Anim. Res. 53 (2004) 177–186
© INRA, EDP Sciences, 2004
DOI: 10.1051/animres:2004009

Original article

Effects of peptidic fractions from *Saccharomyces cerevisiae* culture on growth and metabolism of the ruminal bacteria *Megasphaera elsdenii*

Filippo ROSSI[a]*, Aldo DI LUCCIA[b]**, Donatella VINCENTI[b], Pier Sandro COCCONCELLI[c]

[a] Istituto di Scienze degli Alimenti e della Nutrizione, U.C.S.C., Facoltà di Agraria, Via Emilia Parmense 84, 29100 Piacenza, Italy
[b] Istituto di ricerche sull'adattamento dei bovini e dei bufali all'ambiente del Mezzogiorno, Via Argine 1085, Ponticelli (NA), Italy
[c] Istituto di Microbiologia, U.C.S.C., Facoltà di Agraria, Via Emilia Parmense 84, Piacenza, Italy

(Received 9 September 2002; accepted 2 March 2004)

Abstract – The aim of this study was to evaluate the effects of peptide fractions purified from a *Saccharomyces cerevisiae* product on the growth of the ruminal bacterium *Megasphaera elsdenii*. Such compounds have been shown to affect the growth and metabolism of *M. elsdenii*, the ruminal bacteria which uses lactate the most. A hydrophilic fraction purified by means of HPLC has proven to stimulate cell growth, the production of butyrate (+100%) and valerate (+76.1%) as well as the metabolization of lactate (+ 16.9%). This chromatographic fraction was further characterized and seven positively charged peptides were identified. It was observed that two peptides rich in lysine and histidine were the most effective in stimulating growth (+ 18.5%) and lactate utilization (+ 74.1%) in *M. elsdenii*. These results show that, in addition to previously studied factors such as the supply of nutrients or oxygen removal, the presence of peptidic fractions is involved in the stimulation of bacterial growth by *S. cerevisiae*.

***Megasphaera elsdenii* / rumen / *Saccharomyces cerevisiae* / peptides**

Résumé – Effet des fractions peptidiques purifiées de *Saccharomyces cerevisiae* sur le développement et le métabolisme de la bactérie ruminale *Megasphaera elsdenii.* L'effet de fractions protéiques, purifiées à partir d'un produit commercial à base de cellules de *Saccharomyces cerevisiae,* sur le développement de *Megasphaera elsdenii* a été évalué. Ces composés ont montré une certaine influence sur le développement et sur le métabolisme de la bactérie *M. elsdenii*, qui est la bactérie ruminale qui utilise majoritairement l'acide lactique. Une fraction hydrophile purifiée par HPLC a stimulé l'accroissement cellulaire, la production d'acide butyrique (+ 100 %) et valérique (+ 76,1 %), et l'utilisation d'acide lactique (+ 16,9 %). Cette fraction chromatographique a été ultérieurement caractérisée, et sept peptides chargés positivement ont été identifiés. Deux peptides

* Corresponding author: filippo.rossi@unicatt.it
** Present address: Dipartimento di Produzione Animale, Università degli Studi di Bari, Via Amendola 165/A, 70139 Bari.

riches en lysine et histidine ont stimulé particulièrement la croissance (+ 18,5 %) et l'utilisation d'acide lactique (+ 74,1 %) chez *M. elsdenii*. Ce résultat démontre que, outre l'apport de nutriments et la réduction de la disponibilité en oxygène, d'autres facteurs purifiés à partir de *S. cerevisiae* sont impliqués dans la stimulation du développement bactérien.

***Megasphaera elsdenii* / rumen / *Saccharomyces cerevisiae* / peptides**

1. INTRODUCTION

Ruminal pH is a very important physiological parameter, conditioning the rumen microbial activity. pH values lower than 6.0, due to the intake of high amounts of starch, lead to a reduction in fiber degradation and microbial protein synthesis [9]. In order to prevent pH drop, ruminant diets are supplemented with several types of buffers: sodium bicarbonate, calcium carbonate, magnesium oxide or clays such as zeolite. In some instances *Saccharomyces cerevisiae* culture has been shown to reduce the pH drop which usually follows a meal [21].

