The Journal of Microbiology, February 2005, p.54-61 Vol. 43, No. 1

NOTE

Growth of *Staphylococcus aureus* with Defective Siderophore Production in Human Peritoneal Dialysate Solution

Ra-Young Park[1], Hui-Yu Sun[1], Mi-Hwa Choi[1], Young-Hoon Bae[2] and Sung-Heui Shin[1]*

[1]Research Center for Resistant Cells and
[2]Department of Biology, Chosun University Medical School, Gwangju 501-759, Republic of Korea

(Received December 16, 2004 / Accepted February 4, 2005)

In this study, we attempted to determine the effects of iron-availability and the activity of the bacterial iron-uptake system (IUS) on the growth of *Staphylococcus aureus* in human peritoneal dialysate (HPD) solution. A streptonigrin-resistant *S. aureus* (SRSA) strain, isolated from *S. aureus* ATCC 6538, exhibited defective siderophore production, thereby resulting in ineffective uptake of iron from low iron-saturated transferrin. The growth of both strains was stimulated in HPD solution supplemented with $FeCl_3$ and holotransferrin, but growth was inhibited in HPD solution which had been supplemented with apotransferrin and dipyridyl. The SRSA strain grew less robustly than did its parental strain in both iron-supplemented HPD solution and regular HPD solution. These results indicate that iron-availability and siderophore-mediated IUS activity in particular, the ability to produce siderophores and thus capture iron from low iron-saturated transferrin play critical roles in the growth of *S. aureus* in HPD solution. Our results also indicated that the possibility of using iron chelators as therapeutic or preventive agents warrants further evaluation.

Key words: *Staphylococcus aureus*, human peritoneal dialysate, iron, siderophore

Staphylococci are known to be one of the most common causative agents of nosocomial infections (Emori and Gaynes, 1993; Sieradzki *et al.*, 1999). Among the various staphylococcal infections, continuous ambulatory peritoneal dialysis (CAPD) peritonitis has become a disease of increasing concern, due to the increasing number of patients contracting it. 50~80% of CAPD peritonitis is caused by staphylococci (von Graevenitz and Amsterdam, 1992).

In general, commercially available CAPD solution does not stimulate staphylococcal growth. However, CAPD solution is enriched by plasma ultrafiltrate during the process of dialysis. Although this human peritoneal dialysate solution (HPD solution) is so highly nutritious that it does, to some extent, stimulate staphylococcal growth, HPD solution maintains an iron-restricted condition (Modun *et al.*, 1998), as the free iron in human body fluids is only 10^{-18} M, which is much less than the minimal iron concentration (0.4~4.0 mM) necessary for the initiation of growth in most pathogenic bacteria. Most iron in the human body is bound to iron-binding glycoproteins, including transferrin and lactoferrin, or is intracellularly sequestered. Iron is virtually the only nutrient which limits bacterial growth inside the human host, and is known to be a crucial nonspecific natural defense mechanism. Accordingly, in order for pathogenic bacteria to grow and cause human infections, they must first be able to effectively uptake iron in such sparse environments. For this, most pathogenic bacteria have developed specific high-affinity iron-uptake systems (IUS) (Ratledge and Dover, 2000).

S. aureus also possesses well-developed high-affinity IUS, which allows it to thrive in very low iron-availability environments. These IUS systems can be divided into two classes: siderophore- and hemoprotein receptor-mediated IUS (Cabrera *et al.*, 2001; Sebulsky and Heinrichs, 2001; Mazmanian *et al.*, 2003; Dale *et al.*, 2004). *S. aureus* has also been known to express iron-repressible transferrin-binding proteins (designated StbA or IsdA) (Brown and Holden, 2002; Taylor and Heinrichs, 2002), but, recently, IsdA has been identified as a broad spectrum, iron-regulated adhesion, which binds to a variety of human ligands, including transferrin and fibronectin (Clarke *et al.*, 2004). One of our recent works generated some evidence that the

* To whom correspondence should be addressed.
(Tel) 82-62-230-6352; (Fax) 82-62-233-6052
(E-mail) shsin@chosun.ac.kr

