Characterisation of a sporulation deficient *C. beijerinckii* strain

Mamou Diallo[1], Florent Collas[1], Serve Kengen [2], Ana M. López-Contreras[1]

[1] Wageningen Food and Biobased Research, Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands, e-mail: mamou.diallo@wur.nl [2] Laboratory of Microbiology, Wageningen University, 6703HB Wageningen, The Netherlands

Background and objective

Solventogenic clostridia are able to utilize a wide range of sugars to produce mixes of solvents such as acetone or isopropanol, butanol and ethanol (ABE/IBE). Sporulation is know to be linked to solvent production but little is known about its regulation in solventogenic clostridia. The current model on sporulation in solventogenic clostridia is based research done on *C. acetobutylicum* [1]. To see whether this model is applicable to another solvent producing species, *C. beijerinckii,* we deleted the *spoIIE* gene in *C. beijerinckii* NCIMB 8052. SpoIIE is a phosphatase involved, in *C. acetobutylicum* at the beginning of the sporulation cascade. Previous studies show that *spoIIE* deficient *C. acetobutylicum* strains are asporogenous and produce solvent [2].

Its homologue in *C. beijerinckii* NCIMB 8052 , cbei0097, was disrupted using an inducible CRISPR-Cas9 system for *Clostridium* developed in our laboratory.

The sporulation cascade in *C. acetobutylicum*

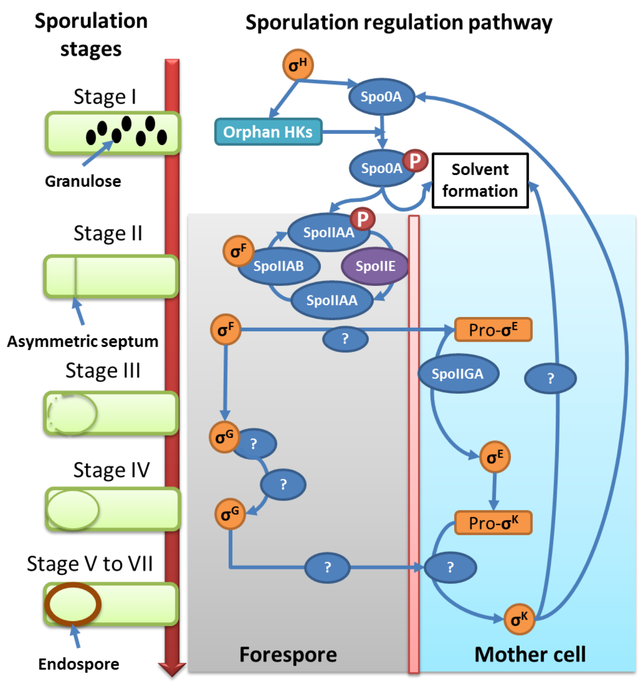

Figure 1. Current model of the sporulation cascade in *C.acetobutylicum* adapted from [1]

The sporulation regulation pathway was first describe in the *Bacillus* genus. Comparative studies between *Bacillus* and *Clostridium* show several differences between the two regulatory networks. This network is different even within the *Clostridium* genus [1]. *C. acetobutylicum* is the most studied solventogenic species. That is why we used *C. acetobutylicum* sporulation model as a reference. Nonetheless this model is not complete, **Figure 1**.

Disruption of the *spoIIE* gene *C. beijerinckii*

The CRISPR-Cas9 system we used needs two plasmids that were transformed sequentially in to the strain. The system was then induced by cultivating the double transformants in a 4% xylose media. We obtained then several colonies bearing a 2.379 kb deletion in the *spoIIE* gene, **Figure 2** and **3** .

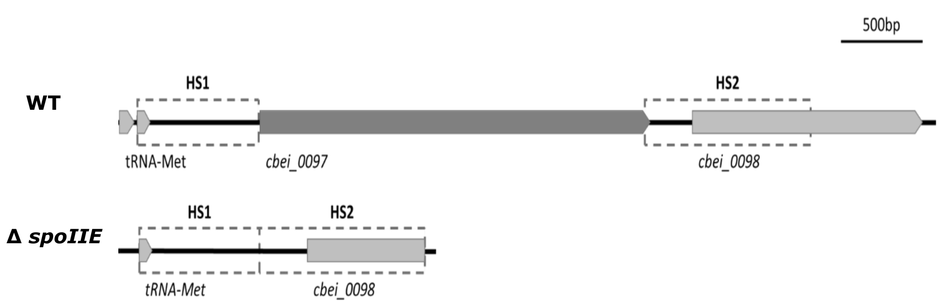

Figure 2. Locus of the *spoIIE* gene and genotype of the mutant strain.

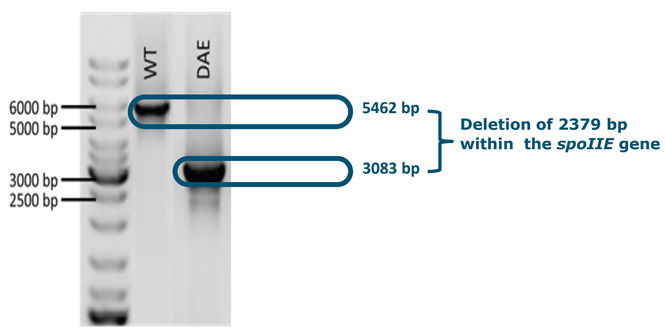

Figure 3. 1% agarose gel of PCR screening of disruption of *spoIIE*

Microscopy observations

The mutant was characterized by phase contrast microscopy. While the wild type (WT) forms spores, **Figure 4a**, the mutant does not sporulate. As described in studies on *spoIIE* deficient *C. acetobutylicum* strains, the cells are stopped at the stage II of the cascade. Indeed the cells seem to be unable to undergo the asymmetric division required to form the spore, **Figure 4b**.

