Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 29, No. 02, pp. 203 - 210, April - June, 2012

DIAUXIC GROWTH OF *Geotrichum candidum* AND *Penicillium camembertii* ON AMINO ACIDS AND GLUCOSE

M. Aziza[1] and A. Amrane[2,3*]

[1]Centre de Développement des Energies Renouvelables, B.P. 62, Route de l'Observatoire,
Village Céleste, Bouzaréah, Algiers, Algeria.
[2]Ecole Nationale Supérieure de Chimie de Rennes, Université de Rennes 1,
Phone: (+33) 2 23 23 81 55, Fax: (+33) 2 23 23 81 20, CNRS, UMR 6226,
Avenue du Général Leclerc, CS 50837, 35708 Rennes Cedex 7, France.
E-mail: abdeltif.amrane@univ-rennes1.fr
[3]Université Européenne de Bretagne, 5 Boulevard Laënnec, 35000 Rennes, France.

(Submitted: August 9, 2010 ; Revised: October 24, 2011 ; Accepted: October 31, 2011)

Abstract - The purpose of this work was to examine physiological differences between the yeast *Geotrichum candidum* and the mould *Penicillium camembertii*, organisms involved in the industrial process of cheese ripening. Three groups of amino acids had previously been characterized, based on their carbon assimilation and dissimilation by the two fungal species. For both of them, a diauxic growth phase had been shown for a group of amino acids, which however had not been examined in light of physiological differences between the two microorganisms. In this work, the higher level of enzymatic activities of *P. camembertii* if compared to *G. candidum* was confirmed since a continuous and sequential use of both carbon substrates, glucose and arginine, was recorded during *P. camembertii* culture; while after glucose depletion, a clear stationary phase was recorded before the assimilation of the considered amino acid as both carbon and nitrogen sources by *G. candidum*. This behaviour was confirmed for the three amino acids tested, *i.e.*, arginine, proline and glutamic acid. Contrarily, during the two growth phases, on glucose and the test amino acid, respectively, higher growth rates were recorded for *G. candidum* compared to *P. camembertii*, showing higher substrate utilisation efficiency by *G. candidum*. Improving the knowledge regarding the metabolization of amino acids might be helpful in designing strategies aiming at improving processes such as cheese ripening. The work should be followed up by similar works using small peptides.
Keywords: *Geotrichum candidum*; *Penicillium camembertii*; Diauxic growth; Amino acids; Carbon substrates; Nitrogen substrates.

INTRODUCTION

Diauxie describes the growth phases of a microorganism as it metabolizes a mixture of substrates, generally sugars. During the first phase, cells preferentially metabolize the substrate whose catabolism is most efficient. Only after exhaustion of the first substrate do the cells switch to the second, owing to the catabolic repression by the first substrate of the key enzyme responsible for the degradation of the second carbon substrate. A lag period is often recorded between the two growth phases, owing to the time required to produce the enzyme needed to metabolize the second substrate.

Since the discovery of the phenomenon of diauxie by Monod in 1942, numerous works dealing with diauxic growth of numerous microorganisms (Egli, 1995; Jones and Kompala, 1999; Ramkrishna *et al*., 1987) on various substrates and the sequential utilization of carbon substrates are available: a

*To whom correspondence should be addressed

mixture of sugars (Basak *et al*., 1995; Kremling *et al*., 2001; Wong *et al*., 1995), a mixture of hydrocarbons (Maachi *et al*., 2001), sugar and alcohol (Jones and Kompala, 1999) or organic acids (De Figuero *et al*., 1996). On the other hand, only a few works have dealt with the sequential use of carbon sources involving amino acids. They were shown to be assimilated as both carbon and nitrogen sources only after lactic acid exhaustion during *Brevibacterium linens* growth (Moreau *et al*., 1998), or after glucose exhaustion, as recently shown during *Penicillium camembertii* (Adour *et al*., 2006; Adour *et al*., 2005) or *Geotrichum candidum* (Amrane *et al*., 2003; Aziza and Amrane, 2007) growth.

The mould *P. camembertii* and the yeast *G. candidum* (Amrane and Prigent, 1997; Fox *et al*., 1993) are the two principal fungal populations responsible of changes occurring in the curd during white soft cheese ripening, which is a complex process (Hynes *et al*., 2002). Both fungi are involved in texture and flavour development through lactic acid catabolism and ammonia release during metabolism of amino acids, leading to curd alkalinization (Engel *et al*., 2001; Fox *et al*., 1990; Greenberg and Ledford, 1979). Consequently, a deeper understanding of fungal physiology and fermentation behaviour, especially concerning the metabolic behaviour of amino acids, may be helpful for the comprehension of the process of cheese ripening and is complementary to the knowledge concerning proteolytic activities of these microorganisms (Bockelmann, 2001; Gripon, 1997).