IsdA-binding of transferrin is not specific for the mediation of iron uptake from transferrin (details will be published elsewhere). Of these IUS, the siderophore-mediated IUS is the basic and essential IUS in most bacteria, with the notable exceptions of the highly human-adaptive *Haemophilus* and *Neisseria* species, which do not produce siderophores (Ratledge and Dover, 2000). *S. aureus*, in particular, possesses a highly developed siderophore-mediated IUS. This allows *S. aureus* to grow even in severely iron-restricted conditions, such as human serum and HPD solution (Trivier and Courcol, 1996). Many studies have demonstrated that siderophore-mediated IUS activity played a critical role in the *in vitro* and *in vivo* growth of *S. aureus* (Rozalska *et al.*, 1998; Chung *et al.*, 2003; Dale *et al.*, 2004). In order to corroborate these findings, it was necessary to develop a mutant strain with a defective siderophore-mediated IUS. However, *S. aureus* produces several types of siderophores, as well as several ATP-binding cassette (ABC) transporter systems for the uptake of iron-siderophore complexes (Brown and Holden, 2002). Strains which do not produce some siderophores compensate for this lack by producing greater amounts of other siderophores. Moreover, each ABC transporter exhibits a marked lack of specificity with regard to siderophore types, and remains versatile, able to bind to and uptake different siderophores. Therefore, it is difficult to evaluate the effects of siderophore-mediated IUS activity simply by the mutation of a specific gene which is related to the expression of a siderophore or iron-uptake transporter.

Accordingly, in the present study, we induced nonspecific multiple mutations via chemical mutagenesis, in order to isolate a *S. aureus* strain which exhibited defective siderophore production. As a result, we isolated a streptonigrin-resistant *S. aureus* (SRSA) strain with defective siderophore production, originating from the *S. aureus* ATCC 6538 strain, by exposing it repeatedly to streptonigrin (Yeowell and White, 1982), which kills bacterial cells with higher IUS activity and higher intracellular iron-storage capabilities. Using this SRSA strain, we attempted to ascertain the effects of iron-availability and bacterial IUS activity on the growth of *S. aureus* on transferrin-bound iron and in HPD solution, an *ex vivo* experimental model, and also attempted to determine the effect of iron-chelators on the growth of *S. aureus* in HPD solution.

Materials and Methods

Laboratory ware, media, reagents, and bacterial strains

The preparation of laboratory ware and the deferration of the medium were described in our previous studies (Chung *et al.*, 2003; Ahn *et al.*, 2004). Unless otherwise noted, all reagents were purchased from Sigma (USA). The residual iron concentrations of the normal Brain Heart Infusion (BHI, Difco, USA) and the deferrated iron-deficient (ID) BHI were about 20.0 μM and 0.4 μM, respectively (Diagnostic Iron and Iron-Binding Capacity kit). When necessary, the ID-BHI was supplemented with 0.5 mg/ml of human-apotransferrin (AT), partially iron-saturated transferrin (PT), and holotransferrin (HT), as well as 10 μM of ferric chloride (FC). In the controls, the ID-BHI was supplemented with an equal volume of phosphate-buffered saline (PBS, pH 7.2). AT, PT, and HT contained iron at concentrations of less than 30, 300~600 and 1,200~1,600 μg per 1 g of protein, respectively. A new SRSA mutant strain, which was unlike the strain described in the previous study (Ahn *et al.*, 2004), was isolated from the *S. aureus* ATCC 6538 strain, using the same method. The nearest colony to the disc containing 125 ng of streptonigrin was picked up and cultured in BHI broth. This bacterium was spread again onto the surface of BHI agar, and exposed to streptonigrin. This procedure was repeated 8 times, until the growth inhibition zone around the streptonigrin disc had completely disappeared.

Human peritoneal dialysate (HPD) solution

The HPD solutions (Daniel PD-2 Peritoneal Dialysis Solution with 1.5% Dextrose, Daxter Healthcare PTE LTD, Singapore) were obtained after dialysis had been performed on patients with chronic renal failure, who were concurrently undergoing CAPD in the nephrology department of the Chosun University Hospital. The HPD solutions were immediately stored at -25°C until use. Immediately prior to use, five samples of the HPD solutions were pooled and filter-sterilized with disposable membrane filters (0.45 μm pore-sized, Milipore), and inactivated at 65°C for 30 minutes. One unused CAPD solution was used as the control. Iron concentrations of the CAPD solution and HPD solutions were quite low, to the extent that they were undetectable (Chung *et al.*, 2003). Protein levels were higher and glucose levels were lower in the HPD solution than in the CAPD solution (Chung *et al.*, 2003). When necessary, 0.5 mg/ml of HT and AT, 10 μM of FC, and 0.5 mM of dipyridyl (DP) were added to the CAPD and HPD solutions. The control was supplemented with an equal volume of PBS.

