Prospecting fat-degrading anaerobes for biotechnology

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The challenge of a sustainable and biobased economy is to develop innovative technologies to recover and reuse energy compounds from waste streams. Lipids present in waste and wastewaters can be converted to **methane** (a biofuel). Hydrolysis of lipids results in the formation of long-chain fatty acids (LCFA) and glycerol. In methanogenic environments LCFA are degraded by syntrophic associations of acetogenic bacteria and methanogens [1]. Glycerol is a fermentable substrate that is ultimately converted to methane in mixed culture anaerobic bioreactors. However, conversion of glycerol by pure or defined cultures of fermentative bacteria can yield important **bulk chemicals**, such as succinate, 1,3-propanediol and hydrogen. This is interesting because glycerol has become an abundant and inexpensive feedstock due to massive production of biodiesel.
Here we present a summary of our work on syntrophic ecophysiology of LCFA conversion and we introduce some of our isolates able to convert glycerol to added-value chemicals.

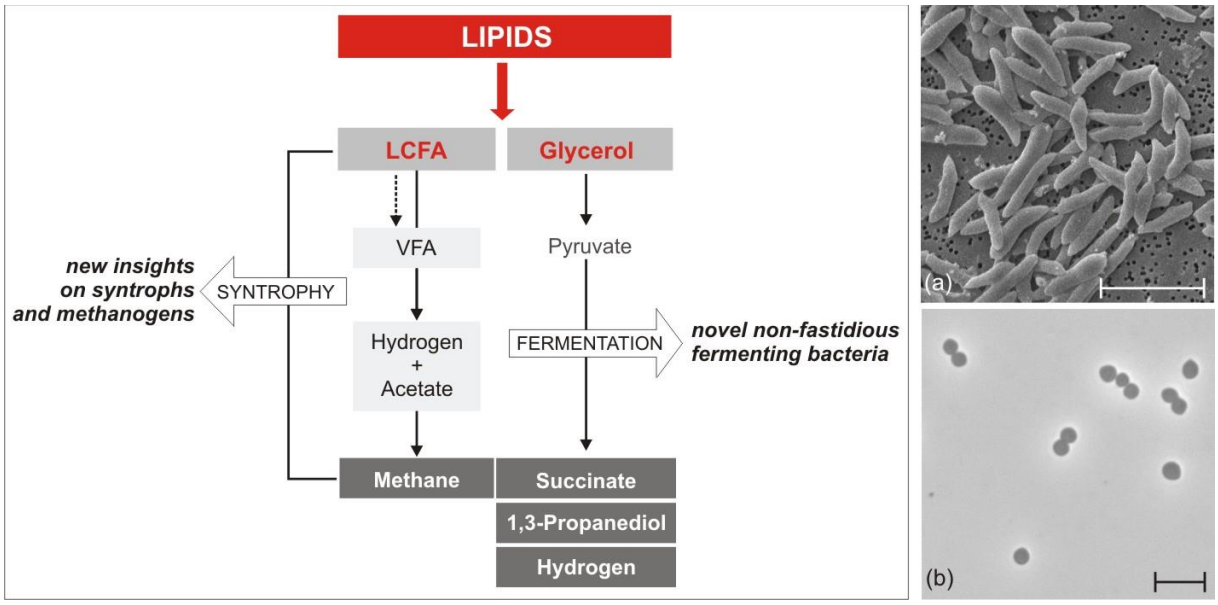

Figure 1: Representation of the microbial processes presented in this work. Microphotographs of novel glycerol-fermenting bacteria: (a) *Ercella succinigenes* strain ZWB^T, and (b) *Thrichococcus* strain ES5 (bar = 5 μm).

Ecophysiology of syntrophic LCFA conversion

Analysis of the 16S rRNA gene from sludge samples converting LCFA revealed the predominance of members of the *Clostridiaceae* and *Syntrophomonadaceae* families [2, 3]. Archaeal populations were mainly comprised of hydrogen-consuming microorganisms belonging to the genus *Methanobacterium*, and acetate-utilizers from the genera *Methanosaeta* and *Methanosarcina*. Enrichments converting oleate ($C_{18:1}$, unsaturated LCFA) and palmitate ($C_{16:0}$, saturated LCFA) showed different bacterial composition, which might be related to the different degrees of saturation of these two LCFA.

A novel obligately syntrophic bacterium, *Syntrophomonas zehnderi* strain OL-4^T, was isolated from an oleate degrading culture [4]. This bacterium was detected by 16S rRNA gene cloning and sequencing in several oleate-degrading sludges.
Activity of methanogenic archaea was more affected by unsaturated LCFA [5]. The degree of tolerance to LCFA was different among distinct species of methanogens. *Methanobacterium formicicum* was able to grow in both oleate- and palmitate-degrading enrichments, whereas *Methanospirillum hungatei* only survived in the palmitate-degrading enrichment. The two acetoclastic methanogens tested, *Methanosarcina mazei* and *Methanosaeta concilii*, could be detected in both enrichment cultures, but with better survival in palmitate-degrading cultures than in oleate-degrading cultures. In general, oleate was more toxic to methanogens than palmitate as further confirmed by live-dead cell viability assays.

Isolation of novel glycerol-converting anaerobes

Fermentative anaerobes can produce valuable organic compounds, which should be exploited for a biobased economy.
We recently isolated a novel anaerobic succinate-producing bacterium, *Ercella succinigenes* strain ZWB^T, from sludge collected from a biogas desulfurization bioreactor (Eerbeek, The Netherlands) (Fig. 1a) [6]. On the basis of 16S rRNA gene sequence similarity, strain ZWB^T belongs to the *Ruminococcaceae* family and it is distantly related to *Saccharofermentans acetigenes* (92% sequence similarity of the rRNA genes). Because of its physiological features and phylogenetic analysis, strain ZWB^T represents a novel species of a new genus. Strain ZWB^T ferments glycerol and several carbohydrates to mainly succinate, H_2, and acetate. Succinate is a valuable energy-rich compound used for organic synthesis of 1,4-butanediol, tetrahydrofuran, butyrolactone, and adipic acid.
Thrichococcus strain ES5 is another glycerol-converting bacterium that was isolated from methanogenic granular sludge (Fig. 1b) [7]. Strain ES5 ferments glycerol to 1,3-propanediol (PDO) as main product, and lactate, acetate and formate as minor products. PDO is an organic chemical that is growing in importance as it can replace ethylene glycol and butylene glycol for the synthesis of polyesters and polyurethanes and it can also be used as solvent, antifreeze or protective agent. Currently the genus *Thrichococcus* includes five established species. Genotypically, all species of this genus have a high (99-100%) 16S rRNA gene similarity, but DNA-DNA hybridization values are inferior to 70%.
Integration of physiology with further genomic and proteomic studies will allow to further understanding the metabolic potential of the different isolates in order to optimize biotechnological processes.

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