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# Genomic Relationships between *Enterococcus faecium* Strains from Different Sources and with Different Antibiotic Resistance Profiles Evaluated by Restriction Endonuclease Analysis of Total Chromosomal DNA Using *Eco*RI and *Pvu*II

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**Forty-seven *Enterococcus faecium* strains from different sources were evaluated by restriction endonuclease analysis (REA) of total chromosomal DNA. Strains from chicken, pork, and humans were clearly divided into separate clusters, whereas strains from different countries, strains with different antibiotic resistance profiles, or clinical and healthy-subject strains were not.**

Infections by *Enterococcus* spp. have become increasingly important during the last decade (14, 24), and there has been much interest in *Enterococcus faecium* since it is prone to take up antibiotic resistance genes (5, 6, 16). Furthermore, enterococci are infamous for their ability to rapidly transfer their resistance genes to other enterococci (14), as well as to bacteria belonging to other genera (12, 17).

It has been proposed that strains are spread from animals to humans (1–3), and since the use of growth promoters and antimicrobial agents in animal husbandry often selects for *E. faecium* (9, 10, 15), it is important to clarify the role of *E. faecium* strains of animal origin in human infections.

*E. faecium* is a homogeneous species (19), and separation of strains belonging to this species requires methods with high discriminatory powers. It has been proposed that restriction endonuclease analysis (REA) is the best method to separate *E. faecium* strains (25), and this method has been used by other workers (7, 11). In these studies, however, only the ability of REA to separate strains and not the ability to reveal relationships between strains was investigated. In the present study, two separate restriction endonuclease digestions were used, compared to the one digestion used in the previous studies (7, 11). Also, use of a computer-based interpretation system allowed us to analyze gels with higher complexity, which meant that a broader range of DNA fragments could be included in the analysis compared to other studies (7, 11).

The aim of this study was to clarify the genomic relationships among *E. faecium* strains from different sources and with different levels of antibiotic resistance. REA of total chromosomal DNA with frequently cutting endonucleases was used due to its great capacity to resolve organisms at the strain level (8).

The strains included in this study are listed in Table 1. All of the strains were previously identified as *E. faecium* strains with methods other than REA (18, 19; data not shown). The test strains were obtained from different specimens and at different times.

The strains were grown overnight in 50 ml of All Purpose Tween broth (Difco Laboratories, Detroit, Mich.). The cells were washed, resuspended in Tris-EDTA buffer (10 mmol of Tris liter$^{-1}$, 1 mmol of EDTA liter$^{-1}$; pH 8.3), and stored at −20°C.

The chromosomal DNA was prepared by enzymatic cell lysis, phenol-chloroform extraction, and dye-buoyant density centrifugation gradient, as previously described (23). This method removed most of the plasmid DNA but left the chromosomal DNA intact.

Restriction endonuclease digestion was performed as described previously for *Lactobacillus* digestion (23), except that two rather than three restriction endonucleases were used to digest the DNA. Two separate digestions were performed, one with *Eco*RI and one with *Pvu*II (Boeringer-Mannheim Scandinavia, Bromma, Sweden). The DNA fragments were visualized by electrophoresis on a submerged 0.9% agarose gel, stained with ethidium bromide, and photographed as previously described for an analysis of *Lactobacillus plantarum* (8).

The gel images were scanned into a computer. Combined gel lanes consisting of the *Eco*RI digestion results and the *Pvu*II digestion results in sequence were constructed, the lanes were compared by using the pattern recognition technique (Pearson coefficient), and dendrograms based on the unpaired group method using arithmetic averages (UPGMA) were constructed. All this was done by using GelCompar 4.0 software (Applied Maths, Kortrijk, Belgium).

Eleven clusters and seven single strains were identified at a similarity level of 45% in the UPGMA dendrogram based on the combined REA profiles obtained from digestion with *Eco*RI and digestion with *Pvu*II (Fig. 1). All but two of the strains tested could be clearly separated. The method exhibited good reproducibility (typically, 90 to 95% similarity) (data not shown); thus, the reproducibility was about the same as that previously obtained with lactobacilli (8). The source of isolation (human, chicken, or pig) could be determined in the clusters obtained from the REA profiles, whereas the specific source of isolation (e.g., feces, blood, or urine) could not be determined (Table 1 and Fig. 1). Furthermore, the antibiotic resistance profiles of the strains (Table 1) were not reflected in the dendrogram, and human strains from healthy persons and clinical specimens did not form separate clusters.

