NOTE**Comparative ribotyping of *Staphylococcus intermedius* isolated from members of the Canoidea gives possible evidence for host-specificity and co-evolution of bacteria and hosts**

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A total of 41 *Staphylococcus intermedius* isolates were isolated from skin of healthy members of six phylogenetic groups within the Canoidea (the dog family, skunk subfamily, weasel subfamily, racoon family, red panda and bear family) of different geographical origin and compared by *EcoRI* ribotyping and cluster analysis. The *S. intermedius* isolates from the different families and subfamilies clustered together in separate groups, almost completely following the phylogenetic relationship of the animal hosts. These ribotype data indicate host-specificity of different types of *S. intermedius* and suggest co-evolution between the animal hosts within the Canoidea and *S. intermedius*.

Keywords: *Staphylococcus intermedius*, host specificity, carnivores

The genus *Staphylococcus* currently consists of 44 validly named species and subspecies (Probst *et al.*, 1998; Kloos & Bannerman, 1999). Staphylococci are considered part of the natural skin flora of most mammalian species. Most of the species demonstrate host specificity and some species even demonstrate habitat or niche specificity on their particular hosts (Kloos, 1980; Kloos & Bannerman, 1999).

Staphylococcus intermedius is the most common cause of canine pyoderma, but is also a part of the resident bacterial skin flora in dogs. *S. intermedius* is also frequently isolated from other carnivores and a certain host specificity of different types of *S. intermedius* has been observed (Hájek, 1976; Kloos, 1980; Hesselbarth & Schwartz, 1995; Chesneau *et al.*, 2000). Detection of restriction fragments containing the rRNA genes has been proposed as a general method for distinguishing bacterial species and has been used successfully to identify species and subspecies within the genus *Staphylococcus* (Grimont & Grimont, 1986; De Buyser *et al.*, 1992; Thomson-Carter *et al.*, 1989; Webster *et al.*, 1994; Kloos *et al.*, 1997).

The Order Carnivora consists of ten major phylogenetic groups (11 families) (Wayne, 1993). Of these, the dog family (Canidae), the weasel family (Mustelidae), the bear family (Ursidae), the seals and walrus (families Phocidae, Otariidae and Odobenidae),

the racoon family (Procyonidae) and the red panda (subfamily Ailurinae) are closely related and are named the Superfamily Canoidea. No studies have been conducted regarding the occurrence or genotypic composition of *S. intermedius* isolated from most of these groups.

The purpose of this investigation was to study and compare the clonal composition of *S. intermedius* isolated from members of the Canoidea.

Bacterial isolates

Bacterial isolates were collected in the period 1993–1997. All isolates were collected by use of sterile cotton swabs. The procedure for collecting samples was as follows: if possible, a sterile cotton swab was inserted into the first 1–2 cm of one nostril and ear and rubbed vigorously, with rotation. The cotton swab was then placed in a sterile plastic bag and the date, animal species, animal origin and place the sample was taken from were noted. If the animal was too small to insert a swab in the nostril or ear, a sterile cotton swab was rubbed vigorously, with rotation, over approximately 8 cm² body sites that had not been in contact with hands. Swabs from other countries were shipped by ordinary mail. Isolates from mink were from the skin of diseased animals submitted to the Danish Vet-

Table 1. Origin of the *S. intermedius* isolates included in this study

Animal species	Country	Year	Origin	Number of isolates	16S rDNA sequenced (n)
Dog family (Canidae)					
Fox	Denmark	1996	Hunting	4	0
	Czech Republic	–	CCM 2885	1	1
Polar fox	Greenland	1993	Hunting	1	0
Wolf	Denmark	1994	Zoo	1	1
Dog	Denmark	1997	Families	3	1
Weasel family (Mustelidae)					
Subfamily Mephitinae					
Hooded skunk	Arizona, USA	1997	Wild, living	2	2
Skunk	Pennsylvania, USA	1994	Road kill	2	1
Subfamily Mustelinae					
Mink	Denmark	1993	Diseased	4	2
	Czech Republic	–	CCM 2618	1	1
Polecat	Denmark	1996	Road kill	2	2
Subfamily Melinae					
Badger	Denmark	1995	Road kill	3	1
	Sweden	1994	Road kill	3	1
	Norway	1996	Wild, living	1	1
Raccoon family (Procyonidae)					
Raccoon	Denmark	1995	Zoo	1	0
	Pennsylvania, USA	1994	Road kill	3	1
	North Carolina, USA	–	pl-426	1	1
	Arizona, USA	1997	Wild, living	1	0
Bear family (Ursidae)					
Bear	Alaska, USA	1997	Wild, living	5	1
Red panda (subfamily Ailurinae)					
Red panda	Denmark	1995	Zoo	2	1
Total				41	18

