The fructose diphosphate/phosphate regulation of carbohydrate metabolism in low G+C Gram-positive anaerobes

J.B. Russell [1][2](*), D.R. Bond [2] and G.M. Cook [3]

[1] Agricultural Research Service, USDA, and [2] Section of Microbiology, Cornell University, Ithaca, New York 14853 and [3] King's College of London, Division of Life Sciences, London

Introduction

In the 1920's, Orla Jensen used lactic acid production as a means of classifying Gram-positive bacteria. Based on his classification scheme, "homofermenters" produced only lactic acid, while the "heterofermenters" produced ethanol and carbon dioxide as well as lactic acid. This scheme was later supported by different pathways of hexose catabolism (DeMoss *et al.*, 1951). Homofermenters utilized the Embden-Meyerhoff enzyme aldolase, while the heterofermentative scheme employed glucose-6-phosphate dehydrogenase and phosphoketolase (Sokatch, 1969).

By the 1950's, it became apparent that lactic acid production was not always a reliable taxonomic tool (Gunsalus and Niven, 1942; White *et al.*, 1955), and by the 1970's the validity of phenotypic traits as phylogenetic indicators was being questioned (Woese, 1987). Based on the observation that rRNA is a complicated molecule relatively free from lateral transfer and environmental selection pressure, 16S rRNA sequencing has become a standard basis of bacterial classification and has allowed bacteriologists to construct phylogenetic schemes of evolution.

The Gram-positive bacteria seem to share a common evolution and have been divided into two major groups commonly referred to as either low or high mol % G+C. The low G+C group of fermentative Gram-positive bacteria includes the streptococci, lactococci, lactobacilli, clostridia, and butyrivibrios, and the phylogenetic relatedness of these bacteria is supported by the observation that many share a pattern of regulatory enzymes and 'energy signals'. This scheme of fructose diphosphate (FDP)/phosphate regulation affects fermentation endproducts, ATP dissipation by non-growth energy spilling reactions, and catabolite regulation.

Phylogeny

In the past, the classification of Gram-positive, fermentative organisms was primarily based upon cell morphology and spore formation (Hardie, 1986), but considerable phenotypic diversity still existed. Gram-positives that produced significant quantities of lactate were often simply defined as "lactic acid bacteria", even though this group included organisms of different morphology and phenotype (Ingram, 1975). While the boundaries of what constituted a "lactic acid bacterium" remained somewhat vague, this definition has survived for more than a century.

With the introduction of 16S rRNA cataloging, the relatedness of lactic acid bacteria became more apparent (Woese, 1987). Streptococci, lactobacilli, and 'clostridial types' have a relatively deep line of descent, often referred to as the low G+C Gram-positive anaerobes. Recent work has subdivided the genus *Lactobacillus* into three distinct clusters (Collins *et al.*, 1991) and the genus *Streptococcus* into three new genera (streptococci, lactococci and enterococci) (Scleifer and Kilpper-Bälz, 1987). The clostridia have proved to be a more heterogeneous group (Collins *et al.*, 1994). A large number of strains are closely related to the 'traditional' spore-forming, solvent-producing clostridia (e.g. *C. butyricum*, *C. acetobutylicum*), but over 20 other clusters of 'clostridial' organisms have been identified. These clusters include such diverse bacteria as the peptostreptococci, ruminococci, butyrivibrios and eubacteria.

FDP/phosphate-dependent lactic acid production

Many species of lactic acid bacteria produce L-lactate, but Wolin (1964) noted that the NADH-dependent L-lactate dehydrogenase (L-LDH) of the

Received February 26, 1996.

(*) Correspondence: J.B. Russell, Wing Hall, Cornell University, Ithaca, NY 14853 (USA).

ruminal bacterium *Streptococcus bovis* could not convert pyruvate to L-lactate. The L-LDH of *S. bovis* required fructose 1,6-diphosphate (FDP) for activation, and similar enzymes have been found in enterococci (Hardman and Pritchard, 1987), lactococci (Crow and Pritchard, 1977), lactobacilli (Hensel *et al.*, 1977), clostridia (Vancanneyt *et al.*, 1990), and butyrivibrios (Van Gylswyk, 1977). FDP-activated NAD-dependent L-LDHs have only been found in Gram-positive bacteria (Garvie, 1980).

FDP-activated L-LDHs normally exist as inactive monomers or dimers (Mayr *et al.*, 1980). FDP induces internal conformational changes in the enzyme after binding to a site distinct from both the NADH and pyruvate sites. This allosteric modification decreases the K_m for pyruvate (Mayr *et al.*, 1982), and in some bacteria, the K_m for NADH (Crow and Pritchard, 1977). L-LDH affinity for FDP is not constant, and *Lactobacillus casei* requires tenfold more FDP than *Lactobacillus curvatus* (Hensel *et al.*, 1977). The pH optima of L-LDHs usually are less than 5.5 (Mayr *et al.*, 1980; Russell and Hino, 1985), and at low pH, FDP is not required as an activator. Protons appear to facilitate tetramer formation, and this pH-induced subunit association promotes internal conformational changes similar to those caused by FDP (Mayr *et al.*, 1982). Divalent cations, such as Co^{2+} and Mn^{2+}, also can induce subunit association and activation (Mayr *et al.*, 1980; Hardman and Pritchard, 1987), but the physiological role of these activators is not clear. Some L-LDHs are not activated by FDP, but many of these enzymes have similar molecular weights, amino acid compositions, and antigenic properties (Crow and Pritchard, 1977; Garvie, 1980). These non-FDP activated L-LDHs appear to be permanently bound together as active tetramers (Mayr *et al.*, 1980), and thus do not require FDP. Wolin (1964) noted that phosphate stabilized the *S. bovis* L-LDH during extraction and purification, but phosphate can also be an inhibitor of L-LDH activity (Yamada and Carlsson, 1975). Free phosphate increases the requirement for FDP, and appears to be a competitive inhibitor of FDP binding (Jonas *et al.*, 1972).

