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Cloning of an *Enterococcus faecalis* Endocarditis Antigen: Homology with Adhesins from Some Oral Streptococci

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Serum from a patient with *Enterococcus faecalis* endocarditis was used to identify the gene *efaA* cloned in Lambda ZapII in *Escherichia coli*. Nucleotide sequence analysis revealed a 924-bp open reading frame encoding a protein with a predicted molecular weight of 34,768. The amino acid sequence of EfaA shows 55 to 60% homology to a group of streptococcal proteins, FimA from *Streptococcus parasanguis*, SsaB from *Streptococcus sanguis*, ScaA from *Streptococcus gordonii*, and PsaA from *Streptococcus pneumoniae*. Members of this group have been shown to be adhesins, and we hypothesize that EfaA may function as an adhesin in endocarditis.

Enterococcus faecalis is a leading cause of endocarditis, accounting for up to 20% of cases of bacterial endocarditis (3, 21, 22). Recent data also suggest that enterococci are the second most commonly reported nosocomial pathogens, with *E. faecalis* accounting for 80 to 90% of all enterococcal infections (28). Comparative studies indicate that native heart valve endocarditis, particularly with involvement of the aortic valve, has a much higher mortality rate than prosthetic valve endocarditis (22).

Despite the aggressiveness of native valve endocarditis, little is known of the underlying pathogenic mechanisms, including the mechanism of adhesion to the valvular epithelium. Guzman et al. (13) have shown that *E. faecalis* strains isolated from urinary tract infections (UTIs) adhered more efficiently to urinary tract epithelial cells, whereas endocarditis isolates exhibited increased adhesion to heart cells. However, growth of the UTI isolates in serum increased adhesion to heart cells by 8- to 10-fold while causing only a 1.5- to 2-fold increase in adhesion to urinary tract cells. The adhesion of UTI isolates to heart cells was then similar to that of endocarditis isolates. Subsequent studies indicated that adhesion to heart cells was mediated principally by D-galactose- and L-fucose-containing residues on the surface of endocarditis isolates and UTI isolates grown in serum (14). These authors suggest that this explains the occurrence of heart infection after bacteremia, with persistence in blood being necessary for expression of endocarditis-specific adhesins.

E. faecalis produces a number of surface protein antigens which can be exploited in the serodiagnosis of *E. faecalis* endocarditis (1, 2, 5, 20). We have shown previously that three cell wall proteins with molecular sizes of 73, 40, and 37 kDa are prominent antigens which are expressed strongly following growth in serum. Antibodies towards them are found in patients with *E. faecalis* endocarditis but not in those with endocarditis due to other streptococci or with *E. faecalis* infections at other sites (2). These antigens have been purified and used in an enzyme-linked immunosorbent assay which could be useful in diagnosing *E. faecalis* endocarditis, particularly in culture-negative cases (26).

In an attempt to determine if the 37-, 40-, and 73-kDa surface antigens have a role in the pathogenesis of *E. faecalis* infective endocarditis, we have screened an expression library in *Escherichia coli* with serum from an endocarditis patient containing high immunoglobulin G titers against these antigens. Genomic DNA from *E. faecalis* EBH1 was extracted (27) and partially digested with *Alu*I, and a library was constructed in Lambda ZapII (Stratagene Cloning Systems, La Jolla, Calif.). Plaque lifts were screened with serum by the protocol described by Stratagene with the *pico*Blue immunoscreening kit. One plaque of 29 initial positives was identified by affinity purification (5). The plaque was purified, plated at high density, and transferred onto nitrocellulose. After blocking and incubation with antiserum, bound antibodies were eluted with 5 ml of 0.2 M glycine–0.15 M NaCl (pH 2.8) for 30 min. The buffer (5 ml) was aspirated, neutralized with 0.4 g of Tris, diluted 1 in 2 with 1% bovine serum albumin in Tris-buffered saline, and used to probe strip blots of *E. faecalis* whole-cell extracts. Affinity-purified antibodies reacting against this plaque reacted against the 37- and 40-kDa proteins from *E. faecalis* EBH1 (Fig. 1A). This plaque was rescued in vivo as a phagemid and designated pGP19. Whole-cell extracts of XL1-Blue/pGP19 with and without induction with 10 mM isopropyl-β-D-thiogalactopyranoside (IPTG) were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (19) on 12% gels and then Western blotting (immunoblotting) (30). One protein of approximately 40 kDa produced in the induced cells reacted strongly with serum from the endocarditis patient (Fig. 1B) and comigrated with the 40-kDa antigen from *E. faecalis* EBH1. The vector control consisting of XL1-Blue/pKS$^-$ was negative.

