Euro Biotechnology 2015 - Systems metabolic engineering of Bacillus subtilis for efficient N-acetyl glucosamine production: Biotechnology Congress 2015 - Long Liu - Jiangnan University

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N-acetyl glucosamine (GlcNAc) is a pharmaceutically and nutraceutically important compound with wide applications and now is mainly produced by hydrolysis from crab and shrimp shells which can cause severe environmental pollution and has potential risk of allergic reactions. In this work, we achieved the over-production of GlcNAc by systems metabolic engineering of Bacillus subtilis, a generally regarded as safe strain. Specifically, GlcNAc synthesis pathway was strengthened by co-over expression of Glucosamine-6-phosphate (GlmS) synthase and GlcNAc-6-phosphate N-acetyl transferase (GNA1) which realized GlcNAc production (240 mg/L). Next, GlcNAc uptake pathway and intracellular degradation pathway were entirely blocked by knockout of all the encoding genes in GlcNAc catabolic pathway to facilitate GlcNAc accumulation. Then, to balance and strengthen GlcNAc synthetic pathway, DNA-guided scaffold system was introduced and increased GlcNAc titer from 1.83 g/L to 4.55 g/L. Synthetic small regulatory RNAs were then employed to optimize expression level of key enzymes in the nodes of GlcNAc-related network including 6-phosphofructokinase (Pfk) and phospho glucosamine mutase (GlmM). GlcNAc titer was improved to 8.30 g/L by modular regulation of the activities of GlcNAc-related modules. In fed-batch fermentation, the GlcNAc titer was further increased to 31.65 g/L which was 3.8-fold that in the shake flask. Finally, to understand kinetics of metabolite changes in GlcNAc synthesis pathway and glycolysis, targeted metabolomics and dynamic labeling were implemented. Inefficient GlcNAc6P dephosphorylation and undesired GlcNAc phosphorylation were identified as rate-limiting step for GlcNAc synthesis which pin-pointed future direction for further pathway optimization. The used systems metabolic engineering strategies may be useful for the construction of versatile B. subtilis cell factories for the production of the other industrially important chemicals. High-strength polymers, such as aramid fibres, are important materials in space technology. To obtain these materials in remote locations, such as Mars, biological production is of interest. The aromatic polymer precursor para -aminobenzoic acid (pABA) can be derived from the shikimate pathway through metabolic engineering of Bacillus subtilis , an organism suited for space synthetic biology. Our engineering strategy included repair of the defective indole-3-glycerol phosphate synthase (trpC), knockout of one chorismate mutase isozyme (aroH) and overexpression of the aminodeoxychorismate synthase (pabAB) and aminodeoxychorismate lyase (pabC) from the bacteria Corynebacterium callunae and Xenorhabdus bovienii respectively. Further, a fusion-protein enzyme (pabABC) was created for channelling of the carbon flux. Using adaptive evolution, mutants of the production strain, able to metabolize xylose, were created, to explore and compare pABA production capacity from different carbon sources. Rather than the efficiency of the substrate or performance of the biochemical pathway, the product toxicity, which was strongly dependent on the pH , appeared to be the overall limiting factor. The highest titre achieved in shake flasks was 3.22 g l^{-1} with a carbon yield of 12.4% [C-mol/C-mol] from an amino sugar. This promises suitability of the system for in situ resource utilization (ISRU) in space biotechnology, where feedstocks that can be derived from cyanobacterial cell lysate play a role.Plastics and polymers are not only omnipresent in our everyday life but are potentially of even greater importance in space technology. Biaxially oriented polyethylene terephthalate (BoPET, trade name Mylar®) is valued for its high tensile strength, chemical and dimensional stability, barrier properties and electrical insulation; layers of metallized BoPET are, for example, used in high-altitude balloons as well as in spacesuits for thermal insulation and radiation resistance. Aramids, like the fabric and sheet material Kevlar®, feature similarly outstanding properties, including high tenacity and strength modulus, low flex fatigue, as well as excellent chemical stability and thermal stability and also radiation resistance. Therefore, they are ideal for a range of specialty applications, including ballistic protection. That these materials are especially suited for construction of environmental suits and habitations in space technology, shows their utilization in inflatable spacecrafts like the ones of Bigelow Aerospace® (NASA, 2017).The feedstocks of aromatic polymers are commonly fossil fuel derived, which is neither sustainable in the long run on Earth, nor available in space or at destinations such as Earth's moon or Mars. Metabolic Engineering may provide the technology to solve this problem, by enabling production of bioreplacement precursors through in situ resource utilization (ISRU). ISRU aims at utilizing synthetic biology to replenish commodities on deep-space exploration missions (Rothschild, 2016).Microbial metabolic pathways give rise to many compounds that can potentially substitute currently petroleum-based chemicals with bio-derived ones or replace them with bio-based alternatives. This includes a multitude of aromatic and aromatic-derived compounds (Averesch and Krömer, 2018). The shikimate pathway intermediate para -aminobenzoic acid (pABA) is one of these aromatics with versatile applicability – it is being used as cross-linking agent for resins and dyes, precursor in the pharmaceutical industry and as a therapeutic itself (e.g. as the drug POTABA®). pABA can also be converted to terephthalic acid (Farlow and Krömer, 2016), as feedstock for production of PET/Mylar®. It may also be possible to convert pABA to para -phenylenediamine (e.g. via Kochi- or Hunsdiecker reaction followed by nucleophilic substitution), which is (besides terephthalic acid) the second monomer of the aramid-fibre Kevlar®. Further, pABA can also be polymerized with itself (Morgan, 1977), potentially yielding a para -aramid with a molecular structure analogous to Kevlar®.The feasibility of producing pABA microbiologically to be used as an industrial precursor was first shown using the yeast Saccharomyces cerevisiae (Krömer et al ., 2013), where a titre of 0.03 g l−1 (0.22 mM) was reached using glucose as the sole carbon source. In a dedicated follow-up study, the titre could be increased to 0.22 g l−1 (1.57 mM) from glycerol/ethanol (Averesch et al ., 2016). Also, bacteria have been utilized for the production of pABA. In Escherichia coli, a concentration of 4.8 g l−1 (35 mM) was reached from glucose (Koma et al ., 2014), while the highest production to date was accomplished with Corynebacterium glutamicum , reaching 43.06 g l−1 (314 mM) from glucose (Kubota et al ., 2016).To leverage this technology in space and ultimately enable the synthesis of aramid fibres, it would be highly desirable to produce pABA in Bacillus subtilis , the organism most suited to space synthetic biology. Bacillus subtilis forms endospores (Nicholson et al ., 2000; Horneck et al ., 2010), which are extremely resistant to several environmental parameters such as drought, salinity, pH and solvents and remain viable for decades; as long as protected from UV radiation, they even endure the vacuum of space (Horneck, 1993).

Biography

Long Liu is currently a life-time professor at School of Biotechnology, Jiangnan University, Wuxi, China. He has been working in the area of bioprocess engineering and metabolic engineering with special reference to bioprocess optimization and control. He has authored 2 books, 3 book chapters, 7 review papers, 20 patents, 37 research papers in SCI journals, and 10 conference papers. He has been a recipient of First prize of Science and Technology progress, Jiangsu, China (2010), First prize of Science and Technology Progress, China Petroleum and Chemical Industry Federation (2011), the Technological Invention Award of China National Light Industry Council (2013), and Excellent Young Teacher of Jiangsu, China (2014).

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