Megasphaera elsdenii is a Gram-negative bacterium which is considered to be the main utilizer of lactate in the rumen [5] and can be used as a rumen probiotic in order to prevent the onset of lactic acidosis. Hibbard et al. [7] increased dry matter intake and reduced lactic acid concentration in the rumen of steers inoculated with *M. elsdenii*; Wiryawan and Brooker [22] reported a lowering in lactate concentration and pH stabilization in the rumen of sheep which were fed a large amount of grain, when *M. elsdenii* was added to their diet. However, Cook et al. [4] were not able to obtain positive effects on daily weight gain following the inoculation of *M. elsdenii* into the rumen of cattle. Robinson et al. [15] and Kung and Hession [10] reported that the inoculation of in vitro rumen cultures with *M. elsdenii*, prevented the accumulation of lactate and the subsequent pH drop.

The addition of *Saccharomyces cerevisiae* culture was shown to improve *M. elsdenii* growth [2, 16], even though the mode of action is not yet fully understood. The aim of this work was to measure the probiotic activity of polypeptides purified from a commercial product containing *S. cerevisiae* (YEA-SACC, Alltech, KY, USA).

2. MATERIALS AND METHODS

2.1. Microorganism and growth conditions

M. elsdenii ATCC 25940 (type strain) was cultured in an anaerobic glove box (Forma Scientific, Marietta, Ohio, USA) at 39 °C in ATCC 566, a medium with Na-lactate as the main energy source containing (per liter) 1.6 g of KH_2PO_4, 3.2 g of K_2HPO_4, 16 mL of Na DL-lactate (60% sol.), 4 g of yeast extract, 500 mg of NH_4Cl, 200 mg of $CaCl_2$, 200 mg of $MgCl_2$, 1 mg of resazurin, 500 mg of cysteine·HCl·H_2O.

2.2. Polypeptidic and peptidic separation

The polypeptidic fractions were purified from a YEA-SACC (Alltech, Lexington, KY, USA) water solution (5 g per 50 mL water, suspended for 1 h) by HPLC by means of the following procedure: 5 g of powder from yeast culture were resuspended in 50 mL of Milli-Q water and filtered on a 0.45 µm filter. The water-soluble components were fractionated by reversed phase high performance liquid chromatography (RP-HPLC) by means of an Acquapore C8 column (Applied Biosystems, Santa Clara, CA, USA). The water soluble yeast extract was diluted 1:100 with developer A (see below) and filtered through 0.45 µm filters (Millipore, Bedford, MS, USA). Then 8 mL of this solution were applied to the column. The chromatogram was developed with three linear gradients

between developer A (water containing 0.1% of trifluoroacetic acid, TFA) and developer B (acetonitrile plus 0.075% TFA) at a flow rate of 1 mL per minute. After running for 9 min without developer B, the first gradient started 0% to 25% developer B for 30 min, followed by 25% to 80% for 15 min and 80% to 100% for 10 min. The column was then held at 100% buffer B for 10 minutes. The chromatographic peaks were detected at 214 nm and computed by a Shimadzu CR-34 integrator (Shimadzu Corporation, Kyoto, Japan). Five peaks, P1-P2-P3-P4-P5, were collected and submitted to biological assay (trial 1). Ultrafiltration by Amicon cell mod. 3 using a 5000 cut off (C.O.) membrane was carried out in order to determine which peaks contained peptidic or polypeptidic components. The ultrafiltrate and retentate were analyzed by RP-HPLC as described above.

The first peak proved to be biologically active; in order to establish if biological activity was due to free amino acids or small peptides, the amino acid composition was determined by RP-HPLC and weak cationic exchange and chelating chromatography was performed.

Both free and total amino acids in peak 1 were determined by *o*-phthaldialdehyde precolumn derivatization according to Umagat et al. [18]. The amino acid separation was performed by using a complete Gilson pumping system consisting of 305 and 306 pumps combined with a Manometric Module, for gradient HPLC, a Dynamic Mixer and a 121 fluorometer. Separation of free amino acids and acid hydrolysis of peak 1 were carried out on a 25 cm × 4.6 mm I.D. ultrasphere column packed with 5 µm ODS particles (Beckman, Berkeley, CA, USA).