DNA was sequenced by the dideoxynucleotide chain termination method (25) with a Sequenase 2.0 kit (U.S. Biochemical) in accordance with the manufacturer's instructions. Reaction products were labelled with ^{35}S-ATP and fractionated on 6% polyacrylamide gels. Nucleotide sequence analysis of pGP19 (Fig. 2) revealed a 924-bp open reading frame encoding a protein with a predicted molecular weight of 34,768 and an isoelectric point of 5.04. The protein, designated *E. faecalis* antigen A (EfaA), is predicted to be hydrophilic (18) except for a putative hydrophobic leader sequence of 19 amino acids (Fig. 2). The mature, hydrophilic protein, with cleavage occurring at the peptide bond between the alanine and cysteine residues at positions 19 and 20, would have an M_r of 32,701. A typical GGAGG ribosome binding site (9) is present 9 bases upstream

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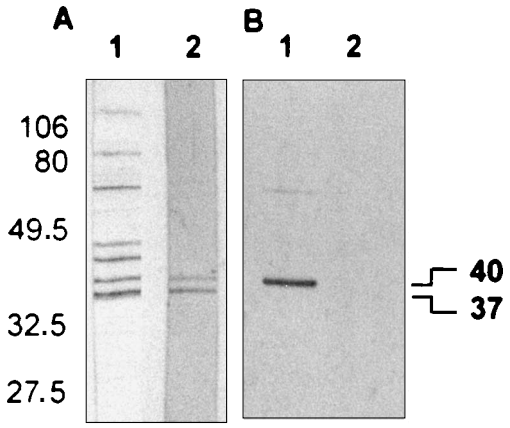

FIG. 1. (A) Immunoblot of *E. faecalis* EBH1 whole cells probed with serum from patient 1 with *E. faecalis* endocarditis (lane 1) and antibodies affinity purified from immunoreactive plaque (lane 2); (B) immunoblot analysis of whole-cell lysates from XL1-Blue/pGP19 (lane 1) and XL1-Blue/pSK$^-$ vector (lane 2), after 1 h of induction with 10 mM IPTG, probed with serum from patient 1 with *E. faecalis* endocarditis.

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          10         20         30         40         50         60
GAATTCCGGCCGGAATTCCGGCTTCTGGTGCGACGATTGTTTTAACCGCCGCCTTATTCT      60

TTTTATTGGCTTTCTTTTTCTCACCAAAGAAAGGCCTAGTATTTGTAAACCGTGAGAAAG     120

AAATGGAGGAATCAACGAATGAAAAAATTTAGTTTATTTTTTTTAACACTTTAGCAGGG     180
                  M  K  K  F  S  L  F  F  L  T  L  L  A  G

TTAACGTTAGCTGCTTGCGGGAATCAAGCCGCTGAAAAGAAAGAAAAATTAGCAATTGTG     240
L  T  L  A  A  C  G  N  Q  A  A  E  K  K  E  K  L  A  I  V

ACAACGAACTCGATCTTATCCGATTTAGTGAAAAATGTTGGGCAAGACAAAATTGAGCTG     300
T  T  N  S  I  L  S  D  L  V  K  N  V  G  Q  D  K  I  E  L

CATAGTATTGTGCCAATTGGGACAGACCCTCACGAATATGAACCGTTACCAGAAGACATT     360
H  S  I  V  P  I  G  T  D  P  H  E  Y  E  P  L  P  E  D  I

