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In Vitro H_2 Utilization by a Ruminal Acetogenic Bacterium Cultivated Alone or in Association with an Archaea Methanogen Is Stimulated by a Probiotic Strain of *Saccharomyces cerevisiae*

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The effects of a live strain of *Saccharomyces cerevisiae* on hydrogen utilization and acetate and methane production by two hydrogenotrophic ruminal microorganisms, an acetogenic bacterial strain and an archaea methanogen, were investigated. The addition of yeast cells enhanced by more than fivefold the hydrogenotrophic metabolism of the acetogenic strain and its acetate production. In the absence of yeasts, and in a coculture of the acetogen and the methanogen, hydrogen was principally used for methane synthesis, but the presence of live yeast cells stimulated the utilization of hydrogen by the acetogenic strain and enhanced acetogenesis.

In the rumen, hydrogen is an intermediate produced particularly during plant cell wall breakdown by cellulolytic microorganisms, such as *Ruminococcus albus*, *Ruminococcus flavefaciens*, and anaerobic fungi (6, 16). H_2 never accumulates in the rumen because it is rapidly used by methanogens, which are the dominant hydrogen-utilizing microorganisms in the rumens of adult ruminants (17). Hydrogenotrophic acetogenic bacteria are also able to utilize hydrogen for acetate production; it has been shown that they are present in high numbers in the rumens of newborn lambs, before establishment of methanogens (11), and also in the rumens of adults fed low-forage diets (9). Acetogenic bacteria could therefore be good candidates to compete with methanogens for hydrogen utilization, and their acetogenesis could represent a beneficial alternative to methanogenesis, which constitutes a loss of energy for the ruminant. The objective of this study was to determine whether a feed additive for ruminants, a *Saccharomyces cerevisiae* strain, could affect in vitro H_2 utilization by an acetogenic bacterial species and an archaea methanogenic strain in pure culture or in cocultivation. The yeast strain used has been shown to stimulate cellulose degradation by ruminal microorganisms in vitro in the rumen-stimulating technique (8), to increase lactate utilization by *Megasphaera elsdenii* (5), and to enhance germination of zoospores and cellulose breakdown by *Neocallimastix frontalis* (4). The methanogenic strain isolated from a sheep rumen in our laboratory, MF_2, shows great similarity with *Methanobrevibacter ruminantium*.

In this study MF_2 was grown in a medium adapted from that described by Balch et al. (1) containing the following (per liter): clarified rumen fluid, 200 ml; mineral 1, 50 ml; mineral 2, 50 ml; trace elements solution, 10 ml; NH_4Cl, 0.5 g; resazurin (0.1%), 1 ml; $NaHCO_3$, 5 g; and cysteine sulfide (1.25% each), 40 ml. The homoacetogenic bacterial strain Ser 8 was isolated in our laboratory from the rumen of a 20-h-old lamb and was representative of the acetogenic species of the laboratory collection, on a morphological and functional basis. It was grown in the same medium as MF_2. The cultures were gassed with a mixture of H_2 and CO_2 (80%/20%, vol/vol) at an initial pressure of 2×10^5 Pa, before being incubated horizontally at 39°C. The cocultures of MF_2 and Ser 8 were performed in the same medium (5 ml of medium per tube). The inocula consisted of 0.25 ml of culture of each microbial strain (optical density at 600 nm = 0.2). The *S. cerevisiae* strain (CNCM. I-1077; Institut Pasteur, Paris, France) was provided by Santel-groupe Agritek and was grown in glucose-peptone-malt extract-yeast extract at 30°C. Live yeast cells were collected from overnight cultures by centrifugation (1,000 × *g*, 10 min). *S. cerevisiae* cells were then resuspended in an appropriate volume of an anaerobic mineral solution (3), counted in a Malassez cell, and added, under strictly anaerobic conditions, either alive or killed after autoclaving (120°C, 20 min), to pure cultures and cocultures at a concentration of 10^8 cells ml^{-1} (in a previous study [results not included] this concentration was the most efficient in stimulating hydrogen utilization by the acetogenic strain, in comparison with 10^6 or 10^7 cells ml^{-1}; with 10^8 cells ml^{-1}, stimulation was threefold higher than with 10^7 cells ml^{-1}). After inoculation, tubes were gassed with H_2-CO_2 at an initial pressure of 2×10^5 Pa and were incubated horizontally at 39°C for 5 days. At the end of incubation, gas consumption was determined by the syringe method (13). H_2 utilization and CH_4 production were measured by gas chromatography after withdrawal of 2 ml of the gas phase in the headspace of the culture tubes. Acetate production was also measured in the culture supernatants by gas chromatography (7). The significance of the yeast effect was determined with a Student's *t* test (15).