Some works were previously carried out on amino acids, peptides or cheese juice and lactate, an available substrate for the secondary cheese ripening micro-flora. Some similarity was found between the behaviours recorded for *G. candidum* and *P. camembertii* growing in pure cultures. Indeed, peptides and amino acids from cheese juice (Aziza *et al*., 2005), peptones (Adour *et al*., 2002) or glutamate (Aziza and Amrane, 2006) were in all cases used by both microorganisms as carbon sources, in addition to being used as nitrogen sources. Contrarily, the second carbon source, lactate, was in all cases only dissimilated by *P. camembertii* for energy supply, while *G. candidum* did not use lactate during growth, but only for energy supply during the stationary phase. For a better characterization of amino acid catabolism, lactate was therefore replaced by another primary carbon source, glucose, even if a system containing glucose is not relevant in terms of food microbiology.

The corresponding work allowed the characterization of three groups of amino acids based on their carbon assimilation and dissimilation by *G. candidum* (Aziza and Amrane, 2007) and *P. camembertii* (Adour *et al*., 2006) in the presence of glucose as the limiting substrate. Physiological differences between both fungi can be noted. Indeed, an energy-saving response was shown by *G. candidum*, since in all cases the organism used glucose as carbon and energy source, while a possible use of the considered amino acid as carbon (and energy) source was only recorded after glucose depletion (Aziza and Amrane, 2007). Contrarily, a simultaneous use of amino acid and glucose for carbon assimilation and dissimilation was found for *P. camembertii*, leading to a clear differentiation between the carbon and energy sources (Adour *et al*., 2006).

In addition, for both fungi, a group of amino acids was characterized based on the sequential use of carbon substrates observed in presence of glucose (Adour *et al*., 2006; Aziza and Amrane, 2007). However, the diauxic growth observed was not examined, in light of physiological differences between the two microorganisms. This is the purpose of this work.

MATERIALS AND METHODS

Microorganisms

Commercial strains of *Geotrichum candidum* Geo17 and *Penicillium camembertii* LV2 (Danisco, Dangé St Romain, France) were used. Freeze-dried spores were stored at +7°C. In order to obtain reliable time lags, before inoculation spores were left to germinate approximately 1 h at 25°C in sterile culture medium. Spore viability was periodically controlled; the number of viable spores was determined by counting the Colony Forming Units that appeared after inoculation with successive decimal dilutions, in triplicate, in pour plates of Yeast-Malt extract agar medium (Difco, Detroit, MI, USA), reconstituted at 38 g L^{-1} and incubated for 3 days at 20°C.

Culture Medium

The medium used throughout this work contained:

- Glucose: 4 g L^{-1} (Merck, Darmstadt, Germany).
- The considered amino acid, arginine, 4 and 8 g L^{-1} during *P. camembertii* and *G. candidum* cultures, respectively, or proline, 12 g L^{-1}, or glutamic acid, 9 g L^{-1} (Acros Organics, New Jersey, USA).
- Inorganics phosphate (Pi): 25 mmol L^{-1} of KH_2PO_4, and 25 mmol L^{-1} of $NaH_2PO_4.H_2O$ (Trinci, 1969).
- A solution of EDTA (ethyleneeiaminetetraacetate) (585 mg L^{-1}) chelated trace elements (mg L^{-1}): (Trinci, 1969) Mg, 25; Fe, 20; Ca, 18; Zn, 4.5; Mo, 2; Cu, 1.3.

The pH of the medium was adjusted to 4.6 with 6 mol L^{-1} HCl, before it was sterilised at 121°C for 20 min.

Culture Conditions

Batch cultures were carried out in a 3 L laboratory-made glass-blown bioreactor (Amrane and Prigent, 1998), which was filled with 2 L of culture medium. The fermentor equipments and the culture medium were sterilized at 121°C for 20 minutes using a 90 L autoclave (Subtil-Crépieux, Chassieu, France).

