvation for relatively long periods of time. In the first hours of starvation, however, the maximal glycolytic and arginine deiminase (ADI) pathway activities decline rapidly. The rate of decrease of the pathway activities diminishes as soon as the cells become depleted of energy-rich intermediates. Loss of glycolytic activity is associated with loss of glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate mutase and pyruvate kinase activities. Upon addition of sugar to starved cultures these enzymatic, and thus the glycolytic, activities can be restored to 100% values. The recovery of enzymatic activities is inhibited by chloramphenicol, indicating that protein synthesis is involved. In contrast, restoration of ADI pathway activity does not require de novo synthesis of proteins. General protein degradation and synthesis have been studied in growing and starving cells using [³⁵S]methionine-labeling of proteins and two-dimensional gel analysis. The breakdown of bulk proteins in exponentially growing cells shows first-order rate kinetics ($t_{1/2}$ of approximately 5 h). Following an initial breakdown of proteins with a $t_{1/2}$ of 5 h during the first hour(s) of starvation, bulk proteins are degraded very slowly in starving energy-depleted cells. The breakdown of proteins during starvation appears to be (largely) nonspecific. The rate of synthesis of proteins decreases rapidly in the first hour(s) of starvation. From the onset of starvation on at least 45 proteins are no longer synthesized. During starvation relative induction of fourteen to fifteen proteins can be observed.

Key words: *Lactococcus lactis* — Growth and starvation physiology — Protein degradation and synthesis

It has been shown that survival of lactococci upon carbohydrate starvation is related to the maintenance of glycolytic capacity (Poolman et al. 1987a). Loss of glycolytic activity results in a decreased ability to synthesize ATP and to generate a proton motive force, and as a consequence, after long periods of starvation the organism fails to accumulate nutrients that are essential for growth even upon renewed addition of sugar. The loss of glycolytic activity in *Lactococcus lactis* subsp. *cremoris* Wg2 has been associated with specific loss of glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate mutase activities (Poolman et al. 1987b). It has also been shown that under certain conditions glyceraldehyde 3-phosphate dehydrogenase can be significantly rate limiting for glycolysis in *L. lactis* (Poolman et al. 1987b), emphasizing the importance of the enzyme in starvation survival.

In the first hour(s) of lactose starvation of *L. lactis*, when culture is still fully viable, ATP levels fall to concentrations below 0.1 mM, and the membrane potential ($\Delta\psi$) and pH gradient (ΔpH) collapse (Otto et al. 1985; Poolman et al. 1987b). In the initial phase of starvation a transient increase in “PEP-pool” intermediates (3-phosphoglycerate, 2-phosphoglycerate and phosphoenolpyruvate) has been observed (Mason et al. 1981; Otto et al. 1985; Poolman et al. 1987a; Thompson and Thomas 1977). The decrease of the PEP-pool intermediates during later phases of starvation has been attributed to the inhibition of pyruvate kinase by inorganic phosphate (Mason et al. 1981; Thompson and Thomas 1977; Thompson 1978, Thompson and Torchia 1984) and the decrease of fructose 1,6-diphosphate (activator of pyruvate kinase; feedforward control of glycolysis) (Konings et al. 1989; Poolman et al. 1987a; Thompson 1978). Under certain conditions the slow utilization of PEP by pyruvate kinase can provide the cells with maintenance energy during prolonged periods of starvation, and also permits the rapid accumulation of phosphoenolpyruvate:sugar phosphotransferase (PTS)-sugars once these become available again (Dawes 1985; Thompson and Thomas 1977). Interestingly, the drop in the energy-rich intermediates in the first hour(s) of starvation coincides with the inactivation of glycolysis resulting in loss of 60–80%

Abbreviations: ADI, Arginine deiminase; ATP, adenosine triphosphate; PEP, phosphoenolpyruvate; $\Delta\psi$, membrane potential; ΔpH , pH gradient; PTS, sugar phosphotransferase system; CDM, chemically defined medium; TCA, trichloro-acetic acid

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of the original activity (Poolman et al. 1987b and this study). The remaining 20–40% activity slowly decreases further. In this paper the nature of the inactivation of the glycolytic pathway was investigated further. Moreover, attempts were made to characterize the bulk protein breakdown in *L. lactis* during starvation, and to analyze the ability of the organism to synthesize new proteins during different stages of starvation.