The pyruvate kinases (PK) of lactic acid bacteria are often allosterically regulated in a manner similar to the L-LDH. These bacteria can have more than one PK (Crow and Pritchard, 1976), and while FDP is not always the sole activator (Collins and Thomas, 1974; Crow and Pritchard, 1976), all PKs appear to be activated by "early" glycolytic intermediates e.g. glucose-6-phosphate (Abbe and Yamada, 1982), fructose-6-phosphate, or galactose-6-phosphate (Thomas, 1976). The PKs of enteric bacteria are often regulated by ATP, ADP, or AMP, but these signals have little effect on the PKs of lactic acid bacteria (Collins and Thomas, 1974). Streptococcal PKs are strongly inhibited by phosphate (Collins and Thomas, 1974; Crow and Pritchard, 1976; Abbe and Yamada, 1982).

Pyruvate-formate lyase (PFL) provides an alternative route of pyruvate catabolism in lactic acid bacteria. PFL is very sensitive to reductions in pH and has a pH optimum as high as 7.5 (Lindmark *et al.*, 1969). PFL is unaffected by FDP or phosphate, but is strongly inhibited by the triose phosphates glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Takahashi *et al.*, 1982). These latter intermediates are in equilibrium with FDP and tend to increase with FDP.

The FDP/phosphate regulation of lactic acid bacteria allows some to shift their fermentation endproducts in an 'energy-dependent' fashion (fig. 1). When energy is limiting (continuous culture), FDP and triose phosphate pools are low, and intracellular phosphate concentrations are high (Thomas *et al.*, 1979; Mason *et al.*, 1981; Fordyce *et al.*, 1984). Under these conditions, the L-LDH is not active, and pyruvate is converted to acetyl-CoA and formate via PFL. NADH arising from glycolysis is oxidized by alcohol dehydrogenase, and flux through acetate kinase of this 'heterofermentative' scheme provides additional ATP. When carbohydrate is plentiful, glycolytic flux can exceed the capacity of

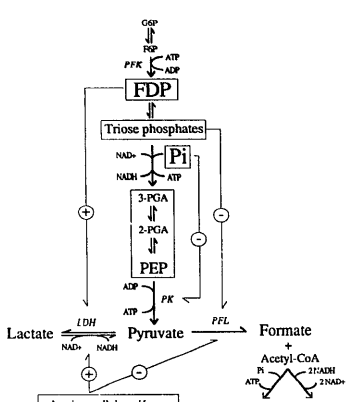

Fig. 1. The catabolism of glucose by lactic acid bacteria showing key regulatory enzymes and energy signals.

PFL. Under these conditions, FDP and triose phosphate concentrations increase (Thompson and Torchia, 1984; Lohmeier-Vogel *et al.*, 1986). FDP activation of L-LDH allows cells to convert excess pyruvate to lactic acid, resulting in classic "homofermentation" (Devries *et al.*, 1970; Thomas *et al.*, 1979).

This regulation allows lactic acid bacteria to maximize their 'rate' of ATP production under both energy-limiting and energy-excess conditions. When energy is limiting, the pathway through PFL is sufficient, and acetate kinase increases the amount of ATP produced per hexose. The PFL pathway, however, is not designed for high rates of hexose catabolism. The lactate pathway decreases ATP per hexose, but this high capacity scheme can provide very high rates of ATP production (fig. 2) (Hungate, 1979).

If lactic acid bacteria are grown at low pH, however, regulation by FDP and phosphate can be circumvented. When extracellular pH is low, intracellular pH declines (Cook and Russell, 1994b), PFL is inhibited, and the LDH is activated in an FDP-independent fashion (Russell and Hino, 1985). This pH-dependent regulation of LDH and PFL promotes pyruvate conversion to lactic acid, and reduces acetate, formate and ethanol production (Rhee and Pack, 1980; Finlayson, 1986). The ability of lactic acid bacteria to decrease their intracellular pH decreases ATP produced per hexose, but the low pH gradient (ΔpH) prevents undissociated fermentation acid anions from accumulating inside the cell (fig. 3). If intracellular pH did not decline, undissociated, membrane-permeable fermentation acids would cross the cell membrane and dissociate in the more alkaline interior in accordance with the Henderson-Hasselblach equation (Russell, 1992).