Cultures were carried out at 25°C and the broth was magnetically stirred at 850 rev min^{-1}. The batch bioreactor was continuously aerated with a constant airflow of 13.0 L h^{-1} (6.5 L of air/L of medium/hour). Inoculation of the culture medium was carried out by an aseptic addition of spore suspension (corresponding to an initial density of 2-3 x 10^8 spores mL^{-1}). The product of the turbidity at 650 nm and the inoculum volume (A_{650}*V) was kept constant at a value of 13 for inocula; the number of spores was adjusted to achieve the considered value for the product A_{650}*V. For spore hydration, the suspension was left approximately 1 h in sterilized culture medium at room temperature before inoculation.

The bioreactor was equipped with a sterilizable combination pH glass electrode (Ingold, Paris, France). The system also contained an aseptic recirculation loop involving a laboratory-made turbidimeter (Amrane and Prigent, 1998). Turbidity was calibrated from dry weight measurement of biomass at the end of culture. The total biomass and broth turbidity were linearly correlated up to a concentration of 5 g L^{-1} (correlation coefficient of 0.98 determined in duplicate experiments) (Amrane and Prigent, 1998). Carbon dioxide in the off-gas was also monitored on-line by a Rubis 3000 IR detector (Cosma, Igny, France) after desiccation in a column of calcium chloride.

Analyses

After centrifugation of the samples (3000 g for 20 minutes), glucose, ammonium and test amino acid concentrations were determined in the supernatants. Glucose was enzymatically determined; it was first oxidized to gluconic acid in the presence of glucose oxidase (GOD), and the produced hydrogen peroxide was measured after 80 minutes by reaction with ABTS (2,2'-Azino-bis[3-ethylbenzthiazoline-6-sulfonic acid]) in the presence of peroxidase (POD) (all from Sigma Diagnostics, St Quentin Fallavier, France). The test amino acid was determined on a 3% sulfonic acid filtrate using a Pharmacia LKB-Alpha Plus series 2 amino acid analyser (Pharmacia Biotech., Saclay, France) (Aziza *et al*., 2005). Ammonium was determined using a Dionex DX-500 high performance liquid chromatographic system (Dionex, F-78354 Jouy-en-Josas, France), with a cation exchange column (CS15 IonPac column, 250 x 4 mm) as described by Gaucheron and co-workers (Gaucheron *et al*., 1996). The observed standard deviations, deduced from triplicate measurements, were 0.3 g L^{-1}, 0.02 g L^{-1} and 4 mg L^{-1} for glucose, test amino acid and ammonium concentrations, respectively.

RESULTS

The longer lag phase recorded during *P. camembertii* culture (Figure 1a), compared to *G. candidum* culture (Figure 1a), was in agreement with previous results (Adour *et al*., 2002; Amrane *et al*., 1999). It should also be related to the earlier colonization of the surface of white soft cheese by *G. candidum*, which develops from the first days of ripening, while *P. camembertii* growth starts later, during the second week of ripening (Aziza and Amrane, 2006; Boutrou *et al*., 2006). Indeed, previous experiments on Camembert juice showed that *G. candidum* immediately degraded large and medium peptides into free amino acids, while *P. camembertii* assimilated large peptides, but medium and small peptides were less consumed (Boutrou *et al*., 2006).

As shown in Figure 1, for both species, a first phase of growth was observed corresponding to the assimilation of glucose and the considered amino acid as carbon and nitrogen substrates, respectively. This trend was confirmed for other amino acids from the same previously characterized group, proline (Figure 2) and glutamic acid (Figure 3), namely amino acids which can be assimilated as carbon sources, in addition to being used as nitrogen sources, by *G. candidum* (Aziza and Amrane, 2007). The absence of ammonium production during this first phase of growth (Figure 1f) confirmed the use of the amino acid only as a nitrogen source, but not as a carbon source (Aldarf *et al*., 2002). Indeed, ammonium release corresponded to the excess nitrogen from amino acids in relation to their carbon content for fungi (Deacon, 1997). Medium acidification (Figure 1c) was recorded as early as the lag phase of growth (Figure 1a), most likely due to an assimilation of arginine in exchange of protons, in agreement with previous results (Amrane *et al*., 2003; Adour *et al*., 2004). Indeed, the initial culture pH was 4.6, namely lower than the isoelectric pH of arginine (10.76), which was thus positively charged. Glucose was assimilated until its exhaustion, observed after nearly 50 and 100 h during *G. candidum* and *P. camembertii* (Figure 1d) cultures, respectively. Growth rates were higher

