MICROBIAL SUCCINATE PRODUCTION

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Introduction. Succinate is considered as an important future platform chemical that can be produced from renewable carbon sources. It can be used as precursor for 1,4-butandiol, tetrahydrofuran, or γ–butyrolactone, succinate salts could be used as deicers, and succinate could serve as precursor for polymers such as poly-butylene succinate [1-2]. In the past decade, numerous studies were performed aiming at the construction of efficient bacterial and yeast-based succinate producers. The most prominent species in this respect are *Escherichia coli*, *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Mannheimia succinici-producens, Corynebacterium glutamicum*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica* [3-6]. Furthermore, various studies on fermentation and downstream processing of succinate or succinic acid were performed [7-8]. Meanwhile, several companies such as Bioamber, Myriant Technologies, Reverdia, or Succinity have started or announced to produce bio-based succinate. The studies in our lab were focused on succinate production with *Corynebacterium glutamicum*, a prominent industrial amino acid producer [9].

Methods. Methods are described in references 10-12.

Results. In a first study, we developed *C. glutamicum* strains that produce succinate under aerobic conditions in a defined minimal medium with glucose as carbon source [10]. The deletion of the succinate dehydrogenase genes (*sdhCAB*) led to an accumulation of 4.7 g/L (40 mM) succinate and high amounts of acetate (125 mM). By deleting the known acetate-producing pathways acetate formatin was reduced by 83% and succinate production increased to 7.8 g/L (66 mM). Whereas overexpression of the glyoxylate shunt genes had only minor effects on succinate production, overproduction of PEP carboxylase and pyruvate carboxylase resulted in a strain that produced 9.7 g/L (82 mM) succinate with a specific productivity of 1.60 mmol/g cell dry weight x h. This value represents the highest productivity among currently described aerobic bacterial succinate producers. Decoupling of succinate production from cell growth using the producer strain *C. glutamicum* Δ*pqo*Δ*pta-ackA* Δ*sdhCAB*Δ*cat*/pAN6-*pyc-ppc* led to an increase of the product yield to 0.45 mol succinate/mol glucose and a titre of 10.6 g/L (90 mM) succinate. Besides glucose, also glycerol could serve as a substrate for aerobic succinate production with *C. glutamicum* [11].
Studies elsewhere had demonstrated the capability of *C. glutamicum* for anaerobic succinate production from glucose under nongrowing conditions [12]. We addressed two shortfalls of this process, the formation of by-products and the limitation of the yield by the redox balance [13]. A derivative of ATCC 13032 was constructed, which lacked all known pathways for acetate and lactate synthesis (strain BOL-1). Chromosomal integration of the pyruvate carboxylase gene *pyc* resulted in strain BOL-2, which catalyzed succinate production with a yield of 1 mol/mol glucose with minor acetate formation. In order to provide additional reducing equivalents from the cosubstrate formate, the *fdh* gene of *Mycobacterium vaccae* for an NAD^+-coupled formate dehydrogenase was integrated chromosomally, leading to strain BOL-3. In an anaerobic batch process with strain BOL-3, a 20% higher succinate yield from glucose was obtained in the presence of formate. A temporary metabolic blockage was prevented by overexpression of the glyceraldehyde 3-phosphate dehydrogenase gene *gapA*. In an anaerobic fed-batch process with glucose and formate, strain BOL-3/pAN6-gap accumulated 134 g/L succinate (1,134 mM) in 53 h with an average succinate production rate of 1.59 mmol per g CDW per h and a succinate yield of 1.67 mol/mol. The by-product level was reduced to 0.10 mol/mol glucose.

Conclusions. The results demonstrate the potential of *C. glutamicum* for aerobic and anaerobic succinate production. By utilization of formate as an additional donor for NADH (and carbon dioxide), very high succinate yields are possible under anoxic conditons.

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