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Note

Effect of a *Saccharomyces cerevisiae* culture on growth and lactate utilization by the ruminal bacterium *Megasphaera elsdenii*

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Summary — This study evaluated the effect of a yeast culture (YEA-SACC) filter-sterilized filtrate on the growth and lactate uptake by the ruminal bacteria *Megasphaera elsdenii*. The levels of filtrate used (v/v) were 0, 1, 2.5, 5%. *M elsdenii* was cultured in ATCC 566 modified medium, under anaerobic conditions, for 24 h at 39°C in batch culture. The treatment caused a linear improvement in lactate utilization and bacterial dry matter production: a 60% increase over the control for a 5% YEA-SACC supplement. The addition of 2.5 and 5% of YEA-SACC resulted in an improvement of the total volatile fatty acids (VFA) production and modified the ratio between the VFA: there was a slight decrease in acetate. The treatment also increased $Y_{LACTATE}$. These results may be partially due to malic acid, present in the YEA-SACC. Malic acid was shown to stimulate the growth rate (after 8 h of incubation) of *M elsdenii* when amounts of 1, 5 or 10 mM were added to the culture medium.

lactate utilization / bacterial growth / *Megasphaera elsdenii* / *Saccharomyces cerevisiae*

Résumé — Effet d'une culture de *Saccharomyces cerevisiae* sur la croissance et l'utilisation de l'acide lactique par la bactérie du rumen *Megasphaera elsdenii*. *Ce travail étudie l'effet d'un filtrat de levure stérilisé sur la croissance et le métabolisme de l'acide lactique par la bactérie du rumen* Megasphaera elsdenii. *Le traitement a été effectué à 4 niveaux 0, 1, 2,5 et 5% (v/v). Pour la préparation du filtrat, 5 g de levure (YEA-SACC) ont été agités pendant 1 h dans 50 ml d'eau bidistillée. La suspension obtenue a été filtrée sur papier filtre et stérilisée par filtres ayant une porosité de 0,45 µm.* M elsdenii *a été cultivée à 39°C pendant 24 h, en anaérobiose. Tous les traitements appliqués ont amélioré la capacité à métaboliser l'acide lactique, et la synthèse de matière sèche bactérienne. Les résultats ont été proportionnels à la quantité du filtrat ajouté. Les 2 paramètres pris en considération ont montré une amélioration de 60% pour le niveau le plus élevé (5%). L'efficacité de croissance ($Y_{LACTATE}$) a été uniquement améliorée par le traitement intermédiaire. La production d'acides gras volatils (AGV) est augmentée à 2,5 et 5%. Les ratios molaires entre AGV ont été également changés montrant une diminution de l'acide acétique. Ces effets peuvent être partiellement dus à la présence d'acide malique dans le filtrat utilisé. En effet, en ajoutant 10 mM d'acide malique, la vitesse de croissance de* M elsdenii *augmente.*

utilisation du lactate / croissance bactérienne* / Megasphaera elsdenii / Saccharomyces cerevisiae

INTRODUCTION

The high amount of starch in ruminant diets stimulates the growth of amylolytic and lactate-producing rumen bacteria, such as *Streptococcus bovis*. The large amount of lactic acid produced by these organisms leads to a decrease in ruminal pH that results in the inhibition of cellulolytic and hemicellulolytic bacteria and, consequently, in rumen disorders (acidosis). Although some ruminal bacteria, such as *Megasphaera elsdenii, Selenomonas ruminantium* and *Veillonella parvula*, can utilize lactate as a carbon source, their metabolic activities are usually not sufficient to reduce the lactate levels to physiological concentrations. *M elsdenii* is considered to be the main utilizer of lactate in the rumen (Counotte *et al,* 1981, 1983). Its metabolization is not inhibited by soluble sugars (Russell and Baldwin, 1978). The addition of yeast culture to the ruminant diet may be an effective tool to modify ruminal fermentative pattern (Williams *et al,* 1991; Carro *et al,* 1992); however, its effect on fiber digestion is still controversial (Chademana and Offer, 1990; Williams *et al,* 1991; Mir and Mir, 1994). The rumen lactate concentration in steers fed rolled barley plus hay (50:50) was lowered by the addition of yeast culture to the diet (Williams *et al,* 1991). Nisbet and Martin (1991) were able to improve the growth and lactate utilization in *S ruminantium* with a supplement of yeast culture filter-sterilized filtrate. Factors affecting lactate uptake by *M elsdenii* have been recently examined by Waldrip and Martin (1993). The aim of this work was to understand the effect of a *Saccharomyces cerevisiae* culture (YEA-SACC) on lactate utilization and growth of *M elsdenii.*