erinary Laboratory for examination. During the hunting season, in January and February 1996, the left forelegs from healthy Danish foxes were sent to the Danish Veterinary Laboratory for examination for *Trichinella spiralis*. The author collected most isolates from badgers, racoons and skunks from road kills in Denmark, Sweden and the USA. Isolates from one brown bear, the wolf, the two red panda and one polecat were from the zoological garden or the zoological museum in Copenhagen.

Isolation and bacterial identification

The skin swabs were streaked on bovine blood agar. From these plates, β -haemolytic staphylococcal isolates were picked, subcultured and identified as *S. intermedius* by using the following scheme (Kloos & Bannerman, 1999): Gram-positive, catalase-positive, oxidase-negative, coagulase-positive, β -galactosidase-positive, hyaluronidase-negative cocci that were sensitive to furazolidon and novobiocin, resistant to bacitracin and produced acid from sucrose, mannose and trehalose and did not produce acid from xylose. *S. intermedius* isolates from 41 different animals from the Canioidea from five countries were included. The origins of the animals are given in Table 1. The following reference strains were included: the type

strain of *S. intermedius* (DSM 20373^T), *S. intermedius* CCM 2885 from a fox, *S. intermedius* CCM 2618 from a mink, the type strain of *Staphylococcus lutrae* (DSM 10244^T) and the type strain of *Staphylococcus schleiferi* subsp. *coagulans* (DSM 6628^T). Also included was *S. intermedius* pl-426 from a racoon, supplied by Wesley E. Kloos (North Carolina State University, Raleigh, NC, USA).

Ribotyping

A single isolate identified as *S. intermedius* from each animal was characterized using ribotyping as previously described (Aarestrup *et al.*, 1995). The banding patterns of the *EcoRI* ribotyping were read using GELCOMP (Applied Maths) and comparison based on the Dice coefficient (Dice, 1945) as described previously (Aarestrup & Jensen, 1996). Clustering of the ribotype patterns was based on the unweighted pair-group method with averages (UPGMA) using GELCOMP.

16S rRNA sequencing

A fragment of the 16S rDNA gene was amplified by PCR with universal primers 8F (positions 8–27 of the *Escherichia coli* chromosome) and 1407R (positions

Fig. 1. Origin, graphic representation and dendrogram obtained from cluster analysis of *EcoRI* ribotypes among 41 isolates of *S. intermedius* from six families and subfamilies within the Canoidea and selected type strains.

1407–1389) from 18 isolates (Table 1). The amplification product was sequenced using primers to conserved regions of 16S rDNA. The amplification product was sequenced on an ABI 373A automatic sequencer using the PrismBig Dye terminator kit (Applied Biosystems) using the primers listed below. All primers are universal (Weisburg *et al.*, 1991) allowing sequencing of both strands. The numbering of the primers refers to the numbering of *E. coli* 16S rDNA (Brosius *et al.*, 1978). Primers were: 8FX, 5'-AGAGTTTGATCCTGGCTNAG-3'; 517F, 5'-CC-AGCAGCCGCGGTAATAC-3'; 535R, 5'-GTATT-ACCGCGGCTGCTGG-3'; 784FX, 5'-GGATTAG-ATACCCNGGTAGTC-3'; 804RX, 5'-GACTTAG-NGGGTATCTAATCC-3'; and 1407RX, 5'-TGAC-GGGCGGTGTGTACAA-3'. 16S rRNA sequences were retrieved from GenBank and aligned with the sequences determined using DNASIS (Hitachi Software Engineering).