Growth conditions

The two bacterial strains grown in normal BHI were inoculated into the normal BHI containing 200 μM of DP, an iron-chelator, then cultured with vigorous shaking (220 rpm) at 37°C overnight, in order to adapt the bacterial strains to the iron-restricted conditions, and to reduce the extent of intracellular iron-storage. About 1×10^6 cfu/ml of these preconditioned bacteria were then inoculated into 15 ml of test media and HPD solutions, and cultured with vigorous shaking (220 rpm) at 37°C for 24 h. During culturing, culture fluids were obtained at 0, 3, 6, 9, 12, 18 and 24 h for the monitoring of bacterial growth, and the culture supernatants obtained at the same times were used

in the other assays applied in this study.

Chrome azurol S (CAS) agar diffusion assay, 6 M urea-gel electrophoresis and bioassay

S. aureus produces several types of siderophores. Therefore, in order to semi-quantitatively determine the production of total siderophores, a CAS agar diffusion assay was carried out, according to the method described by Shin *et al.* (2001). In brief, a CAS agar plate was punched with a gel puncher, creating small holes. Each hole was filled with 100 μl of culture supernatant, and the plates were incubated overnight at 37°C. The size of the yellow or orange haloes which formed around each hole indicated total siderophore activity, regardless of the chemical nature of the siderophores. In order to directly observe the acquisition of iron from transferrin during culturing, equal volumes of the culture supernatants were electrophoresed on 6 M urea-gel (6%), according to the previously described method (Makey and Seal, 1988), then visualized by Coomassie blue staining.

As *S. aureus* produces several types of siderophores, in order to obtain crude fractions containing low-molecular-weight-siderophores, we ultrafiltrated the culture supernatants from the ID-BHI (Vivaspin, MWCO 10,000, Sartorius, Germany). The total siderophore activity of the culture ultrafiltrates of the two strains were compared on CAS agar plate, and were then equalized with demineralized water. In order to compare iron-capturing ability between these equivalent siderophores, the culture ultrafiltrates were allowed to react with 0.5 mg/ml of HT for 10, 30, and 60 min at 37°C, after which, equal volumes of the mixtures were electrophoresed on 6 M Urea-gel. In order to compare the growth-stimulating ability of these equivalent siderophores, about 10^4 cfu/ml of the preconditioned bacteria were then spread onto the MM9 agar (Lindsay and Riley, 1994) supplemented with 0.5 mg/ml of AT, PT, and HT. The discs, each containing 30 μl of the culture ultrafiltrates, were placed onto the surface of the agar, and the plates were incubated for 24 h at 37°C.

All experiments performed in this study were repeated three times. Some of the results were expressed as the mean ± standard error, and the other results were expressed as representatives of each experiment. Student's t-tests were used for statistical analysis.

Results and Discussion

Functional characterization of SRSA strain

It is believed that bacteria exhibiting streptonigrin resistance also tend to exhibit low IUS activity and intracellular iron-storage, due to the fact that streptonigrin kills bacteria with higher levels of IUS activity and intracellular iron-storage (Yeowell and White, 1982). Therefore, streptonigrin was used to select a mutant with defective IUS capacity (Sebulsky *et al.*, 2000). This indicated that

