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Sensitivity to detergents and plasmid curing in *Enterococcus faecalis*

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Abstract This research reports the sensitivity of a clinical isolate of *Enterococcus faecalis* to sodium N-lauroylsarcosinate (sarkosyl) and sodium dodecyl sulfate (SDS), as well as the efficiency of these detergents in curing the strain. Compared to *Escherichia coli*, *Enterococcus faecalis* was very sensitive to both detergents, with minimum inhibitory concentrations (MIC) for the latter being 100 times lower than for *Escherichia coli*. The clinical isolate of *Enterococcus faecalis* used in this study exhibited plasmid-borne resistance to kanamycin (MIC 2 mg/ml) and tetracycline (MIC 50 μg/ml); 3% curing was observed after growth in the presence of sarkosyl but no curing was observed after growth in the presence of either SDS or acridine orange. In contrast, 35% curing of plasmid-bearing *Escherichia coli* was observed after growth in the presence of either SDS or acridine orange, but none was observed after growth in the presence of sarkosyl.

Keywords Antibiotic sensitivity · Detergent sensitivity · Curing · *Enterococcus faecalis* · *Escherichia coli*

Introduction

Enterococcus faecalis, a Gram-positive bacterium, is ubiquitous in the environment. It is a normal inhabitant of the human gastrointestinal tract and it is also found in water, soil, vegetation, food, and the gastrointestinal tract of a variety of other organisms besides humans [9, 15, 19, 20]. It has been used in probiotic cultures such as cheese and other milk product starters [7]. Although for a long time it was not considered as posing any threat to human health [17], *Ent. faecalis* has since been recognized as the leading cause of nosocomial infections, and as the infectious agent in serious diseases such as endocarditis, meningitis, and others [8, 16, 22, 24, 26, 27, 29]. It is a hardy bacterium that can grow at temperatures ranging from 10 to 45°C and over a broad pH range [21]. It survives exposure at 60°C for 30 min and is able to grow in the presence of 6.5% NaCl [11]. Recovery of enterococci has also been reported after prolonged periods on environmental surfaces [23]. Furthermore, *Ent. faecalis* has been shown to adapt to lethal levels of bile salts and sodium dodecyl sulfate (SDS) after brief exposure to sub-lethal levels of either of these detergents [10]. These properties explain, at least in part, the role played by the bacterium in the spread of nosocomial infections. Of greater concern is that *Ent. faecalis* has acquired plasmid-mediated resistance to a number of antibiotics [5, 6, 28], not to mention that some strains also carry genes coding for antibiotic resistance in their genome [11, 15]. Pathogens originating from the native flora of patients are unlikely to possess resistance beyond that intrinsic to the genus [15]. Those that have acquired significant resistance, usually plasmid-mediated, to two or more antibiotics are more likely to cause nosocomial transmission. To rid them of the plasmid(s) they harbor would facilitate control of their propagation by restoring their sensitivity to antibiotics.

In this study, a clinical isolate of *Ent. faecalis* exhibiting resistance to kanamycin with a minimum inhibitory concentration (MIC) of 2 mg/ml and to tetracycline (MIC = 50 μg/ml) was used. The antibiotic resistances were found to be plasmid-mediated, and attempts were made to cure the strain. While the use of previously reported curing agents such as acridine orange or SDS was unsuccessful, the use of sodium N-lauroylsarcosinate (sarkosyl) proved to be useful for curing this *Ent. faecalis* strain.

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Materials and methods

Bacterial strains

The *Escherichia coli* strains HB101, HB101 (pBR322) and HB101 (pBR325) were provided by N.O. Keyhani, Department of Microbiology and Cell Sciences, University of Florida at Gainesville, FL. The *Ent. faecalis* strain used in this study was a clinical isolate provided by S. Lauwers from the Microbiology Department of the Vrije Universiteit Brussel University Hospital, Brussels, Belgium.

pBR322 is a 4.3 kb plasmid carrying Amp^R and Tet^R [4]; pBR325 is a 5.7 kb plasmid carrying Amp^R, Tet^R and Cm^R [3].

Chemicals

SDS, acridine orange, and sarkosyl were purchased from Sigma, Searle, and Fluka, respectively. All other chemicals were from Merck.

Culture media

Luria-Bertani (LB) broth and LB-agar plates were used throughout this study. Peptone, yeast extract and agar were from Merck; antibiotics were from Sigma. All media and antibiotics were prepared according to [25].

Antibiotic sensitivity and detergent sensitivity

Antibiotic sensitivity and detergent sensitivity were determined by the MIC method as described in [1].

