Short Communication

Submerged cultures of Geotrichum candidum and Penicillium camemberti on amino acids and glucose

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Growth and pH variations were examined during the culturing of Geotrichum candidum and Penicillium camemberti on glucose and a single amino acid. Sixteen amino acids were tested and a diauxic growth was observed for the majority of the amino acids tested with G. candidum. In the first phase, the glucose and amino acid considered were used as C and N sources respectively, resulting in a medium acidification due to an amino acid/H+ exchange. In a subsequent phase, the amino acid was used for both C and N sources, resulting in ammonium production, which raised the pH. P. camemberti metabolized less amino acids as both C and N sources than G. candidum. However, when this was the case, and in contrast with G. candidum, P. camemberti simultaneously assimilated the glucose and amino acid considered as carbon sources.

The fungi P. camemberti and G. candidum contribute to the ripening of soft Camembert cheeses (Amrane and Prigent, 1997; Gripon, 1993), given that they are partly responsible for the development of texturization and organoleptic characteristics (Karahadian and Lindsay, 1987; Ribadeau-Dumas, 1984). Much attention has been paid to the biochemistry and enzymatic activities of these microorganisms (Cerning et al., 1987; Fox et al., 1993). On the other hand, very little attention has been paid to the growth of both microorganisms.

Some results are available concerning growth of both fungi on solid cultures of casein hydrolysate (tryp tic casein peptone)-based media (Aldarff et al., 2002). Such media are interesting, since being rather close to the real system (lactic curd), however remaining too complex for specific identification of the nutritional mechanisms related to the carbon and nitrogen source assimilation, especially amino acid metabolism.

Cultivations of G. candidum and P. camemberti in synthetic liquid media containing a single amino acid in the presence or absence of lactic acid have shown that the majority of amino acids are convenient N sources, while some of them can also be metabolized as C and energy sources (Pihon et al., 1998). In both cases, the pH of the medium increased during growth due to lactic acid consumption and ammonia release resulting from amino acid deamination (Karahadian and Lindsay, 1987).

Some results are available concerning the growth of G. candidum (Duran et al., 1973) and P. camemberti (Bockelmann et al., 1999) with glucose and ammonia as C and N sources, respectively. In this case, medium acidification has been observed, and is attributed to a NH4+/H+ exchange (Deacon, 1997). However, to our
knowledge, the growth of the two fungi with glucose and amino acid as the C and N sources has not been previously reported.

The examination of growth of *G. candidum* and *P. camemberti* on different free amino acids and glucose as the carbon and energy sources, as well as the corresponding pH variations, may be very informative and is therefore the main purpose of this work.

The strains *G. candidum* Geo17 and *P. camemberti* LV2 (Rhodia Food, Dangé St. Romain, France) were used. Freeze-dried spores were maintained at +7°C. Spore viability was periodically controlled.

A common medium basis was used for both microorganisms (g L\(^{-1}\)): the required amino acid, 10 (Acros, Geel, Belgium); glucose, 10 (Merck, Darmstadt, Germany); KH\(_4\)PO\(_4\), 6.26; Na\(_2\)HPO\(_4\), 2H\(_2\)O, 0.71; and a solution of EDTA (EthyleneDiamineTetraAcetate) (15 g L\(^{-1}\)) chelated trace elements at ×20 the final strength according to Trinci (1969). The salt concentrations were (g L\(^{-1}\)): MgSO\(_4\), 7H\(_2\)O, 5; CaCl\(_2\), 1; ZnSO\(_4\), 7H\(_2\)O, 0.4; MnSO\(_4\), 4H\(_2\)O, 0.4; CuSO\(_4\), 5H\(_2\)O, 0.1; (NH\(_4\))\(_2\)Fe(SO\(_4\))\(_2\), 7H\(_2\)O, 2; and (NH\(_4\))\(_2\)MoO\(_4\), 6H\(_2\)O, 0.073. The pH was adjusted to 4.5 with 1 M HCl or NaOH. The medium was then sterilized at 121°C for 20 min.