MATERIALS AND METHODS

Organism and culture conditions

The strain type ATCC 25940 of *M elsdenii* was used in this work. The strain was cultured normally in ATCC 566 modified medium, that was composed as follows: (per liter) KH_2PO_4, 1.6 g; K_2HPO_4, 3.2 g; yeast extract, 4.0 g; NH_4Cl, 0.5 g; $CaCl_2$, 0.2 g; $MgCl_2$, 0.2 g; Cysteine-HCl, 0.5 g; sodium D-L lactate solution 60% w/v, 16 ml.

The *M elsdenii* was cultured in batch culture at 39°C in an anaerobic glove box (Forma Scientific, Marietta, Ohio, USA), with an atmosphere composed of 10% CO_2, 5% H_2 and 85% N_2. Glassware and plasticware were exposed to the anaerobic atmosphere within the glove box for at least 48 h before being utilized.

Table I. Effect of *Saccharomyces cerevisiae* culture (YEA-SACC) filter-sterilized filtrate on lactate uptake by whole cells of *Megasphaera elsdenii.*

YEA-SACC % (v/v)	*Lactate utilized (mg/100 ml)*	*Variations compared with control*
0	817.36^{Aa}	
1	1033.45^{ABb}	+26.5%
2.5	1085.07^{B}	+32.8%
5	1335.00^{C}	+63.4%

SE = 64.70; least squares means with different letters are significantly different: $^{a, b}$ ($P < 0.05$); ^{A,B} ($P < 0.01$).

Growth studies

The effect of the YEA-SACC filtrate on the growth of *M elsdenii* ATCC 25940 was determined. Because YEA-SACC contains an insoluble component, a sterile filtrate was prepared as previously described (Nisbet and Martin, 1991). The YEA-SACC filtrate was added under anaerobic condition to the medium in the following concentrations: 0 (control), 1, 2.5 and 5% of the medium ($n = 7$ for each level).

The effects of YEA-SACC filtrate on lactate utilization and $Y_{LACTATE}$ (mg bacterial dry matter produced/mM of lactate metabolized) were studied. To study the effect of L-malate on bacterial growth, 1, 5, 10 mM L-malate, from a 500 mM stock solution, previously filtered and sterilized under anaerobic conditions, were added to the medium ($n = 4$ for each level). Growth studies were performed in 50 ml flasks of ATCC 566 modified medium, inoculated with 0.5 ml of overnight cultures of *M elsdenii* ATCC 25940 and incubated at 39°C in anaerobic glove boxes for 24 h (YEA-SACC).

At the end of the fermentation, cells were recovered by centrifugation at 10 000 x g for 15 min at 4°C, washed with bidistilled sterile water and resuspended in 1 ml of sterile water. The dry weight of the washed cell suspension was determined after drying at 105°C for 3 h as previously described (Russell, 1986). Cell free supernatant was collected and stored a –20°C. Volatile fatty acids (VFA) and lactate were analyzed by gas chromatography (Varian model 3700) according to the method of Fussell and McCailey (1987). The optical density of the cultures, at 600 nm (Shimidzu spectrophotometer), was measured spectrophotometrically against a blank of non-inoculated medium.

The data were analyzed by a one-way analysis of variance, the GLM procedure of the SAS statistical package (SAS, 1988). For growth data analysis, values were assumed to be statistically different ($P < 0.05$; Russell and Chen, 1989) when the coefficient of variability (CV) was less than 10% and the differences among means were greater than 3 times the SD. If CV was greater than 10%, significance ($P < 0.10$) was determined using a Student's *t*-test (Steel and Torrie, 1980).

