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Morphological Significance of *Cladosporium* Contaminants on Materials and Utensils in Contact with Food

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Cladosporium **contaminants on materials and utensils that come into contact with food were morphologically investigated. The most common contaminants, *C. cladosporioides* and *C. sphaerospermum*, were detected on the samples. The morphological changes of the *Cladosporium* species were investigated by using stereoscopic, optical light, fluorescent, and scanning electron microscopes. Microscopically the *Cladosporium* contaminants were observed as aggregated dark brown spots, strongly pigmented, irregularly swollen, and in long chains. Using fluorescent microscopy, the *Cladosporium* mycelia were clearly stained with fluorescein diacetate as viable cells, but the old cells were mostly non-viable, as shown by staining with propidium iodide. The dynamics of the morphological changes showed that the penetrating mycelia were closely attached to the surface of the materials and utensils under investigation. These results provide information about the significance of *Cladosporium* contamination on materials and utensils in contact with food and may contribute to the control of fungal contamination.**

Key words : *Cladosporium* / Contamination / Morphological changes / Food associated utensils.

INTRODUCTION

Cladosporium is widely recognized as a common contaminant in food, wood, plastics, rubber and other environmental materials (Pitt et al., 1997; Samson et al., 2002; Takatori, 2002). It also can be detected in the indoor environment. *Cladosporium* can cause serious problems as an allergen or air- or food-borne contaminant. In addition, the biodeterioration of both instruments and dwellings can be attributed to *Cladosporium* (Adhikari et al., 2000; Aihara et al., 2001; Takatori et al., 2001; Takatori, 2001; Tanaka et al., 2002a; Tanaka et al., 2002b). The genus *Cladosporium* as saprophyte and plant pathogen is often the predominant isolate from airborne microflora (Aihara et al., 2001; Airaudi et al., 1996/1997; Nielsen, 2003). Conidia of *Cladosporium* species are particularly well adapted to aerial dispersal since they are small, dry, heavily pigmented and highly resistant to humid conditions (Inada et al., 2002; Takatori, 2002). Two species, *C. cladosporioides* and *C. sphaerospermum*, are commonly associated with contamination and distribution in food and dwelling materials (Aihara et al., 2002; Takatori, 1993).

In brief, the morphological changes of the contaminating fungi are not known. The current study

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examines the morphological findings of *Cladosporium* contaminating some materials and utensils that come into contact with food. This study confirms that *Cladosporium* contaminants grow and extend as a means of penetration into substrates. Finally, it provides a method for analysis of the control of contamination by *Cladosporium* in materials and utensils that come into contact with food.

MATERIALS AND METHODS

Contaminated materials and utensils

Containers, wrappings and utensils suspected of being contaminated with fungi were collected from food factories. Eight kinds of materials and utensils showed contamination with *Cladosporium*. These included the silicon resin of a food container, epoxy resin of a vegetable package, the polyurethane of a food package, wooden chopsticks, a synthetic resin brush for floor cleaning, a wooden brush with a plant fiber sweeper, the inorganic joint of a kitchen sink drainage system, and multiple towel of fruit wrap.

Isolation and identification

The materials and utensils were cut aseptically into 0.5 × 1.0mm pieces and, 5 pieces per material or utensil were placed on potato dextrose (PD) agar and M40Y agar supplemented with chloramphenicol 50 μg • ml^{-1}. Each sample was incubated at 25℃ for 7days. After incubation, the growing fungi were isolated for identification. Since *Cladosporium* was the target, species identification was limited to this genus. *Cladosporium* isolates were identified by their colonies and morphology as has been described elsewhere (de Vries, 1952; Domsch et al., 1980; Ellis, 1971).