Materials and methods

Organism and culture conditions

Lactococcus lactis subsp. *lactis* ML3 (NCDO 763) was grown in a chemically defined medium (Otto et al. 1983, Poolman and Konings 1988) supplemented with 5 mM galactose and 5 mM arginine unless indicated otherwise. All cultures were grown in flasks semi-aerobically at 30 °C. The arrows in the figures indicate the additions that were made to the culture media in which the cells were growing or starving.

Viable count measurements

Culture samples were diluted stepwise with complex MRS-medium (De Man et al. 1960). Finally, 50 µl of the diluted cell suspension was spread on agar plates containing complex MRS medium, 1.5% agar and 0.2% (wt/vol) galactose. The plates were incubated for 3 days at 30 °C. Viable counts were determined in triplicate from plates containing 20 to 300 colonies.

Glycolysis and arginine deiminase pathway activity

Prior to measurement of metabolic activities culture samples were washed twice and resuspended to a final protein concentration of 0.3 mg/ml in 0.5 mM potassium-phosphate (pH 6.5) containing 70 mM KCl and 1 mM MgSO₄. Following equilibration at 30 °C galactose, lactose or glucose (10 mM each) for glycolytic activity or arginine (2 mM) for arginine deiminase (ADI) pathway activity were added. Acidification (lactic acid production by glycolysis) and alkalization (ammonia production by ADI pathway activity) of the medium were monitored with a sensitive pH-electrode connected to an amplifier and recorder essentially as described (Poolman et al. 1987c). In some experiments, simultaneously with the pH measurements, samples were withdrawn from the vessel and lactate concentrations in the supernatant were determined by gas chromatography (Laanbroek and Pfennig 1981).

Enzymatic activities

Cells were harvested, washed and resuspended to a final A₆₆₀ of 80–100 in either 100 mM potassium-phosphate (pH 7.0) or 100 mM triethanolamine-Cl (pH 7.6) each supplemented with 10% (vol/vol) glycerol. Cells were broken by cycle sonication (8 × 15 s with 45-s intervals; amplitude intensity of 8 µm) under nitrogen at 4 °C. To remove the cell debris the extracts were centrifuged for 60 min at 48000 × g at 4 °C. The enzymatic activities in the cell lysates were determined by standard procedures involving NAD(H)- or NADP-coupled assays (Bergmeier 1974), essentially as described by Poolman et al. (1987b).

Bulk protein degradation

L. lactis ML3 cells were grown in chemically defined medium (CDM), in which the methionine concentration was lowered to 50 µM (normal concentration is 800 µM), supplemented with

10 mM galactose and 10 mM arginine. After two doubling times and at an A₆₆₀ of 0.1, 7.4 MBq [³⁵S]methionine was added to 30 ml of cells for labeling of proteins synthesized during exponential growth. After approximately two doubling times (A₆₆₀ of about 0.4), cells were collected by centrifugation, washed with CDM without methionine but containing 3 mM galactose and 3 mM arginine, and resuspended to an A₆₆₀ of 0.4 in the same medium (growing conditions) or in CDM without methionine and energy source (starving conditions). To prevent protein synthesis from [³⁵S]methionine released during protein breakdown, unlabeled methionine (1 mM) was added to the cultures. Incubations were carried out at 30 °C. Following the washing/resuspension steps, samples (0.6 ml) were taken at different time intervals and the proteins were precipitated with 50% trichloro-acetic acid (TCA) for 10 min at 4 °C. The suspensions were centrifuged and the pellets were washed successively with 10 and 5% TCA solutions and finally with acetone (−20 °C). The radioactivity in the protein was determined by liquid scintillation spectrometry.

Bulk protein synthesis

Since methionine is required for growth of *L. lactis* ML3, cells were harvested and washed twice with CDM without methionine prior to labeling of the proteins. Subsequently, the cells (0.6 ml, A₆₆₀ = 1.0) were incubated for 10 min at 30 °C with 0.37 MBq [³⁵S]methionine. Incorporation of [³⁵S]methionine was stopped by the addition of cold methionine (500 µM, final concentration). Proteins were precipitated and radioactivity was counted as indicated above.