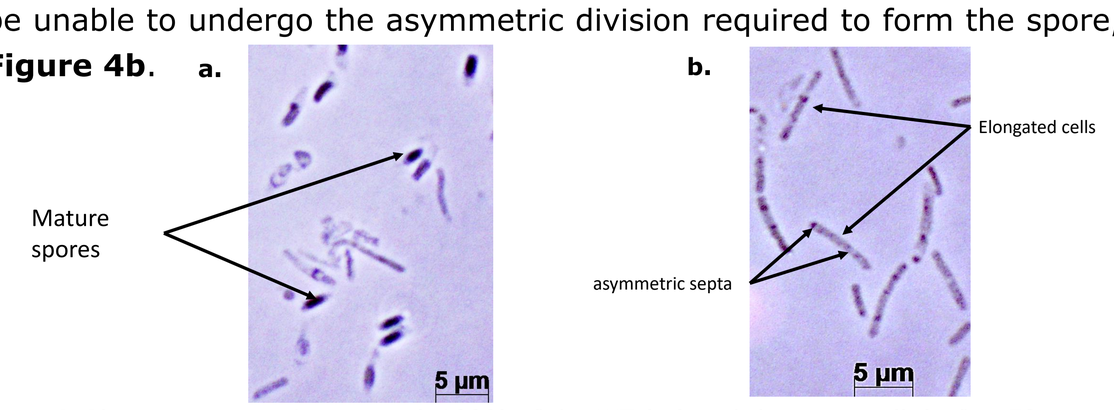

Figure 4. Phase contrast microscopy (x1000) of the WT (a.) and the mutant (b.) after 72 hours of growth

Granulose detection

The granulose produced by the strains was detected by iodine staining. The vapors of iodine bind to the granulose which colors the cells in dark brown. Both the wild type and the mutant produce granulose, **Figure 5.**

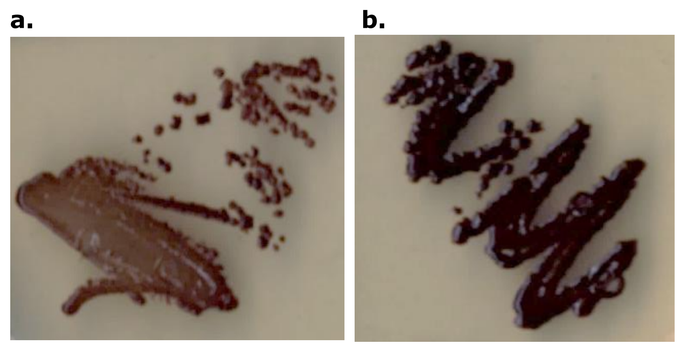

Figure 5. Iodine staining of the wilt type (a.) and the mutant (b.) after 24 hours growth

Fermentation product

Batch fermentations in 400 mL reactors show that *spoIIE*'s disruption does not impair solvent formation, **Table 1**.

strain	Glucose consumed ($g.L^{-1}$)	Acetate consumed ($g.L^{-1}$)	Butyrate ($g.L^{-1}$)	Acetone ($g.L^{-1}$)	Butanol ($g.L^{-1}$)	Ethanol ($g.L^{-1}$)	Total solvent ($g.L^{-1}$)
WT	28.75	1.06	0.51	2.06	7.44	0.04	9.54
DspoIIE	28.69	0.85	0.68	2.52	7.78	0.03	10.32

Table 1. End point concentrations of butyrate, acetone, butanol and ethanol as well as glucose and acetate consumption after 25 hours of 400 mL batch fermentation

Conclusions

- The *spoIIE*'s disruption in *C. beijerinckii* stops sporulation cascade at the stage II.
- Since it does not hinder granulose formation or solvent production. The *spoIIE* mutant produces similar amounts of solvent compared to the wild type strain.
- Those observations show that like in *C. acetobutylicum, spoIIE* encodes for a protein involved in early stage of the sporulation cascade in *C. beijerinckii*

References

1. Al-Hinai, M. A., Jones, S. W. & Papoutsakis, E. T. The Clostridium Sporulation Programs: Diversity and Preservation of Endospore Differentiation. *Microbiol. Mol. Biol. Rev.* **79,** 19–37 (2015).
2. Bi, C., Jones, S. W., Hess, D. R., Tracy, B. P. & Papoutsakis, E. T. SpoIIE is necessary for asymmetric division, sporulation, and expression of sigmaF, sigmaE, and sigmaG but does not control solvent production in Clostridium acetobutylicum ATCC 824. *J. Bacteriol.* **193,** 5130–7 (2011).

Wageningen University & Research
P.O. Box 123, 6700 AB Wageningen
Contact: mamou.diallo@wur.nl
M + 31 (0) 614 32 79 31
https://www.wur.nl/nl/Personen/Mamou-M-Mamou-Diallo-MSc.htm

Acknowledgements

Acknowledgement: This work was financed by the European Union Marie Sklodowska Curie Innovative Training Networks (ITN), Clospore - Contract number 642068