GCGAAAGCTTCTGAAGCGGACATTTTATTCTTTAACGGCTTGAACTTAGAAACAGGCGGA     420
A  K  A  S  E  A  D  I  L  F  F  N  G  L  N  L  E  T  G  G

AATGGCTGGTTTAACAAATTAATGAAAACGGCCAAAAAAGTTGAGAATAAAGATTACTTT     480
N  G  W  F  N  K  L  M  K  T  A  K  K  V  E  N  K  D  Y  F

TCTACAAGCAAAAATGTTACGCCACAATATTTAACAAGTGCCGGTCAAGAACAAACAGAA     540
S  T  S  K  N  V  T  P  Q  Y  L  T  S  A  G  Q  E  Q  T  E

GATCCGCATGCTTGGTTAGACATTGAAAATGGCATCAAATATGTAGAAAACATTCGTGAC     600
D  P  H  A  W  L  D  I  E  N  G  I  K  Y  V  E  N  I  R  D

GTGTTAGTAGAAAAAGATCCAAAAAATAAAGATTTCTATACAGAAAACGCGAAAAATTAT     660
V  L  V  E  K  D  P  K  N  K  D  F  Y  T  E  N  A  K  N  Y

ACCGAAAAACTTAGCAAACTACATGAGGAAGCCAAAGCTAAATTTGCTGATATTCCTGAT     720
T  E  K  L  S  K  L  H  E  E  A  K  A  K  F  A  D  I  P  D

GATAAAAAATTATTAGTTACAAGTGAAGGTGCCTTTAAATATTTCTCCAAAGCTTATGAT     780
D  K  K  L  L  V  T  S  E  G  A  F  K  Y  F  S  K  A  Y  D

TTAAATGCCGCTTATATTTGGGAAATTAACACAGAAAGTCAAGGAACACCTGAACAAATG     840
L  N  A  A  Y  I  W  E  I  N  T  E  S  Q  G  T  P  E  Q  M

ACCACGATTATTGATACCATTAAGAAATCAAAAGCACCTGTGTTATTTGTTGAAACCAGT     900
T  T  I  I  D  T  I  K  K  S  K  A  P  V  L  F  V  E  T  S

GTCGATAAACGTAGTATGGAACGGGTCTCAAAAGAAGTGAAACAGCCAATTTACGATACA     960
V  D  K  R  S  M  E  R  V  S  K  E  V  K  Q  P  I  Y  D  T

CTTTTCACAGACTCCCTTGCCAAAGAAGGAACAGAAGGCGATACGTACTACAGCATGATG    1020
L  F  T  D  S  L  A  K  E  G  T  E  G  D  T  Y  Y  S  M  M

AACTGGAATTTAACAAAAATCCATGATGGCTTAATGAGTAAATAAATAATAAAGAAGAAA    1080
N  W  N  L  T  K  I  H  D  G  L  M  S  K  *
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FIG. 2. Nucleotide sequence of the *E. faecalis* EBH1 *efaA* gene and deduced amino acid sequence of EfaA. A putative ribosome binding site is underlined. The predicated amino acid sequence of EfaA is shown in the one-letter code; an asterisk denotes a translational stop codon. The sequence will appear in the EMBL and GenBank nucleotide sequence data libraries under the accession number U03756.

of the ATG translation start codon, but no putative promoter sequences were identified. We were unable to confirm that the cloned gene product was the *E. faecalis* EBH1 40-kDa antigen by N-terminal amino acid sequence analysis because it was blocked. However, the two proteins comigrate with each other and are immunologically cross-reactive, and the cloned protein has been used in our serodiagnostic assay and was similarly able to discriminate between *E. faecalis* and non-*E. faecalis* endocarditis and *E. faecalis* infections at other sites (unpublished data).