Two-dimensional gel electrophoresis

Protein samples were separated by two-dimensional gel electrophoresis as described (Garrels 1979; Groat et al. 1986), except LKB ampholines were used in the first isoelectric focussing dimension. The second dimension was a sodium dodecyl sulfate-11%-polyacrylamide gel. Equivalent amounts of radioactivity were loaded for each sample (approximately 800000 cpm). Labeled proteins were visualized by autoradiography on XAR-5 film (Eastman Kodak Co.).

Isolation of membrane vesicles

Exponentially growing and galactose/arginine starving cells of *L. lactis* ML3 (1 l cultures, A₆₆₀ of 0.5) were harvested, washed three times and resuspended in minimal volumes of 100 mM potassium-phosphate (pH 7.0). Droplets of the cell suspensions were frozen in liquid nitrogen and immediately stored at −80 °C. Membrane vesicles were prepared from these cells by osmotic lysis as described (Otto et al. 1982).

Transport assays

(i). *Diffusion potential driven uptake.* Membrane vesicles were incubated for 1 h at 4 °C in 20 mM potassium-phosphate (pH 6.5) containing 100 mM potassium-acetate and valinomycin (2 nmol/mg of protein). The membrane vesicles were subsequently pelleted by microfuge centrifugation for 25 min. After resuspension in the same buffer to a final concentration of approximately 25 mg of protein/ml, 2-µl samples were diluted 100-fold into 20 mM sodium-phosphate (pH 6.5) containing 100 mM sodium-HEPES, and ¹⁴C-labeled amino acids (0.7–2.0 µM, final concentration). This imposed a ΔpH and ΔΨ (inside alkaline and negative). As control, potassium-phosphate/potassium-acetate loaded vesicles were diluted into the same buffer, so that no ΔpH and ΔΨ were formed. At chosen time intervals samples were filtered (cellulose nitrate or cellulose acetate

filters, Millipore, 0.45 μ m pore size) and washed twice with 2 ml of ice-cold 0.1 M LiCl. Transport experiments were performed at 30 °C. The initial rate of transport was determined from the uptake after 2.5 s. Radioactivity was determined by liquid scintillation spectrometry.

(ii). *Arginine/ornithine exchange*. Membrane vesicles were incubated for two h at room temperature in 50 mM potassium-phosphate (pH 7.0) containing 500 μ M ornithine. Subsequently, the membrane vesicles were pelleted by microfuge centrifugation and resuspended in the same buffer to a concentration of approximately 25 mg of protein/ml. The exchange reaction was initiated upon 100-fold dilution of 2 μ l of concentrated vesicle suspension into 50 mM potassium-phosphate (pH 7.0) containing [^{14} C]arginine (1.5 μ M, final concentration). Subsequent steps and conditions were as described above.

Miscellaneous

Protein concentrations were measured according to Lowry et al. (1951) or Bradford (1976) using bovine serum albumin as standard.

Results

Metabolic activities

Lactococcus lactis ML3 co-metabolizes galactose and arginine thereby producing almost equimolar amounts of acid (lactic acid) and base (ammonia) which leaves the medium pH largely constant during growth. This property was used to set up several cultures without the necessity of external pH control. During growth of *L. lactis* ML3 in chemically defined medium (CDM) containing 5 mM galactose and 5 mM arginine the pH of the medium decreased from 6.7 to 6.4 (Fig. 1A). When the galactose was consumed, growth stopped, and the pH rose to 6.7 due to the metabolism of residual arginine. Culture viability decreased by less than 10% in the first 12 h of starvation, i.e. after the galactose was consumed (data not shown).

Glycolytic activity

Under the conditions employed more than 98% of the galactose was converted into lactic acid. Consequently, the glycolytic activities inferred from the acidification of the medium (pH recordings) and the production of lactic acid (gas chromatography) were very similar (Fig. 1B). During galactose (and arginine) starvation the maximal glycolytic activity decreased rapidly to approximately 35% of the activity of exponentially growing cells (Fig. 1B). Upon addition of galactose (and arginine) to starved cultures the glycolytic activity could be restored, but the longer the starvation period had lasted the longer it took before the activity was fully restored (Figs. 1B and 2A). Recovery of glycolytic activity required de novo synthesis of proteins since chloramphenicol inhibited the process (Fig. 2A).