during *G. candidum* growth if compared to *P. camembertii* growth (Figure 1a), 0.10 and 0.05 g L^{-1} h^{-1} for maximum growth rates and 0.38 and 0.24 h^{-1} for specific growth rates for *G. candidum* and *P. camembertii* respectively, deduced from growth time-courses (Figure 1a), and in agreement with previous results (Adour *et al*., 2002). Maxima for CO_2 production (Figure 1b) were recorded almost simultaneously with the maxima recorded for growth rates, in agreement with previous results (Adour *et al*., 2004; Aziza and Amrane, 2007). During *P. camembertii* culture, growth continued (Figure 1a) after glucose exhaustion (Figure 1d), at the expense of the sole available carbon and nitrogen source remaining, arginine (Figure.1e), leading to lower growth rates than those recorded on a primary carbon source like glucose. Assimilation of arginine as a carbon source, in addition to being used as a nitrogen source, was illustrated by the production of ammonium (Figure 1f), which corresponded to the excess nitrogen from amino acids in relation to their carbon content for fungi (Deacon, 1997), leading to a rise in pH (Figure 1c). A decline in the growth phase was observed at the end of culture (Figure 1a), owing to the depletion of arginine (Figure 1e), resulting in cessation of ammonium production (Figure 1f). Contrarily, during *G. candidum* culture, glucose exhaustion from the medium resulted in a stationary phase from roughly 50 to 120 h of culture (Figure 1a). The stationary phase was illustrated by the low rate of arginine dissimilation as an energy source (Figure 1e). A second phase of growth was then observed from approximately 120 h of culture, illustrated by an increase of the biomass concentration from 1.9 to 3.3 g L^{-1} (Figure 1a), and accompanied by an important concomitant amount of arginine consumed (Figure 1e), leading to the release of the excess nitrogen as ammonium (Figure 1f).

