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Occurrence of mycotoxin producing fungi in bee pollen

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Abstract

The natural mycobiota occurring in bee pollen is studied in the present report with special attention to analyze the incidence of fungal species that are potential producers of mycotoxins. A total of 90 ready-to-eat bee pollen samples were analyzed. Eighty-seven samples were collected in stores placed in different Spanish areas and three were from Buenos Aires (Argentina). The statistical results (ANOVA) showed that yeasts and *Penicillium* spp. were the predominant fungi. With regard to the potential mycotoxin producing species, *Penicillium verrucosum*, *Aspergillus niger* aggregate, *Aspergillus carbonarius*, *Aspergillus ochraceus*, *Aspergillus flavus*, *Aspergillus parasiticus* and *Alternaria* spp. were found. The last genus was isolated very frequently. The potential ability for producing ochratoxin A (OTA) and aflatoxins B_1, B_2, G_1 and G_2 was studied by culturing in vitro the isolates followed by analysis of these mycotoxins in culture extracts by HPLC with fluorescent detection. It was found that 100%, 53.3%, 33.3% and 25% of the isolates of *A. carbonarius*, *A. ochraceus*, *P. verrucosum* and *A. niger* aggregate, respectively, produced OTA. Moreover, 28.6% of the isolates from the *A. flavus* plus *A. parasiticus* group were able to produce aflatoxin B_1. Aflatoxin B_2 was detected in only 10% of the cultures. Aflatoxins G_1 and G_2 were not detected in cultures under the assayed conditions. This is the first report carried out on the natural mycobiota occurring in bee pollen in general and on the toxigenic capability of these isolates in particular.

Keywords: Bee pollen; Fungi; Mycobiota; Mycotoxins; Ochratoxin A; Aflatoxins

1. Introduction

Nowadays, a change in food habits of consumers is taking place, principally in the developed countries, where people aspire to have more healthy and nutritious diets. These diets include natural products among which the hive products like honey, bee pollen and royal jelly play an important role (Serra Bonvehí and Escolá Jordá, 1997).

Bee pollen consists of plant pollens collected by worker bees combined with plant nectar and bee saliva. The material is compacted into pellets, which are used as food for drone bees. Pollen consists of the male germ seeds of plants, flowers or blossoms on

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trees (PDRHealth, 2005). This product has been used as a nutrient-rich health food for many centuries and its benefits have been widely lauded (Lyngheim and Scagnetti, 1979; Linskens and Jorde, 1997). Bees use pollen as their nutritional source of proteins (25–30%), carbohydrates (30–55%), lipids, including fatty acids and sterols (1–20%), vitamins and minerals. Bee pollen is a rich source of free amino acids, and for this reason its use in the human diet is very highly appreciated (Serra Bonvehí et al., 1991; Block et al., 1994). It is also a potential source of polyphenols and other healthy compounds (Rice Evans and Packer, 1997; Serra Bonvehí et al., 2001).

The German Federal Board of Health has officially recognized bee pollen as a medicine (Linskens and Jorde, 1997). More specifically, ingestion of bee pollen by rats has been shown to decrease the level of the lipid oxidation products (malondialdehyde and conjugated dienes) in the erythrocytes, thus suggesting an antioxidant role for bee pollen. (Dudov and Starodub, 1994; Linskens and Jorde, 1997). Other authors have demonstrated immunostimulation activity on primary and secondary levels of IgM and IgE in rabbits feed on bee pollen containing diet for 1 month (Dudov et al., 1994). It also acts as intestinal regulator favouring digestion and nutrient assimilation (Zhao et al., 1996; Orzaez Villanueva et al., 2002). These and other properties of bee pollen convert this commodity more and more in a regular ingredient in diet, which makes its consumption to increase constantly.

The quality of bee pollen is strongly dependent on its preservation. Pollen has specific characteristics linked to floral species or cultivars it comes from (Serra Bonvehí, 1988). After being collected from hives, bee pollen is carried to packaging centres, where the product is cleaned, dried and packaged. The techniques vary depending on the available machinery. The quality of the end product also depends on these cleaning, drying and packaging processes currently applied by beekeepers or apicultural traders to achieve longer storage life (Serra Bonvehí and Lopez Alegret, 1986). Water activity (a_w) of bee pollen that is ready for human consumption is about 0.268 (0.261–0.280) (Serra Bonvehí and Escolá Jordá, 1997).

Taking into account its nutrient content, a variety of microorganisms could grow in bee pollen. If collection, storage and marketing practices are not appropriate fungi might develop in it as it happens in cereal grains (Magan and Lacey, 1988; Lacey and Magan, 1991). Fungi colonize the terrestrial environment successfully and utilize solid substrates efficiently by growing over their surfaces and penetrating into their matrices. Many fungi produce mycotoxins that can cause acute or chronic intoxication and damage to humans and animals after ingestion of contaminated food and feed (Marasas and Nelson, 1987; Moss, 1996). Among the mycotoxins, aflatoxins and ochratoxin A (OTA) occupy especial places due to its high occurrence and toxicity. The European Commission has established maximum allowable limits for these toxins in some food products (Commission Regulation, 2001, 2002a,b, 2005).

Aflatoxins are hepatotoxic, teratogenic, mutagenic and carcinogenic mycotoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B_1 is listed as a group I carcinogen by the International Agency for Research on Cancer (IARC, 1982). Aflatoxin-producing fungi are widespread worldwide and can produce these toxic compounds either before or after harvest (Miller, 1994).

OTA is nephrotoxic, hepatotoxic, teratogenic and immunotoxic to animals (Dirheimer, 1998) and has been associated to fatal endemic human nephropathies (Plestina, 1992). Due to its carcinogenicity to mice and rats (Pohland et al., 1992), OTA has been classified as possible carcinogen to humans being included in group 2B (IARC, 1993). This mycotoxin is produced by *Aspergillus ochraceus*, *Aspergillus alliaceus* (Bayman et al., 2002), *Aspergillus carbonarius* (Joosten et al., 2001), *Aspergillus niger* (Abarca et al., 1994) and *Penicillium verrucosum* (Frisvad and Filtenborg, 1989). OTA is widely distributed and its occurrence has been reported mainly in cereals and coffee (Trucksess et al., 1999; Vrabcheva et al., 2000), milk (Breitholtz-Emanuelsson et al., 1993), wine (Markaki et al., 2001) and beer (Visconti et al., 2000).

Taking into account that bee pollen is collected in countries with moderate/calid temperate and humid climates during flowering season the major aims of our research were (1) the evaluation of the mycobiota occurring in bee pollen and (2) the study of the potential ability of the isolated fungi to produce mycotoxins. This is the first report about the occurrence of toxigenic fungi in bee pollen.