A copper-chelex 100 resin (Bio-Rad, Hercules, USA) was used to fractionate amino acids and peptides. The sample was dissolved in water, injected and eluted using 100 mL of 1 mM NH_3 first then 200 mL of 5 mM NH_3 with a flow of 1 mL per min. The absorbance of the eluted fractions was measured at 280 nm [1]. Seven fractions, identified as F1-F2-F3-F4-F5-F6-F7, were thus isolated.

2.3. Growth studies

Two different sets of trials were carried out. In trial 1 the effect of peptides from YEA-SACC was studied, while in trial 2 the seven individual fractions isolated from peptide P1, a mixture of all fractions in equal amounts and filter sterilized filtrate of YEA-SACC prepared as described by Nisbet and Martin [14] were tested. The biological activity of the eluted fractions was assessed as follows: equal amounts of the purified fractions were dissolved in Milli-Q water and filtered on 0.45 µm filter. In trial 1, all of the treatments were added to the medium in the amount of 1% of the final volume, while in trial 2 the solutions were added to the medium in the amount of 0.50% of the final volume. The same volume of sterile distilled water was added as a negative control.

The microbial growth was determined by optical density at 600 nm (OD_{600}) by means of a Shimadzu spectrophotometer. The production of volatile fatty acid (VFA) and the concentration of lactate were also measured by gas-chromatography, using the method proposed by Fussell and McCailey [6].

2.4. Experimental design

Data were analyzed using the GLM procedure of the SAS statistical package [17]; two different trials were carried out, with three replicates for each treatment in the trial, so as to have 6 replicates/treatment. Optical density and AGV concentration at 24 hours were performed on 4 samples per treatment. The statistic model was as follows:

$$Y = \mu + T + F + \varepsilon$$

where:

Y = observed parameters (lactate utilization, VFA production, OD_{600})

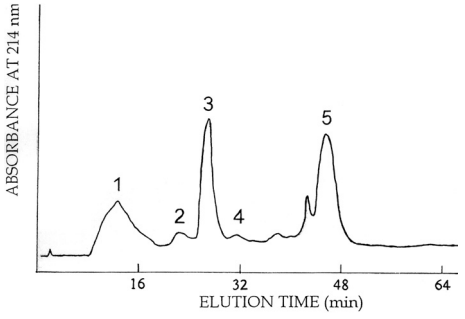

Figure 1. HPLC separation of polypeptides contained in the water-soluble fraction of *S. cerevisiae* culture. Different peaks are identified with numbers.

μ = overall mean
T = treatment effect
F = effect of fermentation
ε = experimental error.

3. RESULTS

3.1. First experiment

3.1.1. HPLC separation of yeast peptides

Since the aim of this work was to identify the active compound from *S. cerevisiae* that is able to stimulate the growth of the anaerobic rumen bacterium *M. elsdenii*, the first approach was the identification of active peptides from yeast cells. Figure 1 shows the HPLC chromatogram of the water-soluble fraction from YEA-SACC; five peaks were detected and the amount of each peak was subsequently determined. Considering that the separation occurred on a column with an aliphatic chain of 8 carbons, the fractionation of water-soluble components was based on the hydrophobic properties of the molecules, depending on the charge/size ratio. Ultrafiltration on 5000 C.O. and subsequent RP-HPLC analysis showed that peaks 1 (37.4% of total fractions), 2 (2.3% of total fractions), 3 (23.4% of total fractions) and 4 (0.4% of total fractions) were detected in the ultrafiltrate, their components having a molecular weight > 5000 Da, whereas peak 5 (36.5% of total fractions) was recorded in the retentate, hence its composition should be ascribed to the polypeptidic components.

Thus, the fast elution of peak 1 is due to the smallest, positively charged components, corresponding to free amino acids and two or three unit peptides. Peak 2 is due to peptides with higher molecular weights than peak 1 and peak 5, corresponding to the polypeptide fraction which was eluted from 100% buffer B. As reported in Table I, fraction 1, is mainly due to lysine, histidine, and valine.

Table I. Amino acid composition of peak 1 (P 1) isolated from filter-sterilized YEA-SACC filtrate.

AMINO ACIDS	% on total amino acids	TOTAL
Negatively charged		11.23
Aspartate	5.94	
Glutamate	5.29	
Positively charged		45.65
Histidine	13.85	
Arginine	6.26	
Lysine	25.54	
Hydrophilic		20.51
Serine	5.99	
Glycine	7.72	
Threonine	6.79	
Tyrosine	0.00	
Hydrophobic		22.61
Alanine	3.42	
Methionine	3.51	
Valine	10.31	
Phenylalanine	0	
Isoleucine	4.55	
Leucine	0.81	

Table II. Effect of polypeptidic fractions, from yeast culture, on the growth of *Megasphaera elsdenii*, expressed as optical density at 600 nm.

