Metabolic shifts in Lactococcus lactis

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Het onderzoek dat in dit proefschrift wordt gepresenteerd, heeft ten doel om meer te weten te komen over het suikermetabolisme, de regulering en de evolutie ervan in de melkzuurbacterie *Lactococcus lactis*. Speciale aandacht voor deze processen gaat uit naar de individuele cel. Het hoofddoel was om de rol van populatieheterogeniteit tijdens verschillende substraatveranderen te beoordelen. We hebben ontdekt dat een complex systeem van specifieke en globale reguleringsfactoren het gedrag van een enkele cel in de populatie bepaalt en dat de populatie cellen uit veel metabolische fenotypen bestaat. Ons evolutionair model suggereert dat de waargenomen fenotypische heterogeniteit een voorbeeld kan zijn van een bet-hedging strategie die een evolutionair voordeel kan opleveren.

Dit proefschrift was ook toegewijd om de onopgeloste intrigerende fenomenen in *L. lactis*, zoals de groei van lactose-/cellobiose- of galactose-negatieve stammen op respectievelijk lactose, cellobiose of galactose te bestuderen. Al deze gebeurtenissen zijn al eerder waargenomen, echter zijn ze nog nooit toegeschreven aan specifieke metabolische systemen. Verschillende activatiemechanismen bleken vereist te zijn om de genclusters die plantsuikers kunnen utiliseren weer in gebruik te stellen, die vervolgens nieuwe functies konden verwerven en de cellen helpen om aan stressvolle situaties te ontsnappen. Om de adaptieve capaciteit van deze nieuwe stam met opnieuw geactiveerde lactose-utilisatievermogen verder te onderzoeken, is deze voortdurend in een chemisch gedefinieerd medium met lactose gepropageerd. Alhoewel a priori veranderingen in suikeropname- en metabole systemen voor de hand liggen, hebben wij ontdekt dat de geëvolueerde stammen hun stikstofmetabolisme aangepast hebben. Deze experimentele evolutiestudie illustreert het belang van arginine in het energiemetabolisme van *L. lactis* en biedt aanvullend bewijs dat 'carbon catabolite repression' van bepaalde genen onder invloed van fluctuerende omstandigheden de microbiële fitheid kan verlagen.

Een regulatoire schakelaar die het metabolisme voor pyrimidine koppelt aan de biosynthese van de celwand, werd ook opgehelderd. De competitie tussen twee

enzymen voor hun gemeenschappelijke substraat asparaginezuur bepaalt de plasticiteit van de celwand en dient als een zeer gevoelige regulatoire schakelaar. Verder geven we een overzicht van verschillende groen-fluorescerende eiwitten die beschikbaar zijn voor de Gram-positieve bacteriën *L. lactis*, *S. pneumoniae* en *B. subtilis*. Naast GFP varianten van *Aquorea victoria* werd ook een verzameling *Obelia* sp. GFPs getest in *L. lactis* om meer te weten te komen over het codongebruik van deze bacterie.

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