Curing experiments

Curing of plasmid-carrying *Escherichia coli* and *Ent. faecalis* strains was attempted by growth in the presence of either acridine orange (final concentrations ranging from 25 to 200 μg/ml), SDS (final concentrations ranging from 0.01 to 6% for *Escherichia coli* strains and from 0.001 to 0.25% for the *Ent. faecalis* strain) or sarkosyl (final concentrations ranging from 0.25 to 10% for *Escherichia coli* strains and from 0.003 to 0.5% for the *Ent. faecalis* strain). Cured bacteria were identified by replica plating and further tested by culture in LB medium supplied with increasing antibiotic concentrations.

Plasmid DNA extraction and isolation

Plasmid DNA was extracted from plasmid-carrying strains either by the alkaline lysis method [2] or the boiling method [13]. In addition, for *Ent. faecalis*, a novel method similar to the boiling method but replacing lysozyme (10 mg/ml) with sarkosyl (10%) was used.

Transformation experiments

The *Escherichia coli* strain HB101 was transformed with plasmid DNA isolated from the *Ent. faecalis* strain used in this study by the calcium chloride procedure [25]. Transformed bacteria were identified by replica plating and further tested by culture in LB medium supplied with increasing antibiotic concentrations.

Agarose gel electrophoresis

Plasmid DNA preparations were electrophoresed in 1% agarose gels as described in [25], except that ethidium bromide was added to the electrophoresis buffer only, at a final concentration of 0.5 μg/ml. The agarose was type-II, low-endo-osmotic agarose from Merck. Gels were 8×10 cm.

Characterization of *Ent. faecalis*

The *Ent. faecalis* strain was tested for the presence of catalase by adding a few drops of 30% H_2O_2 to 0.1 ml bacterial suspension and watching for bubble formation. The *Escherichia coli* strains were used as catalase-positive controls. The *Ent. faecalis* strain was also tested for growth in the presence of 6.5% NaCl, growth at pH 9.6, and growth on bile-aesculin agar with black discoloration of the agar. Cells were also stained according to the Gram-stain procedure.

Results and discussion

The *Ent. faecalis* strain provided to us was Gram-positive and catalase-negative when tested as described in Materials and methods. It grew in the presence of 6.5% NaCl at pH 9.6 and also on bile-aesculin agar with black discoloration of the agar. Since *Ent. faecalis* has been found to develop resistance to SDS [10], we tested the sensitivity of this strain to SDS as well as to sarkosyl, another anionic detergent, and we compared it to the sensitivity of *Escherichia coli* to these detergents.

Detergent sensitivity

The *Ent. faecalis* strain was much more sensitive than *Escherichia coli* to both sarkosyl and SDS. No growth of *Ent. faecalis* was observed in the presence of 0.1% sarkosyl while growth of *Escherichia coli* remained unaffected even in the presence of 0.5% sarkosyl. The detergent MIC was 0.1% for *Ent. faecalis* and 10% for

Escherichia coli. The effect of SDS on the strains was similar to that of sarkosyl. While no growth was observed for *E. faecalis* in 0.05% SDS, *Escherichia coli* growth was still unaffected in the presence of 0.25% SDS. The MIC was 0.05% SDS for *Ent. faecalis* and 6% SDS for *Escherichia coli*.

The extreme sensitivity to detergents of an enteric Gram-positive bacterium compared to that of an enteric Gram-negative bacterium was not unexpected given the frequency of detergent resistance found in Gram-negative bacteria [18]. Incidentally, the SDS MIC against *Ent. faecalis* identified in this study was three times higher than that reported by others (0.017%; [10]). The same group had shown induced tolerance of *Ent. faecalis* to their reported SDS MIC of 0.017%, and had also shown SDS-induced cross-protection and bile salts-induced cross-protection [10]. Since the strain used in this study was a clinical isolate, it may have benefited from bile salts-induced detergent tolerance, hence the higher SDS MIC observed. No further cross-protection was induced by sarkosyl.

High sensitivity to detergents provides a way of controlling pathogen spread, at least on environmental surfaces. Infected patients, however, must be treated with antibiotics; consequently, the resistance exhibited by pathogens must be examined carefully.

Antibiotic sensitivity

The *Escherichia coli* strains HB101, HB101 (pBR322), HB101 (pBR325), and the *Ent. faecalis* strain used in this study were tested for their sensitivity to kanamycin, tetracycline, ampicillin and chloramphenicol.