Loss of glycolytic activity in *L. lactis* subsp. *cremoris* Wg2 was associated with loss of glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate mutase activity (Poolman et al. 1987b). Similarly, these enzymatic activities rapidly decreased during starvation of *L. lactis* ML3 (Fig. 3A and B). In addition, loss of pyruvate kinase activity was observed (Fig. 3C). The activities of all three enzymes were restored to 100% values upon addition of galactose to starving cultures. The activities were not restored in the presence of chloramphenicol (data not shown).

Since galactose/arginine grown cells of *L. lactis* are also induced for the arginine-ornithine antiporter and the ADI enzymes, the effect of starvation on ADI pathway activity was examined. The ADI pathway activity also decreased during starvation but, surprisingly, the recovery of activity upon addition of galactose (and arginine) was not affected by chloramphenicol (Fig. 2B).

Fig. 1A, B. Growth of *L. lactis* ML3 and glycolytic activity during growth and starvation. A Exponentially growing cells were diluted into chemically defined medium containing 5 mM galactose and 5 mM arginine to an A_{660} of 0.1 (○). After growth for approximately 4 h, galactose was exhausted from the medium and the cells entered the stationary phase (starving conditions; 0). After 4 h of starvation galactose was added to a final concentration of 5 mM (indicated by arrow; open triangles). During growth and starvation the pH

was monitored continuously (closed symbols; ■, upon addition of galactose). B Effect of starvation on maximal glycolytic activity as estimated from the pH changes of the medium (open symbols) and the determination of lactic acid (closed symbols). The arrow indicates the addition of galactose and arginine to the culture medium to final concentrations of 5 mM each. Zero time denotes the onset of starvation

Fig. 2. Effect of starvation and chloramphenicol on glycolytic (A) and arginine deiminase pathway (B) activities in *L. lactis* ML3. At zero time cells entered the stationary phase (starving conditions; 0). Where indicated galactose and arginine were added to the culture medium to final concentrations of 5 mM each. The response of the pathway activities upon addition of galactose plus arginine to starving cultures is shown for cells that were starved in the absence (closed circles) and presence of 100 µg/ml chloramphenicol (closed triangles). Pathway activities were estimated from the pH recordings, and converted into nmol H⁺/min × mg of protein (glycolysis) or nmol NH₃/min × mg of protein (ADI pathway activity)

Fig. 3A–C. Effect of starvation on the activity of glycolytic enzymes. Samples were taken from cultures of *L. lactis* ML3 and prepared for estimation of enzymatic activities as described under "Materials and methods". Cells entered the stationary phase (starving conditions) at zero time. After 4 h of starvation galactose was added to the culture medium to a final concentration of 5 mM (indicated by arrows). A Pyruvate kinase (1.78, closed circles);

lactate dehydrogenase (2.38, open triangles). B Glyceraldehyde 3-phosphate dehydrogenase (1.92, closed triangles); Enolase (3.15, open squares). C Aldolase (8.53, open circles); phosphoglycerate mutase (4.83, closed triangles). The 100% specific activity values, in µmol min⁻¹ mg⁻¹ of protein, are given above between parentheses

Bulk protein degradation

The effect of starvation on the activities of the three glycolytic enzymes suggested that proteins were specifically degraded in the initial phase of starvation. To investigate this possibility, proteins were labeled during growth in chemically defined medium supplemented with [³⁵S]methionine, and degradation of proteins in exponentially growing and starving cultures was compared (Fig. 4; open and closed circles, respectively). By adding an excess of unlabeled methionine to a growing culture, the decrease in labeled proteins/A₆₆₀ represents the resultant of label distribution over dividing cells and protein breakdown. After correction for the label distribution, estimated from the doubling time (about 2.5 h), concentration-dependent breakdown of bulk proteins was observed with a t_{1/2} of

approximately 5 h (Fig. 4, dotted line). Upon addition of chloramphenicol to exponentially growing cells protein degradation occurred with a t_{1/2} of approximately 4.5 h (not shown). Starving cells degraded significant amounts of proteins only in the first 60–90 min of starvation after which the pool of [³⁵S]-labeled polypeptides was stably maintained for more than 4 h (t_{1/2} > 40 h) (Fig. 4, open circles).