The method used to prepare DNA for the REA included a large number of steps for obtaining pure, unfragmented, essentially plasmid-free DNA. The efficiency of plasmid DNA removal by this DNA preparation method was demonstrated

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TABLE 1. Clusters of *E. faecium* strains obtained at the 45% similarity level: sources of strains, identification method(s) used, and antibiotic resistance

| Cluster | Isolate | Source[a] | Identification method(s)[b] | Antibiotic resistance[c] |
|---|---|---|---|---|
| I | SE ch 4c | Swedish chicken | phen + RAPD | TC |
| I | SE ch 17d | Swedish chicken | phen + RAPD | TC |
| I | DK ch 5a | Danish chicken | phen + RAPD | EM, VA |
| I | DK ch 6b | Danish chicken | phen + RAPD | EM, PV, VA |
| II | NCC 218[d] | The Netherlands, human, hosp | RAPD | EM |
| II | SE hum B42[d] | Sweden, human blood, hosp | phen + RAPD | PV |
| II | DK hum 5/3b[d] | Denmark, human feces, healthy | RAPD | EM |
| II | DK hum 27b[d] | Denmark, human feces, healthy | RAPD | NX |
| II | DK hum 24a[d] | Denmark, human feces, healthy | RAPD | EM, NX |
| II | CCUG 542[Te] | Type strain, human feces | phen + RAPD | |
| II | SE hum B50[d] | Sweden, human feces, hosp | phen + RAPD | TC |
| II | G | Danish probiotic yoghurt | phen + RAPD | |
| II | DK hum 19a[d] | Denmark, human feces, healthy | RAPD | NX, VA |
| III | SE hum B36[d] | Sweden, human urine, comm | phen + RAPD | AM, EM, NX, PV, TC |
| III | SE humET45[d] | Sweden, human urine, comm | phen + RAPD | AM, EM, NX, PV, TC |
| III | SE hum B49[d] | Sweden, human wound, comm | phen + RAPD | AM, EM, PV, TC |
| III | SE hum B60[d] | Sweden, human feces, hosp | phen + RAPD | |
| III | SE humET33[d] | Sweden, human urine, hosp | phen + RAPD | AM, EM, PV |
| III | SE humET47[d] | Sweden, human urine, comm | phen + RAPD | AM, EM, PV, TC |
| III | SE hum multi[d] | Sweden, human blood, hosp | phen + RAPD | AM, CL, EM, PV, PP, TC, TS, VA |
| IV | SE hum ET144[d] | Sweden, human urine, hosp | phen + RAPD | AM, EM, NX, PV, TC |
| IV | SE hum 19a[d] | Sweden, human normal flora | phen + RAPD | |
| V | SE ch 14a | Swedish chicken | phen + RAPD | TC |
| V | SE ch 2a | Swedish chicken | phen + RAPD | NX, TC |
| VI | NCC 212[d] | Probiotic strain | RAPD | CL, EM, PV |
| VI | DK hum 30b[d] | Denmark, human feces, healthy | RAPD | EM |
| VI | MC74[d] | Probiotic strain | RAPD | EM |
| VI | SF68[d] | Probiotic strain | RAPD | EM |
| VI | DK hum 26/2a[d] | Denmark, human feces, healthy | RAPD | EM |
| VI | NCC 270[d] | Probiotic strain | RAPD | EM |
| VI | DK hum 23a[d] | Denmark, human feces, healthy | RAPD | EM, PV |
| VII | DK hum 12a[d] | Denmark, human feces, healthy | RAPD | EM |
| VII | DK hum 20/2b[d] | Denmark, human feces, healthy | RAPD | NX |
| VIII | DK hum 1a[d] | Denmark, human feces, healthy | RAPD | EM |
| VIII | DK hum 17a[d] | Denmark, human feces, healthy | RAPD | EM |
| IX | DK po 4d | Danish pork | phen + RAPD | |
| IX | DK po 24a | Danish pork | phen + RAPD | EM |
| X | DK hum 22/2a[d] | Denmark, human feces, healthy | RAPD | CL, EM, TC |
| X | DK hum 11a[d] | Denmark, human feces, healthy | RAPD | NX |
| XI | DK hum 3b[d] | Denmark, human feces, healthy | RAPD | TC |
| XI | DK hum 8b[d] | Denmark, human feces, healthy | RAPD | NX |
| Straggler | DK hum 25a[d] | Denmark, human feces, healthy | RAPD | EM, PV, TC |
| Straggler | DK ch 6d | Danish chicken | phen + RAPD | EM, PV, VA |
| Straggler | DK ch 7c | Danish chicken | phen + RAPD | EM, VA |
| Straggler | DK po 5d | Danish pork | phen + RAPD | |
| Straggler | SE po 1c | Swedish chicken | phen + RAPD | |
| Straggler | DK po 24b | Danish pork | phen + RAPD | |
| Straggler | DK hum 18b[d] | Denmark, human feces, healthy | RAPD | |