Many clostridia have FDP-dependent LDHs, but lactic acid is not normally a major endproduct of clostridial fermentations. Clostridia typically produce weak acids (e.g. butyrate and acetate) and solvents (e.g. butanol and acetone). Clostridia and lactic acid bacteria have different patterns of intracellular pH regulation. Clostridia buffer their intracellular pH decline to a greater extent, and the increase in ΔpH restricts them to a less acidic niche (Huang *et al.*, 1985). Most clostridia lack PFL, and instead utilize pyruvate-ferridoxin oxidoreductase (PFO) (Uyeda and Rabinowitz, 1971; Meinecke *et*

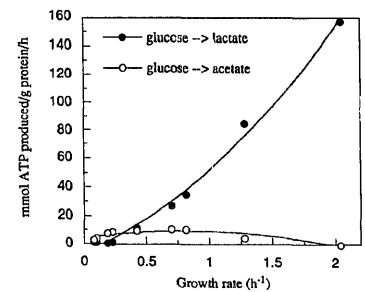

Fig. 2. The ATP production of *Streptococcus bovis* in continuous culture showing the amount produced by glucose conversion to either lactate or acetate formate and ethanol (data taken from Russell and Baldwin, 1979).

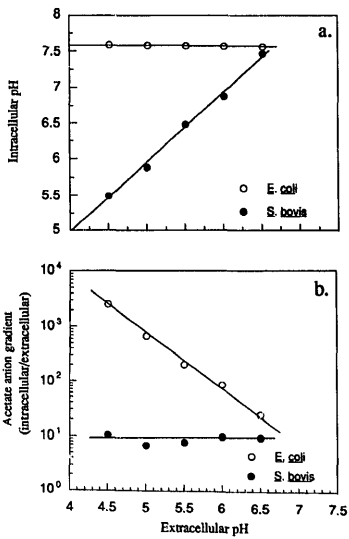

Fig. 3. The effect of extracellular pH on the intracellular pH (a) or acetate anion accumulation (b) of *S. bovis* and *Escherichia coli* (data taken from Russell, 1992 and Kaback, 1990).

al., 1989). High PFO activity and low LDH expression circumvent the influence of FDP and phosphate, and keep clostridia from producing lactate, a strong acid that would decrease pH even more.

FDP-stimulated ATPase activity

Because low G+C Gram-positive anaerobes are not normally respiratory, the primary purpose of the F_1F_O ATPase is to create a protonmotive force. The F_1F_O ATPase has a relatively high affinity for ATP (Rimpiläinen *et al.*, 1989) but is inhibited by the Δp that it creates (Maloney, 1977, 1990). ATPase regulation is usually thought to be transcriptional, and reductions in pH can induce ATPase synthesis (Kobayashi, 1985; Belli and Marquis, 1991). Recent work with *S. bovis* indicated that the F_1F_O ATPase could also hydrolyse 'excess' ATP. If *S. bovis* was starved for nitrogen or treated with the antibiotic chloramphenicol, the rate of ATP production (glycolysis) exceeded the rate of ATP utilization (protein synthesis). The accumulation of intracellular ATP promoted lactic acid production, and a non-growth mechanism of ATP spilling (Russell and Strobel, 1990; Russell, 1993a).

Energy-limited *S. bovis* cells spilled little, if any, ATP, but a sudden increase in energy caused a rapid increase in non-growth energy dissipation (Cook and Russell, 1994a). Because the non-growth ATP hydrolysis was enhanced by protonophores and completely inhibited by DCCD, it appeared that the F_1F_O ATPase was catalysing a futile cycle of protons through the cell membrane (fig. 4). Excess energy caused an increase in intracellular ATP, but these accumulations were often transient (Cook and Russell, 1994a). Based on the observation that energy spilling cells were always homolactic, it appeared that FDP might be a regulatory signal for the F_1F_O ATPase. This conclusion was supported by recent observations: (1) energy spilling cells had high intracellular FDP, and (2) the F_1F_O ATPase activity could be increased 2-fold by FDP addition (Bond and Russell, 1996). FDP-stimulated energy spilling has only been characterized in *S. bovis*, but *L. cremoris* and *L. lactis* also dissipate ATP in non-growth reactions (Otto, 1984; Fordyce *et al.*, 1984).

The ability of energy spilling reactions has not been precisely defined, but there are several potential advantages (Russell and Cook, 1995). Bacteria are often provided with a transient supply of energy, and bacteria that can re-initiate growth quickly have a distinct growth advantage. A high rate of energy source turnover might allow cells to maintain higher intracellular concentrations of critical precursor metabolites (Marr, 1991). Because the $\Delta G'p$ of ATP hydrolysis can be as much as 3-fold higher than Δp (Cook and Russell, 1994a), the ATPase has the potential to distort the cell membrane via dielectric effects (Russell and Cook, 1995). In this light, energy spilling reactions, by decreasing membrane resistance, may serve as a "safety valve". Lastly, energy spilling, by turning over ATP, may provide the ADP which is needed for normal glycolytic catabolism. It has long been recognized that *E. coli* and other bacteria can produce the toxic endproduct methylglyoxal (Cooper, 1984) and that this alternative pathway of hexose catabolism, which does not require ADP, is fostered by an imbalance of catabolism and anabolism (Russell, 1993b).

