Solvent production by a sporulation deficient *Clostridium* mutant 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

Natural solvent producing bacteria belong to the *Clostridium* genus. They form spores and live in anaerobic conditions. Most are able to utilize a wide range of sugars to produce mixes of solvents such as acetone or isopropanol, butanol and ethanol (ABE/IBE). Studies show a link between sporulation and solvent production [1] but none was able to explain the involved mechanism. The current model on sporulation in solventogenic clostridia is based on *C. acetobutylicum* [2]. To prove that 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 several *Clostridium* strains at the beginning of the sporulation cascade. Previous studies show that *spoIIE* deficient *C. acetobutylicum* strains are asporogenous but still produce solvent [3].

Its homologue in *C. beijerinckii* NCIMB 8052 , cbei0097, was disrupted using a novel very efficient CRISPR-Cas9 system for *Clostridium* developed in our laboratory. This new method uses two plasmids to couple the inducible expression of the cas9 nuclease from *Streptococcus pyogenes* carried on one plasmid and the transcription of a guide RNA carried on another. The system is then activated by the addition of xylose into the media.

The sporulation cascade in *C. acetobutylicum*

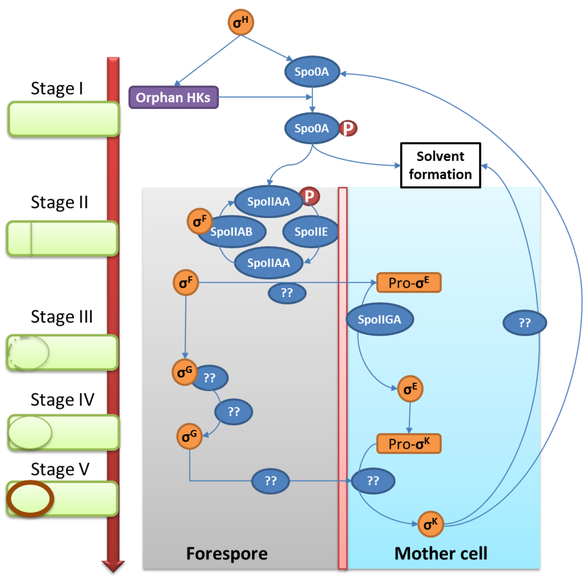

Figure 1. Current model of the sporulation cascade in *C.acetobutylicum*

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 [2]. *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** .

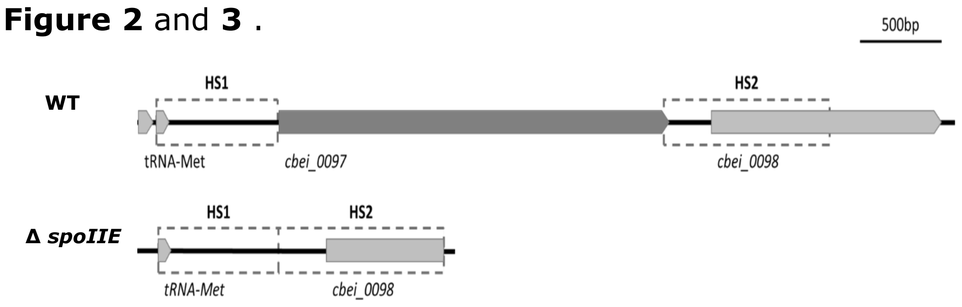

Figure 2. Locus of the *spoIIE* gene and genotype of the mutant strain.

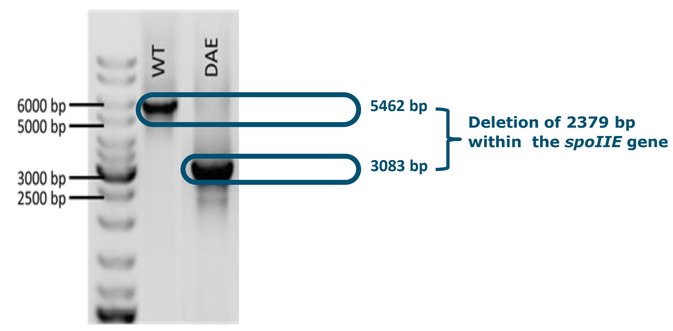

Figure 3. 1% agarose gel of PCR screening of disruption of *spoIIE*

Cell morphology

The mutant was characterized by transmission electron microscopy (TEM). While the wild type (WT) follows normally the sporulation cycle, **Figure 4**, 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 5**.

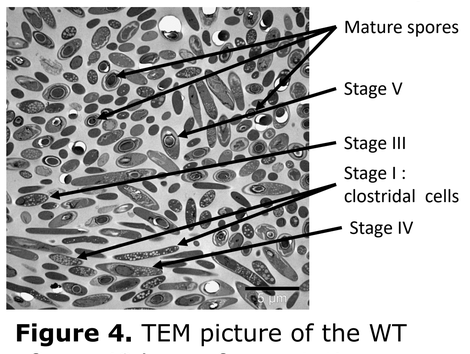

Figure 4. TEM picture of the WT after a 48 hours fermentation

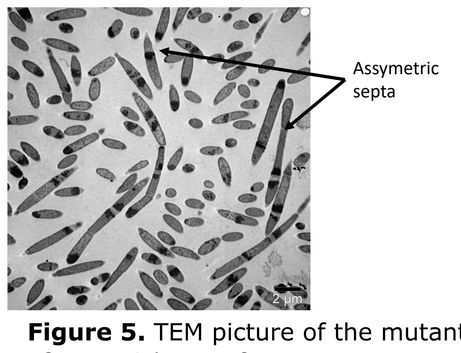

Figure 5. TEM picture of the mutant after a 48 hours fermentation

Fermentation profile

- The metabolism is affected by this disruption, the mutant is not able to consume as much glucose, **Figure 6**, and acetate as the wild type (WT)
- The product balance is also disrupted, the mutant strain cannot assimilate the acids to produce solvents, **Figure 7**

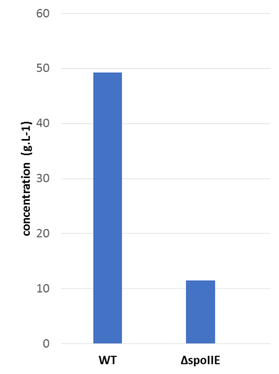

Figure 6. Concentration of the glucose metabolized by the cells after 145 hours

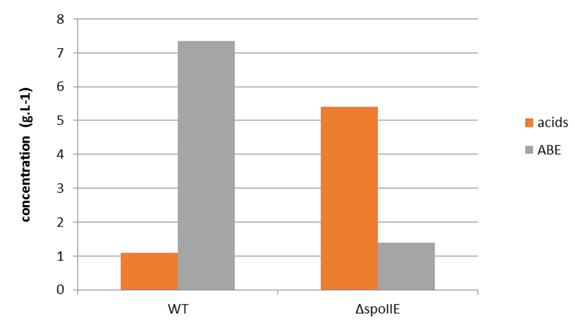

Figure 7. Acid (acetate and butyrate) and acetone, butanol and ethanol (ABE) production after 145 hours of fermentation at 35°C

Conclusions

- The *spoIIE*'s disruption has a major impact on the metabolism and on the sporulation. Unlike the wild type strain, the *spoIIE* mutant produces mainly acids and little solvent.
- Moreover the mutant does not perform asymmetric division.
- Those observations show that like in *C. acetobutylicum, spoIIE* encodes for a protein involved in septum formation in *C. beijerinckii* but that its disruption also inhibits solvent production.
- Thus this result suggests that the sporulation model established for *C. acetobutylicum* is not fitted for *C. beijerinckii*.

References

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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