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APPARENT CROSS-INFECTION WITH A SINGLE STRAIN OF *MALASSEZIA PACHYDERMATIS* ON A PIG FARM

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Twenty-nine isolates of *Malassezia pachydermatis* were recovered from a single farm of 100 pigs in Croatia. In contrast, 290 farm pigs from other locations (northern parts of Croatia and Slovenia) yielded only two non-lipid dependent isolates of *M. pachydermatis* using the same swabbing procedure. Ten of the 29 isolates from a single farm had their identity confirmed by karyotyping, and were typed by restriction fragment length polymorphism (RFLP) analysis. All but one of these isolates sub-typed were indistinguishable, one isolate produced a slightly different RFLP profile. Control isolates recovered from dog skin gave RFLP profiles that were easily distinguished from those produced by the pig isolates. These results suggest that a single strain of *M. pachydermatis* had colonised this pig herd.

Key words: DNA restriction, *Malassezia pachydermatis,* pig

For quite a long time, members of the genus *Malassezia* isolated from humans have been classified in two different entities, *Pityrosporum ovale* and *Pityrosporum orbiculare* and those isolated from animals have been named *Pityrosporum canis*. Since 1995 the taxonomy of the genus *Malassezia* has been revised according to morphology and molecular biological techniques (Guillot and Gueho, 1995; Guillot et al., 1995). As a result of this the genus *Malassezia* has been enlarged to include seven distinct species, three former taxa (*M. pachydermatis*, *M. furfur* and *M. sympodialis*) and four new taxa (*M. globosa*, *M. obtusa*, *M. restricta* and *M. slooffiae*). All species except *M. pachydermatis* are lipid-dependent (basidiomyceteous) yeasts. Members of this genus belong to the normal mammalian cutaneous microflora and are also believed to act as opportunistic human and animal pathogens (causing seborrhoeic dermatitis, pityriasis versicolor, folliculitis, otitis and some forms of atopic dermatitis).

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The majority of *Malassezia* isolates recovered from pig skin belong to the lipid-dependent species (Gustafson, 1959; Gustafson, 1960; Guillot et al., 1994). Non-lipid dependent *Malassezia* yeasts are only occasionally isolated from pig skin (Kuttin and Glas, 1985; Melzig, 1989). It has been demonstrated that all isolates of *M. pachydermatis* share a remarkably homogeneous chromosome profile by karyotyping and therefore karyotyping provides an effective method of confirming the species of isolates (Anthony et al., 1994). Restriction digestion has been shown to discriminate between different strains of *M. pachydermatis* isolated from dogs and humans and increased discrimination has been described with the use of a simple repeat poly (GT) probe (Anthony et al., 1994).

The purpose of this study was to determine the frequency of *Malassezia* isolates at the external ear canal of healthy pigs and to characterise the isolates. Here we report the results of isolation, cultural and DNA identification of *M. pachydermatis* from a large number of pigs and the use of restriction fragment length polymorphism (RFLP) typing on a number of these isolates in order to understand the epidemiology of this yeast in these pigs.

Materials and methods

The examined 390 animals were kept in three different farms (two in Croatia and one in Slovenia) for two years and constituted both males and females with different ages and breeds. The animals were submitted to clinical examination and specimens collected by swabbing of ear canals. Cultures were performed on Sabouraud's dextrose agar supplemented with chloramphenicol (50 mg/l) and cycloheximide (200 mg/l) at 37 °C and observed at last for three weeks. The colonies were evaluated macroscopically and microscopically according to their morphometric features. Control dog and cat *M. pachydermatis* strains were obtained from the collection of the Mycological Laboratory of the Department of Microbiology and Infectious Diseases, Veterinary Faculty in Zagreb.

Electrophoretic karyotyping

Karyotyping was carried out as described previously (Anthony et al., 1994) with ten randomly chosen isolates. They were harvested after 24 h growth on Sabouraud's dextrose agar. DNA was extracted by a modification of the method described by Carl and Olson (1985) and gels run as described previously (Anthony et al., 1994).