Microscopic examination

Microscopic examinations were done with stereoscopic, optical light, fluorescent and scanning electron microscopes. A stereoscopic microscope (Olympus SZX9) was used to observe the materials or utensil surface. An optical light microscope (Olympus BX51) was used for the inner parts of samples that were stained with lactophenol or periodic acid Schiff reagent. Fluorescent microscopes (Olympus BX51WI) were used to examine the viable and nonviable cells of the contaminating *Cladosporium*. The fluorescein diacetate reagent (FDA) detected viable cells and propidium iodide (PI) detected nonviable cells (Yang et al. 1995). Scanning electron microscopes (SEM) (Hitachi S4000) were used to examine the surface of materials or utensils. The samples were cut longitudinally and transversely using a double-edged razor blade to produce small sections approximately 2-3mm thick for SEM. Sections were fixed in 3% glutaraldehyde, washed in sodium cacodylate, followed by distilled water, and dehydrated in 20 to 100% ethanol series with 20% concentration intervals. Sections were critical point dried, mounted on the aluminium stubs, coated with gold using a sputtering device and observed using SEM operated at 20kV.

RESULTS AND DISCUSSION

Detection of *Cladosporium* on materials and utensils in contact with food

As shown in Table1, types of materials and utensils were almost solely and heavily contaminated with the *Cladosporium* species . Seven of the 8 were contaminated exclusively with either *C. cladosporioides* or *C. sphaerospermum*. Only the inorganic joint was contaminated with both species. The fungal diversity in food environments depended upon the atmospheric conditions as well as the materials and utensils associated with food. *Cladosporium* populates the food and food environment settings (Adhikari et al. 2000). The physiological characteristics of *Cladosporium* have been described by Aihara et al. (2000), Tanaka et al. (2002c) and Takatori (2002).

Morphological observations by stereoscopic microscope

TABLE 1. Tested materials and utensils contaminated with *Cladosporium* species.

Food associated utensil	Main contaminants	Other isolates
Plastic (Silicon resin)	*C. sphaerospermum*	
Plastic (epoxy resin)	*C. cladosporioides*	
Polyurethane	*C. sphaerospermum*	*Penicillium* sp.
Wooden chopsticks	*C. cladosporioides*	*Rhodotorula* sp.
Synthetic resin brush	*C. sphaerospermum*	*Aureobasidium pullulans*
Wooden brush	*C. sphaerospermum*	
Inorganic joint	*C. cladosporioides*	
	C. sphaerospermum	
Multiple towel	*C. cladosporioides*	*Rhodotorula* sp.

The contaminated mycelia were likely to form a mosaic pattern that spread irregularly toward the outside. The mycelia had a thick width in places in the silicon resin (Fig.1-a). The patterns on the polyurethane implements contaminated with *C. sphaerospermum* were dark spots that were aggregations primarily of clumps of mycelia (Fig.1-b). Furthermore the black colored spots of aggregations of concentrated mycelia were scattered on the wooden brush (Fig.1-c). The wooden brush became darker more rapidly than either the plastic or the synthetic resin brush. The ease of adsorption of water by the wood resulted in the humid conditions that favored contamination. *Cladosporium* penetration into synthetic substrates has been documented (Gravesen et al., 1999; Gu, 2003). *Cladosporium* and other fungi degrade many kinds of materials (Han et al., 2002; Park et al., 2001).