Comparison of the 1,085-bp sequence with the GenBank and EMBL databases revealed four sequences which exhibited a high degree of homology. These were the type 1 fimbrial gene (*fimA*) from *Streptococcus parasanguis* FW213 (8), the *Streptococcus sanguis ssaB* gene encoding adhesin B (11), the *Streptococcus pneumoniae psaA* gene coding for pneumococcal surface adhesin A (24), and the *Streptococcus gordonii scaA* gene (17). An alignment of the deduced peptide sequences of EfaA, FimA, SsaB, PsaA, and ScaA is shown in Fig. 3. The amino acid homologies with EfaA were 60.5, 57.7, 60.5, and 55.5%, respectively, with an overall similarity of 71%. Studies of the functions of FimA and SsaB suggest that these proteins constitute a group of streptococcal adhesins involved in adhesion to components of the oral cavity. Antibodies directed against fimbriae isolated from *S. parasanguis* (*S. sanguis*) FW213, encoded by the *fimA* gene, inhibited binding of the organism to saliva-coated hydroxyapatite (7), while the purified SsaB protein has been shown to inhibit attachment of *S. sanguis* to saliva-coated hydroxyapatite (12). FimA, SsaB, and PsaA are 80 to 90% homologous and exhibit greatest diversity at their N-terminal ends. This is also true for comparisons with EfaA identified in this study (Fig. 3). However, all deduced proteins have the sequence Leu-X-X-Cys near the N-terminal end. Jenkinson (15), studying the adhesin SarA from *S. gordonii*, has identified this sequence as a lipoprotein consensus sequence and the site for cleavage of the signal peptide. Kolenbrander et al. (17) have recently reported the genetic organization of a 6-kb *Eco*RI fragment of *S. gordonii* PK488 chromosomal DNA, including *scaA*. The sequences of open reading frames ORF1 and ORF2, upstream of *scaA*, are consistent with their encoding an ATP-binding protein and hydrophobic membrane protein, respectively. The resultant genetic organization of ORF1, ORF2, and *scaA* suggests an ATP-binding cassette-lipoprotein-dependent transport system which is similar to the periplasmic-binding-protein-dependent transport systems of gram-negative bacteria. Open reading frames flanking *fimA* (10), *ssaB* (11), and *psaA* (24) show homology with the *scaA* operon. Analysis of the amino acid translation of the limited sequence data upstream of the *efaA* transcriptional start site at base 139 in this work does show homology to the open reading frames upstream of *fimA*, *psaA*, and *scaA* (data not shown). However, translation is in another reading frame and continues to a stop codon (TAG) at base 150, whereas the *fimA*, *psaA*, and *scaA* flanking open reading frames stop 12, 12, and 22 bases, respectively, upstream of the adhesin gene start sites. Although no transport functions have been assigned to these systems to date, Jenkinson (16) has proposed that they could function as adhesins if the lipoprotein ligand-binding compo-

