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Characterization of Plasmids Determining Hemolysin and Bacteriocin Production in *Streptococcus faecalis* 5952

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Two plasmids designated pOB1 and pOB2 were isolated from *Streptococcus faecalis* strain 5952 and found to have molecular weights of approximately 46 × 10^6 and 28 × 10^6, respectively. pOB1 was found to determine hemolytic activity and was transmissible, whereas pOB2 appeared to determine a bacteriocin that is specifically inhibitory to *S. faecalis* strains harboring the 26-megadalton plasmid pAM539.

We have recently isolated and characterized a plasmid designated pAM539, harbored by *Streptococcus faecalis* subsp. *liquefaciens* strain ND539 (4, 10). The plasmid was shown to confer sensitivity to a bacteriocin produced by *Streptococcus faecalis* strain 5952. The latter strain is hemolytic on horse blood agar (and thus corresponds to an *S. zymogenes* variant) and is a clinical isolate obtained from E. Britt of St. Joseph Mercy Hospital, Ann Arbor, Mich. In this communication, we examine the plasmid content of strain 5952 and show that the hemolytic and bacteriocin activities are plasmid associated.

The strains used in this study are listed in Table 1. The technical procedures and materials used were as detailed elsewhere (3, 6, 7, 10). For isotope labeling experiments, an M9-glucose medium supplemented with 0.3% yeast extract and 0.5% Casamino Acids (M9-YE) was used. Matings were done in Oxoid nutrient broth no. 2 (NB2).

Strain 5952 was grown in the presence of radioactive thymidine, harvested, and lysed by the lysozyme-Pronase-Sarskosyl procedure (6). Covalently closed circular plasmid deoxyribonucleic acid (DNA) was evident as a satellite component when the lysate was centrifuged to equilibrium in a CsCl-ethidium bromide buoyant density gradient. Table 2 summarizes the results of analyses of this DNA using sucrose density gradient centrifugation and electron microscopy (6). Two plasmids were identified with approximate molecular weights of 46 × 10^6 and 28 × 10^6 and were designated pOB1 and pOB2, respectively. Each of these plasmids was present in the cell to the extent of one to two copies per chromosomal genome equivalent.

Incubation of broth (NB2) cultures of strain 5952 at 45°C for several days resulted in the loss of hemolytic activity (as monitored on horse blood agar [7]) in as many as 3% of the surviving cells. DNA analyses of six such nonhemolytic derivatives showed that all six were devoid of pOB1 but retained pOB2. It appears, therefore, that pOB1 determines hemolysin synthesis—an observation that is not surprising in view of previous reports of the plasmid control of *S. faecalis* hemolysins (7, 8, 11). The strains devoid of pOB1 were found to still produce the bacteriocin to which *S. faecalis* strains harboring pAM539 are specifically sensitive. (This bacteriocin assay made use of strains RT1 and DT 34 [which are isogenic except for the presence or absence of pAM539] as indicators, as described in references 4 and 10.) This bacteriocin will subsequently be referred to as streptocin 101.

Mating experiments carried out as previously described (7), using strain 5952 as a donor and the plasmid-free strain JH2-2 as a recipient, resulted in hemolytic transconjugants that were subsequently shown to have received pOB1 but not pOB2. Such transconjugants were good donors of the hemolytic trait, thus establishing the transmissible nature of pOB1. These strains (i.e., harboring only pOB1) also failed to produce the bacteriocin activity to which pAM539 confers sensitivity.