Restriction fragment length polymorphism (RFLP) analysis

For RFLP analysis, fungal masses were subcultured in Sabouraud's broth and incubated in an orbital incubator at 30 °C overnight. DNA was extracted as

described previously (Anthony et al., 1994). Briefly: cell walls were disrupted by incubating about 0.2 g of the washed cells in 0.5 ml of LET buffer [0.5 M EDTA, 0.01 M Tris and 2% NaOH (pH 7.5)] containing 1 mg of Zymolyase 20T and 1 mg of Novozym, mixing well and holding at 30 °C for 5 h. The spheroplasted cells were centrifuged at 10,000 *g* and the supernatant discarded then lysed by re-suspending with vigorous mixing in 0.5 ml of GES reagent (5 M guanidium thiocyanate in 100 mM EDTA plus 0.5% *N*-laurylsarcosine) and holding at room temperature for 20 min. Dissolved protein was precipitated by adding 100 µl of 5 M potassium acetate and holding on ice for 30 min. To each tube was added 0.5 ml of chloroform 2-pentanol mixture (24:1) and the phases mixed well. The tubes were centrifuged at 10,000 *g* for 5 min and the upper phase transferred to a new microcentrifuge tube, the lower phase and cell debris were discarded. The extracted DNA was precipitated and washed in 70% ethanol and dissolved in 50 µl of TE.

A 1% agarose mini gel was run at 65 volts for 2 h to assess the quality of the extracted DNA and to check for the presence of any extra-chromosomal nucleic acid. Twenty µl of the DNA prepared in this way was restricted with *Bgl*II to the manufacturers instructions and run on an 0.8% agarose gel at a constant 30 volts for 18 h. For Southern hybridisations DNA was capillary blotted onto Stratagene nylon membranes. Hybridisations were carried out using biotin-labelled poly (GT) (Anthony et al., 1994).

Results

Clinical examination of the animals revealed different accumulation of cerumen in different age categories of pigs. The majority of pigs over two years of age more often had accumulation of cerumen in comparison to the younger animals.

While swabbing the pigs from the farm with greatest number of fungal isolates it was noted that approximately one-third of the pigs had mild to moderate inflammation in the external ear canal and marked accumulation of cerumen. All pigs from other farms, including two with *M. pachydermatis* isolates, had no signs of any unusual mucosal changes.

Twenty-nine isolates of *M. pachydermatis* were recovered in pure or mixed cultures together with different saprophytic moulds from a single heard of 100 pigs on one farm; in contrast, 290 pigs from other locations yielded only two non-lipid dependent isolates of *M. pachydermatis.* Ten of these 29 non-lipid dependent isolates from a single farm had their identity confirmed by karyotyping. These 10 strains were then typed using RFLP analysis and poly (GT) hybridisation.

All isolates karyotyped displayed the typical chromosome profile for *M. pachydermatis* (Fig. 1). The RFLP patterns obtained from 9 of these 10 isolates were indistinguishable (Fig. 2, lanes 1 to 9) but quite different to the dog isolate (Fig. 2, lane 11) analysed. The remaining isolate had a profile that dif-

fered by a single band at approximately 15 kbp (Fig. 2, lane 10). None of the isolates recovered were carrying dsRNA plasmids previously reported from dog isolates.