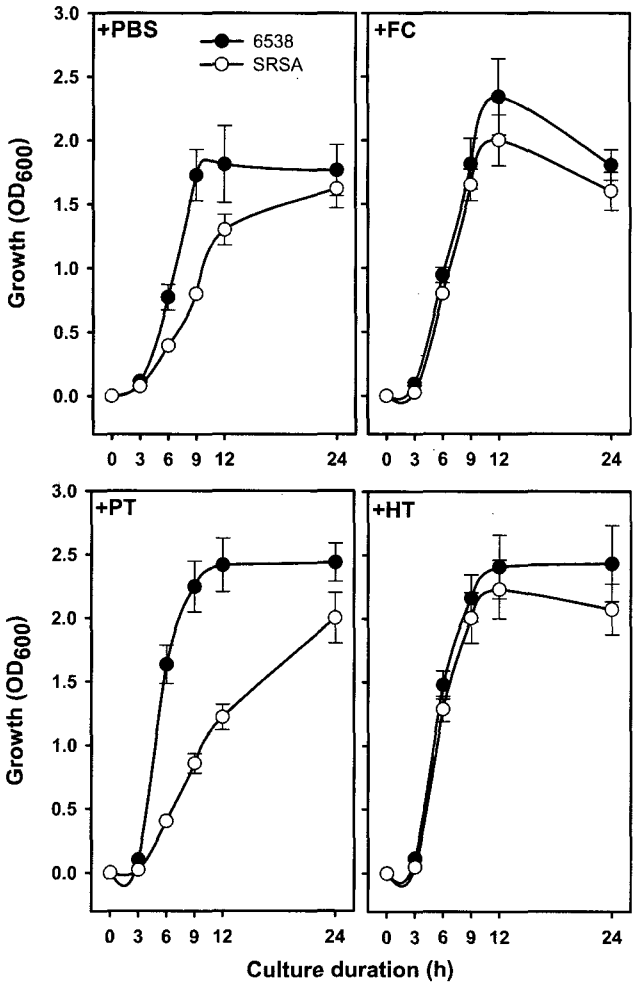

Fig. 1. Comparison of bacterial growth between the streptonigrin-resistant strain (SRSA) and its parental strain (6538) in iron-deficient BHI supplemented with phosphate-buffered saline (PBS), 10 μM of $FeCl_3$ (FC), 0.5 mg/ml of holotransferrin (HT), and partially iron-saturated transferrin (PT). About 1×10^6 cfu/ml of the bacteria was inoculated into the media, and cultured for 24 h with vigorous shaking (220 rpm) at 37°C. Bacterial growth was monitored by the measurement of optical density (O.D.) at a wavelength of 600 nm of the culture fluids obtained at indicated times.

the SRSA strain exhibited lower IUS activity and intracellular iron-storage than did its parental strain.

S. aureus has been established to possess well-developed high affinity IUS. *S. aureus* produces various siderophore types, and also has the ability to use heterologous hydroxamate-siderophores. It is additionally capable of utilizing hemoproteins as a sole source of iron, via specific receptors on the cell wall (Trivier and Courcol, 1996; Sebulsky *et al.*, 2000; Mazmanian *et al.*, 2003). In addition, *S. aureus* is known to express IsdA on its cell wall, allowing it to bind directly to transferrin. However, this IsdA-mediated transferrin-binding is not considered to be involved in the uptake of iron from transferrin (Clarke *et al.*, 2004).

