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Safety assessment of dairy microorganisms: *Geotrichum candidum* ☆

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Abstract

Geotrichum candidum is a ubiquitous filamentous yeast-like fungus commonly isolated from soil, air, water, milk, silage, plant tissues, digestive tract in humans and other mammals. This species is widely used as adjunct culture in the maturation of cheese. The genus *Geotrichum* is composed of 18 species. A recent taxonomic revision concluded that the old *Galactomyces geotrichum*/*G. candidum* complex contained four separate species of which *Galactomyces candidus* sp. nov./*G. candidum*. M13 primer can be used for identifying species of the *Geotrichum* genus. Used in combination, RAPD-PCR and RAM-PCR permit strains to be differentiated. The species can be unambiguous differentiated from the two species most frequently described in human pathology: *Geotrichum clavatum* (reclassified *Saprochaete clavata*) and *Geotrichum capitatum* (reclassified *Magnusiomyces capitatus*/*Saprochaete capitata*). Sources of exposure are food ingestion – cheese consumption playing a major role – inhalation and contact. A bibliographic survey was conducted to assess corresponding hazards and risks. *G. candidum* infections (mainly pulmonary or bronchopulmonary, but also cutaneous, oral, disseminates) are very rare: fewer than 100 cases reported between 1842 and 2006. Moreover, cases were not all confirmed by repeated isolations and demonstration of the fungus' presence in tissues, a prerequisite to establish a true diagnosis of geotrichosis. Immunocompromised population was recently shown as a target for opportunistic infection. The most effective treatments include either azole drogs as ketonazole, iconazole and clotrimazole, or polyene antibiotics as amphotericin B, nystatin and pimaricin, or voriconazole–amphotericin B association. Less than 1 case/year of disease was possibly caused by *G. candidum* and it never included dairy products or foodborne infection. The risk of developing an infection due to *G. candidum* in connection with its technological use and consumption of dairy products is virtually nil. For these reasons, *G. candidum* should be proposed for QPS status.

Keywords: *Geotrichum candidum*; Dairy products; Safety

1. Generalities/presentation

Geotrichum candidum, formerly *Oidium lactis*, is a filamentous yeast-like fungus (for the other yeasts *see* Part X, the Hemiascomycetous yeasts by Jacques and Casaregola, 2008). *G. candidum* strains display wide phenotypic variability, from the yeast-like form to the mould-like form. It is a ubiquitous fungus found in a wide range of habitats such as plant tissues, silage (O'Brien et al., 2005), soil, milk, air and water. It is also a component of the natural flora of the digestive tract in humans and many other mammals (Heinic et al., 1992; Samson et al., 1996). Vasei and Imanieh (1999) note that "the number of (yeast) species which appear to be relatively consistent residents of the bowel lumen is limited, the most common being *Candida*, *Rhodotorula* spp. and *Trichosporon*, and one of the less common being *G. candidum*. This last species is also found naturally in cheese and fermented milk, but in France it has also been added as a ripening agent for at least thirty years. It is reported by the International Dairy Federation and European Food and Feed Culture Association as a microorganism with a documented history of use in dairy products (Mogensen et al., 2002).

In retrospect, no foodborne disease has been linked to the consumption of products containing *G. candidum*. The French regulation on biological safety levels does not list it in any class of biological pathogen (Journal Officiel de la République Française, 1994). It does not appear on the official list of biological agents published by the Advisory Committee on Dangerous Pathogens (2004).

☆ Contribution to the safety assessment of technological microflora found in fermented dairy products, PART XI.

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The major aim of the review is to assess hazards and possible risks, if any, due to *G. candidum* associated with cheese consumption.

1.1. Taxonomy

G. candidum is classed by Kurtzman and Fell (1998) and Barnett et al. (2000) among the yeasts. Although some other authors cite it amongst the moulds (Wouters et al., 2002), it must be borne in mind that the terms "yeast" and "mould" do not have any formal taxonomic significance (Kirk et al., 2001). In fact all species of *Geotrichum* genus are considered as filamentous yeast-like fungi (De Hoog and Smith, 2004).

G. candidum species formerly belonged to the Deuteromycetes (Fungi imperfecti) because of their asexual reproduction. At present, however, taxonomists tend to consider that this class has no taxonomic meaning and that *G. candidum* should therefore be reclassified among the Hemiascomycetes (Jacques and Casaregola, 2008). Current nomenclature of *G. candidum* is mentioned in Fig. 1.