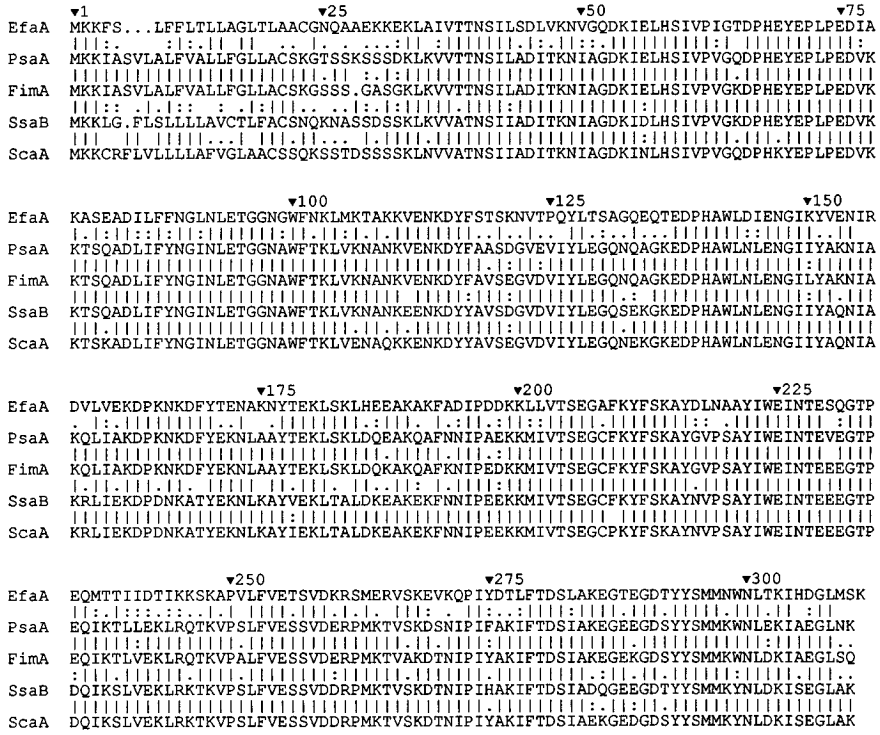

FIG. 3. Alignment of the amino acid sequences of *E. faecalis* EBH1 EfaA and the corresponding homologs *S. pneumoniae* PsaA, *S. parasanguis* FimA, *S. sanguis* SsaB, and *S. gordonii* ScaA. Colons represent highly conserved amino acids, and periods represent moderately conserved amino acids (6).

nent encounters its ligand in a complexed form immobilized on a cell surface.

Southern analysis of representative streptococci using an internal 0.4-kb *Hin*dIII fragment from *efaA* failed to give any hybridization even under low-stringency conditions (data not shown), suggesting that EfaA is not as closely related to FimA, SsaB, ScaA, and PsaA as they are to each other. Andersen et al. (4) found no hybridization with DNA from *E. faecalis* when probes from *scaA* and *ssaB* were used, and antiserum against the 38-kDa adhesin from *S. gordonii* also failed to react with sonicated cell extracts of *E. faecalis.*

Our previous Western blotting studies have shown that expression of the 37- and 40-kDa antigens is regulated by serum (2). To determine whether this regulation was at the transcriptional level, Northern (RNA) analysis was performed on total RNA extracted from *E. faecalis*. An overnight culture of *E. faecalis* EBH1 in 1% yeast extract (Difco) was diluted 1:100 into prewarmed medium and grown to the mid-log phase (optical density at 600 nm, 0.5). Total RNA was prepared by the hot phenol method (29), and Northern blotting was performed as described by Sambrook et al. (23) with a ^{32}P-labelled probe in 10 ml of Northern prehybridization buffer overnight at 45°C. The filters were washed twice for 5 min at room temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% SDS and then twice again for 15 min at 65°C in 0.1× SSC containing 0.1% SDS. The ^{32}P-labelled 0.4-kb internal *Hin*dIII fragment was generated with a kit based on random hexanucleotide primers (Boehringer-Mannheim) in accordance with the manufacturer's instructions. As shown in Fig. 4, no message was detected in cells grown in a simple yeast extract medium. However, 15 min after the addition of fetal calf serum to 1%, message was detected which continued to increase up to 2 h. Parallel Western blotting experiments indicated that expression of the 37- and 40-kDa antigens, detected with patient 1 antiserum, followed that of *efaA* message (data not shown). The nature of the inducing component in serum is unknown at present, although no message was detected when the serum was dialyzed against water, suggesting that the inducing component in serum is less than 10 kDa. The transcript size was approximately 3 kb and is similar to that reported for PsaA and FimA (10, 24). If these proteins are the lipoprotein ligand-binding components of ABC-type transport systems proposed Kolenbrander et al. (17), then it is possible that the individual components may be expressed from a single message. Analysis of the sequence data of Kolenbrander et al. (17) indicates that if ORF1, ORF2, *scaA*, and ORF4 are tran-