In order to functionally characterize the IUS of the

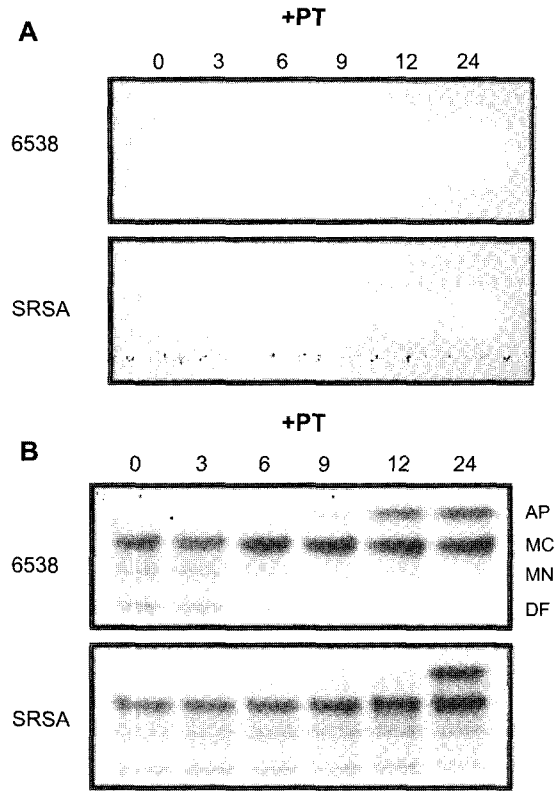

Fig. 2. Comparison of the production of total siderophores (A) and the ability to utilize iron from transferrin (B) between the streptonigrin-resistant strain (SRSA) and its parental strain (6538) in iron-deficient BHI supplemented with 0.5 mg/ml of partially iron-saturated transferrin (PT). Bacterial growth is shown in Fig. 1. Using the culture supernatants obtained at 0, 3, 6, 9, 12 and 24 h, CAS agar diffusion assay and 6 M urea-gel electrophoresis were performed. The size of yellow or orange haloes indicates siderophore activity. According to the level of iron-saturation, transferrin was separated into four forms: apotransferrin (AP), monoferric C-terminal (MC), monoferric N-terminal (MN) and diferric (DF) forms.

SRSA strain, we first cultured the SRSA strain and its parental strain were cultured in ID-BHI with PBS, $FeCl_3$, and transferrin-bound iron (AT, PT and HT). No significant differences were observed with regard to growth rates between the two strains in the ID-BHI with $FeCl_3$ and HT ($p>0.05$). However, the growth rate of the SRSA strain in the ID-BHI containing PBS and PT was noticeably lower than that of its parental strain ($p<0.05$, Fig. 1). The growth of the SRSA strain was more severely retarded when the ID-BHI was supplemented with AT (data not shown). In contrast, the retarded growth of the SRSA strain in the ID-BHI supplemented with PT recovered to a level commensurate with that of its parental strain, when the ID-BHI was supplemented with deferoxamine, a well-known heterologous hydroxamate-type siderophore (data not shown). This indicated that the ability of the SRSA strain to produce siderophores was defective under relatively lower iron-availability conditions, compared with that of its parental strain. In addition, the hemoprotein receptor-mediated IUS of the SRSA strain did not appear to be defective, as the growth rate between the two strains in the ID-BHI containing 0.5 mg/ml of hemoglobin was not significantly different ($p>0.05$, data not shown).

When total siderophore production was compared between the two strains via CAS agar diffusion assays, no differences were observed in the total siderophore production between the two strains in the ID-BHI containing FC and HT (data not shown), whereas the SRSA strain produced a smaller amount of siderophores in the ID-BHI containing PBS and PT than did its parental strain, and produced them more slowly (Fig. 2A). Similar results were also observed in other reports, which utilized a rapid-growing strain and a slow-growing strain of *S. aureus* (Trivier *et al.*, 1995; Chung *et al.*, 2003). Due to the iron-chelating activity of ID-BHI itself, and the sensitivity of the CAS agar diffusion assay, siderophore production was masked in the early growth phase, and became detectable only in a late growth phase (Trivier *et al.*, 1995). The total siderophore activities of both strains were dependent on iron-availability: the highest level in the ID-BHI supplemented with PBS and the lowest level in the ID-BHI supplemented with FC (data not shown), and were also inversely related to bacterial growth (Lindsay and Riley, 1994; Trivier *et al.*, 1995).

When the ability to capture iron from PT was compared between the two strains on 6 M urea-gel, the SRSA strain was determined to have an impaired ability to capture iron from PT as compared to its parental strain (Fig. 2B). The ability of the two strains to capture iron from PT was consistent with their total siderophore production. However, no differences were detected with regard to the strains' ability to capture iron from HT, with the exception of a slight delay in the iron-capturing ability of the SRSA strain (data not shown).

The ability of bacteria to capture iron from transferrin-bound iron is known to be dependent on the types of siderophores produced, due to variances in the affinity of siderophores for iron, in addition to the variances in the preference of siderophores for diverse iron sources (Brock *et al.*, 1991). Therefore, first, to compare the ability to capture iron from transferrin between the equivalent siderophores obtained from the two strains, we allowed the equivalent siderophores to react with HT (or PT) (Fig. 3A). We found there to be no difference with regard to iron-capturing ability between the two (Fig. 3B). Secondly, we compared the growth-stimulating ability on transferrin-bound iron between the equivalent siderophores obtained from the two strains. When discs containing the equivalent siderophores were applied to MM9 agar supplemented with AT, PT, and HT, the growth of both strains was stimulated on agar with AT or PT, as well as HT, by the siderophores alone (Fig. 3C). Although the parental strain grew better on MM9 agar than did the