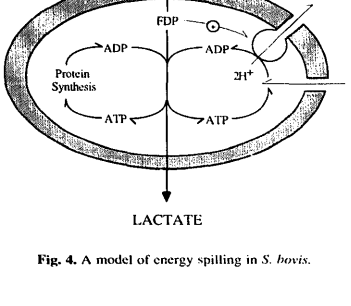

Fig. 4. A model of energy spilling in *S. bovis*.

Phosphate-dependent PEP conservation

The viability of bacteria has often been correlated with an energized membrane (Dawes, 1985), but the membrane potential of lactic acid bacteria declines rapidly when sugar is depleted (Otto *et al.*, 1984). The ability of lactic acid bacteria to remain viable for many hours, or even days, after starvation, is regulated by intracellular phosphate (fig. 1). Lactic acid bacteria commonly use the phosphoenolpyruvate (PEP) phosphotransferase system (PTS) as a method of transport, and the PTS does not require either a membrane potential or ATP. The PTS is driven by PEP, and lactic acid bacteria conserve a PEP pool by rapidly inhibiting PK. When exogenous energy sources are depleted, FDP declines, inorganic phosphate accumulates, and PK inhibition prevents further conversion of PEP to pyruvate (Thompson and Thomas, 1977). While both FDP and phosphate can affect PK activity, ^{31}P NMR and ^{14}C fluorography experiments indicated that phosphate was the more important regulator (Lohmeier-Vogel *et al.*, 1986). Cells treated with iodoacetate

only depleted their PEP pools when phosphate declined (Thompson and Torchia, 1984), and PEP concentrations were more closely correlated with changes in phosphate than FDP.

FDP-dependent inducer expulsion

Bacteria often utilize some energy sources preferentially, and sequential patterns of energy source utilization minimize protein synthesis and maximize growth rates (Magasanik, 1961). In enteric bacteria, catabolite regulation is often mediated by cAMP-dependent control of transcription, as well as PTS-mediated inhibition of active transport (inducer exclusion) (Postma *et al.*, 1993). In low G+C Gram-positive anaerobes, the PTS is the dominant method of sugar transport, and some PTS sugars can inhibit the activity and synthesis of other PTS systems (Thompson, 1987). In these bacteria, cAMP is not a key regulatory molecule, and PTS-mediated inducer exclusion appears to be due to a straightforward competition for HPr, a non-sugar-specific soluble phosphocarrier of the PTS.

In 1980, Reizer and Panos demonstrated that *Streptococcus pyogenes* had a unique mechanism of catabolite regulation (Reizer and Panos, 1980). When *S. pyogenes* and other lactic acid bacteria transport the non-metabolizable lactose analog, methyl-β-D-thiogalactopyranoside (TMG), the intracellular pool of TMG-6-P is stable for long periods of time. If glucose is added, however, the TMG-6-P is dephosphorylated and expelled. Based on these results, it appeared that "inducer expulsion" involved a glucose-dependent activation of a sugar phosphate phosphatase (Reizer *et al.*, 1983; Thompson and Chassy, 1983a,b; London *et al.*, 1985; Sutrina *et al.*, 1988; Ye *et al.*, 1994). Because inducer expulsion could not be triggered by 2-deoxyglucose (2-DG), a non-metabolizable glucose analog, it appeared that inducer expulsion was indeed an energy-dependent phenomenon (Cook *et al.*, 1995).

When glucose is rapidly taken up by lactic acid bacteria, FDP accumulates (Thompson and Torchia, 1984) and intracellular phosphate declines (Mason *et al.*, 1981). FDP activates an ATP-dependent protein kinase (Reizer *et al.*, 1984), and the work of Deutscher and Saier (1983) and Reizer *et al.* (1983) showed that this kinase was able to phosphorylate HPr in a unique position, serine 46. A mutant form of HPr (HPrS46D) having a negatively charged aspartyl group at position 46 elicited TMG expulsion in the absence of FDP, suggesting that the phosphorylation of HPr at this unique site was regulating inducer expulsion. A mutant HPr with a neutral alanine at position 46 could not trigger expulsion of TMG, even if FDP was present (Reizer *et al.*, 1989). Recent work in *L. lactis* (Ye and Saier, 1995) and *S. bovis* (Cook *et al.*, 1995) has demonstrated that a membrane-associated, sugar phosphate-phosphatase is stimulated 10-fold by HPr(ser-P). Since HPr(ser-P) can be dephosphorylated by a phosphate-activated phosphatase (Reizer *et al.*, 1989, 1993), phosphate and FDP are both determinants of inducer expulsion.

All bacteria possessing a PTS have HPr, but HPr is not always able to facilitate inducer expulsion. Only the metabolite-activated protein kinase appears to phosphorylate HPr at serine 46, and this protein has only been demonstrated in Gram-positive bacteria. *Lactobacillus brevis* does not use a PTS system per se (Reizer *et al.*, 1988), but this bacterium still has HPr, a metabolite-activated protein kinase, and a glucose-dependent mechanism of TMG expulsion (Romano *et al.*, 1987). Until recently, inducer expulsion had only been demonstrated in lactic acid bacteria, but *Clostridium acetobutylicum*, a bacterium with an FDP-dependent L-LDH, also expelled TMG when glucose was added (Diez-Gonzalez and Russell, 1996).