Hours of incubation	Optical density (600 nm)						
	Control	P1+ P 2	P 2	P 3	P 5	Mix	SE
4	0.327^{BCc}	0.491^{Ee}	0.294^{Bb}	0.095^{Aa}	0.353^{Cc}	0.396^{Dd}	0.011
6	0.819^{Bb}	1.081^{Cd}	0.843^{Bbc}	0.416^{Aa}	0.812^{Bb}	0.866^{Bc}	0.014
7	1.040^{C}	1.297^{E}	1.019^{C}	0.801^{A}	0.939^{B}	1.120^{D}	0.017
8	1.112^{Cc}	1.377^{Ee}	1.122^{CDc}	0.883^{Aa}	0.980^{Bb}	1.188^{Dd}	0.017
24	0.649^{Cc}	0.706^{Cd}	0.600^{Bb}	0.564^{Bb}	0.839^{De}	0.487^{Aa}	0.014

Means in the same row with different letters are significantly different: ^{a,b,c,d,e}: $P<0.05$; ^{A,B,C,D,E}: $P<0.01$. Mix: mix of all polypeptidic fractions, added in equal volume; SE: standard error.

3.1.2. Growth experiment

Bacterial growth was increased when a mixture of fractions 1 and 2 was added to the medium: higher values of OD_{600} (Tab. II) and VFA production were observed both at 8 and 24 h of growth, lactate utilization after 24 h of incubation was also increased (Tab. III). Higher VFA production, concerning fatty acids derived directly from lactate dissimilation, like butyrate, or indirectly as valeric acid, which is synthesized through propionate, was mainly observed after 8 h of growth (Tab. III).

The addition of the polypeptide mixture 2 and 3 did not affect the bacterial metabolism as compared to the control, while the addition of fraction 5 reduced the microbial growth, even when mixed with other treatments.

3.2. Second experiment

3.2.1. HPLC fractionation of peak 1

The results achieved suggest that fraction 1 contains biologically active compounds that stimulate the growth and metabolism of *M. elsdenii*. In order to further investigate on active peptides, peak 1 was fractionated by a low pressure weak cation exchange and chelating liquid chromatography. Seven peaks were thus isolated and collected. Peaks 1-2-3 were due to free amino acids negatively charged or without charge, while peaks 4-5-6-7 were caused by positively charged free amino acids and peptides. Since chromatographic separation was performed at an alkaline pH, from 1mM to 5 M ammonia solution, the amino acids and peptides with negative net charge were first eluted. When the positive charge was predominant, the amino acids were eluted first, followed by the peptides. Thus, peaks 6 and 7 were due to a higher amount of positively charged peptides than the others.

3.2.2. Growth experiment

The supplementation of the medium with peptides 5-6-7, their mixture and YEA-SACC led to an increase in growth, expressed as $OD_{600\ nm}$, after 4 hours of incubation when compared with the control. The growth rate of the microbial culture supplemented with peptides 1-2-3 was increased after 7 hours of incubation (Tab. IV). The growth of bacteria cultured in the medium supplemented with F6, F7, their mixture and YEA-SACC was the same as in the control. The differences in $OD_{600\ nm}$ detected after 24 h of growth are meaningless and

Table III. Effect of polypeptides, purified from the soluble fraction of YEA-SACC, on lactate utilization (%) and VFA production (mM·L^{-1}) of *Megasphaera elsdenii*.

