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Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

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Citation (APA):
Redl, S. M. A. (2016). Gas Fermentation using Thermophilic Moorella Species for production of Biochemicals. Novo Nordisk Foundation Center for Biosustainability.

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GAS FERMENTATION USING THERMOPHILIC
MOORELLA SPECIES FOR PRODUCTION OF
BIOCHEMICALS

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PhD thesis

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English abstract

Gas fermentation is a promising technology which gained increasing attention over the last years. In this process, acetogenic bacteria convert gases rich in H₂, CO₂, and CO, into compounds of higher value. The gas can derive from industrial off-gas or from waste streams via gasification. In the gas fermentation processes that are nearly on commercial level, mesophilic acetogens are used to mainly produce ethanol and butanediol. However, thermophilic acetogens, such as *Moorella thermoacetica* would allow for easy downstream processing when producing volatile products such as acetone.

This thesis starts with a review of the feedstock potential for gas fermentation and how thermophilic production strains as well as unconventional fermentation processes such as mixotrophy can help to exploit this potential. I analyzed a process with respect to thermodynamic and economic considerations, in which acetone is produced from corn-stover derived syngas with *M. thermoacetica* hypothetically overexpressing the respective heterologous pathway. To make such a process feasible, some challenges have to be overcome. A system for genetic manipulation has been published in 2013, and I describe my attempts to extend the published genetic toolbox. One crucial tool is a robust selection marker. We characterized a thermostable beta-galactosidase and its application as screening marker in thermophiles. In 1981, strain *M. thermoautotrophica*, reported to be closely related to *M. thermoacetica*, was isolated. Together

with collaborators, I identified *M. thermoautotrophica* to be a mixed culture of *M. thermoacetica* strains. We *de novo* sequenced the genome of several *M. thermoacetica* strains to shed light on the taxonomic relations within the genus *Moorella*. Although well studied, some aspects of the physiology of *M. thermoacetica* are still unknown. I performed an RNA-seq study of *M. thermoacetica* grown on sugar and gaseous substrates (H_2/CO_2 and $H_2/CO_2/CO$) to obtain insights into the transcription profile. To facilitate the research with anaerobes and thermophiles such as *Moorella* species, I developed together with a colleague a device that enables the fully automated generation of growth curves in mid-sized cultures. In the respective chapter, we elaborate on the role of 3D printing in the construction of novel lab equipment and present the aforementioned solution for automated tracking of bacterial growth.

In conclusion, this thesis describes several projects which help to pave the way for biochemical production with the thermophile *M. thermoacetica* on in an economically competitive way.

Dansk sammenfatning

Gasfermentering er en lovende teknologi, der har opnået øget opmærksomhed de senere år. I denne proces omdanner acetogene bakterier gasser, der er rige på H_2 , CO_2 og CO , til forbindelser med højere værdi. Gassen kan stamme fra industriel off-gas eller fra affaldsstrømme via gasifikation. I gasfermenteringsprocesser, der er næsten på kommercielt niveau, bruges mesofile acetogener til hovedsagligt at producere ethanol og butandiol. Termofile acetogener, såsom *Moorella thermoacetica*, ville dog tillade nem downstream-behandling ved produktion af volatile produkter såsom acetone.

Denne afhandling starter med et review af feedstock-potentialet for gasfermentering og hvorledes termofile produktionsstammer, såvel som ukonventionelle fermenteringsprocesser fx mixotrofi, kan hjælpe udnyttelsen af dette potentiale. Jeg analyserede en proces med hensyn til termodynamiske og økonomiske overvejelser, hvori acetone produceres fra majsstrå-afledet syngas med *M. thermoacetica*, der teoretisk set overudtrykker den respektive heterologe reaktionsvej. For at muliggøre en sådan proces skal visse udfordringer overvindes. Et system til genetisk manipulation er blevet publiceret i 2013, og jeg beskriver mine forsøg på at udvide den publicerede genetiske værktøjskasse. Et afgørende værktøj er en robust selektionsmarkør. Vi karakteriserede en termostabil beta-galactosidase og dens anvendelse som screening-markør i termofiler. I 1981 isoleredes stammen *M. thermoautotrophica*, der er rapporteret som nært

beslægtet til *M. thermoacetica*. Sammen med samarbejdspartnere identificerede jeg stammen *M. thermoautotrophica* til at være en mikset kultur af *M. thermoacetica* stammer. Vi *de novo*-sekventerede genomet af flere *M. thermoacetica*-stammer for at kaste lys på de taxonomiske relationer indenfor slægten *Moorella*. Selvom organismen er velstuderet, er visse fysiologiske aspekter ved *M. thermoacetica* stadig ukendte. Jeg foretog et RNA-seq studie af *M. thermoacetica*, dyrket på sukker og gasholdige substrater (H_2/CO_2 og $H_2/CO_2/CO$) for at opnå indsigt i den transkriptionelle profil. For at facilitere forskning med anaerobere og termofiler, såsom *Moorella*-arter, udviklede jeg sammen med en kollega et apparat, der tillader den fuldt automatiserede dannelse af vækstkurver for mellemstore kulturer. I det respektive kapitel uddyber vi rollen for 3D-printning i konstruktionen af nyt laboratorie-udstyr og fremlægger den førnævnte løsning til automatiseret sporing af bakteriel vækst.

I konklusion beskriver denne afhandling flere projekter, som kan hjælpe til at bane vejen for biokemisk produktion med den termofile *M. thermoacetica* på en økonomisk konkurrencedygtig måde.