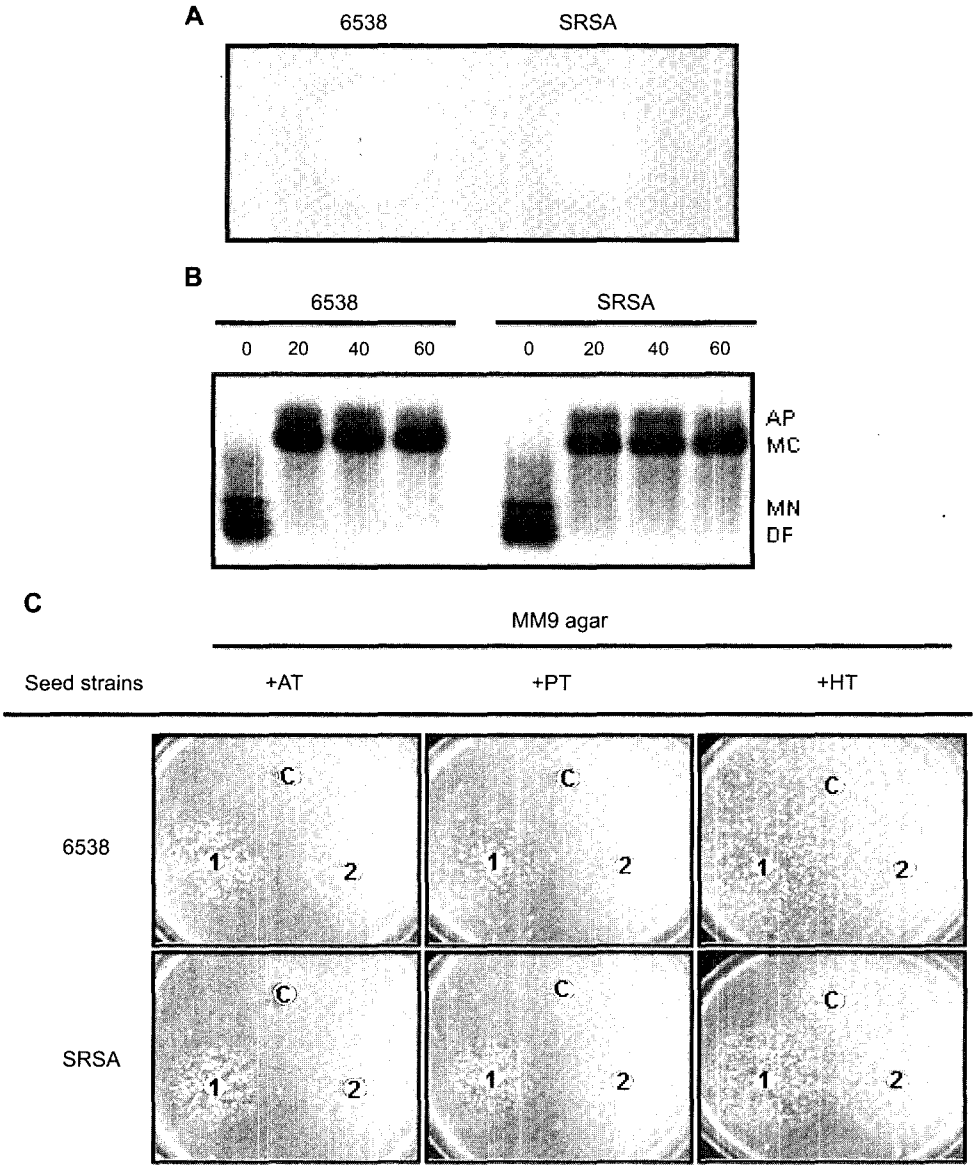

Fig. 3. Comparison of the iron-capturing ability of the siderophores from transferrin (B) and the growth-stimulating ability of the siderophores on transferrin-bound iron (C) between the streptonigrin-resistant strain (SRSA) and its parental strain (6538). B: The equivalent siderophores (A) were allowed to react with 0.5 mg/ml of holotransferrin (HT) at 37°C for 20, 40 and 60 min, and were then electrophoresed on 6 M urea-gel and visualized with Coomassie blue. Four forms of transferrin are referred to in Fig. 2. C: About 1×10^4 cfu/ml of the two strains were spread onto the surface of the MM9 agar with 0.5 mg/ml of apotransferrin (AT), partially iron-saturated transferrin (PT) and HT. Discs containing the equivalent siderophores (A) from the SRSA (1) and its parental strains (2) were placed on the surface of the MM9 agars, and incubated overnight at 37°C.