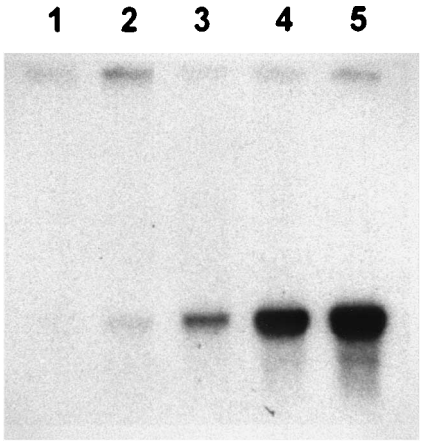

FIG. 4. Northern blot analysis of total RNA extracted from *E. faecalis* EBH1 grown in 1% yeast extract (lane 1) and after addition of 1% horse serum for 15 min (lane 2), 30 min (lane 3), 60 min (lane 4), and 120 min (lane 5).

scribed together, the message would be approximately 3.2 kb. However, a potential rho-independent terminator was identified immediately downstream of *scaA* (17), which would result in an approximately 2.5-kb message. Northern analysis using probes from the adjacent open reading frames is required to resolve this question.

In summary, we have cloned a gene from *E. faecalis* which encodes a dominant antigen in patients with infectious endocarditis and which shows homology to a family of adhesins from some oral streptococci. At present, we are performing experiments to determine if EfaA has a role in adhesion in endocarditis and to test the hypothesis that it forms part of a ligand-inducible ABC-type transport system.

Nucleotide sequence accession number. The nucleotide sequence in Fig. 2 has been submitted to the GenBank and EMBL databases and assigned the accession number U03756.

