Staphylococcal Enterotoxin A Gene (sea) Expression Is Not Affected by the Accessory Gene Regulator (agr)

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The goal of this work was to determine whether staphylococcal enterotoxin type A gene (sea) expression is regulated by an accessory gene regulator (agr). The Tn551 insertionally inactivated agr allele of Staphylococcus aureus ISP546 was transferred to three Sea+ S. aureus strains. Each of the Agr- strains produced as much staphylococcal enterotoxin A (SEA) as its parent strain. These results suggest that sea expression is regulated differently from that of seb, sec, and sed, which previously have been shown to require a functional agr system for maximal expression.

Staphylococcal enterotoxins cause the intoxication staphylococcal food poisoning; in addition, they have been implicated as virulence factors in some cases of toxic shock-like syndromes (4, 9-11, 13, 25, 32). Characterized enterotoxins are classified into five major serological types (A through E, designated SEA through SEE) (4).

The accessory gene regulator (agr) was initially identified as a locus that when inactivated resulted in a change in expression of several exoproteins and cell surface-associated proteins (e.g., toxic shock syndrome toxin 1, α-hemolysin, and protein A [6, 15, 28]). Subsequently, agr has been shown to be a polycistronic locus that has two divergent transcripts, RNAII and RNAIII (3.5 and 0.5 kb in length, respectively [1, 2, 16, 18, 20, 24, 27]). The agr region corresponding to RNAII has at least four open reading frames including agrA and agrB (1, 2, 16, 18, 20, 24, 27). agrA expression is required for RNAIII expression (18). RNAIII, which includes the gene for delta-hemolysin, is essential for agr regulation of agr target genes (14).

SEB, SEC, and SED are among those exoproteins whose expression is affected by agr. More SEB (3- to 5-fold), SEC (16- to 32-fold), and SED (4- to 5-fold) are produced by Staphylococcus aureus strains that have an active agr allele than by derivatives that have an insertionally inactivated agr allele (3, 12, 30). The agr effect is at the level of steady-state seb and sec mRNAs (12, 30). In addition, among several S. aureus strains, a correlation between the level of RNAIII and seb mRNA was observed; on the basis of these data, the authors suggested that strain differences in agr expression may be one reason to account for variation in the amount of SEB produced among strains (8). The goal of this study was to determine whether, like the other enterotoxins genes, sea expression is affected by agr.

The S. aureus strains used in this study are described in Table 1. Staphylococcal cultures were grown in Erlenmeyer flasks that contained medium consisting of 3% (wt/vol) N-Z amine type A (Sheffield Products, Norwich, N.Y.) and 1% (wt/vol) yeast extract (Difco Laboratories, Detroit, Mich.) at 37°C with agitation (31). Samples for Northern (RNA) blot analysis of agr transcripts were prepared from cells harvested when the cultures had attained an optical density at 540 nm of 6 (Spec 20; Bausch and Lomb, as rebuilt by Update Instrument, Madison, Wis.), which represented cultures in the transition between the exponential growth and stationary phases. For Western blot (immunoblot) analysis, samples were prepared from cultures incubated for 16 to 18 h.

The conditions and medium used for propagation of phase 80a (obtained from P. A. Pattee [21]), preparation of transducing lysates, and transduction and isolation of tetracycline- and erythromycin-resistant (Tmm' and Em', respectively) transductants have been previously described (30).

The RNA extraction procedure, criteria for judging the quality of the RNA, RNA quantification, and the Northern blot procedure have been reported (30). Preparation of staphylococcal whole-cell DNA and the Southern blot protocol using high-stringency hybridization conditions were the same as previously described (5).

Procedures for culturing Escherichia coli, isolation of E. coli plasmid DNA, DNA endonuclease restriction digests, isolation of DNA fragments from agarose gels, and labeling of double-stranded DNA with 32P have been given elsewhere (30). The probe used in Southern blot analysis to confirm that derivatives had acquired the Tn551 insertionally inactivated agr from S. aureus ISP546 was the insert fragment of pRN6656; this 1.2-kbp HincII fragment contained only agr DNA, including the portion of the agr which has the Tn551 insertion in ISP546 (obtained from R. P. Novick [27]). The probe used in Northern blot analysis to detect agr-encoded transcripts was a 952-bp ClaI-PstI fragment of pRN6397, which included 378 and 403 bp corresponding to regions of RNAII and RNAIII, respectively (obtained from R. P. Novick [24, 27]).

Preparation of culture supernatants, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and Western blot procedures were performed as reported (30). The intensity of the signals on Western blots was quantified by densitometry (GS 300 densitometer; Hoefer Scientific Instruments, San Francisco, Calif.). Antiserum prepared against SEA and SEB was kindly provided by M. S. Bergdoll (University of Wisconsin—Madison).