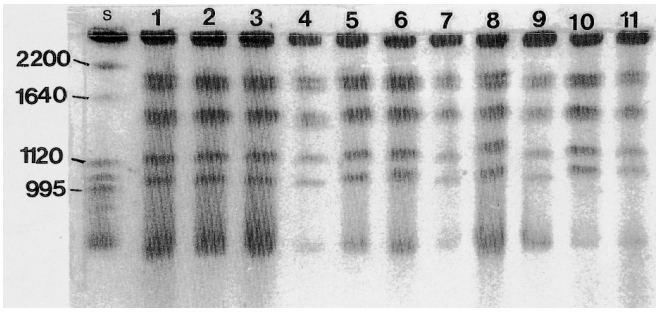

Fig. 1. Karyotypes of pig *Malassezia pachydermatis* (tracks 1 to 10) and dog *M. pachydermatis* isolate (track 11) showing identical profiles consistent with those previously reported for this species. The *Saccharomyces cerevisiae* size standard is marked S, the size of selected bands is indicated in kbp

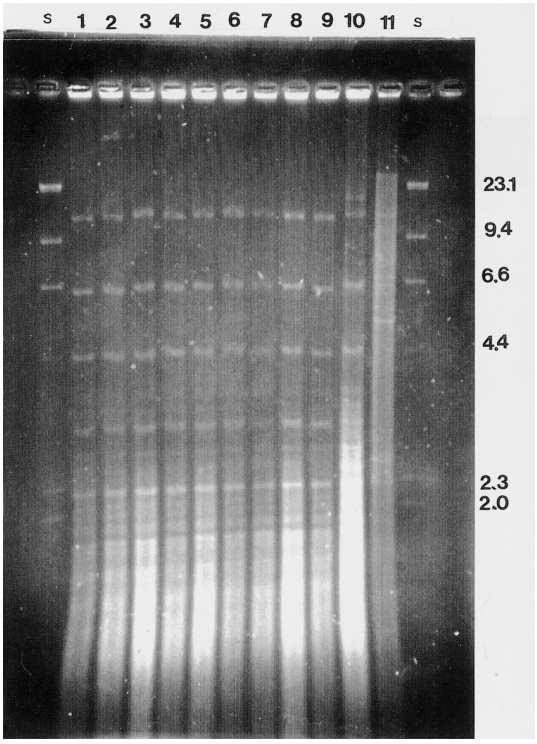

*Fig. 2. Hpa*II digestion of DNA from 10 isolates of *Malassezia pachydermatis* obtained from pigs (tracks 1 to 10), and one isolate from a dog (track 11). Lambda *Hin*dIII size standards are marked S, the size of selected bands is indicated in kbp

Discussion

The genus *Malassezia* consists of a closely related group of fungi which share many cultural, morphological, serological and immunological similarities (Gueho et al., 1996). These especially morphological similarities make it difficult to conduct epidemiological investigations and therefore determine the pathogenicity of individual species. It is hoped that the use of DNA analyses can reveal differences or similarities to both saprophytic and pathogenic strains (Guillot and Gueho, 1995). In this paper we describe the use of karyotyping and DNA restriction for the epidemiological study of *Malassezia* yeasts.

Cultural results showed that non-lipid dependent strains of the genus *Malassezia* that we were screening for are relatively uncommon on pigs (Glumac, 1995). The opposite is true for dog skin for which to the authors' knowledge no unequivocal isolation of lipid-dependent *Malassezia* has been described (Bond and Anthony, 1995). Thus the most striking finding was the high rate of isolation of this species from a single group of pigs. The RFLP data indicate that a single strain of *M. pachydermatis* was colonising these pigs. Approximately one-third of the pigs in this group had mild to moderate inflammation in the external ear canal and marked accumulation of cerumen. Recently a similar correlation of increased colonisation with *Malassezia* species in Brazilian cattle with otitis has been reported. In cattle with otitis a predominance of *Malassezia globosa* was observed (Duarte et al., 1999), in contrast to *M. pachydermatis* from the pigs studied here. These observations suggest a role for *Malassezia* yeasts in the aural health of these mammals although bacteriological examinations were not performed in both cases and possible bacterial involvement was not examined. As the newly developed phenotypic and genotype methods for the identification of *Malassezia* species are applied in more studies a better understanding of these important members of mammalian skin flora will hopefully emerge.

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