J. Microbiol. Biotechnol. (2008), **18**(1), 124–127

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Assimilation of Peptides and Amino Acids and Dissimilation of Lactate During Submerged Pure Cultures of *Penicillium camembertii* and *Geotrichum candidum*

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Received: August 3, 2006 / Accepted: September 23, 2006

The behavior of *Penicillium camembertii* and *Geotrichum candidum* growing in submerged pure cultures on simple (glutamate) or complex (peptones) substrates as nitrogen and carbon sources and lactate as a second carbon source was examined. Similar to the behavior previously recorded on a simple substrate (glutamate), a clear differentiation between the carbon source and the energy source was also shown on peptones and lactate during *P. camembertii* growth, since throughout growth, lactate was only dissimilated, *viz.*, used for energy supply by oxidation into CO_2, whereas peptides and amino acids from peptones were used for carbon (and nitrogen) assimilation. Because of its deaminating activity, *G. candidum* preferred peptides and amino acids to lactate as energy sources, in addition to being assimilated as carbon and nitrogen sources. From this, on peptones and lactate, *G. candidum* grew faster than *P. camembertii* (0.19 and 0.08 g/l/h, respectively) by assimilating the most readily utilizable peptides and amino acids; however, owing to its lower proteolytic activity, the maximum biomass was lower than that of *P. camembertii* (3.7 and 5.5 g/l, respectively), for which continuous proteolysis and assimilation of peptides were shown.

Keywords: Carbon sources, energy supply, *Geotrichum candidum*, *Penicillium camembertii*, nitrogen sources, pure cultures

Growth of the mold *Penicillium camembertii* and the yeast *Geotrichum candidum* at the surface of white soft cheeses induces the neutralization of the curd [11–13], leading to texture and flavor development.

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The catabolism of amino acids plays an important role during ripening, since they are involved in medium alkalinization through ammonia production [11, 15] and in the production of many flavor compounds [6, 14]. A screening of the behavior of each microorganism during growth on a single amino acid (and lactate) showed that a lower number of amino acids was convenient carbon sources for *P. camembertii*, if compared with *G. candidum* [19]. Subsequent work on a single amino acid, glutamate, shown to be a convenient carbon and nitrogen source amino acids for both species, and lactate showed that *P. camembertii* assimilated lactate throughout growth [2], whereas *G. candidum* assimilated lactate only at the end of culture for energy supply for cell maintenance [4].

To roughly simulate the composition of the aqueous phase of a Camembert cheese [5], growth of *G. candidum* and *P. camembertii* on peptones and lactate was previously examined [1]. However, the behavior recorded during *G. candidum* growth concerning carbon assimilation for cellular biosynthesis, and especially carbon dissimilation, *viz.*, used for energy supply, was not previously examined in detail and was therefore one of the main objectives of the paper. Owing to the absence of mass transfer limitations [3] and the shorter incubation time [8, 16], submerged cultures were considered in this work.

A clear differentiation between the carbon source and the energy source was shown during *P. camembertii* growth on glutamate and lactate [2]; namely, glutamate was used as a carbon (and a nitrogen) source for biosynthesis, whereas lactate was assimilated for energy supply, as deduced from carbon and nitrogen yield examinations. The behavior recorded on more complex substrates, like peptones, concerning carbon and energy sources was not previously examined in detail [1], and is therefore also discussed in this paper.

MATERIALS AND METHODS

Microorganisms

The commercial strains *Penicillium camembertii* LV2 and *Geotrichum candidum* Geo 17 (Danisco, Dangé St. Romain, France) were used.

Media

The synthetic media used in this work contained the same base compoments:

- Ten g/l of sodium L (+)-lactate (Prolabo, Paris, France).
- Inorganic phosphates (Pi): 25 mM of KH_2PO_4 and 25 mM of $NaH_2PO_4{\cdot}H_2O$ [18].
- A solution of EDTA (585 mg/l) chelated trace elements (mg/l) [21]: Mg, 25; Fe, 20; Ca, 18; Zn, 4.5; Mo, 2; Cu, 1.3.

In addition, the GL medium contained 14 g/l glutamic acid (Acros Organics, NJ, U.S.A.), and the PL medium contained 5 g/l of tryptic casein peptones and 5 g/l of pancreatic casein peptones (Biokar, Pantin, France).

Before sterilization at 121°C for 20 min, the pH of the media were then adjusted to 4.6, with 10 M NaOH for the glutamate-lactate medium and with 6 M HCl for the peptones-lactate medium.