RESULTS

Supplementing the growth medium with YEA-SACC improved the lactate utilization of growing cells of *M elsdenii*. As shown in table I, increasing the concentration of the YEA-SACC filtrate in the medium led to increased lactate utilization. When 5% filtrate was added, the lactate utilization was much higher than the control (+63.4%); however, it also increased with 1% supplement of YEA-SACC to the medium (26.5%). Higher rates of lactate fermentation are confirmed by the acetate, propionate, butyrate and valerate production, which are consistent with the known end products of lactate metabolism by *M elsdenii* (Rogosa, 1984). Supplementing the growth medium with 2.5 and 5% of YEA-SACC filtrate increased total VFA production (table II), with a higher production of VFA being found at the higher concentration of YEA-SACC. The molar % of fatty acids were also slightly different. There was a tendency for the acetate production to decrease when 2.5% of YEA-SACC was added to the medium (table II).

The YEA-SACC supplementation stimulated the growth of *M elsdenii* on lactate, calculated as the increase of microbial dry matter production (table III). This increment was linearly related to YEA-SACC filtrate concentration (table III). The same results were obtained when *M elsdenii's* growth was monitored as the optical density of the bacterial culture (table IV). The optical density was 25% (6 h) and 50% higher (9 h) for the 5% supplement as compared to the control.

The $Y_{LACTATE}$ values after treatment with 1, 2.5 and 5% of YEA-SACC filtrate are reported in table V. The $Y_{LACTATE}$ of *M elsdenii* was 10.46 g of cells per mole of lactate when 1% YEA-SACC was supplemented. It was 11.78 when the 2.5% of YEA-SACC filtrate was added. When 5% of YEA-SACC

Table II. Effect of *Saccharomyces cerevisiae* culture (YEA-SACC) filter-sterilized filtrate on volatile fatty acid (VFA) production by *Megasphara elsdenii* culture.

YEA-SACC % (v/v)	*Molar percentage of VFA*				*VFA production (mg/100 ml)*
	Acetic acid	*Propionic acid*	*Butyric acid*	*Valeric acid*	
0	33.06[a]	46.09	10.89	9.96	774.10[a]
1	31.83	48.03	9.59	10.55	782.41[a]
2.5	30.74[b]	48.79	10.60	9.87	858.60[b]
5	31.28	49.41	10.08	9.22	869.52[b]
SE	0.64	1.513	0.843	1.263	51.62

Least squares means, in the same column, with different letters are significantly different: [a, b] ($P < 0.10$).

filtrate was added, the $Y_{LACTATE}$ decreased to a value similar to the control. *M elsdenii* growth, after 8 h of fermentation, was linearly increased when adding different concentrations of malic acid (table VI). The increment responses of OD_{600} were 5.62, 11.36 and 12.21%, respectively, for 1, 5 and 10 mM of malate concentration.

DISCUSSION

When adding 2.5% of YEA-SACC filtrate, the improvement in the amount of bacterial dry matter production was higher than the increase in lactate utilization, suggesting a better efficiency of lactate utilization for microbial synthesis. This hypothesis is sup-

Table III. Effect of *Saccharomyces cerevisiae* culture (YEA-SACC) filter-sterilized filtrate on bacterial dry matter (DM) production by *Megasphaera elsdenii* culture.

YEA-SACC % (v/v)	*Bacterial DM production (mg/100 ml)*	*Variations compared with control*
0	95.01[a]	
1	115.82[b]	+21.9%
2.5	142.13[b]	+49.6%
5	157.69[b]	+66.0%

SE = 20.15; Least squares means with different letters are significantly different: [a, b] $P < 0.05$.

Table IV. Effect of *Saccharomyces cerevisiae* culture (YEA-SACC) filter-sterilized filtrate on the growth of *Megasphaera elsdenii* on lactate.

Time of incubation (h)	*Absorbance at 600 nm*	
	Control	*YEA-SACC**
0	0.046	0.053
2	0.108	0.113
4	0.422	0.449
6	0.788	0.996
8	1.021[a]	1.426[b]
9	1.012[a]	1.517[b]
24	1.285[a]	1.476[b]

* 5% v/v; least squares means with different letters are significantly different: [a, b] $P < 0.05$.

