Ann Zootech (1996) 45, Suppl, 355

Taxonomical and physiological characteristics of H_2/CO_2-utilizing acetogenic bacteria from the human colon

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In human, the ingested substrates which are not absorbed in the upper-digestive tract reach the colon where they are fermented by a complex anaerobic microflora. A large part of the hydrogen produced during these fermentations is used in situ by H_2-consuming microorganisms. methanogenesis, sulphate-reduction and reductive acetogenesis. This latter process which leads to the production of acetate from H_2 and CO_2, is potentially benefit for the host and has been recently recognized as an active process in the colon of non-methane excretors (Lajoie et al, 1988, Appl Environ Microbiol, 54, 2723-2727; Bernalier et al, 1994, Nouvelles Tendances en Écologie Microbienne, SFM, ed, Paris, 110-117). Isolation of acetogenic bacteria was therefore performed in our laboratory from fecal samples of three non methanogenic subjects. The taxonomy of these strains was determined and their physiology was studied.

Enrichments of acetogenic bacteria were performed from 10^{-5}-10^{-7} dilution of human feces using a modified AC21 medium with H_2/CO_2 as sole energy source. Among the fifteen acetogenic strains isolated, nine were Gram$^+$ sporulated rods while the six others corresponded to Gram$^+$ cocci. The phylogenetical approach, consisting on the analysis and comparison of the complete 16S rRNA sequence, has demonstrated that seven of the *Clostridium* species were closely related to *C. mayombei* and *C. glycolycum* while the two other strains would correspond to a new species of the same Clostridial cluster. Two strains of Gram$^+$ cocci were assumed to be a new *Peptostreptococcus* species while another one had high 16S rRNA sequence homology with *C. coccoides*. The other Gram$^+$ cocci which mainly fermented glucose to lactate were identified as *Streptococcus* species.

All these species were able to grow autotrophically using H_2/CO_2 to produce acetate following the stochiometric equation of reductive acetogenesis :

$4H_2 + 2CO_2 = 1CH_3COOH + 2H_2O$.

Measurements of $^{13}CO_2$ incorporations by these bacteria have demonstrated that, in presence of H_2, acetate labelled on both methyl and carboxyl group was mainly produced. The kineticss of H_2/CO_2 utilization by these microorganisms were followed owing to a pressure sensors system fixed to cultures tubes. Gas consumption and acetate production occured during the exponential and stationary growth phases and the doubling time of the strains was about 20 h. Furthermore, H_2/CO_2 metabolism of some Clostridia appeared strictly dependent on the presence of Na^+ in the medium in contrast to their glucose fermentation. During autotrophic growth, energy is mostly generated by electron transfert phosphorylation which involves or not Na^+ depending on the strains. In contrast, ATP is mainly synthesized by substrate level phosphorylation during glucose fermentation. These acetogens were also able to use a large variety of organic compounds. This nutritional versatility gives acetogens an important ecological advantage compared to methanogens or sulfate-reducers which possess a more limited range of substrates. First results concerning the regulation of H_2/CO_2 metabolism of these acetogens by an organic substrate have demonstrated that some strains could co-metabolize H_2/CO_2 and glucose while a diauxic growth was shown for other ones. These regulations constitute one of the important factors influencing the hydrogenotrophic populations balance in the human colon.