Ent. faecalis exhibited resistance to kanamycin (MIC 2,000 μg/ml) and tetracycline (MIC 50 μg/ml) but not to ampicillin (MIC 5 μg/ml) and chloramphenicol (MIC 5 μg/ml). The MICs of the four antibiotics for the *Escherichia coli* strains tested are given for comparison. For *Escherichia coli* strain HB101, which was sensitive to all four antibiotics, MICs were 50 μg/ml for kanamycin, 2 μg/ml for tetracycline, 10 μg/ml for ampicillin, and 10 μg/ml for chloramphenicol. As expected, plasmid pBR322 conferred resistance to tetracycline (MIC 100 μg/ml) and ampicillin (MIC 3,000 μg/ml) while plasmid pBR325 conferred resistance to tetracycline (MIC 100 μg/ml), ampicillin (MIC 1,000 μg/ml) and chloramphenicol (MIC 100 μg/ml) to strain HB101.

Ent. faecalis is reputed to be sensitive to ampicillin, making the latter a medication that was often used against the bacterium in a number of infections [15]. Tetracycline resistance in *Ent. faecalis* has been reported to be encoded within its genome [7, 15] but also on a 58 kb conjugative plasmid [5]. Resistance to aminoglycosides (e.g., kanamycin) has been attributed to the ability of enterococci to block the uptake of the drug at the cell wall [12]. However, resistance to high levels of aminoglycosides has been reported to be plasmid-borne [14]. Thus we investigated the presence of plasmid(s) in the *Ent. faecalis* strain under study using rapid plasmid isolation techniques.

Rapid plasmid isolation

Use of the alkaline lysis method or the boiling method [25] gave consistently good results for plasmid isolation from HB101 (pBR322) and HB101 (pBR325) as tested by electrophoresis in 1% agarose gels. When the alkaline lysis method was used for plasmid isolation from *Ent. faecalis*, the resulting preparation produced only a faint band in agarose gels in one out of three preparations (Fig. 1a, lanes 2, 3). When the boiling method was used, one band was almost always detectable in agarose gels (Fig. 1b, lanes 1, 2). However, when we modified the boiling method by replacing the lysozyme with sarkosyl, the plasmid DNA preparation obtained consistently showed two sets of bands, revealing the presence of two plasmids in the *Ent. faecalis* strain studied (Fig. 1c, lanes 1, 2). Thus, using a novel rapid plasmid isolation method involving sarkosyl, two small plasmids (~7 and 5.7 kb), conferring kanamycin and tetracycline resistance, were found in this strain of *Ent. faecalis*. The plasmid DNA

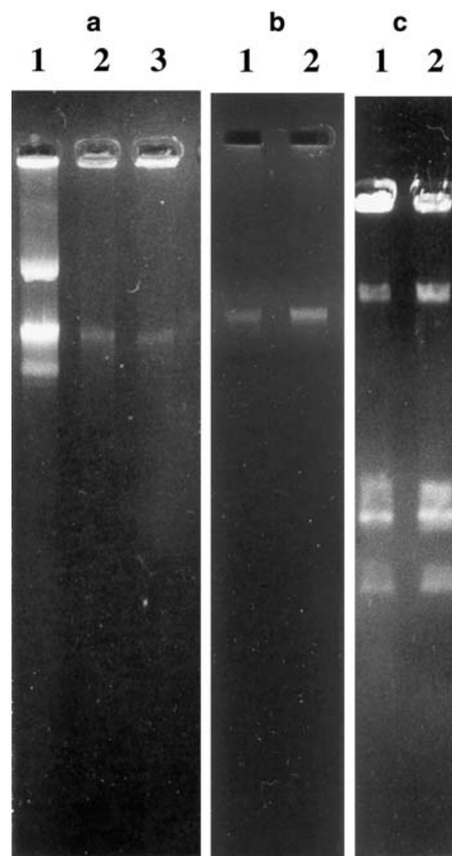

Fig. 1a–c Agarose gel electrophoresis of plasmid DNA preparations. **a** Alkaline lysis method. Lanes: *1* pBR322; *2, 3 Enterococcus faecalis* plasmid. **b** Boiling method. Lanes: *1, 2 Ent. faecalis* plasmid. **c** Novel method using sarcosyl. Lanes: *1, 2 Ent. faecalis* plasmids (~7 and 5.7 kb, respectively)

thus prepared was used to transform *Escherichia coli* strain HB101 in order to further establish that the antibiotic resistance exhibited by *Ent. faecalis* was plasmid-mediated.