Items[1]	VFA concentration (mM·L^{-1})				Total VFA	Lactate utilization (%)
	Acetate	Propionate	Butyrate	Valerate		
			8 hours of growth			
Control	28.8^{b}	43.0^{b}	7.1^{A}	6.7^{A}	85.6^{b}	77.7^{bc}
P 1+P 2	29.5^{Bb}	47.5^{B}	14.2^{Bc}	11.8^{B}	103.3^{Bc}	90.8^{Bc}
P 2	30.3^{Bb}	44.6^{b}	6.4^{Aab}	5.6^{A}	86.8^{b}	73.9^{b}
P 3	31.8^{Bb}	44.3^{b}	6.9^{Aab}	6.5^{A}	89.7^{b}	75.6^{b}
P 5	23.1^{a}	32.8^{Aa}	5.5^{Aa}	5.7^{A}	67.2^{Aa}	61.3^{Aa}
Mix	22.4^{Aa}	34.4^{Aa}	7.9^{Ab}	6.9^{A}	71.6^{Aa}	69.6^{Aab}
SE	0.512	0.682	0.484	0.508	0.868	4.448
			24 hours of growth			
Control	30.3^{Bb}	36.2^{Bb}	21.7^{Bb}	23.3^{B}	113.7^{B}	93.9^{Aa}
P 1+P 2	24.3^{b}	36.8^{Bb}	14.4^{ABab}	21.7^{B}	98.1^{B}	98.1^{ABb}
P 2	27.2^{b}	34.5^{b}	15.1^{ABab}	21.0^{B}	99.2^{B}	99.2^{ABb}
P 3	28.3^{b}	33.1^{b}	14.5^{ABab}	16.0^{B}	85.3^{B}	97.8^{ABb}
P 5	14.4^{Aa}	14.5^{Aa}	9.0^{Aa}	11.6^{A}	49.7^{A}	98.8^{ABb}
Mix	15.6^{Aa}	19.6^{a}	9.0^{Aa}	11.7^{A}	56.3^{A}	99.2^{Bb}
SE	1.025	1.521	1.398	0.731	2.018	0.196

Means in the same row with different letters are significantly different: $^{A, B}$: $P < 0.01$; $^{a, b, c}$: $P < 0.05$.
[1] Numbers show several peaks.
Mix: mix of all polypeptidic fractions, added in equal volume, SE: standard error.

mainly due to aging and starvation of the bacterial population.

3.3. VFA production

After 8 hours of growth, VFA concentration (Tab. V) was higher in the bottles containing peptides 6 and 7 than in the control, while no positive effects were induced by F4 supplementation. Lactate disappearance was improved by supplementation with peptides 4-5-6-7, their mixture and YEA-SACC, and no differences were observed between these treatments. Except for F1, all the treatments led to increased synthesis of butyric acid, the supplementation with a mixture of all seven peptides led to a higher production of this acid when compared with the individual supplementation of F1-F2-F3-F4. An increase in valeric acid production was observed only in the bacterial cultures supplemented with peptide 7, the peptide mixture and with YEA-SACC. F6 and F7 increased acetate production, while propionate synthesis was improved by F7 only.

The hypothesis that lactate is converted to butyrate and valerate, is supported by the positive relationship between the percentage of utilized lactate and the production of butyrate ($r^2 = 0.74$; $P < 0.01$) and valerate ($r^2 = 0.58$; $P < 0.01$). No significant relationship was detected between lactate uptake and acetate ($r^2 = 0.12$; $P < 0.318$) and propionate ($r^2 = 0.036$; $P < 0.600$) production.

Table IV. Effect of fractions, isolated from peak 1, on the growth of *M. elsdenii* expressed as optical density at 600 nm ($O.D._{600\ nm}$) of the culture.

Hours of growth	$OD_{(600\ nm)}$										
	Control	Fractions									
		F 1	F 2	F 3	F 4	F 5	F 6	F 7	Mix	YEA-SACC	SE
2	0.073	0.067	0.066	0.068	0.070	0.076	0.078	0.080	0.080	0.077	0.0054
4	0.235^{a}	0.236^{a}	0.234^{a}	0.236^{a}	0.244^{ab}	0.262^{bc}	0.273^{cd}	0.275^{cd}	0.291^{d}	0.282^{d}	0.0068
6	0.434^{a}	0.489^{bc}	0.477^{ab}	0.488^{bc}	0.522^{bcd}	0.532^{cde}	0.538^{cde}	0.571^{de}	0.577^{e}	0.567^{de}	0.0179
7	0.701^{a}	0.786^{b}	0.774^{b}	0.798^{bc}	0.821^{bcd}	0.795^{b}	0.850^{cde}	0.879^{de}	0.861^{d}	0.870^{d}	0.0186
9	0.883^{a}	0.962^{b}	0.970^{b}	0.991^{bc}	0.988^{bc}	1.036^{cd}	1.022^{cd}	1.046^{d}	1.034^{cd}	1.031^{cd}	0.0174
24	1.255^{e}	1.241^{cde}	1.248^{de}	1.237^{bcd}	1.241^{cde}	1.224^{ab}	1.232^{abc}	1.231^{abc}	1.219^{a}	1.219^{a}	0.0048

Means in the same row with different letters are significantly different: $^{a, b, c, d, e}$: $P < 0.05$.
Mix: mix of all polypeptidic fractions, added in equal volume; YEA-SACC: a commercial product containing *S. cerevisiae*; SE: standard error.