Table V. Effect of *Saccharomyces cerevisiae* culture (YEA-SACC) filter-sterilized filtrate on growth yields of lactate grown cells of *Megasphaera elsdenii.*

YEA-SACC % (v/v)	$Y_{LACTATE}$ *(mg cells/mM lactate metabolized)*
0	10.46
1	10.09
2.5	11.78
5	10.63

SE = 1.20.

ported by a parallel behavior of $Y_{LACTATE}$, which reached a maximum at 2.5% supplementation. A similar result was obtained by Nisbet and Martin (1991), who studied the effect of YEA-SACC on lactate uptake by *S ruminantium.*

The effects of YEA-SACC filtrate on VFA pattern and production are partially in agreement with other studies, which show an increase in VFA production and a decrease in acetate and propionate molar percentage, following addition of YEA-SACC to pure cultures of different rumen microorganisms (Harrison *et al,* 1988; Nisbet and Martin, 1991).

Using *Aspergillus oryzae* (Amaferm) fermentation extract filtrate, Waldrip and Martin (1993) obtained a reduction in acetate production and an increase in the molar percentage of valeric acid by *M elsdenii.*

Based on the effects on lactate utilization, VFA production and growth stimulation, the addition of YEA-SACC filtrate must provide one or more soluble factors which stimulate lactate metabolism of *M elsdenii.* The role of amino acids is not clear. Waldrip and Martin (1993) showed that Amaferm stimulated growth of *M elsdenii* in the absence of Trypticase only. According to this data, stimulation seems to be due to the amino acid content of the filtrate.

According to Wallace (1986), amino acids are probably not involved, in growth stimulation, because they meet only 37% of the energy maintenance requirements of *M elsdenii.*

Table VI. Effect of different concentrations of malic acid on the growth of *Megasphaera elsdenii* on lactate.

Time of incubations (h)	*Absorbance at 600 nm*			
	Control	*Malate*		
		1 mM	*5 mM*	*10 mM*
2	0.061	0.069	0.098[b]	0.033
4	0.341	0.343	0.359	0.176
6	0.863	0.861	0.936[b]	0.875
7	1.057	1.082*	1.150*	1.085
8	1.138[a]	1.202[b]	1.267[b]	1.277[b]
24	1.244	1.250	1.286	1.341

[a, b] Means significantly different from the control ($P < 0.05$); * means significantly different from the control ($P < 0.10$).

Lactate uptake by *M elsdenii* was unchanged when sugars (Hino *et al,* 1994) or Na^+ and K^+ (Waldrip and Martin, 1993) were added to the medium. Uncoupling agents causing diffusion of protons into the cell strongly reduced lactate utilization by *M elsdenii,* whereas ionophores and ATPase inhibitors had a poor inhibitory effect on lactate utilization (Waldrip and Martin, 1993). Based on their results, Waldrip and Martin (1993) suggested that a proton motive force-driven mechanism may be involved in L-lactate uptake by *M elsdenii.*

Since Nisbet and Martin (1991) showed that organic acids from the YEA-SACC filtrate, particularly malate, stimulate *S ruminantium* growth, we investigated the influence of malic acid on *M elsdenii* growth. Our experiment demonstrated that growh rate of *M elsdenii* was increased slightly by adding malic acid (table VI).

The YEA-SACC filtrate was assayed for L-malate content and found to contain a 4.2 mM of L-malate. Thus, when adding 2.5% of YEA-SACC, the final concentration of malate was only 0.21 mM, which would therefore play a minor role in stimulating the growth of *M elsdenii.*

CONCLUSION

YEA-SACC filter-sterilized filtrate increased the growth, lactate uptake, pattern and VFA production in *M elsdenii* cultures. An improvement in microbial synthesis efficiency, as a function of supplementation level, was also observed.The malic acid content of YEA-SACC filtrate explains only a small part of the probiotic activity observed. Further studies will be necessary to detect which substances in YEA-SACC are the most important in stimulating the metabolic activity of *M elsdenii.*

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