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Generation of non-protein nitrogen and volatile compounds by *Penicillium chrysogenum* Pg222 activity on pork myofibrillar proteins

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Abstract

A non-toxigenic strain of proteolytic *Penicillium chrysogenum* Pg222 isolated from dry-cured ham was tested for its ability to generate non-protein nitrogen (NPN) and volatile compounds from muscle myofibrillar proteins. The activity of mold led to higher accumulation of non-protein and amino acidic nitrogens than control samples. Volatile compound analysis revealed the presence of branched compounds, such as 3-methylbutanal and 3-methylbutanol only in samples inoculated with the mold. Similarly, compounds such as ethanol, propanol and 2-methoxy ethanol were detected only in inoculated samples at all sampling time. 3- and 2-methylpentane, benzoic and acetic acids, 2-butanone and 2-ethylhexanol, pyridine and 3-carene were detected occasionally, but only in the Pg222 batch. The proteolytic activity of *P. chrysogenum* Pg222 lead to accumulation of soluble NPN compounds, in addition to the generation of volatile compounds of great interest for dry-cured meat products. Therefore, this mold could be appropriate to be used as a non-toxigenic starter culture during the ripening of dry-cured meat products to stimulate proteolysis and flavour development.

Keywords: *Penicillium chrysogenum*; Volatile compounds; Non-protein nitrogen; Dry-cured meat products

1. Introduction

Proteolysis is considered to be one of the major processes involved in aroma development in dry-cured meat products (Ordóñez et al., 1999; Martín et al., 2001). During ripening, protein breakdown yields small peptides and free amino acids (Córdoba et al., 1994a, b; Martín et al., 1998b), which are involved in taste and flavour development (Flores et al., 1997; Ruiz et al., 1999; Ordóñez et al., 1999).

The proteolysis in dry-cured meat products has been attributed mainly to endogenous enzymes (Toldrá, 1998), but these enzymes may be inhibited by salt and curing agents during the ripening process (Sárraga et al., 1989; Rico et al., 1991; Toldrá et al., 1993). On the other hand, micro-organisms isolated from dry-cured meat products, mainly molds, have shown high proteolytic activity during ripening time (Rodríguez et al., 1998; Bruna et al., 2001), and could be used to accelerate proteolysis. However, control of the fungal population growing on these products is essential, because most of the molds isolated from dry-cured meat products are toxigenic (Núñez et al., 1996, 2000; Sosa et al., 2002). Therefore, selected non-toxigenic strains could be used as starter cultures to make sure of the positive contribution of molds in the ripening of dry-cured meat products.

A non-toxigenic strain of *Penicillium chrysogenum* isolated from dry-cured ham (Núñez et al., 1996) has shown high hydrolytic activity for meat proteins (Rodríguez et al., 1998; Martín et al., 2001; Benito et al., 2003). However, before this strain is proposed as a starter culture, it should be tested for its ability to

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contribute to taste and flavour development. Several proteolytic micro-organisms used as starter cultures in dry-cured meat products are able to hydrolyse meat proteins, but do not contribute to the accumulation of free amino acids and the generation of volatile compounds (Ordoñez et al., 1999). The further transformation of free amino acids, released as a consequence of proteolytic activity, into volatile compounds is essential for the development of the characteristic flavour of dry-cured meat products. It is known that some mold strains are able to degrade amino acids into branched aldehydes and alcohols (Karahadian et al., 1985; Bruna et al., 2001). The ability of proteolytic *P. chrysogenum* to generate volatile compounds from meat proteins should be assayed. Since myofibrillar proteins are the most hydrolysed proteins in dry-cured meat products (Córdoba et al., 1994a), a direct test on those proteins might be very valuable to know the contribution of the proteolytic activity of *P. chrysogenum* Pg222 to accumulation of compounds related to taste (soluble non-protein nitrogen (NPN) compounds) and flavour development (volatile compounds).

The aim of this work was to know the contribution of proteolytic activity of *P. chrysogenum* Pg222 to the generation of NPN and volatile compounds, to determine its potential to be used as a starter culture for dry-cured meat products.

2. Materials and methods

2.1. Mold strain

The strain of *P. chrysogenum* Pg222 has been isolated from dry-cured ham and it has not shown toxicological effects in some biological assays, including brine shrimp test and cytotoxicity against VERO cells (Núñez et al., 1996). This strain has shown proteolytic activity when growing on meat slices (Rodríguez et al., 1998).

2.2. Extraction of myofibrillar muscle proteins

Pork loins were removed from carcasses immediately after slaughter. The exterior surface of the muscles was sterilized by searing as described by Dainty and Hibbard (1980). The burnt tissues were removed down to a depth of ca. 5 mm, using sterile instruments in a laminar flow cabinet Bio Flow II (Telstar, Spain). Sterile tissues were then cut into small pieces, and ca. 20 g samples were placed in Stomacher bags. The extraction of proteins was carried out following the method described by Rodríguez et al. (1998). Sarcoplasmic proteins were removed from muscle after homogenization and washing three times with 200 ml of sterile 0.03 M, pH 7.4 potassium phosphate buffer. Myofibrillar proteins were extracted with 200 ml of sterile 0.55 M IK, 0.05 M sodium phosphate buffer pH 7.4 containing 200 mg/l of chloramphenicol (Sigma, St. Lois, Missouri) to prevent bacterial growth.

2.3. Culture conditions

Culture medium was obtained by mixing the above myofibrillar proteins extract with sterile nutrient broth and NaCl to reach a final concentration of 1.6 mg of protein per ml, 0.1% (wt/vol) of nutrient broth and 5% (wt/vol) NaCl. This medium was designed to favour proteolysis by mold adding low amount of other nutrients, and to reach a salt concentration similar to dry-cured meat products.

P. chrysogenum Pg222 was inoculated with 0.1 ml of a suspension containing ca. 10^7 spores/ml in 15 ml of medium, and incubated at 25 °C for 4 days. Non-inoculated medium was also incubated as a control. The sterility of the control batch was confirmed by determining the absence of the growth of micro-organisms on plate count agar (Oxoid, Unipath, Basingstoke, UK). All samples were taken in triplicate every 4 h after an initial incubation of 24 h.

2.4. Analysis of total protein

The concentration of protein was determined following the Bradford method (Bradford, 1976) using 100 μl of culture media.

2.5. Analysis of non-protein nitrogen

NPN was determined by the method of Johnson (1941) using 4 ml of culture media after protein precipitation with 0.6 M perchloric acid, as described by De Ketelaere et al. (1974).

2.6. Analysis of amino acid nitrogen

Amino acid nitrogen (AN) was determined from the 0.6 N perchloric protein precipitation fraction after peptide precipitation with sulfosalicilic acid 10%, according to Martín et al. (1998a).

2.7. Extraction of volatile compounds

Samples of 1 ml of culture media were put into a 10 ml headspace vial (Hewlett-Packard, Palo Alto, California, USA) and sealed with a PTFE butyl septum (Perkin-Elmer, Foster City, California, USA) in an aluminium cap. Volatile compounds were extracted by Solid Phase Micro-Extraction technique (SPME) (Ruiz et al., 1998) with a 10 mm long, 100 μm thick fibre coated with carboxen-poly dimethylsiloxane (Supelco Co., Bellefonte, Pennsylvania, USA). Prior to collection of