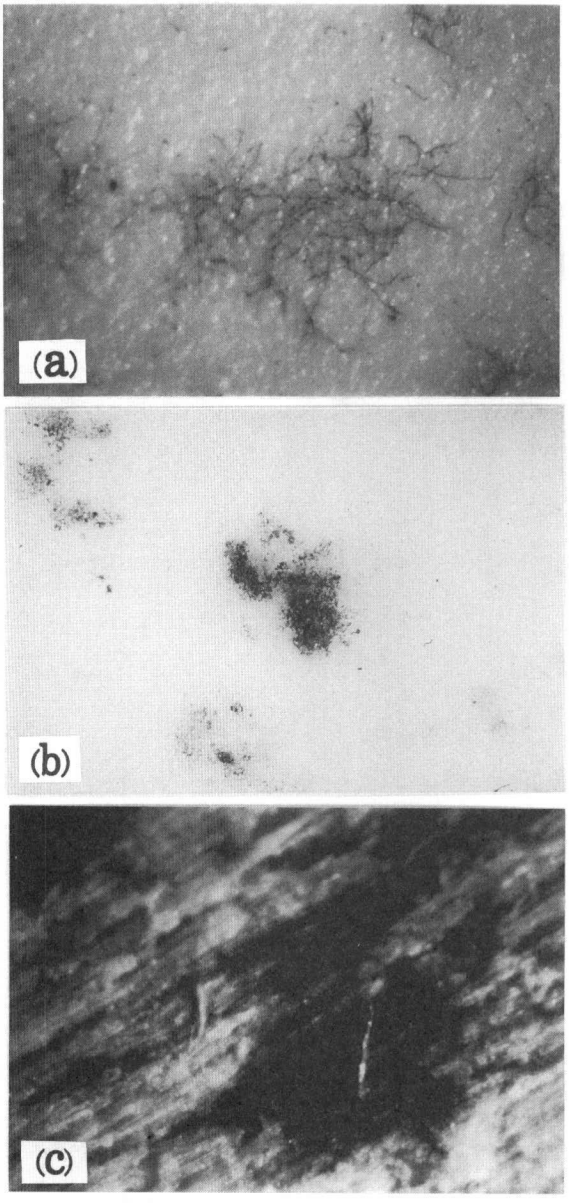

FIG. 1. *Cladosporium* contamination by stereoscopic findings in food associated utensils. The mycelia with mosaic pattern of *C. sphaerospermum* in silicon resin. The dark spots by clumps of *C. sphaerospermum* in polyurethane. The black colored spots of *C. sphaerospermum* mycelia in wooden brush.

Morphological observations by optical light microscope

The morphology of the *Cladosporium* on contaminated samples shows various changes (Perhkar et al. 2001, Shirakawa et al. 2003, Vember et al. 1999). First, the morphological patterns of *C. sphaerospermum* were demonstrated on the wooden implement. *C. sphaerospermum* conidia were evenly scattered on the wooden brush (Fig.2).

Tissue-like clumps were observed in silicon resin contaminated with *C. sphaerospermum* and epoxy resin with *C. cladosporioides*. The clumps of mycelia

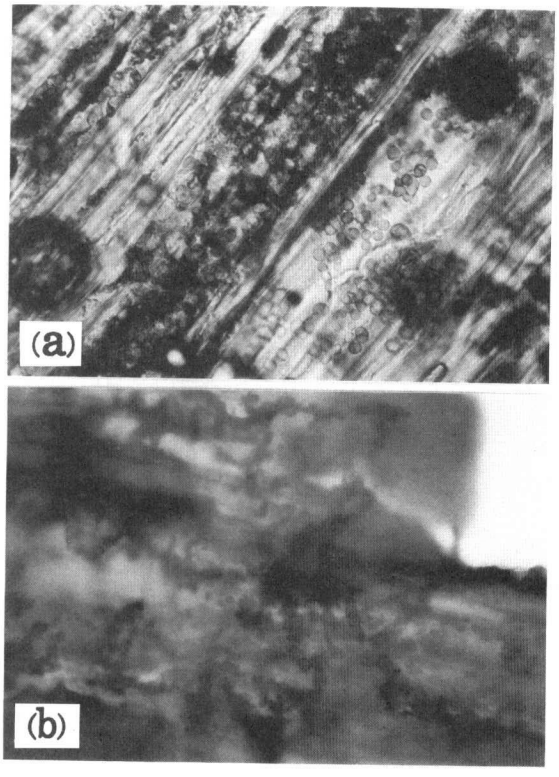

FIG. 2. *Cladosporium* contamination by optical microscopic findings in food associated utensils. The morphological patterns of *C. sphaerospermum* in wooden brush. The mature conidia were evenly scattered across the wooden fiber. The long chained mycelia of *C. sphaerospermum* in wooden brush. The fungal elements were differentially stained by periodic acid Schiff staining.

were 150-200 μm in diameter (Fig.3-a, b). The constitutional cells were thick and ciger-shaped, in irregular chains and with chlamydospore type mycelia (Fig.3-c). There were morphological characteristics that were consistent with the spore sheath of *C. sphaerospermum* in silicon resin and the wooden brush (Fig.2-a) when samples were subjected to periodic acid Schiff staining. The fungal sheath was differentially stained with a violet color in the wooden debris (Fig.2-b).