Culture Conditions

Batch fermentations were carried out in a 3-l laboratory-made glass-blown bioreactor filled with 2 l of synthetic culture medium (the bioreactor with the medium was sterilized at 121°C during 20 min), or in a 700-ml sterilized (121°C during 20 min) laboratory-made glass-blown bioreactor filled with 300 ml of culture medium. More details on the bioreactor equipment can be found in previous papers [1, 4].

Spores were added to 10 ml of sterile juice. The product of the turbidity at 650 nm and the inoculum volume (A_{650}*V) was kept constant at values of 13 and 100 for the inocula of the 700-ml and the 3-l bioreactors, respectively; the number of spores was adjusted to achieve the considered value for the product A_{650}*V.

Analyses

The glutamic acid concentration corresponding to its primary α-amino group was measured by the TNBS method [20].

Ammonium concentration was determined spectrophotometrically by the Nessler method. Total nitrogen was determined in a 10401 digestion unit (Bioblock, Illkirch, France), after mineralization in a mixture of concentrated sulfuric acid and hydrogen peroxide, followed by a colorimetric measurement of the formed ammonium using the Nessler method. The peptones concentration was obtained by subtracting the ammonium concentration from the total nitrogen concentration [1].

Lactic acid was determined enzymatically: it was first oxidized in pyruvate in the presence of lactate oxidase (LOD), and the produced hydrogen peroxide was measured after reaction with ABTS (AZINO-bis[3-ethylbenzthiazoline-6-sulfonic acid]) in the presence of peroxidase (POD) (all from Sigma Diagnostics, St. Quentin Fallavier, France).

Calculus

Carbon and nitrogen yields corresponded to the slope of the linear fit of the corresponding graph carbon from the released product versus carbon from the consumed substrate.

For rate calculations, to obtain reliable derivatives from the raw data, the corresponding time-courses were fitted using the following logistic function before time-differentiation:

$$y=\frac{y_0-y_f}{1+\left(\frac{t}{k_1}\right)^{k_2}}+y_f \qquad (1)$$

where y is the consumption or production of the considered parameter, y_0 and y_f are its initial and final values, respectively, and k_1 and k_2 are coefficients.

RESULTS AND DISCUSSION

Yields of carbon from biomass on carbon from peptones $Y_{C_X C_P}$ on the one hand, and carbon from CO_2 on carbon from lactate $Y_{C_{CO_2} C_S}$ on the other hand, during *P. camembertii* growth on peptones and lactate based medium [1] were close to unity (1.07±0.04), as shown in Fig. 1. Throughout growth, peptones were therefore assimilated as carbon (and nitrogen) substrate for cellular biosynthesis, whereas lactate was dissimilated (*i.e.*, used for energy supply by oxidation into CO_2). This result shows a clear differentiation between the carbon source and the energy source, as also previously shown on glutamate and lactate [2]. A direct assimilation of peptides, mediated by peptides permease [9], has to be considered to account for the similar behavior recorded on peptones and on a convenient carbon (and nitrogen) source amino acid, like glutamate.

Contrarily, on both media used, GL or PL, *G. candidum* used peptides and amino acids instead of lactate for both assimilation and dissimilation [1, 4], at least until the end of growth, as shown during growth on peptones and lactate (Fig. 2A). It resulted in an increase of the pH (Fig. 2A),

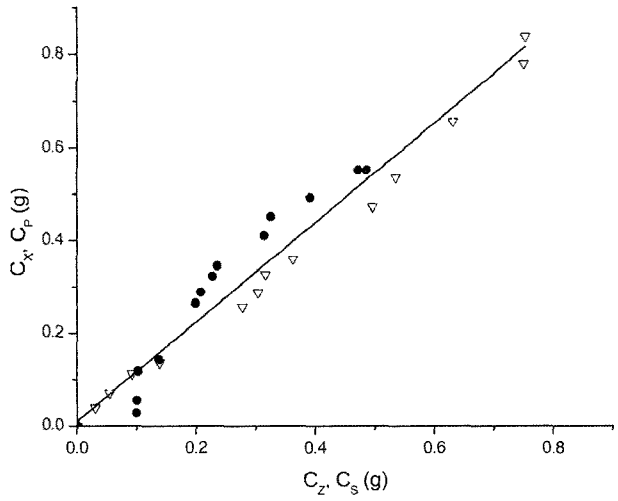

Fig. 1. Yield of carbon from biomass on carbon from peptones consumed $Y_{C_X C_P}$ (●) and yield of carbon from CO_2 released on carbon from lactate consumed $Y_{C_{CO_2} C_S}$ (▽) for *Penicillium camembertii* growing on peptones and lactate based medium; (—) linear fit.