Transformation of *Escherichia coli* strain HB101 with *Ent. faecalis* plasmid

Escherichia coli strain HB101 was transformed with the plasmid DNA preparation obtained using the novel method described from *Ent. faecalis*. Transformed colonies that were resistant to kanamycin (MIC 2,000 μg/ml) and tetracycline (MIC 50 μg/ml) were obtained.

Having established that the *Ent. faecalis* strain under study had acquired plasmid-borne antibiotic resistance, the efficiency of detergents in curing the strain was investigated and compared to that of acridine orange, a well known curing agent.

Curing

Under our experimental conditions, growth in various concentrations of acridine orange led to the cure of plasmid-carrying *Escherichia coli* strains HB101 (pBR322) and HB101 (pBR325) but not of *Ent. faecalis* (Table 1).

Growth in SDS led only to the cure of HB101 (pBR322); not a single cured colony was found out of 500 HB101 (pBR325) colonies or *Ent. faecalis* colonies tested (Table 1). However, growth in sarkosyl produced a small number of cured *Ent. faecalis* colonies; no cured *Escherichia coli* colony was found out of 500 tested for each strain (Table 1). Thus, sarkosyl was much more efficient than SDS in curing *Ent. faecalis*. Compared to *Escherichia coli* strain HB101, the cured *Ent. faecalis* strain was more sensitive to ampicillin (MIC 5 μg/ml compared to 10 μg/ml against HB101) and chloramphenicol (MIC 5 μg/ml compared to 10 μg/ml against HB101), had the same sensitivity to kanamycin (MIC 50 μg/ml), but retained some resistance to tetracycline (MIC 25 μg/ml compared to 2 μg/ml against HB101). Compared to the original *Ent. faecalis* strain, the kanamycin MIC against the cured strain was 40 times less (MIC 50 μg/ml compared to 2,000 μg/ml against the original strain) while the tetracycline MIC was halved (MIC 25 μg/ml compared to 50 μg/ml against the original strain); ampicillin and chloramphenicol MICs were the same for both the original and cured *Ent. faecalis* (5 μg/ml in all cases).

Attempts to isolate plasmids from the cured *Ent. faecalis* strain were unsuccessful. The preparations obtained using the rapid plasmid isolation method described in this paper no longer gave any plasmid band in agarose gels and would not transform *Escherichia coli* strain HB101.

Conclusions

We have established the sensitivity of a clinical isolate of *Ent. faecalis* to two anionic detergents, sarkosyl (MIC 0.1%) and SDS (MIC 0.05%), and its resistance to kanamycin (MIC 2 mg/ml) and tetracycline (MIC 50 μg/ml). We developed a novel and efficient method involving the use of sarkosyl for rapid plasmid isolation from the strain studied. We established that resistance to kanamycin and tetracycline is carried on small plasmids (~7 and 5.7 kb) in this *Ent. faecalis* isolate. While attempts to cure the strain with SDS or acridine orange remained unsuccessful, we were able to cure the strain with sarkosyl, reducing the MIC of kanamycin from 2 mg/ml to 50 μg/ml and that of tetracycline from 50 to 25 μg/ml. Thus the use of sarkosyl proved to be very efficient in handling *Ent. faecalis*, a Gram-positive bacterium responsible for nosocomial and other serious infections. In addition to facilitating rapid plasmid isolation from the strain, sarkosyl was a curing agent while SDS and acridine orange were not.

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Table 1 Curing effects of acridine orange, sodium dodecyl sulfate (SDS) and sodium N-lauroylsarcosinate (sarkosyl)

Strain	Percentage cured colonies produced after growth in the following concentrations[a]							
Acridine orange (μg/ml)	**25**	**50**	**75**	**100**	**150**	**200**		
HB101 (pBR322)	0	26	26	35	0	0		
HB101 (pBR325)	0	0	15	15	0	0		
Enterococcus faecalis	0	0	0	0	NG[b]	NG		
SDS (%)	**0.001**	**0.005**	**0.01**	**0.05**	**0.25**	**0.5**	**1**	**2**
HB101 (pBR322)	–	–	0	0	27	27	35	0
HB101 (pBR325)	–	–	0	0	0	0	0	0
Ent. faecalis	0	0	0	NG	NG	NG	NG	NG
Sarkosyl (%)	**0.005**	**0.02**	**0.1**	**0.5**	**1**	**2**	**4**	
HB101 (pBR322)	0	0	0	0	0	0	0	
HB101 (pBR325)	0	0	0	0	0	0	0	
Ent. faecalis	0	3	NG	NG	NG	NG	NG	

[a]Five-hundred colonies tested for each strain
[b]No growth

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