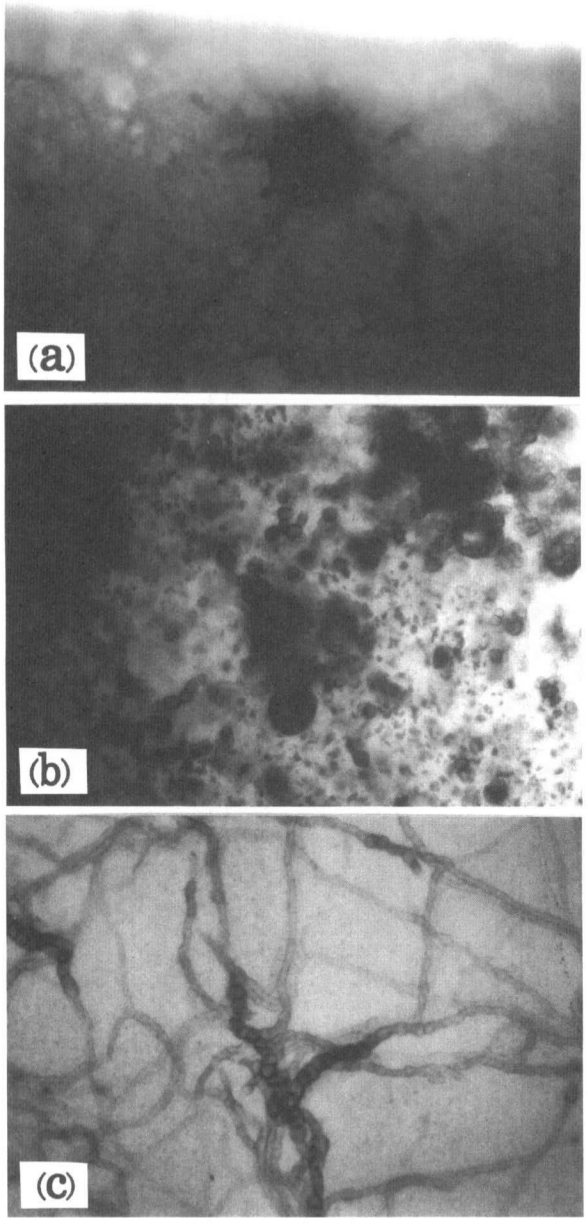

FIG. 3. The polymorphological changes of contaminated *Cladosporium*. (a) The tissue-like clumps of *C. sphaerospermum* with hard and tight form in silicon resin. (b) The clumps of *C. cladosporioides* mycelia, around 150-200 μm in diameter in epoxy resin. (c) The constitutional cells with thick, cigar-shaped and irregular chains, and chlamydospore type mycelia in synthetic resin brush.

Morphological observations by fluorescence microscope

The microscopic fluorescent lectin assay can distinguish fungi from other microbes (Potts et al., 2001). In this investigation, fungal cells were experimentally divided into viable and nonviable cells. The fungal cells which invaded into substrates gradually decreased in viability. The other mycelial activities also decreased together. The cell activity at the initial stage of contamination was quite strong, but at the mature stage began to decline to inactivity. The fungal cells of *Cladosporium* and other fungi became extinct with long period of contamination (Kemp et al., 2001; Takatori, 2001).

Under observation with a fluorescence microscope, *C. sphaerospermum* in polyurethane reacted with PI