After 5 days of incubation and in the absence of *S. cerevisiae*, the acetogenic strain Ser 8 used only 156.7 ± 25.5 μmol of H_2; the addition of live yeast cells highly stimulated hydrogen utilization ($P < 0.0001$) and acetate production ($P < 0.001$) by the bacterium (Table 1). The number of live yeast cells recovered after 5 days was very low (1.2×10^3 ml^{-1}). The addition of autoclaved yeasts led to the same stimulation on the acetogen metabolism. In the methanogenic monoculture, gas utilization was very variable; however, MF_2 consumed more H_2 than did Ser 8. In the presence of *S. cerevisiae*, hydrogen

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TABLE 1. Effects of *S. cerevisiae* on hydrogen utilization and methane and acetate production by the acetogenic strain Ser 8 and the archaea methanogen MF_2[a]

Culture	H_2 utilization (μmol)	CH_4 production (μmol)	Acetate production (μmol)	H_2 recovery (%) in production of:	
				CH_4	Acetate
Ser 8	156.7 ± 25.5	0	37.8 ± 4.2	0	96.5
Ser 8 + live *S. cerevisiae*	821.5 ± 34.5	0	250.7 ± 9.2	0	122.0
Ser 8 + autoclaved *S. cerevisiae*	846.8 ± 46.5	0	258.2 ± 9.7	0	121.9
MF_2	701.2 ± 201.2	176.7 ± 38.7	Traces	99.2	0
MF_2 + live *S. cerevisiae*	1,103.9 ± 132.0	209.8 ± 19.9	Traces	76.1	0
Ser 8 + MF_2	844.7 ± 45.5	151.6 ± 11.4	40.2 ± 9.9	71.8	19.0
Ser 8 + MF_2 + live *S. cerevisiae*	994.5 ± 85.6	128.4 ± 24.6	174.0 ± 9.6	51.6	70.0
Live *S. cerevisiae*	0	0	<10	0	0

[a] *S. cerevisiae* (live or autoclaved) was used at 10^8 cells ml^{-1}. Results are means ± standard deviations for five assays.

utilization by MF_2 was stimulated ($P < 0.01$), but the increase did not have a significant effect on CH_4 production by this strain.

In the coculture, in the absence of *S. cerevisiae*, levels of hydrogen consumption and methane production were close to those measured in MF_2 pure culture; only 19% of hydrogen was used in acetate synthesis, while 72% was implicated in methane formation. The addition of yeasts led to a stimulation ($P < 0.01$) of H_2 utilization; the methane concentration remained relatively stable. In contrast, in the presence of yeast cells, the acetate concentration was significantly increased ($P < 0.0001$), indicating that the acetogenic bacterial species was stimulated and more efficient in hydrogen utilization (70% of the hydrogen was used for acetate production).

These preliminary results are the first report of the effect of fungal feed additives used in ruminant nutrition on an acetogenic bacterial strain; the *S. cerevisiae* strain used in this experiment was able to stimulate an acetogenic species even in the presence of methanogens. Further research is needed to identify the mode of action of *S. cerevisiae* on acetogenic bacteria; as autoclaved yeasts were as efficient as viable yeasts in the stimulation of the hydrogenotrophic function of the acetogenic bacterial strain, a heat-resistant factor could be in part implicated in this stimulation. Some previous reports have evidenced the role of B vitamins (4) and organic acids (12) provided by *S. cerevisiae* in the stimulation of rumen microorganisms. Furthermore, yeast extract has been shown to be a growth factor for an acetogenic strain isolated from the rumen of a deer (14).

In the rumen, acetogens are not able to compete with methanogens, contrary to the situation observed in some other ecosystems, such as the termite gut or the colons of nonmethanogenic human subjects, where acetogens are more abundant and more efficiently able to metabolize hydrogen (2, 10). The use of yeasts as ruminant feed additives could help these bacteria to compete or at least to cometabolize hydrogen with methanogens; this type of feed supplementation could therefore be an interesting way to reduce methane emissions, to optimize rumen metabolism, and to promote ruminant performance and animal health.

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