The taxonomy of the *Geotrichum* link genus has been studied by Smith, De Hoog and co-workers for more than twenty years (De Hoog et al., 1986). *Galactomyces geotrichum* Redhead et Malloch has been considered as the teleomorphic state of *G. candidum* from 1977 (Redhead and Malloch, 1977) to 2004 (De Hoog and Smith, 2004). By studying the DNA melting curves and by performing DNA–DNA hybridization experiments, Smith et al. (1995) distinguished four subgroups of *G. geotrichum*: *G. geotrichum* sensu stricto, *G. geotrichum* group A, group B and group C. Smith et al. (2000) presented a key to identify taxa of the genera: *Geotrichum*, *Galactomyces* and *Dipodascus*. A taxonomic revision of *Geotrichum* and its teleomorphs has been proposed by De Hoog and Smith in December 2004. It was concluded that the *G. geotrichum*/*G. candidum* complex contained four separate species (Table 1).

At present (De Hoog and Smith, 2004 ; Pimenta et al., 2005 ; Suh and Blackwell, 2006 ; Wuczkowski et al., 2006), the genus *Geotrichum* is composed of 18 species (including 6 sp. nov.). Six species, among which *G. candidum*, have a teleomorph in *Galactomyces* Redhead and Malloch, six in *Dipodascus* de Lagerheim and six an unknown teleomorph.

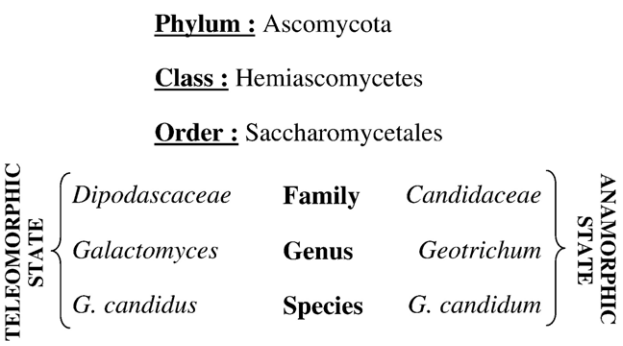

Fig. 1. Present nomenclature of *Galactomyces candidus*/*Geotrichum candidum*. Teleomorphic state = perfect state = sexual form; anamorphic state = imperfect state = asexual form. (From Kurtzman and Fell, 1998; Barnett et al., 2000; De Hoog and Smith, 2004).

Table 1
Geotrichum candidum and teleomorph before and after taxonomic revision from De Hoog and Smith (2004)

Before December 2004		Since December 2004	
Galactomyces geotrichum complex		Four separate species	
Teleomorphic state	Anamorphic state	Teleomorphic state	Anamorphic state
G. geotrichum sensu stricto	*Geotrichum candidum*	*G. geotrichum*	Unnamed *Geotrichum* species
G. geotrichum group A	*G. candidum*	*Galactomyces candidus*	*G. candidum*
G. geotrichum group B	*G. candidum*	*Galactomyces pseudocandidus* sp. nov	*Geotrichum pseudocandidum*
G. geotrichum group C	*G. candidum*	Unknown	*Geotrichum europaeum* sp. nov.

Type strains proposed (De Hoog and Smith, 2004) are : CBS 178.71 ex-holotype strain of teleomorph, and CBS 615.84 neotype of anamorph designated by the authors.

1.2. Identification tools

Galactomyces candidus/*G. candidum* is identifiable by coupling phenotypic and genotypic approaches (Gente et al., 2006). It is firstly necessary to ensure that it is a fungus with holothallic spore production (hyphae splitting at the septum to form individual cells called arthrospores). The M13 primer can be used to discriminate the species belonging to the genus *Geotrichum* and teleomorphs. *G. candidus*/*G. candidum* is characterized by the presence of a common three bands profile, among which a major band at 860 bp was observed (Gente et al., 2006). For traceability prospects, strains can be differentiated using three RAPD probes (OPA 19, OPT15, DB18) and GATA4, a RAM probe (Gente et al., 2002). More recently, Florez et al. (2007) proposed a typing method based on RAPD primers, ari1 and omt1.

Considering the recent taxonomic revision proposed by De Hoog and Smith (2004), *G. candidum* strains previously included in the *G. geotrichum*/*G. candidum* complex, need a confirmation step based on phenotypic tests (growth at 35 °C and D-mannitol assimilation) to differentiate it from the closely-related, newly-described species (Table 1) *G. geotrichum*/*Geotrichum* sp., *Galactomyces pseudocandidus*/*Geotrichum pseudocandidum* and *Geotrichum europaeum*.

For safety assessment, *G. candidum* must be differentiated from the two species most frequently described in human pathology (Lacroix and Feuilhade de Chauvin, 2005): *Geotrichum clavatum* (reclassified *Saprochaete clavata* De Hoog and Smith, comb. nov.) and *Geotrichum capitatum* (first reclassified as *Blastoschizomyces capitatus* and then as *Magnusiomyces capitatus*/*Saprochaete capitata* De Hoog and Smith, comb. nov). For this purpose, the M13 primer is effective (Gente et al., 2006). Furthermore, *G. candidum* assimilate D-xylose as a carbon source and cannot grow at 40°C when the others two species are respectively negative and positive for these two tests (De Hoog and Smith, 2004).