Conclusions

Because rRNA genes encode relatively complicated molecules which appear to evolve slowly and are not laterally transferred from one species to another, changes in rRNA are thought to provide an evolutionary clock that runs at a more or less constant speed. True phylogenists have been dismayed by the observation that many simple biochemical traits (e.g. antibiotic resistance) can be easily changed or transferred from one species to another, and microbial physiology has been treated as only the 'flesh' that adorns the true 'phylogenetic skeleton' (Kandler, 1994). Given the observation that the FDP/phosphate regulatory web (1) confers many metabolic advantages, (2) is a complicated scheme, and (3) only appears to be found in bacteria

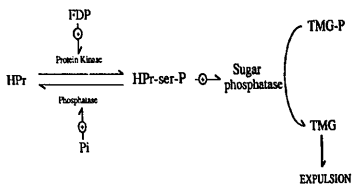

Fig. 5. The mechanism of inducer expulsion in lactic acid bacteria.

sharing a common 16S rRNA ancestry, it may be possible to relate these aspects of macrophysiology to the phylogeny of other low G+C Gram-positive anaerobes.

References

Abbe, K. & Yamada, T. (1982), Purification and properties of pyruvate kinase from *Streptococcus mutans*. *J. Bacteriol.*, 149(1), 299-305.

Belli, W.A. & Marquis, R.E. (1991), Adaptation of *Streptococcus mutans* and *Enterococcus hirae* to acid stress in continuous culture. *Appl. Environ. Microbiol.*, 57, 1134-1138.

Bond, D.R. & Russell, J.B. (1996), A role for fructose 1,6-diphosphate in the energy spilling reaction of *Streptococcus bovis*. *Appl. Environ. Microbiol.*, 62, 62095-62099.

Collins, L.B. & Thomas, T.D. (1974), Pyruvate kinase of *Streptococcus lactis*. *J. Bacteriol.*, 120(1), 52-58.

Collins, M.D., Rodrigues, U., Ash, C., Aguirre, M., Farrow, J.A.E., Martinez-Murcia, A., Phillips, B.A., Williams, A.M. & Wallbanks, S. (1991), Phylogenetic analysis of the genus *Lactobacillus* and related lactic acid bacteria as determined by reverse transcriptase sequencing of 16S rRNA. *FEMS Microbiol. Lett.*, 77, 5-12.

Collins, M.D., Lawson, P.A., Willems, A., Cordoba, J.J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H. & Farrow, J.A.E. (1994), The phyiogeny of the genus *Clostridium*: Proposal of five new genera and eleven new species combinations. *Int. J. Sys. Bacteriol.*, 44 4, 812-826.

Cook, G.M. & Russell, J.B. (1994a), Energy-spilling reactions of *Streptococcus bovis* and resistance of its membrane to proton conductance. *Appl. Environ. Microbiol.*, 60, 1942-1948.

Cook, G.M. & Russell, J.B. (1994b), The effect of extracellular pH and lactic acid on pH homeostasis in *Lactococcus lactis* and *Streptococcus bovis*. *Curr. Microbiol.*, 28, 165-168.

Cook, G.M., Kearns, D.B., Russell, J.B., Reizer, J. & Saier, M.H., Jr. (1995), Regulation of the lactose phosphotransferase system of *Streptococcus bovis* by glucose: independence of inducer exclusion and expulsion mechanisms. *Microbiology*, 141, 2261-2269.

Cooper, R.A. (1984), Metabolism of methylglyoxal in microorganisms. *Annu. Rev. Microbiol.*, 38, 49-68.

Crow, V.L. & Pritchard, G.G. (1976), Purification and properties of pyruvate kinase from *Streptococcus lactis*. *Biochim. Biophys. Acta*, 438, 90-101.

Crow, V.L. & Pritchard, G.G. (1977), Fructose 1,6-diphosphate-activated L-lactate dehydrogenase from *Streptococcus lactis*, kinetic properties and factors affecting activation. *J. Bacteriol.*, 131(1), 82-91.

Dawes, E.A. (1985), Starvation, survival and energy reserves, *in* "Bacteria in Their Natural Environments" (M.F. a. G.D. Floodgate) (pp. 43-79). Academic Press, London.

DeMoss, R.D., Bard, R.C. & Gunsalus, I.C. (1951), The mechanism of the heterolactic fermentation: a new route of ethanol formation. *J. Bacteriol.*, 62, 499-510.

Deutscher, J. & Saier, M.H., Jr. (1983), ATP dependent protein kinase-catalyzed phosphorylation of a seryl residue in HPr, a phosphate carrier protein of the phosphotransferase system in *Streptococcus pyogenes*. *Proc Natl. Acad. Sci.* USA, 80, 6790-6794.

DeVries, W., Kapteijn, W.M.C., Van der Beck, E.G. & Stouthamer, A.H. (1970), Molar growth yields and fermentation balances of *Lactobacillus casei* L3 in batch cultures and in continuous cultures. *J. Gen. Microbiol.*, 63, 333-345.

Diez-Gonzalez, F. & Russell, J.B. (1996), The regulation of thiomethylgalactoside transport in *Clostridium acetobutylicum* P262 by inducer exclusion and inducer expulsion mechanisms. *FEMS Microbiol. Lett.*, 136, 123-127.