To analyze whether or not specific proteins were degraded in the initial phase of starvation, protein samples of cells that had been starved for various periods of times were subjected to two-dimensional gel electrophoresis. In order to detect possible specific breakdown of proteins equal amounts of radioactivity were loaded on each gel. However, careful examination of two-dimensional autoradiograms of polypeptides obtained from cells at different

Fig. 4. Protein degradation in exponentially growing (●) and starving (○) cells of *L. lactis* ML3. Experimental details are described under "Materials and methods". The time required for the washing and resuspension of the cells, subsequent to labeling with [^{35}S]methionine, is indicated by the filled bar. The decay in labeled proteins/ A_{660} in the presence of unlabeled methionine (1 mM) is shown. The dotted line indicates net protein degradation in the presence of methionine, i.e. after correction for growth (label distribution over dividing cells).

stages of starvation did not reveal significant differences (data not shown), indicating that protein degradation was nonspecific in the early hours of starvation.

Bulk protein synthesis

Protein synthesis requires metabolic energy. Since lactococci are largely depleted of metabolic energy after a few hours of starvation (Poolman et al. 1987a), it was of interest to determine the protein synthesis capacity in the transition period from growth to starvation. Specific incorporation of [^{35}S]methionine into polypeptides decreased rapidly (>15-fold) during the first 1–2 h of starvation (Fig. 5). Upon addition of galactose and arginine the specific incorporation rate increased within 2–3 h to values of growing cells. Similar results were obtained when cells were starved for glucose (data not shown).

It has been shown that *E. coli* and other bacteria synthesize starvation specific proteins to differentiate into a stress resistant state (Matin et al. 1989; Matin 1991). To determine whether starvation-specific proteins were synthesized by *L. lactis*, protein samples (indicated by A and B in Fig. 5) were analyzed by two-dimensional gel electrophoresis. During starvation at least 45 polypeptides, that were present in exponentially growing cells, were no longer synthesized or their levels were too low to be detected (Fig. 6). In the first hour of starvation at least two proteins were relatively induced (data not shown; no. 5 and 9 in Fig. 6). Based on 2D-gel analysis of samples taken after different periods of starvation and data from three independent experiments, additional 14–15 proteins exhibited markedly increased synthesis compared to growing cells.

Fig. 5. Effect of starvation on protein synthesis by *L. lactis* ML3. Rates of incorporation of [^{35}S]methionine into polypeptides during exponential growth and galactose/arginine starvation (starting at zero time). The specific rate of incorporation was determined after 10 min of incubation. A and B refer to samples that were taken for two-dimensional gel analysis (see Fig. 6). Arrow indicates the time at which galactose and arginine were added to the culture medium (5 mM each)

Transport of amino acids

One-dimensional SDS-PAA gel analysis of membranes proteins of exponentially growing and starving cells indicated major changes in protein composition of the cytoplasmic membrane (data not shown). Since most of the membrane proteins of *L. lactis* serve a transport function, the activity of a number of well-characterized (Konings et al. 1989) amino acid carriers was determined. Membrane vesicles were isolated from growing and starving cells, and from cells recovering from starvation. The activities of the proton-linked branched-chain amino acid (leucine), lysine, methionine, phenylalanine, serine and alanine carriers (Fig. 7A), and the activity of the arginine/ornithine antiporter (Fig. 7B) were not or only moderately affected by starvation.

Discussion

The data presented in this paper indicate that the period in which *Lactococcus lactis* can respond to starvation stress is restricted to a few hours following the exhaustion of carbohydrates (energy) from the medium. In this period the capacities of the glycolytic and ADI pathways decrease. Although the loss of glycolytic activity could be correlated with specific loss of glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate mutase and pyruvate kinase activity, two-dimensional gel analyses indicated that overall protein degradation is non-specific during starvation. In a previous study (Poolman et al. 1987b) it has been shown that during lactose starvation of *L. lactis* subsp. *cremoris* Wg2 glyceraldehyde 3-phosphate dehydrogenase is inactivated but not (specifically) degraded. A similar situation may hold for *L. lactis*

Fig. 6. Two-dimensional autoradiograms of polypeptides synthesized during growth and starvation of *L. lactis* ML3. The different autoradiograms correspond to samples **A** and **B**, indicated in Fig. 5.

For **A** and **B** approximately 800000 cpm of labeled proteins were applied on the gel. Circles indicate proteins that are induced in starving relative to growing cells

ML3. The nature of the decrease of phosphoglycerate mutase and pyruvate kinase activities is unknown.