After 24 hours of growth (Tab. V), a reduction in the concentration of acetate and an increase in butyrate and valerate were recorded. This could be due to the accumulation of H_2 in aged bacterial culture, resulting in the accumulation of reduced coenzymes.

Microorganisms can use two molecules of acetate to produce butyrate [8] or obtain valerate by the combination of acetate and propionate; both reactions allow the re-oxidation of reduced coenzymes [5, 11]. A positive relationship ($r^2 = 0.428$; $P < 0.05$) was observed between the concentration of valerate at 24 h and the decrease in acetate + propionate from 8 to 24 h.

4. DISCUSSION

Since peptide 2 did not stimulate *M. elsdenii* growth and since it represents only a small percentage of the peptides purified from YEA-SACC (Tab. I), the positive effect observed was probably due to the hydrophilic fraction 1. Chen et al. [3] observed higher growth of *M. elsdenii* in the presence of hydrophilic peptides.

The addition of a small hydrophilic peptide (463 Da) to the culture medium improves the growth of gram-negative bacteria, such as *M. elsdenii*, more than the addition of its free amino acids [23].

The uptake of charged compounds could allow ATP synthesis [12], and although it is not known whether *M. elsdenii* has ATPase activity or not, the use of an ATPase inhibitor reduced L-lactate utilization by *M. elsdenii* by approximately 30% [19].

According to Zaho et al. [23], hydrophilic peptides could enhance the assimilation of its constituent amino acids through the stimulation of a particular peptide transport system depending on the peptide structure.

As reported in Table I, the peptidic fraction 1 is made up of positively charged amino acids, such as histidine and lysine, and this fact probably plays an important role in the growth stimulation of *M. elsdenii*. Peptides 4-5-6-7 and the mixture of all seven fractions led to an increase in the utilization of lactate similar to that obtained with the sterile filtrate of YEA-SACC. Furthermore fraction 7 promoted the synthesis of acetate and propionate.

Table V. Effect of fractions, purified from peak 1, on lactate utilization (%) and VFA production ($mM \cdot L^{-1}$) of *Megasphaera elsdenii*.