Finlayson, H.J. (1986), The effect of pH on the growth and metabolism of *Streptococcus bovis* in continuous culture. *J. Appl. Bacteriol.*, 61, 201-208.

Fordyce, A.M., Crow, V.L. & Thomas T.D. (1984), Regulation of product formation during glucose or lactose limitation in nongrowing cells of *Streptococcus lactis*. *Appl. Environ. Microbiol.*, 48(2), 332-337.

Garvie, E.I. (1980), Bacterial lactate dehydrogenases. *Microbiol. Rev.*, 44(1), 106-139.

Gunsalus, I.C. & Niven, C.F., Jr. (1942), The effect of pH on the lactic acid fermentation. *J. Biol. Chem.*, 145, 131-136.

Hardie, J.M. (1986), Genus *Streptococcus*, *in* "Bergey's manual of systematic bacteriology, Vol. 2" (P.H.A. Sneath, N.S. Mair, M.E. Sharpe and J.G. Holt) (p. 1043). Williams and Wilkins, Baltimore.

Hardman, M.J. & Pritchard, G.G. (1987), Kinetics of activation of L-lactate dehydrogenase from *Streptococcus fecalis* by fructose 1,6-bisphosphate and by metal ions. *Biochim. Biophys. Acta*, 912, 185.

Hensel, R., Mayr, U., Stetter, K.O. & Kandler, O. (1977), Comparative studies of lactic acid dehydrogenases of lactic acid bacteria. — I. Purification and kinetics of the allosteric L-lactic acid dehydrogenase from *Lactobacillus casei* ssp. *casei* and *Lactobacillus curvatus*. *Arch. Microbiol.*, 112, 81-93.

Huang, L., Gibbins, L.N. & Forsberg, C.W. (1985), Transmembrane pH gradient and membrane potential in *Clostridium acetobutylicum* during growth under acetogenic and solventogenic conditions. *Appl. Environ. Microbiol.*, 50, 1043-1047.

Hungate, R.E. (1979), Evolution of a microbial ecologist. *Annu. Rev. Microbiol.*, 33, 1-20.

Ingram, M. (1975), The lactic acid bacteria-a broad view, *in* "Lactic acid bacteria in beverages and food" (J.G. Carr, C.V. Cutting, and G.C. Whiting) (pp. 1-13). Academic Press, New York.

Jonas, H.A., Anders, R.F. & Jago, G.R. (1972), Factors affecting the activity of the lactate dehydrogenase of *Streptococcus cremoris*. *J. Bacteriol.*, 111, 397-403.

Kaback, H.R. (1990), Active transport: membrane vesicles, bioenergetics, molecules and mechanisms, *in* "Bacterial energetics", (T.A. Krulwich) (pp. 515-193). Academic Press, New York.

Kandler, O. (1994), Cell wall biochemistry and the three-domain concept of life. *Syst. Appl. Microbiol.*, 16, 501-509.

Kobayashi, H. (1985), A proton-translocating ATPase regulates pH of the bacterial cytoplasm. *J. Biol. Chem.*, 260, 72-76.

Lindmark, D.G., Paolella, P. & Wood, N.P. (1969), The pyruvate formate-lyase system of *Streptococcus faecalis*. *J. Biol. Chem.*, 244(13), 3605-3612.

Lohmeier-Vogel, E.M., H-Hägerdahl, B.& Vogel, H.J. (1986), Phosphorus-31 NMR studies of maltose and glucose metabolism in *Streptococcus lactis*. *Appl. Microbiol. Biotechnol.*, 25, 43-51.

London, J., Hausman, S.Z. & Thompson, J. (1985), Characterization of a membrane-regulated sugar phosphate phosphohydrolase from *Lactobacillus casei*. *J. Bacteriol.*, 163, 951-956.

Magasanik, B. (1961), Catabolite repression. *Cold Spring Harbor Symp. Quant. Biol.*, 26, 249-256.

Maloney, P.C. (1977), Obligatory coupling between proton entry and the synthesis of adenosine 5'-triphosphate in *Streptococcus lactis*. *J. Bacteriol.*, 132, 564-575.

Maloney, P.C. (1990), Microbes and membrane biology. *FEMS Microbiol. Rev.*, 87, 91-102.

Marr, A.G. (1991), Growth rate of *Escherichia coli*. *Microbiol. Rev.*, 55, 316-333.

Mason, P., Carbone, D.P., Cushman, R.A. & Waggoner, A.S. (1981), The importance of inorganic phosphate in regulation of energy metabolism of *Streptococcus lactis*. *J. Biol. Chem.*, 256(4), 1861-1866.

Mayr, U., Hensel, R. & Kandler, O. (1980), Factors affecting the quaternary structure of the allosteric L-lactate dehydrogenase from *Lactobacillus casei* and *Lactobacillus curvatus* as investigated by hybridization and ultracentrifugation. *Eur. J. Biochem.*, 110, 527-538.

Mayr, U., Hensel, R., Deparde, M., Pauly, H.E., Pfeiderer, G. & Trommer, W.E. (1982), Structure-function relationship in the allosteric L-lactate dehydrogenases from *Lactobacillus casei* and *Lactobacillus curvatus*. *Eur. J. Biochem.*, 126, 549-558.