Shake cultures were carried out (duplicates) in 500 ml Erlenmeyer flasks containing 50 ml of the medium. The cultures were incubated at 20°C on an orbital shaker (Unimax 2010, Heidolph, Kelheim, Germany) with an agitation speed of 200 rpm. Inoculation was made by an aseptic addition of 1 ml of spore suspension in the same medium (corresponding to a turbidity of 1.2 at 600 nm).

Once or twice a day, samples of broth were harvested for pH measurements. At the end of culturing, total biomass was determined by dry weight measurement after filtration of the whole of the remaining broth on GF/C glass microfiber filters (Whatman, Maidstone, England). Error bars of ±0.5 g L\(^{-1}\) and ±0.1 unit were observed for the final biomass concentration and pH, respectively.

After centrifugation of the sample, glucose was determined spectrophotometrically on the supernatant by the phenol-sulfuric acid method for total sugars (Herbert et al., 1971).

In Fig. 1, the order chosen for the 16 amino acids tested corresponds from left to right to decreasing final biomass concentrations observed for *G. candidum* in the presence of glucose as the carbon and energy source.

As expected, in the presence of glucose, the majority of the tested amino acids were convenient nitrogen sources for both fungi. *G. candidum* growth always gave final biomass concentrations superior to 1 g L\(^{-1}\) except with tryptophan, for which no noticeable biomass was recorded even after 6 days of shake culturing. No noticeable biomass was recorded during *P. camemberti* growth on cysteine and phenylalanine, while a low final biomass was observed with tryptophan. All the other amino acids tested gave final biomass concentrations superior to 1 g L\(^{-1}\) with the presence of glucose in the medium.

A comparison with previous results obtained with lactate as the carbon source (Pihon et al., 1998) shows that, as expected, more amino acids became convenient nitrogen sources when glucose was the carbon source: His, Met, Thr, Phe and Cys for *G. candidum* and Met for *P. camemberti*.
Growth of *G. candidum* and *P. camembertii* was always accompanied by pH variations (Fig. 1). A low medium acidification was observed in case of low growth (*P. camembertii* on tryptophan) or even when no noticeable biomass was observed (*G. candidum* on tryptophan and *P. camembertii* on cysteine). A total absence of pH variation was only noticed during the culturing of *P. camembertii* on phenylalanine.

Acidification was recorded for the majority of the amino acids during *G. candidum* growth and for about half of the amino acids tested as the nitrogen source for *P. camembertii* (Fig. 1). Medium acidification has been previously reported for these fungi growing on glucose and ammonia as C and N sources, respectively (Bockelmann et al., 1999; Duran et al., 1973), and attributed to a NH$_4^+$/H$^+$ exchange (Deacon, 1997). Amino acids are positively charged since the initial culture pH was 4.5, namely lower than the isoelectric pH of amino acids (except both acidic amino acids, glutamic and aspartic acids). Due to this positive charge, it can be assumed that amino acids were taken up in exchange for H$^+$, to maintain cellular electroneutrality. Fungi need only one amino acid, and from this they can produce the other amino acids by transamination reactions (Deacon, 1997).

No acidification was observed during *G. candidum* growth on glutamic acid or aspartic acid as the nitrogen source, due to their negative charge at the initial culture pH (isoelectric pHs are 3.22 and 2.98 for Glu and Asp, respectively). A medium acidification was recorded for all the other amino acids tested. The pH decrease could reach up to 2 pH units for lysine and cysteine, corresponding to a pH slightly higher than 2.5. A pH increase followed this acidification step, which can most likely be attributed to the metabolization of the amino acid as the carbon and energy source as well as the nitrogen source, except for threonine, phenylalanine, histidine, cysteine and tryptophan, which were not therefore convenient carbon and energy sources for *G. candidum*, which is in agreement with previous results (Pilhon et al., 1998). Amino acids contain excess nitrogen in relation to their carbon content for fungi, so ammonium is released during their metabolization as C and N sources and can raise the pH (Deacon, 1997).