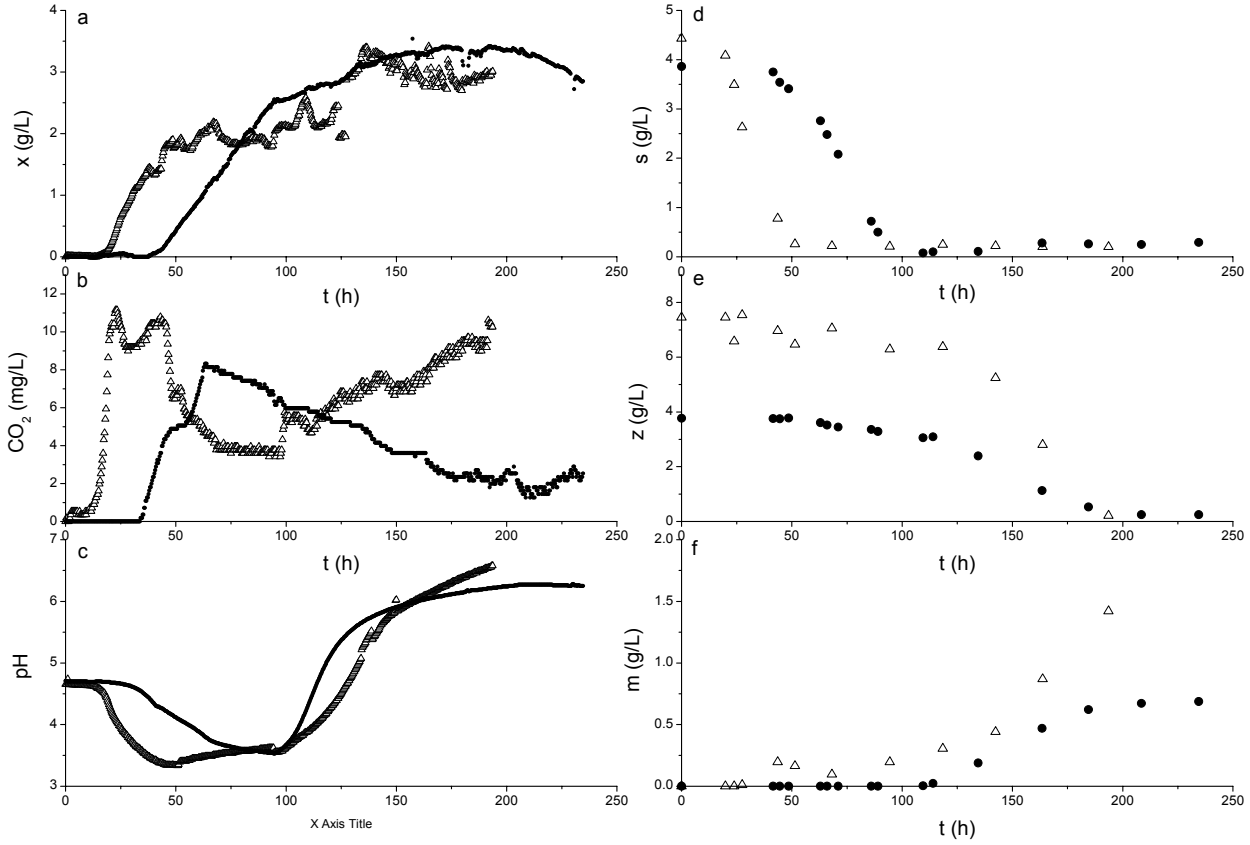

Figure 1: Online biomass concentration *x* (a), CO_2 emission (b) and pH (c) time-courses, as well as offline glucose *s* (d), arginine *z* (e) and ammonium *m* (f) time-courses during culture of *Geotricum candidum* (Δ) and *Penicillium camembertii* (●) on glucose (4 g L^{-1}) and arginine (8 and 4 g L^{-1} during *G. candidum* and *P. camembertii* cultures) based medium.

The trend recorded during *G. candidum* growth was confirmed for proline (Figure 2a) and glutamic acid (Figure 3). Indeed, a stationary phase followed glucose exhaustion from the medium, recorded from roughly 75 to 175 h for proline (Figure 2a) and from 70 to 130 h for glutamic acid (Figure 3a). It was then followed by a second growth phase, corresponding to an increase of the biomass concentration from roughly 5.0 to 6.5 g L^{-1} and 3.5 to 5.0 g L^{-1} during growth on proline (Figure 2a) and glutamic acid (Figure 3a), respectively; and illustrated by the important concomitant consumption of proline (Figure 2b) and glutamic acid (Figure 3b), leading to a high production of ammonium (Figs. 2b and 3b), and hence a medium alkalinisation (Deacon 1997) during growth on proline (Figure 2a) and glutamic acid (Figure 3a) (Deacon, 1997). It should be noted, however, that during growth on glutamic acid, a pH increase was recorded from the beginning of growth (Figure 3a), namely during the first phase of growth corresponding to amino acid assimilation only as a nitrogen source, with glucose being the carbon and energy source. This was the consequence of its negative charge from the beginning of culture (isoelectric pH 3.22), contrary to arginine (pHi = 10.76) and proline (6.3).

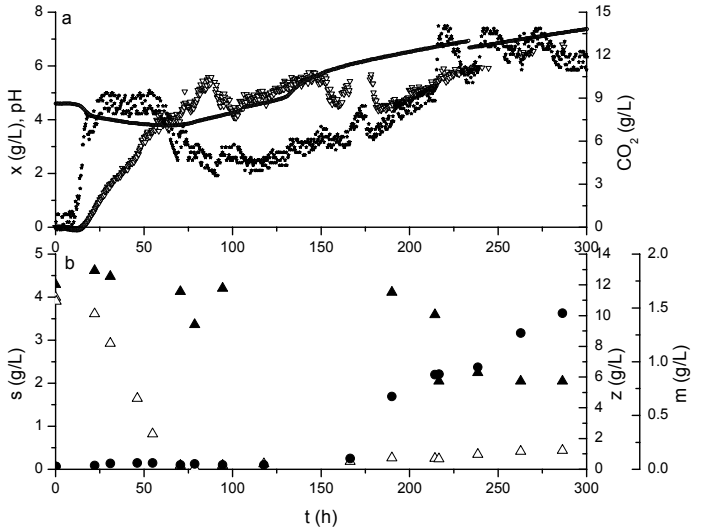

Figure 2: Online parameter monitoring (a): biomass concentration *x* (▽), CO_2 emission (☆) and pH (❍) time-courses, as well as offline parameter monitoring (b): glucose *s* (Δ), arginine *z* (▲) and ammonium *m* (●) time-courses, during culture of *Geotricum candidum* on glucose (4 g L^{-1}) and proline (12 g L^{-1}) based medium.