The strategy for determining whether an agr product affected sea expression was to construct Agr- derivatives and compare the amount of SEA produced by the Agr- derivative to that produced by its Agr+ parent strain. Acquisition of an inactive agr allele was expected to result in several phenotypic changes. Therefore, Tmm' was used as an outside marker. The Tmm' marker of ISP484 was transferred...
TABLE 1. S. aureus strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype (phenotype)</th>
<th>Source or origin</th>
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<tbody>
<tr>
<td>FR1100</td>
<td>sea+ (Tmnr Hem+ Em+), Agr+</td>
<td>M. S. Bergdoll, University of Wisconsin—Madison</td>
</tr>
<tr>
<td>MJB389</td>
<td>sea+ (Tmnr Hem+ Em+), Agr-</td>
<td>FR1100 × 80μg/ISP484 select for Tmnr' (this study)</td>
</tr>
<tr>
<td>MJB408</td>
<td>sea+ (Tmnr Hem- agr::Tn551), Agr-</td>
<td>MJB389 × 80μg/ISP546 select for Em' (this study)</td>
</tr>
<tr>
<td>FR1281A</td>
<td>sea+ seb+ (Tmnr Hem+ Em+), Agr+</td>
<td>M. S. Bergdoll</td>
</tr>
<tr>
<td>MJB394</td>
<td>sea+ seb+ (Tmnr Hem- agr::Tn551), Agr+</td>
<td>FR1281A × 80μg/ISP484 select for Tmnr' (this study)</td>
</tr>
<tr>
<td>FR1759</td>
<td>sea+ (Tmnr Hem+) Agr+</td>
<td>M. S. Bergdoll, Iowa State University, Ames (26)</td>
</tr>
<tr>
<td>MJB386</td>
<td>sea+ (Tmnr Hem+), Agr-</td>
<td>P. A. Pattee (19)</td>
</tr>
<tr>
<td>MJB406</td>
<td>sea+ (Tmnr Hem- agr::Tn551), Agr-</td>
<td>P. A. Pattee, Iowa State University, Ames (26)</td>
</tr>
<tr>
<td>ISP484</td>
<td>(Tmnr)</td>
<td>R. F. Novick, Public Health Research Institute, New York (22)</td>
</tr>
<tr>
<td>ISP546</td>
<td>(Sea- Tmnr agr::Tn551 Hem-), Agr-</td>
<td></td>
</tr>
<tr>
<td>RN450</td>
<td>(Sea- Agr+)</td>
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* Hem+ indicates that there was a zone of hemolysis surrounding the colonies after incubation on medium containing sheep erythrocytes.
* FR1100 was transduced with a lysate of phage 80x that had been prepared on strain ISP484.
* Tn551 encodes a determinant for erythromycin resistance (Em' [23]).

To S. aureus FR1100 and FR1759, resulting in MJB389 and MJB386, respectively, FR1281A was naturally Tmnr.

The Tn551 (containing the Em' marker) insertionally inactivated agr allele of ISP546 was transduced to FR1281A, MJB386, and MJB389, and Em' transductants were isolated (MJB394, MJB406, and MJB408, respectively). Whole-cell RNA samples from each of the three pairs of Agr+ or Agr- strains (Agr+-Agr- pairs) were examined by a Northern blot procedure for agr transcripts RNAII and RNAIII (Fig. 1). The blotting patterns were consistent with FR1281A, MJB386, and MJB389 producing transcripts that correspond to RNAII and RNAIII, and their respective Em' transductants did not contain these agr transcripts (Fig. 1). FR1281A, MJB386, and MJB389 were hemolytic when grown on Trypticase soy agar with 5% (vol/vol) sheep erythrocytes (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, Md.); this was phenotypic evidence that these parent strains were Agr+.

In contrast, each Em' derivative did not produce detectable hemolysins, as expected for Agr- strains. SEB analysis provided additional evidence that MJB394 was Agr-, unlike its parent strain, FR1281A. MJB394 produced at least 32-fold less SEB than its parent strain, FR1281A (Fig. 2A). Gaskill and Khan have reported that three- to fivefold less SEB is produced in an Agr- strain than in an Agr+ strain (12). The discrepancy in the magnitude of the agr effect on SEB production between this and the previous study may be due to host background differences or to the fact that Gaskill and Khan used recombinant plasmids that contained seb (12).

For two of the Agr+-Agr- pairs, Southern blot analysis was used to obtain physical evidence that acquisition of the Em' marker correlated with a change in agr. Southern blots containing whole-cell DNA from MJB389, MJB408, FR1281A, and MJB394 that had been digested with BglII or EcoRI were reacted with an agr-derived probe (1.2-kbp HindII-RsaI fragment). Neither agr nor Tn551 has an EcoRI site (18, 23). Examination of these Southern blots revealed that MJB408 and MJB394 had larger EcoRI fragments that contained agr than those of their parent strains (data not shown). There are no BglII sites within agr, and there is one site within Tn551 (18, 23). Analysis of the BglII-digested DNA revealed that the banding patterns of MJB408 and MJB394 were identical to that of ISP546 and different from those of their parent strains (data not shown). These results were consistent with MJB408 and MJB394 having acquired the Tn551 insertionally inactivated agr allele of ISP546.

A Western blot procedure was used to compare the amounts of extracellular SEA produced by each of the three Agr+-Agr- pairs. Culture supernatants from each of the Agr- derivatives contained as much SEA as similar samples obtained from their respective Agr+ parent strains (Fig. 2B). The lack of an agr effect on SEA production is in contrast to that observed for SEB, SEC, and SED, in which the Agr- derivatives produced less enterotoxin than the Agr+ strains (3, 12, 30). This suggests that sea expression is regulated differently from that of seb, sec, and sed.

Previously, we reported that some environmental conditions (e.g., (i) alkaline pH and (ii) glucose under conditions of nonmaintained pH) affect agr expression and thereby expression of at least one agr target gene, sec (29, 31). Given that there is a difference between enterotoxin types with respect to agr expression, it seems plausible that some environmental conditions may favor production of agr-regulated enterotoxins and that other conditions would favor production of SEA. Perhaps this difference in agr regulation...
among the enterotoxins is one factor contributing to (i) the fact that a much higher percentage of nonmenstrual cases of toxic shock-like illness are due to SEB and SEC producers than to SEA producers (7) and (ii) the fact that SEA causes most staphylococcal foodborne disease outbreaks (for a summary, see reference 17). Alternatively, the association of different types of enterotoxins with various diseases may be a reflection of the distribution of enterotoxin-producing S. aureus strains in nature.

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REFERENCES