Therefore, except for glutamic and aspartic acids, amino acids were first metabolized by *G. candidum* as nitrogen sources and glucose as the carbon and energy source, resulting in medium acidification due to amino acid/proton exchange. Then, when the available glucose was depleted, as shown from glucose determination on the sample taken at the more acidic pH (less than 0.2 g glucose L$^{-1}$ remained in the broth), some of the amino acids tested (Pro, Leu, Val, Met, Arg, Ala, Ser, Lys and Gly) were metabolized as carbon and energy sources as well as nitrogen sources, resulting in a pH increase due to ammonia release (Fig. 1a).

It should be observed that the lowest final biomass concentrations were obtained for the amino acids only metabolizable as N sources (Fig. 1a).

For glutamic and aspartic acids, this diauxic growth, if it occurred, could not be easily pointed out due to the absence of a first step of medium acidification. To show this possible diauxic growth, shake culturing does not seem to be the most convenient method. Indeed, the culture volume was too low to allow a representative number of samples to determine the various consumption kinetics. Moreover, growth kinetics deduced from turbidimetric measurements on samples do not seem representative due to the mycelial growth of both microorganisms. Consequently, more confident conclusions may be deduced from submerged cultures in fermenters, with on-line turbidimetric monitoring of biomass concentration specially adapted to the case of filamentous microorganisms (Amrane and Prigent, 1998). Corresponding work is in progress in our laboratory.

During *P. camembertii* growth on glucose and one of the various amino acids tested (Fig. 1b), the following behavior could be observed:

No growth nor pH variations on phenylalanine.

Only acidification during growth on Val, Met, Lys, His, Cys and Trp since amino acids were taken up in exchange for H$^+$. Glucose was the carbon source, while these amino acids were only used as nitrogen sources and not assimilated as carbon sources. It should be observed that in the absence of an extra carbon source, very weak growth was recorded on these amino acids as both C and N sources (Pilhon et al., 1998).

Diauxic growth on arginine and leucine. In the first phase, glucose and the amino acid considered were used as both C and N sources, respectively, resulting in medium acidification due to an amino acid/H$^+$ exchange. Then, when glucose was depleted, as shown from glucose determination, the amino acid was used as both the C and N.
sources, resulting in an increase of the pH due to the ammonium produced from amino acid deamination.

Only an increase of the pH on Pro, Glu, Ala, Ser, Asp, Gly and Thr. The most probable assumption to account for this behavior being that both the glucose and amino acid considered were simultaneously used as carbon sources, resulting in a pH increase due to the ammonium produced. The final biomass concentrations recorded with this group of amino acids, and the above arginine, were higher than those recorded for the other amino acids (3.8 g L⁻¹ at less, Fig. 1b), and agreed with previous results recorded for a single amino acid in the absence of an extra carbon source (Pilhon et al., 1998).

Therefore, higher final biomass concentrations were recorded during culturing on the C and N source amino acids.

Not as many amino acids can be metabolized as both C and N sources by P. camemberti. However, when this was the case, the enzymatic system for amino acid assimilation as a C source did not have to be induced by glucose depletion (except for Arg and Leu) as was the case for G. candidum, resulting in the simultaneous assimilation of the glucose and amino acid considered as carbon sources.

It has been previously demonstrated that pH variations are linearly correlated with growth; thus, fungal growth may be monitored by pH measurements (Amrane et al., 1999). Therefore, pH has been monitored and displayed for both microorganisms growing on glucose and some representative amino acids in Fig. 2.

As observed, G. candidum diauxic growth started after a 1-day lag phase. During the second day of culturing, the glucose and amino acid considered were then metabolized as C and N sources, respectively. Afterwards, pH increased due to amino acid utilization as both C and N sources until the stationary state was reached after 3–4 days of growth (Fig. 2a).

According to previous results (Amrane and Prigent, 1997), lower growth rates were recorded for P. camemberti, when compared to G. candidum; P. camemberti growth started after a 3-4-day lag phase and ceased at best after 7 days (Fig. 2b).

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References


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