All isolates could be typed by *EcoRI* ribotyping, assigning the isolates to 22 different types (Fig. 1). Based on 50% similarity, the three type strains and the *S. intermedius* isolates from Canoidea clustered separately. The *S. intermedius* isolates from the racoon family, the weasel subfamily, the skunk subfamily, the bear family, the dog family and red panda formed separate clusters with more than 70% similarity. Although the badger (subfamily Melinae) is classified as a member of the weasel family (Mustelidae), the *S.*

intermedius isolates from badger clustered with those isolated from the racoon family (Procyonidae). This conforms to the phylogenetic study performed by Wayne (1993) based on DNA homology of members of the Canoidea. The 16S rRNA sequences of the 18 selected isolates were more than 99% identical to each other. Comparison to sequences available in GenBank confirmed close identity to *S. intermedius*.

The results of the present study indicate considerable genetic diversity within the species *S. intermedius*, as observed previously (Hesselbarth & Schwarz, 1995; Chesneau *et al.*, 2000). The *S. intermedius* isolates in the present study most likely represented part of the normal skin flora among six groups within the Canoidea. Isolates obtained from hosts within a family were found to cluster together. This indicates either co-evolution of the bacteria and the host or host adaptation. Co-evolution is frequently observed among host and parasites such as lice, mites and flukes (Kellog, 1913; Hegner, 1928; Barker, 1994; Fain, 1994; Gibson & Bray, 1994), yeasts of cacti (Starmer *et al.*, 1978), oncornaviruses of primates (Benveniste & Todaro, 1977), digeneans of crocodilians (Brooks, 1979) and bacteria of nematodes (Fischer-Le Saux *et al.*, 1998).

At present, it is not known at what time the genus *Staphylococcus* evolved. The finding of *Staphylococcus succinus* in 25 to 40 million-year-old amber (Lambert

et al., 1998) shows that staphylococci have been present for several million years. The Canoidea separated into the different families and subfamilies between 40 and 50 million years ago (Wayne, 1993). If the genetic diversity of *S. intermedius* from different groups is a result of co-evolution, these clusters must have separated at the same time. Regardless of whether the clusters observed are a result of co-evolution or adaptation, the differences in the genotypes of *S. intermedius* found among the different groups, based on *EcoRI* ribotyping, indicate a high degree of host specificity. If the finding of the present study can be confirmed using other methods and including more isolates, the species *S. intermedius* might be divided into subgroups or subspecies.

DNA–DNA homology studies on *S. intermedius* from horse, pigeons, mink and dogs have previously showed higher homology between isolates obtained from the same host species compared to that observed between hosts (Meyer & Schleifer, 1978). However, the number of isolates examined in that study was too low to make any firm conclusions.

In addition to *Staphylococcus aureus* and *S. intermedius*, two other coagulase-positive staphylococcal species have been isolated from members of the Canoidea; *S. schleiferi* subsp. *coagulans* from dogs (Igimi *et al.*, 1990) and *S. lutrae* from otters (Foster *et al.*, 1997). The *S. intermedius* isolates in this study differed biochemically from *S. schleiferi* subsp. *coagulans* in the ability to produce acid from trehalose and from *S. lutrae* in the inability to produce acid from xylose. In addition, ribotyping revealed a distant relationship to these species.

The *S. intermedius* type strain clustered separately from the other *S. intermedius* isolates examined in this study. This confirms the results of previous studies that have indicated that the *S. intermedius* type strain may not be representative of the species (Chesneau *et al.*, 2000).

It has not been possible for the author to isolate *S. intermedius* from members of the seal family (Phocidae). If the theory of co-evolution holds true, an *S. intermedius* subgroup might be present on seals. However, seals live under environmental conditions that are so different from those experienced by the other members of the Canoidea that *S. intermedius* might have been lost during evolution.

In conclusion, this study indicates host-specificity of different types of *S. intermedius* and suggests co-evolution between animal hosts within the Canoidea and *S. intermedius*.

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