

Nucleotide sequence of a *nuc* gene encoding the thermonuclease of *Staphylococcus intermedius*

Olivier Chesneau and Névine El Solh*
Laboratoire des Staphylocoques et des Streptocoques, National Reference Center for Staphylococci, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France

Submitted August 19, 1992 EMBL accession no. X67678

A recombinant plasmid, pIP1607, containing the thermonuclease gene (*nuc*) of *S.intermedius* was detected in a pUC18 library, constructed by cloning cellular DNA of *S.intermedius* strain LRA076 partially digested with *Sau*3A in the *Bam*HI site. *E.coli* strain TG1 was used as a recipient for DNA transformation. The screening method consisted in replica-plating the ampicillin-resistant colonies on DNA-toluidine blue agar plates (1, 2). Of the 3000 transformants tested, one exhibited a nuclease activity that was further demonstrated as thermostable by boiling 5 ml of a cell suspension (1).

The 1.4 kbp insert of the recombinant plasmid pIP1607 was sequenced on both strands by the Sanger dideoxy chain termination method performed on denatured double strand DNA in the presence of SSB protein (3). The G+C content of this DNA fragment (36 mol%) is similar to those observed among staphylococci. Computer-assisted sequence analysis revealed only one potential translational reading frame of 168 amino acids (Figure 1). The free energy of interaction of the most stable structure between the putative RBS sequence, located 4 bp upstream from the ATG codon, and the 3′ terminus of the 16S rRNA (4, 5) was calculated according to Tinoco (6) and was evaluated as −55,2 kJ/mol. This value reflects a stable association and falls within those previously determined among Gram-positive bacteria (4, 5). The N-terminal region of the predicted amino acid sequence, ^{1}**MKKITTGVLILAIAIVVLIFQY-INGDG**^{27}, had all the features (7) of a leader peptide: two positively charged residues (lysine) at positions 2−3, an unbroken stretch of 13 hydrophobic residues at positions 8 to 20, and a 'glycine-aspartic acid-glycine' sequence at positions 25−26−27 which might correspond to the consensus sequence preceding the cleavage site of the signal peptidase. Within the *nuc* gene product of *S.intermedius* strain LRA076, the cleavage site would therefore be located between glycine and proline at positions 27−28, yielding a secreted protein of 141 amino acids (Figure 1). The sequence of this putative secreted protein displayed a significant homology with that of the mature *S.aureus* thermonuclease, also named nuclease A (1). In addition, all the amino acids involved in the biological activity of the nuclease A (8), except one threonine at position 113, matched at the same relative positions (Figure 1).

REFERENCES

1. Shortle,D. (1983) *Gene* **22**, 181−189.
2. Lachica,R.V.F., Genigeorgis,C. and Hoeprich,P.D. (1971) *Appl. Microbiol.* **21**, 585−587.
3. Mabilat,C., Goussard,S., Sougakoff,W., Spencer,R.C. and Courvalin,P. (1990) *Plasmid* **23**, 27−34.
4. McLaughlin,J.R., Murray,C.L. and Rabinowitz,J.C. (1981) *J. Biol. Chem.* **256**, 11283−11291.
5. Moran,C.P.Jr, Lang,N., LeGrice,S.F.J., Lee,G., Stephens,M., Sonenshein,A.L., Pero,J. and Losick,R. (1982) *Mol. Gen. Genet.* **186**, 339−346.
6. Tinoco,I.Jr, Borer,P.N., Dengler,B., Levine,M.D., Uhlenbeck,O.C., Crothers,D.M. and Gralla,J. (1973) *Nature* **246**, 40−41.
7. Pugsley,A.P. and Schwartz,M. (1985) *FEMS Microbiol. Rev.* **32**, 3−38.
8. Hynes,T.R. and Fox,R.O. (1991) *Proteins* **10**, 92−105.

```
                          *                 *   * *
S.aureus     ATSTKKLHKEPATLIKAIDGDTVKLMYKGQPMTFRLLLVDTPETKHPKK   49
               :     :      :::::      ::    ::  ::::::  :
S.intermedius PFKKSSTDVRGESYLVKRVIDGDTIIIDKDGQDERVRLIGVDTPETVKPNT   78

                                  ** *
S.aureus     GVEKYGPEASAFTKKMVENAKKIEVEFDKGQRTDKYGRGLAYIYADGKMVN  100
              :  ::  :: ::::   :          ::::: :::     : :
S.intermedius PVQPYGKAASNFTKKHLTNQRVRLEYDREPK--DKYGRTLAYVWLGDEMFN  127

                    * *
S.aureus     EALVRQGLAKVAYVYKPNNTHEQHLRKSEAQAKKEKLNIWSEDNADSGQ  149
              :  :::  :  : ::          : :  ::::
S.intermedius VKLAKEGLAR-AKFYPPNDKYRILIEQAQKEAQKKQLNIWER          168
```

Figure 1. Alignment of the amino acid sequences of the putative secreted Nuc protein of *S.intermedius* strain LRA076 and the nuclease A of *S.aureus* strain Foggi (1). All the amino acids involved in the biological activity of this latter protein (8) are marked with asterisks. Amino acid identities are indicated by colons.

* To whom correspondence should be addressed