Meinecke, B., Bertram, J. & Gottschalk, G. (1989), Purification and characterization of the pyruvate-ferredoxin oxioreductase from *Clostridium acetobutylicum*. *Arch. Microbiol.*, 152, 244-250.

Otto, R. (1984), Uncoupling of growth and acid production in *Streptococcus cremoris*. *Arch. Microbiol.*, 140, 225-230.

Otto, R., Klont, B., ten Brink, B. & Konings, W.N. (1984), The phosphate potential, adenylate energy charge, and proton motive force in growing cells of *Streptococcus cremoris*. *Arch. Microbiol.*, 139, 338-343.

Postma, P.W., Lengeler, J.W. & Jacabson, G.R. (1993), Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol. Rev.*, 57, 543-594.

Reizer, J. & Panos, C. (1980), Regulation of β-galactoside phosphate accumulation in *Streptococcus pyogenes* by an expulsion mechanism. *Proc. Natl. Acad. Sci. USA*, 77, 5497-5501.

Reizer, J., Novotny, M.J., Panos, C. & Saier, M.H., Jr. (1983), Mechanism of inducer expulsion in *Streptococcus pyogenes*, a two-step process activated by ATP. *J. Bacteriol.*, 156, 354-361.

Reizer, J., Novotny, M.J., Hengstenberg, W. & Saier, M.H., Jr. (1984), Properties of ATP-dependent protein kinase from *Streptococcus pyogenes* that phosphorylates a seryl residue in HPr, a phosphocarrier protein of the phosphotransferase system. *J. Bacteriol.*, 160. 333-340.

Reizer, J., Deutscher, J., Grenier, F., Thompson, J., Hegenstenberg, W. & Saier, M.H., Jr. (1988), The phosphoenolpyruvate:sugar phosphotransferase system in Gram-positive bacteria: properties, mechanism and regulation. *CRC Crit. Rev. Microbiol.*, 15, 297-338.

Reizer, J., Deutscher, J. & Saier, M.H., Jr. (1989), Metabolite-sensitive, ATP-dependent, protein-kinase catalyzed phosphorylation of HPr, a phosphocarrier protein of the phosphotransferase system in Gram-positive bacteria. *Biochimie*, 71, 989-996.

Reizer, J., Romano, A.H. & Deutscher, J. (1993), The role of phosphorylation of HPr, a phosphocarrier protein of the phosphotransferase system, in the regulation of carbon metabolism in Gram-positive bacteria. *J. Cell. Biochem.*, 51, 19-24.

Rhee, S.K. & Pack, M.Y. (1980), Effect of environmental pH on fermentation balance of *Lactobacillus bulgaricus*. *J. Bacteriol.*, 144(1), 217-221.

Rimpiläinen, M.A., Mettänen, T.T., Nikasaari, K. & Forsén, R.I. (1988), The F_1-ATPase from *Streptococcus cremoris*: isolation, purification, and partial characterization. *Int. J. Biochem.*, 20, 1117-1124.

Romano, A.H., Brino, G., Peterkofsky, A. & Reizer, J. (1987), Regulation of β-galactoside transport and accumulation in heterofermentative lactic acid bacteria. *J. Bacteriol.*, 169, 5589-5596.

Russell, J.B. & Baldwin, R.L. (1979), Comparison of maintenance energy expenditures and growth yields among several rumen bacteria grown on continuous culture. *Appl. Environ. Microbiol.*, 37, 537-543.

Russell, J.B. & Hino, T. (1985), Regulation of lactate production in *Streptococcus bovis*: A spiraling effect that leads to rumen acidosis. *J. Dairy Sci.*, 68, 1712-1721.

Russell, J.B. & Strobel, H.J. (1990), ATPase-dependent energy spilling by the ruminal bacterium, *Streptococcus bovis*. *Arch. Microbiol.*, 153, 378-383.

Russell, J.B. (1992), Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. *J. Appl. Bacteriol.*, 73, 363-370.

Russell, J.B. (1993a), Effect of amino acids on the heat production and growth efficiency of *Streptococcus bovis*: Balance of anabolic and catabolic rates. *Appl. Environ. Microbiol.*, 59, 1747-1751.

Russell, J.B. (1993b), The glucose toxicity of *Prevotella ruminicola*: methylglyoxal accumulation and its effect on membrane physiology. *Appl. Environ. Microbiol.*, 59, 2844-2850.

Russell, J.B. & Cook, G.M. (1995), Energetics of bacterial growth: Balance of anabolic and catabolic reactions. *Microbiol. Rev.*, 59, 48.

Schleifer, K.H. & Kilpper-Bälz, R. (1987), Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci, and lactococci: a review. *Syst. Appl. Microbiol.*, 10, 1-19.

Sokatch, J.R. (1969), Fermentation of sugars, *in* "Bacterial physiology and metabolism" (pp. 74-93). Academic Press, New York.

Sutrina, S.L., Reizer, J. & Saier, M.H., Jr. (1988), Inducer expulsion in *Streptococcus pyogenes*: properties and mechanism of the efflux reaction. *J. Bacteriol.*, 170, 1874-1877.