We next tried to determine whether streptocin 101 was plasmid borne. A derivative of strain 5952 devoid of pOB1, designated as strain 5952-C1, was grown overnight in the presence of acridine orange or grown and incubated at 45°C (for a few days) in an effort to derive bacteriocin-negative variants; however, these attempts were unsuccessful. (The frequency of variants would have had to have been greater than 10^{-3}

TABLE 1. *Bacterial strains*

Strain	Plasmid content	Comments and/or related reference
5952	pOB1, pOB2	Hemolytic clinical isolate that produces a bacteriocin activity to which cells harboring plasmid pAM539 are sensitive
5952-C1	pOB2	Hemolytic-negative variant of 5952
5952-C1S	pOB2	Streptomycin-resistant mutant of 5952-C1
JH2-2	None	Has chromosomal mutations conferring resistance to rifampin and fusidic acid (9)
DT34	pAMγ1, pAMα1	JH2-2 transconjugant that received the two plasmids originally from DS-5 (6)
RT1	pAMγ1, pAMα1, pAM539	Essentially identical to DT34 except for the presence of pAM539
HD1	pAMγ1	JH2-2 transconjugant that received the plasmid from DS-5 (6)
DS-5	pAMγ1, pAMβ1, pAMα1	Original clinical isolate containing three plasmids (6)

TABLE 2. *Physical properties of plasmid DNA isolated from S. faecalis strain 5952*

Plasmid	s_{CCC}[a]	Mol wt × 10^6[b]	Contour length[c]	Mol wt × 10^6 from contour length	No. of copies/ chromosomal genome equivalent[d]
pOB1	65*s*	46.4	23.3 ± 0.83	45.4 ± 1.61	0.89
pOB2	52*s*	27.6	14.41 ± 0.41	28.0 ± 0.78	1.73

[a] Sedimentation constant of covalently closed circular (CCC) form.
[b] The equation $s_{CCC} = 0.037\ M^{0.428}$ was used to determine-molecular weight (2).
[c] Average of five molecules each ± standard deviation.
[d] Calculations were based on the assumption that the genome size of *S. faecalis* DNA is 1.47×10^9 daltons as determined by Bak et al. (1). The amount of CCC DNA recovered from the dye-CsCl gradients averages about 6.0%. Of this, the relative amounts of pOB1 and pOB2, as determined by sucrose-density gradient analysis, are 45 and 55%, respectively.

for us to detect them.) As another approach, we reasoned that we might be able to mobilize pOB2 by using the pAMγ1-pAMα1 transfer system (7) in an effort to construct an isogenic pair of strains that differed only with respect to the presence or absence of pOB2. (pAMγ1 is a 35-megadalton transmissible plasmid that determines hemolysin production [7], whereas pAMα1 is a 6-megadalton nontransmissible plasmid that determines tetracycline resistance [5]. This system has been used previously to construct isogenic pairs with or without pAM539 [10].) However, it was subsequently found that when pAMγ1 and pAMα1 were both introduced into strain 5952-C1S in a mating experiment (strain DT-34 was used as a donor), tetracycline-resistant, hemolytic transconjugants were found to be devoid of pOB2. (Three isolates were analyzed for plasmid content, and all three harbored only pAMγ1 and pAMα1.) These transconjugants also failed to exhibit streptocin 101 production. (The bacteriocin assay was carried out as described in reference 10.) In a different experiment, pAMγ1 alone was introduced into 5952-C1s, using donor strain HD1, which harbors only pAMγ1. Five hemolytic transconjugants were examined for plasmid content and streptocin 101 production. Three of the strains harbored both pAMγ1 and pOB2 and produced streptocin 101; however, two of the strains harbored only pAMγ1 and failed to produce streptocin 101. These data may be interpreted as follows. Plasmid pOB2 may be unstable in the presence of pAMγ1. Evidence against this, however, is represented by a failure to see instability upon further culturing of the transconjugants containing both plasmids. If unstable coexistence is involved, it may relate to a time during or immediately after transfer of pAMγ1 into the recipient. Alternatively, those transconjugants which lack pOB2 may represent a small fraction of the recipients that had previously lost (were spontaneously cured of) pOB2 and in turn somehow became preferred recipients. Another possibility is that pOB1 integrated into the chromosome and in so doing lost the ability to produce bacteriocin.

In summary, we have provided evidence that *S. faecalis* strain 5952 harbors two plasmids, one of which (pOB1) is transmissible and determines a hemolysin, whereas the other (pOB2) determines a bacteriocin that has a specific activity against strains harboring pAM539.

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