A Metabolic Model of Exopolysaccharide Production in *Lactobacillus delbrueckii* subsp. *bulgaricus*

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ABSTRACT

Exopolysaccharides (EPS) from lactic acid bacteria (LAB) play an important role in enhancing the rheology and texture of fermented dairy foods. Whilst LAB are attractive vehicles for the production of probiotics due to their GRAS (Generally Recognized as Safe) status, the economic production of EPS by LAB remains constrained by their metabolism. Rational metabolic engineering studies aimed at altering the production of EPS and lactic acid require an understanding of the contributions of whole biosynthetic networks, or segments thereof, to the end–products. *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2483 was confirmed to follow a homofermentative pattern of catabolism, and to export exopolysaccharide (EPS), lactate, and galactose as principal metabolites concurrently with cell growth. The EPS formed was found to diminish after a period of time, probably due to degradation of the polymer. Intracellular glucose resulting from the splitting of lactose taken up into the cell was the principal source of carbon for EPS, lactate and biomass. Kinetic models which were applied to describe the production of EPS, lactate and galactose in batch fermentation suggested, however, that a small percentage of carbon from galactose could have been diverted towards the formation of EPS, lactate, and biomass. Emphasis was placed upon developing a rational screening programme in order to generate a mutant of *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2483 with a higher specific yield of EPS production than the parent strain, for the purposes of understanding the flux to sugar-nucleotides associated with raised levels of EPS production. This process resulted in the isolation of a chemically induced mutant of *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2483 with a higher specific EPS yield than the parent strain. In addition, it was demonstrated that by changing the environmental conditions viz. by reducing the water activity of the growth medium, carbon flux to the EPS-synthesizing pathways could be raised in batch culture. Detailed information on the enzymatic activities and metabolite levels associated with EPS formation at steady state metabolic conditions was derived from continuous culture studies. The formation of EPS, lactate, and galactose, as well as the distribution of carbon fluxes through the glucose 6-phosphate and glucose-1-phosphate branch points in *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2483 was measured at different growth rates in continuous culture. This investigation revealed an enhanced carbon flux to the EPS-synthesizing pathway being associated with higher growth rates, despite a limitation imposed by a "bottleneck" at the glucose-6-phosphate branch-point. Results of similar metabolic flux studies in continuous culture with *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2483, grown under conditions of osmotic stress supported this deduction. Comparative metabolic flux investigations between the chemically induced mutant of *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2483, previously isolated, and the parent strain, provided further evidence of this constriction of carbon flow.

Collectively, these investigations suggested that any improvement in the flux of carbon towards EPS formation in *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2483 could be effected by the enhancement of the activities of phosphoglucomutase, UDP-glucose pyrophosphorylase, and UDP-galactose 4-epimerase. In all of the metabolic studies undertaken in continuous culture, raised levels of carbon flux toward EPS sugar-nucleotides occurred in conjunction with raised levels of specific lactate production rates. In these instances, raised levels of ATP which were measured could be associated with raised levels of glycolysis and the biosynthesis of certain sugar-nucleotides. These findings implied that the cell dissipated excess cellular energy by the formation of sugar-nucleotides, however it may be that the raised flux toward the sugar nucleotides was a direct response to excess energy available in the cell. Any strategy aimed at enhancing carbon flow to EPS by the diversion of carbon away from glycolysis would need to be counterbalanced by the cell's requirement to generate ATP via this pathway, including its need to maintain its redox balance.

FOREWORD

Taking this dissertation to press represents a satisfying stage of what has been my goal for a number of years.

I would like to acknowledge the excellent contribution of Assoc. Prof. Ian Maddox, who has served as an excellent supervisor and remained a steadfast navigator throughout this period of research. In addition, Dr Vaughan Crow of the New Zealand Dairy Research Institute (Fonterra Research Centre) who, with his impressive wealth of knowledge, has served as a superb co–supervisor and consultant. I refer as well to Dr Richard Archer of Fonterra Cooperative Group Limited, whose co–supervisorship sponsored challenging intellectual excursions, which are so important for this process, and whose capital investment in an HPLC allowed for so much of the progress made in this study. I wish to thank my co–supervisor Dr Derek Haisman of the Institute of Food, Nutrition and Human Health, whose intellect and humour remained a constant beacon. Last, but by no means least, I wish to acknowledge Prof. Harjinder Singh of the Institute of Food, Nutrition, and Human Health who has co-supervised as an interested and knowledgeable party.

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