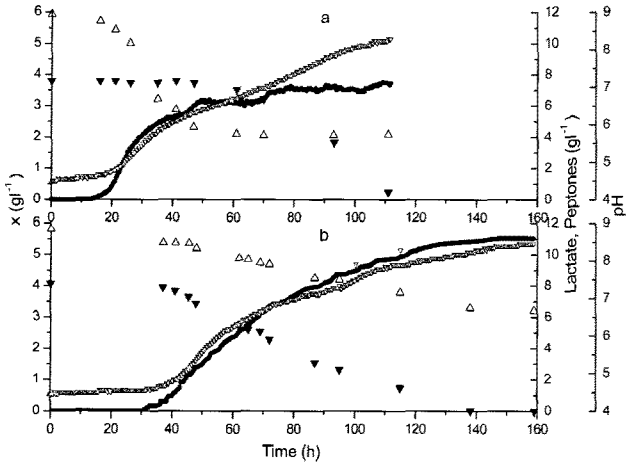

Fig. 2. Culture data of *Geotrichum candidum* (a) and *Pencillium camembertii* (b) growing on peptones and lactate based medium. On-line monitored growth (●) and pH (∨); off-line monitored peptones (∧) and lactate (▼) concentrations.

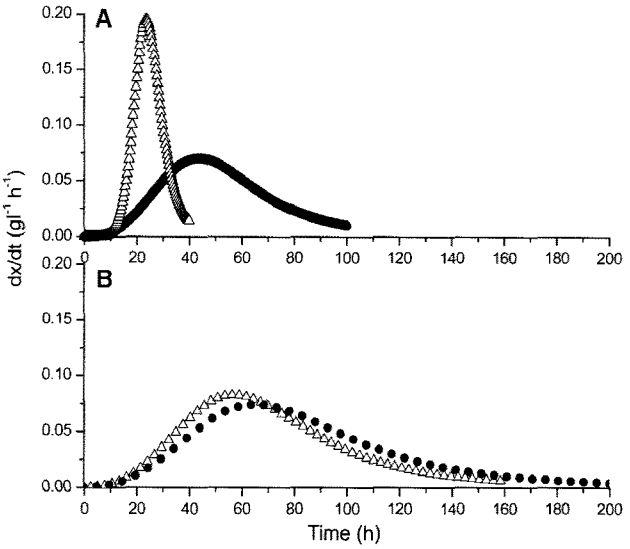

Fig. 3. Growth rates during *Geotrichum candidum* (**A**) and *Pencillium camembertii* (**B**) cultivation on peptones-lactate (△) and on glutamate-lactate (●) based media.

due to ammonium release during their metabolization as C and N sources, owing to the excess nitrogen in relation to their carbon content for fungi [10]. Lactate was only dissimilated for energy supply for cell maintenance during the stationary state (Fig. 2A), leading to an alkalinization that continued during the stationary state (Fig. 2A).

A deaminating activity on glutamic and aspartic acids as well as on leucine, phenylalanine, and methionine was previously reported for *G. candidum* [13]. Oxidative deamination is ensured by oxidoreductases and leads to α-keto acids production [6]. In the case of glutamic acid, deamination resulted in α-ketoglutaric acid, which can be directly fed into the tricarboxylic acid cycle to generate energy [10]. From this, its dissimilation for energy supply, in addition to its assimilation for cellular biosynthesis, was not surprising [4].

On peptones-lactate based medium, various peptides and amino acids were available for *G. candidum* and *P. camembertii* growth. Contrarily, during growth on GL medium, the two microorganisms have to produce the other amino acids by transamination reactions [10], leading to lower growth rates, as shown in Fig. 3.

The higher maximum growth rates recorded on peptones and lactate during *G. candidum* growth compared with *P. camembertii* growth, 0.19 and 0.08 g/l/h, respectively (Fig. 3), have to be related to the sooner colonization of the medium by *G. candidum* during cheese ripening [18]. However, since the proteolytic activity of *G. candidum* was clearly lower than that of *P. camembertii* [17], a decrease of the growth rate was observed earlier during *G. candidum* culture, after exhaustion of the more readily utilizable peptides and amino acids, until the stationary state was achieved after less than 80 h of growth (Fig. 2A). Contrarily, during *P. camembertii* culture, the decrease of the growth rate was less pronounced, and growth continued until one major substrate became limiting after about 160 h of culture (Fig. 2B) [1], in agreement with the continuous proteolysis and assimilation of peptides shown on cheese juice [7], and maximum biomass concentration was therefore higher of 5.5 g/l (3.7 g/l for *G. candidum*) (Fig. 2).

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