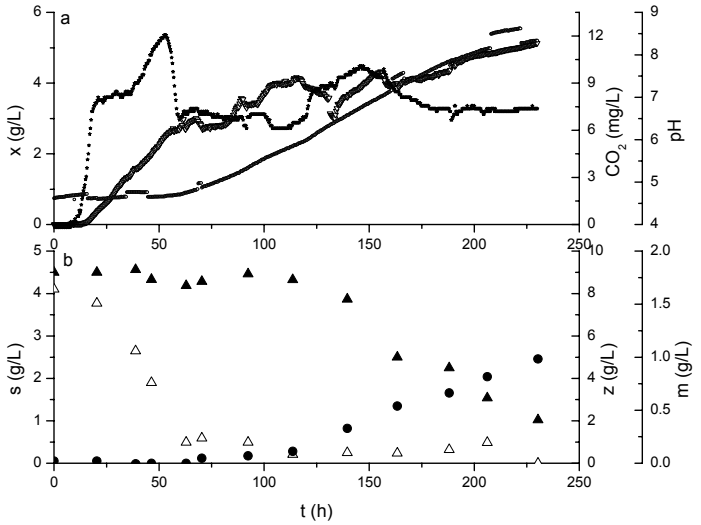

Figure 3: Online parameter monitoring (a): biomass concentration *x* (▽), CO_2 emission (☆) and pH (❍) time-courses, as well as offline parameter monitoring (b): glucose *s* (Δ), arginine z (▲) and ammonium *m* (●) time-courses, during culture of *Geotricum candidum* on glucose (4 g L^{-1}) and glutamic acid (9 g L^{-1}) based medium.

DISCUSSION

Both species exhibited diauxic behaviour for the amino acids considered, in agreement with previous results (Aziza *et al*., 2004; Aziza and Amrane, 2007). Three groups of amino acids were previously characterized given their ability to be assimilated as a carbon source by *P. camembertii*. Amino acids from the first group (Cys, His, Lys, Met, Trp and Val) are convenient nitrogen sources, but cannot be assimilated as carbon sources. However, they are also dissimilated, namely used for energy supply by oxidation into CO_2, during the stationary phase, after glucose depletion. Growth exhibited diauxic behaviour for the second group of amino acids (Arg, Leu), since they can be assimilated as carbon sources, in addition to their assimilation as nitrogen sources, but only after glucose depletion. A clear differentiation between the assimilated and the dissimilated carbon was demonstrated for the third group of amino acids (Ala, Asp, Glu, Gly, Pro, Ser, Thr); it was shown that the carbon from glutamic acid was assimilated, while the carbon from glucose was dissimilated (Adour *et al*. 2006). Arginine belonged to the intermediate group, namely used as a carbon source only after glucose depletion (Aziza *et al*., 2004). Contrarily, during *G. candidum* growth, amino acids can be assimilated as carbon sources (in addition to being used as nitrogen sources) only after glucose depletion at best (Aziza and Amrane, 2007), illustrating glucose repression (Ronne, 1995). Moreover, a clear stationary phase was recorded before a second *G. candidum* growth phase took place, using the considered amino acid as both carbon and nitrogen sources, as shown for arginine (Figure 1), proline (Figure 2) and glutamic acid (Figure 3). The stationary phase corresponded to the time needed for the activation of the enzymatic complex required to assimilate the considered amino acid, which was subsequently used as a carbon source for cytoskeleton synthesis (Deacon, 1997; Jones and Kompala, 1999).

Contrarily, no significant time was needed for the activation of the enzymatic complex required for the use of arginine as carbon source by *P. camembertii*, since no stationary phase was observed between both growth phases during culture on glucose and arginine (Figure 1b). This result could be related to the possible simultaneous use of amino acid and glucose for carbon assimilation and dissimilation by *P. camembertii*, contrary to *G. candidum* (Aziza and Amrane, 2007). Indeed, a glucose repression (Ronne 1995) has to be considered to account for the behaviour recorded during *G. candidum* growth on glucose and a single amino acid from the second or the third groups; the amino acid under consideration was used for carbon assimilation and dissimilation only after glucose depletion (Aziza and Amrane 2007). This result has also to be related to the clear differentiation between the carbon source and the energy source when *P. camembertii* was cultivated on a convenient carbon (and nitrogen) source such as peptides and/or amino acids (used for carbon assimilation) and an extra carbon source, like lactate (Adour *et al*., 2004; Adour *et al*., 2002) or glucose (Adour *et al*., 2006), used for carbon dissimilation.

During the two sequential growth phases, on glucose and on arginine, higher growth rates were recorded during *G. candidum* culture (Figure 1a), compared to *P. camembertii* culture (Figure 1a), in agreement with previous results (Adour *et al*., 2002; Aldarf *et al*., 2002) and with the quicker colonization of the medium by *G. candidum* during cheese ripening (Molimard *et al*., 1995). The higher growth rates recorded during *G. candidum* growth on arginine, compared to *P. camembertii*, has to be related to the deaminating activity on some amino acids previously reported for *G. candidum* (Greenberg and Ledford, 1979). Improving the knowledge concerning the metabolization of amino acids is not directly related to cheese ripening, but may be helpful owing to the lack of related physiological studies and should be subsequently followed by similar works on small peptides.