[a] hosp, hospitalized patient; comm, community-based patient; healthy, healthy individual.
[b] Identification procedures were performed by workers in our laboratory. phen, phenotypic identification; RAPD, randomly amplified polymorphic DNA identification.
[c] AM, ampicillin; CL, chloramphenicol; EM, erythromycin; NX, norfloxacin; PP, piperacillin; PV, penicillin V; TS, trimethoprim-sulfamethoxazole; TC, tetracycline; VA, vancomycin.
[d] Received as *E. faecium* (phenotypically identified).
[e] Identified as *E. faecium* by workers at the Culture Collection, University of Gothenburg, Gothenburg, Sweden.

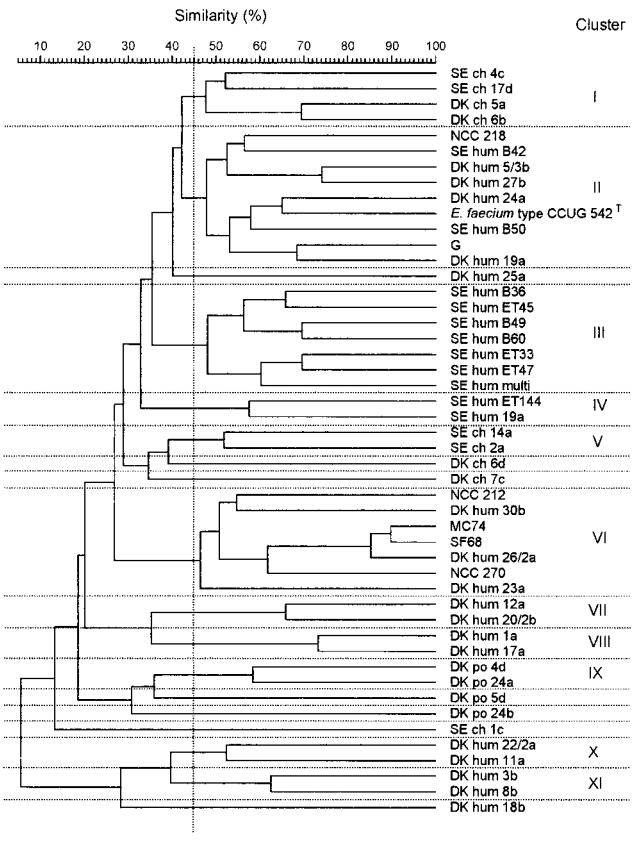


FIG. 1. UPGMA dendrogram based on the REA profiles of *E. faecium* strains from different sources.

by the fact that the existence of antibiotic resistance genes in many of the strains tested did not affect the clustering (Table 1 and Fig. 1).

The question of whether *Enterococcus* strains and/or their antibiotic resistance genes are spread between animals and humans has not been clearly answered. Strong evidence that animal enterococci are capable of infecting humans has been presented by Das et al. (3). Several other workers have also suggested that resistant strains are spread from animals to humans (1, 2, 13). Seyed-Akhavani et al. (21) have suggested the possibility that resistance is spread via plasmid transfer from resistant animal strains to previously susceptible human strains. Indeed, the fact that human *E. faecium* strains are able to receive resistance genes from donor strains from chickens has been demonstrated. For instance, strains DK ch 6d and DK ch 5a, which were used in this investigation, were found to transfer both erythromycin resistance and vancomycin resistance to strain CCUG 542 (18) and to strain G (19a). There are also workers who claim that they have not been able to find any valid evidence for the spread of strains or resistance from animals to humans (4, 20).

The present finding that strains cluster according to their hosts suggests that the strains are host specific. Such specificity has also been observed in *Lactobacillus reuteri* strains by Ståhl and Molin, who found that strains from humans or pigs could be separated from strains from rats by REA (22). The fact that no strains from animals were found to cluster together with human strains in the present study does not necessarily mean that animal-to-human spread does not occur. In the present study, 48 isolates representing only a minute part of the immense number of strains occurring in nature were investigated.

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