Items	VFA concentration ($mM \cdot L^{-1}$)					
	Acetate	Propionate	Butyrate	Valerate	Total VFA	Lactate utilized
			8 hours of growth			
Control	25.39^{Aa}	37.44^{a}	2.28^{Aa}	0.74^{Aa}	65.84^{Aa}	35.33^{ABab}
F 1	30.04^{Aba}	47.02^{ab}	2.61^{ABab}	0.94^{Aab}	80.62^{ABabc}	34.08^{Aa}
F 2	31.81^{ABab}	47.84^{ab}	3.57^{BCDc}	1.13^{ABab}	84.34^{ABabc}	41.58^{ABCabc}
F 3	32.63^{ABab}	48.44^{ab}	3.45^{BCbc}	1.07^{ABab}	85.60^{ABabc}	$49.05^{ABCDbcd}$
F 4	27.19^{Aa}	38.68^{a}	3.45^{BCbc}	1.36^{ABabc}	70.68^{ABa}	59.27^{CDd}
F 5	31.29^{ABab}	42.23^{a}	4.22^{CDcd}	1.31^{ABabc}	79.06^{ABabc}	57.08^{CDd}
F 6	39.82^{Bb}	52.81^{ab}	3.99^{CDcd}	1.35^{ABabc}	97.98^{ABbc}	58.86^{CDd}
F 7	34.95^{ABb}	59.08^{b}	4.28^{CDcd}	1.98^{Bc}	100.28^{Bc}	54.72^{BCDcd}
Mix	30.38^{Aba}	47.41^{ab}	4.69^{Dd}	1.93^{Bc}	84.41^{ABabc}	61.50^{Dd}
YEA-SACC	27.76^{Aa}	40.83^{a}	4.07^{CDcd}	1.60^{ABbc}	74.27^{ABab}	53.58^{BCDcd}
SE	1.3508	2.1623	0.4061	0.5703	2.3417	1.6708
			24 hours of growth			
Control	27.53^{ab}	45.28^{ab}	18.29^{BCbc}	19.83^{ab}	113.15^{ab}	95.56^{ABbc}
F1	24.10^{ab}	40.12^{ab}	15.60^{ABb}	15.00^{a}	97.05^{a}	96.58^{ABbc}
F2	23.51^{ab}	40.20^{ab}	16.73^{BCb}	18.24^{ab}	100.12^{ab}	94.37^{ABab}
F3	21.68^{a}	38.84^{a}	15.38^{ABb}	15.17^{a}	92.55^{a}	96.40^{ABbc}
F4	26.65^{ab}	46.88^{ab}	11.55^{Aa}	17.14^{ab}	104.57^{ab}	90.18^{Aa}
F5	26.26^{ab}	40.40^{ab}	18.04^{BCbc}	19.45^{ab}	106.14^{ab}	97.44^{Bbc}
F6	29.57^{b}	50.98^{b}	16.91^{BCb}	18.39^{ab}	118.52^{b}	96.03^{ABbc}
F7	26.81^{ab}	45.28^{ab}	16.93^{BCb}	15.58^{a}	106.68^{ab}	99.04^{Bc}
Mix	24.14^{ab}	38.08^{a}	17.88^{BCbc}	17.82^{ab}	99.89^{ab}	95.42^{ABbc}
YEA-SACC	27.15^{ab}	42.61^{ab}	20.15^{Cc}	22.43^{b}	114.90^{ab}	95.93^{ABbc}
SE	4.4866	7.5817	2.0766	3.8394	14.3503	3.0816

Means in the same column with different letters differ: $^{a, b, c, d}$: $P < 0.05$; $^{A, B, C, D}$: $P < 0.01$.
Mix: mix of all polypeptidic fractions, added in equal volume, YEA-SACC: a commercial product containing *S. cerevisiae*; SE: standard error.

The improvement of lactate uptake could be due to the generation of a proton gradient; a proton motive force is in fact involved in L-lactate transport by *M. elsdenii* [21] and the uptake of highly charged polypeptides, could generate a proton gradient.

Free amino acids are probably not involved in growth stimulation since they only meet 37% of the energy maintenance requirements of *M. elsdenii* [20].

Lactate is fermented by *M. elsdenii* to propionate and butyrate [5], both of these pathways allowing the extrusion of H^+ and the re-oxidation of reduced coenzymes. Since the synthesis of butyrate requires acetyl-CoA, derived from acetate, the production of butyric acid reduces acetate production; this could explain why the addition of polypeptides 1 + 2 did not improve acetic acid production. Hino et al. [8], were able

to reduce H_2 production by *M. elsdenii* and to increase the synthesis of butyrate by adding acetic acid to the culture.

Different modes of action have been suggested to explain the probiotic effects of *S. cerevisiae* culture on rumen bacteria: the supply of organic acids, amino acids, B vitamins [14, 19], or oxygen removal [13].

Our results show that other factors are involved in the stimulation of bacterial growth, and the soluble peptides contained in *S. cerevisiae* culture could improve the growth of *M. elsdenii*.

AKNOWLEDGEMENTS

The authors wish to thank Maria Luisa Callegari for her helpful comments on the manuscript.

REFERENCES

[1] Armstead I.P., Ling J.R., Chromatographic separation of mixed peptides from amino acids in biological digest with volatile buffers, J. Chromatogr. 586 (1997) 259–263.

[2] Callaway T.R., Martin S.A., Effect of a *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose, J. Dairy Sci. 80 (1997) 2035–2044.

[3] Chen G., Strobel H.J., Russell J.B., Sniffen C.J., Effect of hydrophobicity on utilization of peptides by ruminal bacteria in vitro, Appl. Environ. Microbiol. 53 (1987) 2021–2025.