Similar to glycolysis the activity of the arginine deiminase (ADI) pathway decreases in the initial hours of starvation. In contrast to glycolysis the recovery of ADI pathway upon readdition of an energy source does not require de novo synthesis of proteins, indicating that the ADI pathway enzymes are not irreversibly inactivated. The analysis of arginine/ornithine exchange activities in membrane vesicles isolated from growing and starving cells indicates that the transport system is not inactivated during starvation; rather a slight increase in exchange activity is observed (Fig. 7B). The loss of ADI pathway

activity during starvation could be due to reduced levels of cofactors (nucleotides) that effect ADI enzymes allosterically, e.g. ornithine carbamoyltransferase (Cunin et al. 1986; Poolman et al. 1987c).

Kinetic studies of protein turnover in bacteria have revealed the presence of a labile protein fraction decaying with half-lives of less than 1 h, and a stable fraction decaying with a half-life of several h (Chaloupka and Strnadova 1982; Goldberg 1972; Goldberg and St. John 1976; Goldschmidt 1970; Kowit and Goldberg 1977; Pine 1970). The labile protein fraction usually constitutes up to 5–6% of the total cellular protein content. The experiments described in this paper could not detect the

Fig. 7A, B. Effect of starvation on amino acid transport in *L. lactis* ML3. **A** Artificially imposed Δp -driven transport of amino acids was analyzed in membrane vesicles isolated from (exponentially) growing and starving cells. The arrow indicates the time at which galactose (5 mM) was added to a starving culture. The final amino acid concentrations in the uptake assay were Leucine, 0.8 μ M; L-lysine, 0.7 μ M; L-methionine, 1.4 μ M; L-phenylalanine, 0.9 μ M; L-serine, 1.0 μ M and L-alanine, 1.4 μ M. **B** Arginine/ornithine exchange in growing and starving cells (for details, see **A**). The final L-arginine concentration in the transport assay was 1.5 μ M

rapid decay of a possible labile protein fraction in *L. lactis*. Evidence has been presented indicating that the stable fraction of intracellular proteins in *E. coli* may not be subject to breakdown at all even during prolonged starvation (Goldberg and St. John 1976). The protein degradation studies in growing *L. lactis* cells indicated a component which decayed with a half-life of about 5 h. A similar component of protein degradation was observed in the first 1 to 2 h of starvation. Since protein breakdown is to a certain extent a composite of first-order decay rates for a large number of proteins, it is difficult to establish whether this decay represents a specific protein fraction or not. The extent of the decay in growing cells, i.e. monoexponential decay upto at least 3 h (Fig. 4), suggests that the overall pool of proteins is subject to breakdown. In addition, very little selectivity in the degradation is observed, i.e. most of the proteins appear to be degraded during the initial phase of starvation. The kinetics of bulk protein degradation in the exponential phase of growth and in the initial phase of starvation is very similar (Fig. 4). Starving cells degrade their proteins in one to two hours following the onset of carbohydrate starvation. After this period the polypeptide pool is stable with half-lives of more than 40 h. Since the pool of energy-rich intermediates ("PEP-pool", ATP and proton motive force) gradually decreases to "zero" (levels below the detection limits) in the first one to two hours of starvation (Otto et al. 1985; Poolman et al. 1987a), it is most likely that the degradation (turnover) of proteins is an energy-requiring process. The metabolic energy for protein degradation may be required for the unfolding of the polypeptides, the activation of proteases and/or the cleavage of peptide-bonds (Hershko and Ciechanover 1982; Olden and Goldberg 1978; Waxman and Goldberg 1986).

It has become increasingly clear in recent years that the so-called non-differentiating bacteria, i.e. those that do not form endospore-like structures in response to starvation, also differentiate to more resistant forms upon starvation (Mandelstam 1960; Matin et al. 1989; Matin 1991). It has been shown particularly in *E. coli*, but also in other bacteria, that at the onset of starvation growth proteins are degraded to create a pool of amino acids from which starvation-specific protein are synthesized (Groat et al. 1986; Reeve et al. 1984a, b). These proteins include those specifically synthesized by bacteria in response to other stresses, and as a result starved bacteria develop a general resistant state, i.e. they become more resistant not only to starvation but also to stresses such as oxidation, heat and hyperosmosis (Jenkins et al. 1988, 1990). We show here that several proteins are induced in the starvation state in *L. lactis*. Thus, it is conceivable that *L. lactis* also develops an enhanced resistant state as a result of starvation protein synthesis. Work in progress is directed at addressing this question.

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