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A Transferable Plasmid Associated with AS-48 Production in *Enterococcus faecalis*

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***Enterococcus faecalis* S-48 produces a peptide antibiotic, AS-48, and a bacteriocin, Bc-48. We have isolated mutants that lack these inhibitory characteristics. Further analysis of the mutants indicates that a plasmid of 56 kilobases (pMB2) may harbor the genes for AS-48. In conjugation experiments, pMB2 has been transferred into a plasmid-free OG1X strain of *E. faecalis*. The OG1X(pMB2) transconjugant produces the antibiotic AS-48 in solid medium, and the MIC of AS-48 for this strain is the same as that of the donor strain.**

AS-48 is a peptide antibiotic produced by *Enterococcus faecalis* S-48, with a molecular mass of about 8,000 daltons (2, 4) and a broad inhibitory spectrum (3). Agents which activate the SOS repair system do not induce its synthesis (J. Lara, M. Martínez-Bueno, A. Gálvez, M. Maqueda, and E. Valdivia, Folia Microbiol., in press). In recent reports (3, 6, 7), we have described the bactericidal effect of AS-48, which acts primarily at the cytoplasmic membrane level.

In this paper, we report on the genetic location of the peptide antibiotic AS-48, which is encoded by a conjugative plasmid of 56 kilobases, pMB2, and is responsible for both AS-48 production and immunity. No other phenotypic characteristics associated with this plasmid have been described.

The bacterial strains used in this study, together with their phenotypic characteristics, are listed in Table 1.

Test for AS-48 production. Rapid AS-48 screening by cross-streaking on brain-heart agar (BHA) plates has been described previously (5).

Mutant isolation. The wild-type strain, S-48, was cured of AS-48 production by growth with plasmid-curing agents (M. Martínez-Bueno, A. Gálvez, M. Maqueda, and E. Valdivia, Folia Microbiol., in press). Colonies that did not produce inhibitory activity were isolated after replica plating. By continuous treatment with ethidium bromide, we have also obtained a high frequency (1.8%) of mutants lacking AS-48 (mutants B1 to B9). Their phenotypic characteristics are also shown in Table 1.

Plasmid isolation. Small-scale and preparative plasmid isolations were based on the method of Anderson and McKay (1). Agarose gel (0.6%) electrophoresis was performed according to the method of Maniatis et al. (10).

The plasmid profiles of the S-48 strain and its mutants appear in Fig. 1. All of the AS-48$^-$ strains isolated from the parent strain (mutants B1 to B9, A-48-15, A-48-9, B-48-2, and B-48-47) had lost a plasmid of approximately 56 kilobases (named pMB2) or had a new deleted plasmid (mutant B-48-28). In all cases, the mutants lacking the ability to produce AS-48 had also lost resistance to this antibiotic.

Furthermore, the nonbacteriocinogenic mutants A-48-32, B-48-2, and B-48-47 had deleted the pMB1 plasmid (Fig. 1, lanes B, G, and H).

Conjugal transfer of peptide antibiotic AS-48. The derivative nonbacteriocinogenic strain *E. faecalis* A-48-32 was used as a donor in mating experiments with a plasmid-free *E. faecalis* OG1X (Sm^r, gelatinase negative; 8) by the filter method of Reichelt et al. (11). Transconjugants were selected according to their resistance to streptomycin and AS-48.

The transfer frequency of AS-48 was 2.6×10^{-7} per donor cell. The new strains were identified as transconjugants of *E. faecalis* OG1X according to their resistance to AS-48 and streptomycin, nonproduction of gelatinase, and inhibition of the same strain (OG1X).

Mating experiments were also made in the presence of DNase I, and no reduction of transfer frequencies was observed. Neither transconjugant was detected in these experiments with chloroform-treated cells or cell-free supernatants, which is consistent with the transfer being by conjugation rather than transformation or transduction.