[4] Cook M.K., Cooley J.H., Edens J.D., Goetsch D.D., Das N.K., Huber T.L., Effect of ruminal lactic acid-utilizing bacteria on adaptation of cattle to high-energy rations, Am. J. Vet. Res. 38 (1977) 1015–1017.

[5] Counotte G.H.M., Prins R.A., Janssen R.H.A.M., DeBie M.J.A., Role of *Megasphaera elsdenii* in the fermentation of DL-[2-^{13}C]lactate in the rumen of dairy cattle, Appl. Environ. Microbiol. 42 (1981) 649–655.

[6] Fussell R.J., McCailey D.V., Determination of volatile fatty acids (C_2-C_5) and lactic acid in silage by gas-chromatography, Analyst 112 (1987) 1213–1216.

[7] Hibbard B., Robinson J.A., Greening R.C., Smolenski W.J., Bell R.L., Peter P.J., The effect of route of administration of isolate 407A (UC-12497) on feed intake and selected ruminal variables of beef steers in an acute acidosis inappetence model, Proceedings of the 22nd Biennal Conference on Rumen Function, Chicago, USA,1992, p. 19.

[8] Hino T., Miyazaki K., Kuroda S., Role of extracellular acetate in the fermentation of glucose by a ruminal bacterium, *Megasphaera elsdenii*, J. Gen. Appl. Microbiol. 37 (1991) 121–129.

[9] Hoover W.H., Chemical factors involved in ruminal fiber digestion, J. Dairy Sci. 69 (1986) 2755–2766.

[10] Kung L. Jr., Hession A.O., Preventing in vitro lactate accumulation in ruminal fermentations by inoculation with *Megasphaera elsdenii*, J. Anim. Sci. 73 (1995) 250–256.

[11] Ladd J.N., The fermentation of lactic acid by a gram-negative coccus, Bioch. 71 (1959) 16–22.

[12] Maloney P.C., Microbes and membrane biology, FEMS Microbiol. Rev. 87 (1990) 91–102.

[13] Newbold C.J., Wallace R.J., McIntosh F.M., Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants, Brit. J. Nutr. 76 (1996) 249–261.

[14] Nisbet D.J., Martin S.A., Effect of *Saccharomyces cerevisiae* culture on lactate utilization by the ruminal bacterium *Selenomonas ruminantium*, J. Anim. Sci. 69 (1991) 4628–4633.

[15] Robinson J.A., Smolenski W.J., Greening R.C., Ogilvie M.L., Bell R.L., Barsuhn K., Peter P.J., Prevention of acute acidosis and enhancement of feed intake in the bovine by *Megasphaera elsdenii* 407 A, J. Anim. Sci. 70 (Suppl. 1) (1992) 310.

[16] Rossi F., Cocconcelli P.S., Masoero F., Effect of a *Saccharomyces cerevisiae* culture on growth and lactate utilization by the ruminal bacterium *Megasphaera elsdenii*, Ann. Zootech. 44 (1995) 403–409.

[17] Statistical Analysis System Institute, SAS/STAT user's guide, Version 6, Vol. 1. SAS Institute Inc., Cary, NC, USA, 1988.

[18] Umagat H., Kucera P., Wen L.F., Total amino acid analysis using pre-column fluorescence derivatization, J. Chromatogr. 239 (1982) 463–474.

[19] Waldrip H.M., Martin S.A., Effects of an *Aspergillus oryzae* fermentation extract and other factors on lactate utilization by the ruminal bacterium *Megasphaera elsdenii*, J. Anim. Sci. 71 (1993) 2770–2776.

[20] Wallace R.J., Catabolism of amino acids by *Megasphaera elsdenii* LC1, Appl. Environ. Microbiol. 51 (1986) 1141–1143.

[21] Williams P.E.V., Tait C.A.G., Innes G.M., Newbold C.J., Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers, J. Anim. Sci. 69 (1991) 3016–3026.

[22] Wiryawan K.G., Brooker J.D., Probiotic control of lactate accumulation in acutely grain-fed sheep, Aust. J. Agric. Res. 46 (1995) 1555–1568.

[23] Zhao Q.Y., Piot J.M., Gautier V., Cottenceau G., Isolation and characterization of a bacterial growth-stimulating peptide from a peptic bovine hemoglobin hydrolisate, Appl. Microbiol. Biotechnol. 45 (1996) 778–784.

To access this journal online:
www.edpsciences.org