CONCLUSION

G. candidum and *P. camembertii* species exhibited diauxic behaviour when growing on some amino acids and glucose. However, no stationary phase, namely a period of time with constant cell concentration, was observed between the two growth phases during *P. camembertii* culture, in agreement with the continuous proteolysis and assimilation of peptides by *P. camembertii* (Boutrou *et al*., 2006); in contrast, a clear glucose repression was observed during *G. candidum* growth on glucose and a single amino acid, shown to be an energy-saving response (Ronne, 1995), leading to a stationary phase after glucose exhaustion from the medium and before the second growth phase with the considered amino acid as both carbon and nitrogen source. Even if glucose is not a relevant carbon source in terms of food microbiology, a clear physiological difference was highlighted regarding the assimilation of some amino acids by the two fungi, which are involved in association in the ripening of soft Camembert cheeses.

NOMENCLATURE

m	ammonium concentration	g L^{-1}
s	carbon substrate (glucose) concentration	g L^{-1}
x	biomass concentration	g L^{-1}
z	amino acid (arginine or proline) concentration	g L^{-1}
t	time	h

REFERENCES

Adour, L., Aziza, M., Couriol, C. and Amrane, A., Amino acids as carbon, energy and nitrogen sources for *Penicillium camembertii*. J. Chem. Technol. Biotechnol., 81, 573-579 (2006).

Adour, L., Couriol, C. and Amrane, A., Diauxic growth of *Penicillium camembertii* on glucose and arginine. Enzyme Microb. Technol., 36, 198-202 (2005).

Adour, L., Couriol, C. and Amrane, A., The effect of lactate addition on the growth of *Penicillium camembertii* on glutamate. J. Biotechnol., 114, 307-314 (2004).

Adour, L., Couriol, C., Amrane, A. and Prigent, Y., Growth of *Geotrichum candidum* and *Penicillium camemberti* in liquid media in relation with the consumption of carbon and nitrogen sources and the release of ammonia and carbon dioxide. Enzyme Microb. Technol., 31, 533-542 (2002).

Aldarf, M., Amrane, A. and Prigent, Y., Reconstruction of the biomass history from carbon and nitrogen substrate consumption, ammonia release and proton transfer during solid cultures of *Geotrichum candidum* and *Penicillium camembertii*. Appl. Microbiol. Biotechnol., 58, 823-829 (2002).

Amrane, A., Pestel, V. and Prigent, Y., Submerged cultures of *Geotrichum candidum* and *Penicillium camembertii* on amino acids and glucose. J. Gen. Appl. Microbiol., 49, 251-256 (2003).

Amrane, A., Plihon, F. and Prigent, Y., Proton transfer in relation to growth of *Geotrichum candidum* and *Penicillium camembertii* in synthetic liquid media. Enzyme Microb. Technol., 24, 561-568 (1999).

Amrane, A. and Prigent, Y., Comparison of growth parameters for *Geotrichum candidum* Geo 17 and *Penicillium camemberti* LV2 in solid and liquid cultures. Lait, 77, 641-648 (1997).

Amrane, A. and Prigent, Y., A new turbidimetric device for on-line monitoring of growth of filamentous microorganisms. J. Microbiol. Methods, 33, 37-43 (1998).

Aziza, M., Adour, L., Couriol, C. and Amrane, A., Analysis of batch submerged cultivations of *Geotrichum candidum* growing in lactate with either glutamate or lysine. J. Chem. Technol. Biotechnol., 79, 1412-1416 (2004).

Aziza, M. and Amrane, A., Carbon assimilation and dissimilation during growth of *Geotrichum candidum* on amino acids and glucose. J. Chem. Technol. Biotechnol., 82, 796-801 (2007).

Aziza, M. and Amrane, A., Commensalism during submerged mixed culture of *Geotrichum candidum* and *Penicillium camembertii* on glutamate and lactate. Process Biochem., 41, 2452-2457 (2006).

Aziza, M., Couriol, C., Amrane, A. and Boutrou, R., Evidences for synergistic effects of *Geotrichum candidum* on *Penicillium camembertii* growing on cheese juice. Enzyme Microb. Technol., 37, 218-224 (2005).

Basak, S., Velayudhan, A. and Ladish, M. R., Simulation of diauxic production of cephalosporin C by *Cephalosporium acremonium:* lag model for fed-batch fermentation. Biotechnol. Prog., 11, 626-631 (1995).