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REFERENCES

1. **Aitchison, E. J., P. A. Lambert, and I. D. Farrell.** 1986. Antigenic composition of an endocarditis-associated isolate of *Streptococcus faecalis* and identification of its glycoprotein antigens by ligand blotting with lectins. J. Med. Microbiol. **21:**161–167.
2. **Aitchison, E. J., P. A. Lambert, G. Smith, and I. D. Farrell.** 1987. Serodiagnosis of *Streptococcus faecalis* endocarditis by immunoblotting of surface protein antigens. J. Clin. Microbiol. **25:**211–215.
3. **Almirante, B., M. P. Tornos, M. Gurgui, M. Pujol, and J. M. Miro.** 1991. Prognosis of enterococcal endocarditis. Rev. Infect. Dis. **13:**1248–1249.
4. **Andersen, R. N., N. Ganeshkumar, and P. E. Kolenbrander.** 1993. Cloning of the *Streptococcus gordonii* PK488 gene, encoding an adhesin which mediates coaggregation with *Actinomyces naeslundii* PK606. Infect. Immun. **61:** 981–987.
5. **Burnie, J. P., and I. Clark.** 1989. Diagnosing endocarditis with the cloned 112 kDa antigen of *Enterococcus faecalis*. J. Immunol. Methods **123:**217–225.
6. **Dayhoff, M. O., R. V. Eck, and C. M. Park.** 1972. A model of evolutionary change in proteins, p. 89–99. *In* M. O. Dayhoff (ed.), Atlas of protein sequence and structure. National Biomedical Research Foundation, Silver Spring, Md.
7. **Fachon-Kalweit, S., B. L. Elder, and P. Fives-Taylor.** 1985. Antibodies that bind to fimbriae block adhesion of *Streptococcus sanguis* to saliva-coated hydroxyapatite. Infect. Immun. **48:**617–624.
8. **Fenno, J. C., D. J. LeBlanc, and P. Fives-Taylor.** 1989. Nucleotide sequence analysis of a type I fimbrial gene of *Streptococcus sanguis* FW213. Infect. Immun. **57:**3527–3533.
9. **Ferretti, J. J., and R. Curtiss III.** 1987. Compilation of nucleotide sequences that signal the initiation of transcription and translation in streptococci, p. 293. *In* J. J. Ferretti and R. Curtiss III (ed.), Streptococcal genetics. American Society for Microbiology, Washington, D.C.
10. **Fives-Taylor, P., J. C. Fenno, E. Holden, L. Linehan, L. Oligino, and M. Volansky.** 1991. Molecular structure of fimbriae-associated genes of *Streptococcus sanguis*, p. 240–243. *In* G. M. Dunny, P. P. Cleary, and L. L. McKay (ed.), Genetics and molecular biology of streptococci, lactococci, and enterococci. American Society for Microbiology, Washington, D.C.
11. **Ganeshkumar, N., P. M. Hannam, P. E. Kolenbrander, and B. C. McBride.** 1991. Nucleotide sequence of a gene coding for a saliva-binding protein (SsaB) from *Streptococcus sanguis* 12 and possible role of the protein in coaggregation with actinomyces. Infect. Immun. **59:**1093–1099.
12. **Ganeshkumar, N., M. Song, and B. C. McBride.** 1988. Cloning of a *Streptococcus sanguis* adhesin which mediates binding to saliva-coated hydroxyapatite. Infect. Immun. **56:**1150–1157.
13. **Guzman, C. A., C. Pruzzo, G. Lipira, and L. Calegari.** 1989. Role of adherence in pathogenesis of *Enterococcus faecalis* urinary tract infection and endocarditis. Infect. Immun. **57:**1834–1838.
14. **Guzman, C. A., C. Pruzzo, M. Plate, M. C. Guardati, and L. Calegari.** 1991. Serum dependent expression of *Enterococcus faecalis* adhesins involved in the colonisation of heart cells. Microb. Pathog. **11:**399–409.
15. **Jenkinson, H. F.** 1992. Adherence, coaggregation, and hydrophobicity of *Streptococcus gordonii* associated with expression of cell surface lipoproteins. Infect. Immun. **60:**1225–1228.
16. **Jenkinson, H. F.** 1994. Adherence and accumulation of oral streptococci. Trends Microbiol. **2:**209–212.
17. **Kolenbrander, P. E., R. N. Andersen, and N. Ganeshkumar.** 1994. Nucleotide sequence of the *Streptococcus gordonii* PK488 coaggregation adhesin gene and ATP-binding cassette. Infect. Immun. **62:**4469–4480.
18. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Biol. Chem. **157:**105–132.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
20. **Lambert, P. A., P. J. Shorrock, E. J. Aitchison, P. A. G. Domingue, M. E. Power, and J. W. Costerton.** 1990. Effect of *in-vivo* growth conditions upon expression of surface protein antigens in *Enterococcus faecalis*. FEMS Microbiol. Immunol. **64:**51–54.
21. **Megran, D. W.** 1992. Enterococcal endocarditis. Clin. Infect. Dis. **15:**63–71.
22. **Rice, L. B., S. B. Calderwood, G. M. Eliopoulos, B. F. Farber, and A. W. Karchmer.** 1991. Enterococcal endocarditis: a comparison of prosthetic and native valve disease. Rev. Infect. Dis. **13:**1–7.
23. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Sampson, J. S., S. P. O'Connor, A. R. Stinson, J. A. Tharpe, and H. Russell.** 1994. Cloning and nucleotide sequence analysis of *psaA*, the *Streptococcus pneumoniae* gene encoding a 37-kilodalton protein homologous to previously reported *Streptococcus* sp. adhesins. Infect. Immun. **62:**319–324.
25. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
26. **Shorrock, P. J., P. A. Lambert, E. J. Aitchison, E. G. Smith, I. D. Farrell, and E. Gutschik.** 1990. Serological response in *Enterococcus faecalis* endocarditis determined by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **28:** 195–200.
27. **Skjold, S. A., P. G. Quie, L. A. Fries, M. Barnham, and P. P. Cleary.** 1987. DNA fingerprinting of *Streptococcus zooepidemicus* (Lancefield group C) as an aid to epidemiological study. J. Infect. Dis. **155:**1145–1150.
28. **Tailor, S. A. N., E. M. Bailey, and M. J. Rybak.** 1993. Enterococcus, an emerging pathogen. Ann. Pharmacother. **27:**1231–1242.
29. **Tanimoto, K., and D. B. Clewell.** 1993. Regulation of the pAD1-encoded sex pheromone response in *Enterococcus faecalis*: expression of the positive regulator TraE1. J. Bacteriol. **175:**1008–1018.
30. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.