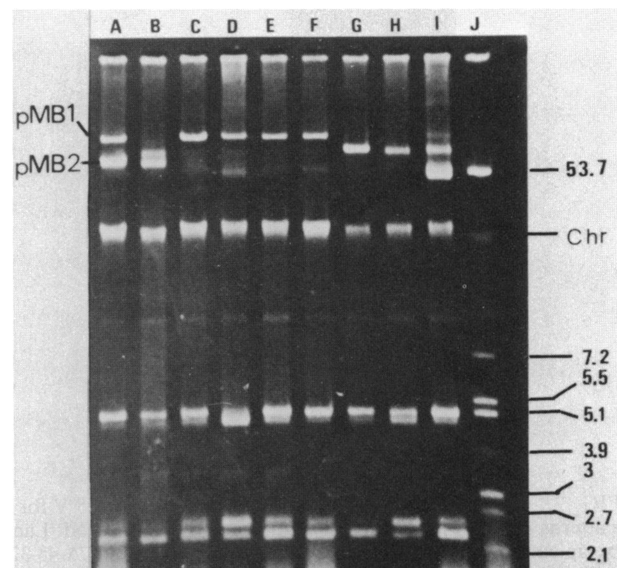

FIG. 1. Plasmid DNA profiles of *E. faecalis* S-48 and mutants. Lane A, *E. faecalis* S-48, wild-type strain; lane B, mutant A-48-32 (pMB1 deleted); lanes C to F, mutants B1, B2, A-48-9, and A-48-15 (cured of pMB2); lanes G and H, mutants B-48-2 and B-48-47 (pMB1 deleted and cured of pMB2); lane I, mutant B-48-28 (pMB1 deleted and cured of pMB2); lane J, *Escherichia coli* V517 reference plasmids. The numbers to the right denote the molecular sizes of the reference plasmids in kilobases. Chr, Position of chromosomal DNA. pMB1 and pMB2 are the plasmids of the wild-type strain S-48. The DNA plasmids were separated electrophoretically in 0.6% agarose gel at 75 V for 4 h.

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TABLE 1. Strains of *E. faecalis* used in this study

Strain	Relevant phenotype[a]	Source or reference
E. faecalis		
S-48	AS-48$^+$ Bac$^+$ Gel$^+$ Sm^s	4
A-48-32	AS-48$^+$ Bac$^-$ Gel$^+$ Sm^s	Martínez-Bueno[b]
B-48-2	AS-48$^-$ Bac$^-$ Gel$^+$ Sm^s	Martínez-Bueno[b]
B-48-47	AS-48$^-$ Bac$^-$ Gel$^+$ Sm^s	Martínez-Bueno[b]
B-48-28	AS-48$^-$ Bac$^+$ Gel$^+$ Sm^s	Martínez-Bueno[b]
A-48-9	AS-48$^-$ Bac$^+$ Gel$^+$ Sm^s	Martínez-Bueno[b]
A-48-15	AS-48$^-$ Bac$^+$ Gel$^+$ Sm^s	Martínez-Bueno[b]
B1 to B9	AS-48$^-$ Bac$^+$ Gel$^+$ Sm^s	This study
OG1X	AS-48$^-$ Gel$^-$ Sm^r	8
OG1X(pMB2)	AS-48$^+$ Gel$^+$ Sm^r	This study
E. coli V517	Plasmid marker	9

[a] AS-48$^+$, AS-48 producer; AS-48$^-$, AS-48 nonproducer; Bac$^+$, Bc-48 bacteriocin producer; Bc-48$^-$, Bc-48 bacteriocin nonproducer; Gel$^+$, gelatinase positive; Gel$^-$, gelatinase negative; Sm^r, streptomycin resistant; Sm^s, streptomycin sensitive.

[b] Martínez-Bueno et al., in press.

The plasmid profiles obtained by small-scale rapid screening of transconjugant (Fig. 2, lane C) showed that they all harbored one plasmid that comigrated with pMB2 from the donor strain (Fig. 2). Also, digestion of the pMB2 plasmid from A-48-32 and OG1X(pMB2) transconjugant strains with *Eco*RI showed identical fragment patterns on agarose gels (data not shown).

MIC testing and immunity. The MIC was defined as being the lowest concentration of AS-48 antibiotic preventing colony formation in BHA plates.

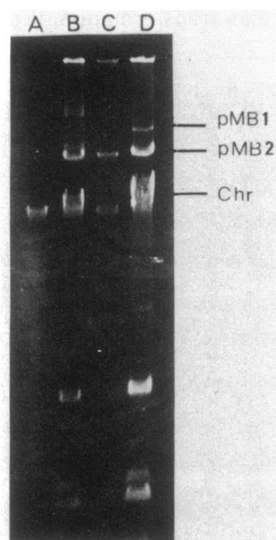

FIG. 2. Agarose gel electrophoresis (0.6% agarose at 75 V for 4 h) patterns of DNA plasmids isolated by small-scale method. Lane A, recipient *E. faecalis* OG1X; lane B, AS-48 gene donor, A-48-32; lane C, transconjugant OG1X(pMB2); lane D, the wild-type strain *E. faecalis* S-48. pMB2 plasmid is shown in lanes B to D. Chr, Chromosomal fragment area. pMB1 and pMB2 are S-48 plasmids.

The resistance of AS-48 expressed by the OG1X(pMB2) transconjugants was equivalent to that observed in the parent strain S-48 and its derivative donor strain, A-48-32 (>460 arbitrary units per ml). The mutants lacking the pMB2 plasmid had, however, also lost resistance to this antibiotic (MIC of 57 arbitrary units per ml). The OG1X parent strain was more sensitive to AS-48 (MIC of 11 arbitrary units per ml).

These results confirm that the genes for AS-48 are of a common origin and are harbored in the pMB2 plasmid. Thus, OG1X(pMB2) shows the same MIC as the producer donor strains and also overproduces the antibiotic AS-48 in solid medium.

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