Bockelmann, W., Secondary cheese cultures. In Technology of cheesemaking, Law, B. A., (Ed.), Academic Press, Sheffield, p. 123 (2001).

Boutrou, R., Aziza, M. and Amrane, A., Enhanced proteolytic activities of *Geotrichum candidum* and *Penicillium camembertii* in mixed culture. Enzyme Microb. Technol., 39, 325-331 (2006).

Deacon, J. W., Modern Mycology. (3rd Ed.) Blackwell Science, Oxford (1997).

De Figuero, R. M., Benito de Cerdenas, I. L., Sesma, L., Alvarez, F., De Ruiz Holgado, A. P. and Oliver, G., Inducible transport of citrate in *Lactobacillus rhamnosus* ATCC 7469. J. Appl. Bacteriol., 81, 348-354 (1996).

Egli, T., The ecological and physiological significance of the growth of heterotrophic microorganisms with mixtures of substrates. Adv. Microb. Ecol., 14, 797-806 (1995).

Engel, E., Tournier, C. and Le Quéré, J. L., Evolution of the composition of a selected bitter Camembert cheese during ripening: release and migration of taste-active compounds. J. Agric. Food Chem., 49, 2940-2947 (2001).

Fox, P. F., Law, J., Mc Sweeney, P. L. H. and Wallace, J., Biochemistry of cheese ripening. In Cheese: Chemistry, Physics and Microbiology, Major cheese groups. Fox, P. F., (Ed.), Chapman & Hall, Glasgow, Vol. 2, p. 389 (1993).

Fox, P. F., Lucey, J. A. and Cogan, T. M., Glycolysis and related reactions during cheese manufacture

and ripening. Crit. Rev. Food Sci. Nutr., 29, 237-253 (1990).

Gaucheron, F., Le Graët, Y., Piot, M. and Boyaval, E., Determination of anions of milk by ion chromatography. Lait, 76, 433-443 (1996).

Greenberg, R. S. and Ledford, L. S., Deamination of glutamic and aspartic acids by *Geotrichum candidum*. J. Dairy Sci., 62, 368-372 (1979).

Gripon, J. C., Flavour and texture in soft cheeses. In Microbiology and Biochemistry of Cheeses and Fermented Milks, Law, B. A., (Ed.), Academic Press, London, p. 95 (1997).

Hynes, E., Ogier, J. C., Lamberet, G. and Delacroix-Buchet, A., The influence of starter and adjunct lactobacilli culture on the ripening of washed curd cheeses. Braz. J. Chem. Eng., 19, 397-402 (2002).

Jones, K. D. and Kompala, D. S., Cybernetic model of the growth dynamics of *Saccharomyces cerevisiae* in batch and continuous cultures. J. Biotechnol., 71, 105-131 (1999).

Kremling, A., Bettenbrock, K., Laube, B., Jahreis, K., Lengeler, J. W. and Gilles, E. D., The organization of metabolic reaction networks. III. Application for diauxic growth on glucose and lactose. Metabolic Eng., 3, 362-379 (2001).

Lenoir, J., The proteasic activity in Camembert type soft cheeses. Rev. Lait. Fr., 275, 231-243 (1970).

Maachi, R., Abousseoud, M. and Chaabane, T., Kinetics of biodegradation of petroleum by *Pseudomonas* sp. Desalination, 139, 367 (2001).

Molimard, P., Bouvier, I., Vassal, L. and Spinnler, H. E., Growth of *Penicillium camemberti* and *Geotrichum candidum* in pure and mixed cultures on experimental mold ripened cheese of Camembert-type. Lait, 75, 3-16 (1995).

Moreau, A., Fillon, M., Truong-Meyer, X. M., Pingaud, H. and Strehaiano, P., Study of lactic acid and amino acid catabolism of *Brevibacterium linens*. Microbiologie, Aliments, Nutrition, 16, 251-258 (1998).

Ramkrishna, D., Kompala, D. S. and Tsao, G. T., Are microbes optimal strategies? Biotechnol. Prog., 3, 121-126 (1987).

Ronne, H., Glucose repression in fungi. Trends Gen., 11, 12-17 (1995).

Trinci, A. P. J., A kinetic study of the growth of *Aspergillus nidulans* and other fungi. J. Gen. Microbiol., 57, 11-24 (1969).

Wong, T. Y., Pei, H., Bancroft, K. and Childers, G. W., Diauxic growth of *Azotobacter vinelandii* on galactose and glucose: regulation of glucose transport by another hexose. Appl. Environ. Microbiol., 61, 430-433 (1995).