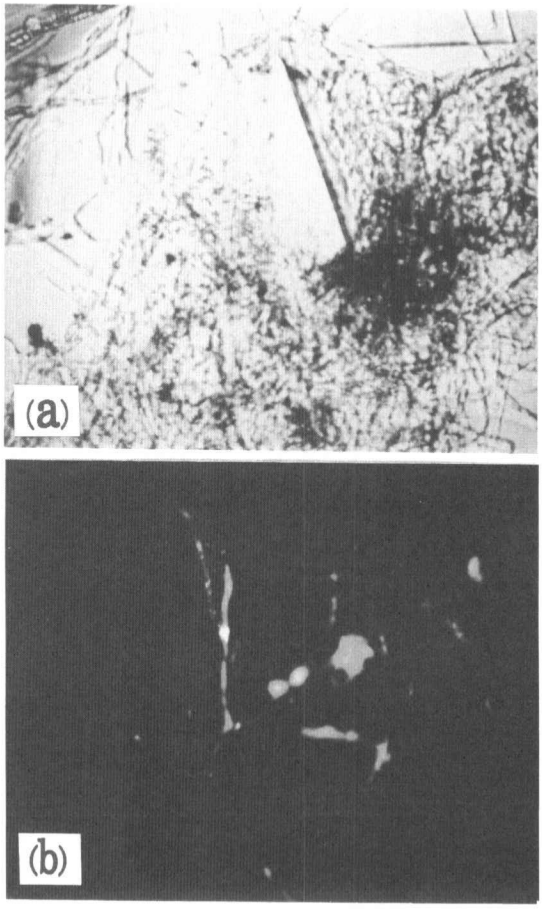

FIG. 4. It showed that (a) and (b) are the same portion of polyurethane by optical light and fluorescent microscope, respectively. Though the contaminated fungus could hardly detect by the optical microscope, it was clearly reacted with PI rather than FDA by fluorescent microscope.

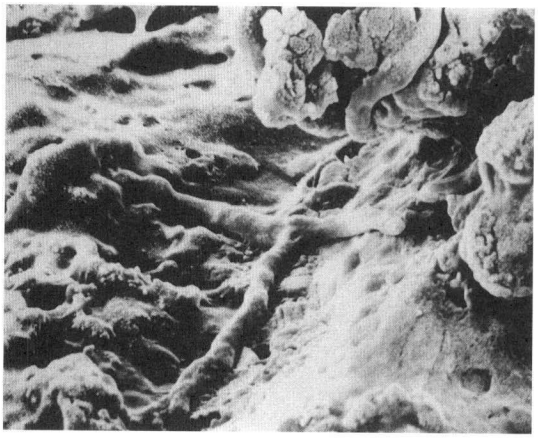

FIG. 5. The surface of wooden chopstick invaded with *C. cladosporioides*.

rather than FDA. This result meant that the most cells of *C. sphaerospermum* contaminating the materials and utensils in contact with food were the inactive or dead (Fig.4).

It was generally confirmed that most of the contaminating mycelia reacted with PI and that the staining showed the non-viability of contaminating fungal cells.

Morphological observations by scanning electron microscope

The three dimensional structures of fungi invading the materials and utensils associated with food were investigated by SEM. The surface of a wooden chopstick invaded by *C. cladosporioides*, visualized by SEM, is shown in Fig.5. The invading mycelia were creeping closely on the surface. Therefore, the creeping mycelia were likely to produce some extracellular metabolites as like enzymes or secondary metabolites that allowed a tight adherence to the surfase and penetration into the substrate. As contamination progressed, the penetration into the substrate took place around the contaminated areas. As shown in Fig.5, the surface of the wooden chopstick contaminated with *Cladosporium* was observed, showing the inequality of penetrating hyphal width and then deterioration.

Cladosporium, the main isolate from foods and, materials and utensils in contact with food, causes severe damage to the object of contamination (Davis et al., 1975; Hill et al., 1984; Mislivec et al., 1979). According to the composition of the contaminated object, the morphological characteristics of the fungal growth were different. Evaluation of fungal growth on synthetic materials has been discussed by Karunasena et al. (2001). Cellulose-containing material was also degraded by fungi, including *Cladosporium*. Furthermore, the *Cladosporium* cell viability in contaminated objects was clearly demonstrated by fluorescent microscopic observations, as well as the dynamic findings of *Cladosporium* mycelia on the utensil surfaces by SEM.

These results expand the knowledge of the morphological changes of *Cladosporium* during contamination and information may be derived for the control of such contamination in foods and materials and utensils which come into contact with food.

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