Takahashi, S., Abbe, K., & Yamada, T. (1982), Purification of pyruvate formate-lyase from *Streptococcus mutans* and its regulatory properties. *J. Bacteriol.*, 149(3), 1034-1040.

Thomas, T.D. (1976), Regulation of lactose fermentation in group N streptococci. *Appl. Environ. Microbiol.*, 32(4), 474-478.

Thomas, T.D., Ellwood, D.C. & Longyear, V.M.C. (1979), Change from homo- to heterolactic fermentation by

Streptococcus lactis resulting from glucose limitation in anaerobic chemostat cultures. *J. Bacteriol.*, 138(1), 109-117.

Thompson, J. & Thomas, T.D. (1977), Phosphoenolpyruvate and 2-phosphoglycerate: endogenous energy source(s) for sugar accumulation by starved cells of *Streptococcus lactis*. *J. Bacteriol.*, 130(2), 583-595.

Thompson, J. & Chassy, B.M. (1983a), Novel phosphoenolpyruvate-dependent futile cycle in *Streptococcus lactis*: 2-deoxy-glucose uncouples energy production from growth. *J. Bacteriol.*, 151, 1454-1465.

Thompson, J. & Chassy, B.M. (1983b), Intracellular hexose-6-phosphate:phosphohydrolase from *Streptococcus lactis*, purification properties and function. *J. Bacteriol.*, 156, 70-80.

Thompson, J. & Torchia, D.A. (1984), Use of ^{31}P nuclear magnetic resonance spectroscopy and ^{14}C fluorography in studies of glycolysis and regulation of pyruvate kinase in *Streptococcus lactis*. *J. Bacteriol.*, 158(3), 791-800.

Thompson, J. (1987), Regulation of sugar transport and metabolism in lactic acid bacteria. *FEMS Microbiol. Rev.*, 46, 221-231.

Uyeda, K. & Rabinowitz, J.C. (1971), Pyruvate-ferridoxin oxioreductase. III. Purification and properties of the enzyme. *J. Biol. Chem.*, 246(10), 3111-3119.

Van Gylswyk, N.O. (1977), Activation of NAD-dependent lactate dehydrogenase in *Butyrivibrio fibrisolvens* by fructose 1, 6-diphosphate. *J. Gen. Microbiol.*, 99, 441-443.

Vancanneyt, M., De Vos, P., Vennens, L. & De Ley, J. (1990), Lactate and ethanol dehydrogenase activities in continuous cultures of *Clostridium thermosaccharolyticum* LMG 6564. *J. Gen. Microbiol.*, 136, 1945-1951.

Virolle, M.J. & Bibb, M.J. (1988), Cloning, characterization and regulation of an α-amylase gene from *Streptomyces limosus*. *Mol. Microbiol.*, 2, 197-208.

White, A.G.C., Steele, R.H. & Pierce, W.A., Jr. (1955), The effect of pH on the fermentation of glucose and galactose by *Streptococcus pyogenes*. *J. Bacteriol.*, 70, 82-86.

Woese, C.L. (1987), Bacterial evolution. *Microbiol. Rev.*, 51, 221-271.

Wolin, M.J. (1964), Fructose-1,6-diphosphate requirement of streptococcal lactic dehydrogenases. *Science*, 146, 775-777.

Yamada, T. & Carlsson, J. (1975), Regulation of lactate dehydrogenase and change of fermentation products in streptococci. *J. Bacteriol.*, 124(1), 55-61.

Ye, J.J., Reizer, J., Cui, X. & Saier, M.H., Jr. (1994), Inhibition of the phosphoenolpyruvate:lactose phosphotransferase system and activation of a cytoplasmic sugar-phosphate phosphatase in *Lactococcus lactis* by ATP-dependent metabolite-activated phosphorylation of serine HPr in the phosphocarrier protein HPr. *J. Biol. Chem.*, 269, 11837-11844.

Ye, J.J. & Saier, M.H., Jr. (1995), Purification and characterization of a small membrane-associated sugar-phosphate phosphatase that is allosterically activated by HPr(ser-P) of the phosphotransferase system in *Lactococcus lactis*. *J. Biol. Chem.* (*in press*).

Carbon metabolism and its regulation in *Streptomyces* and other high GC Gram-positive bacteria

I.T. Paulsen

Department of Biology, University of California at San Diego, La Jolla, CA, 92093-0116 (USA)

Introduction

The high GC Gram-positive bacteria are a diverse group of bacteria, which typically have GC molar ratios in the 60-70% range. They include a large number of organisms of commercial or medical importance, ranging from human pathogens such as *Mycobacterium tuberculosis* to antibiotic-producing *Streptomyces* species.

Carbon catabolite repression (CR) is the phenomenon whereby the synthesis of proteins is repressed by the presence of a catabolite, often generated from a rapidly metabolizable exogenous carbon source such as glucose. CR has been observed for organisms from many genera within the high GC Gram-positive bacteria, e.g., *Mycobacterium* (Bowles and Segal, 1965), *Eubacterium* (Sharak-Genther and Bryant, 1987), *Bifidobacterium* (Degnan and Mac-

Received February 26, 1996.