SRSA strain, in a manner similar to that observed in broth (Fig. 1), no significant differences were observed in the growth-stimulating ability of the equivalent siderophores obtained from the two strains ($p>0.05$). These results indicated that there were no differences in the siderophore types, nor were there any differences in the ability of the two strains to capture iron from transferrin and to utilize iron-siderophore complexes.

Overall, the SRSA strain exhibited defective siderophore-mediated IUS activity, resulting from its delayed production of low levels of siderophores in response to low iron-availability, and thus exhibited insufficient uptake of iron from low iron-saturated transferrin. No further molecular characterization of the SRSA strain was undertaken in the present study, due to the fact that the IUS of *S. aureus* was composed of a complex and sophisticated set of interactions between a variety of proteins (Brown and Holden, 2002). It is likely that streptonigrin caused nonspecific multiple mutations in the complex IUS of *S. aureus,* via the generation of free radicals (Yeowell and White, 1982).

***S. aureus* growth in the HPD solution**

HPD solution can be a useful *ex vivo* experimental model for the study of staphylococcal infections (Modun *et al.*, 1998; Chung *et al.*, 2003). Therefore, in this study, we used HPD solution in our assessment of the effects of iron-availability and the siderophore-mediated IUS activity on *S. aureus* growth, and in our evaluation of the potential of iron-chelators for use as therapeutic or preventive agents for staphylococcal infections.

It was previously known that staphylococci did not grow in commercially available CAPD solution, but grew readily in the HPD solution obtained from patients after

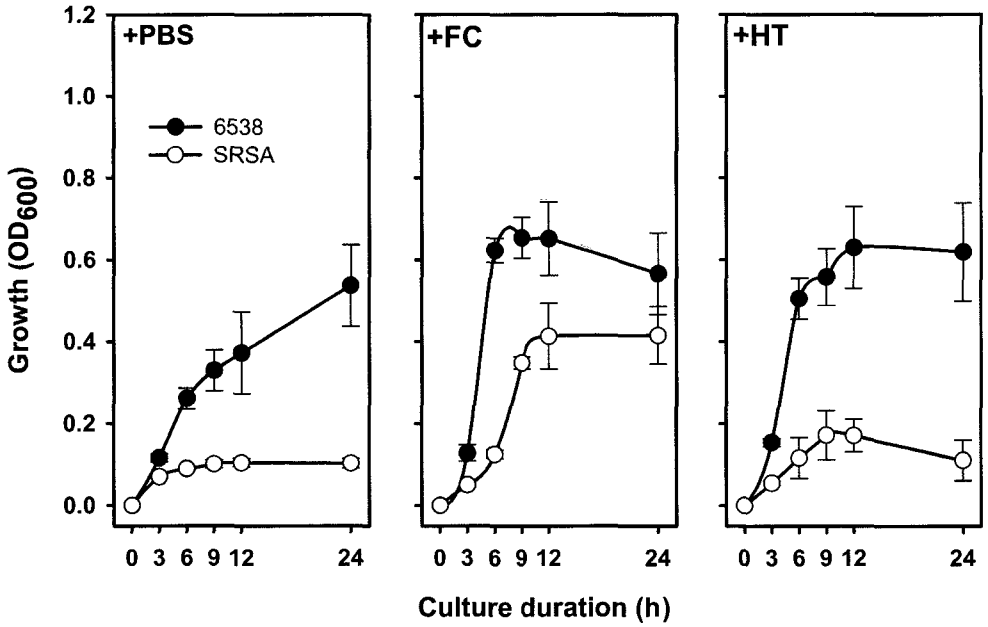

Fig. 4. Comparison of bacterial growth between the streptonigrin-resistant strain (SRSA) and its parental strain (6538) in human peritoneal dialysate solution supplemented with phosphate-buffered saline (PBS), 10 μM of $FeCl_3$ (FC), and 0.5 mg/ml of holotransferrin (HT). Bacterial growth was monitored by measuring the optical density (O.D.) at a wavelength of 600 nm of the culture fluids obtained at the indicated times.

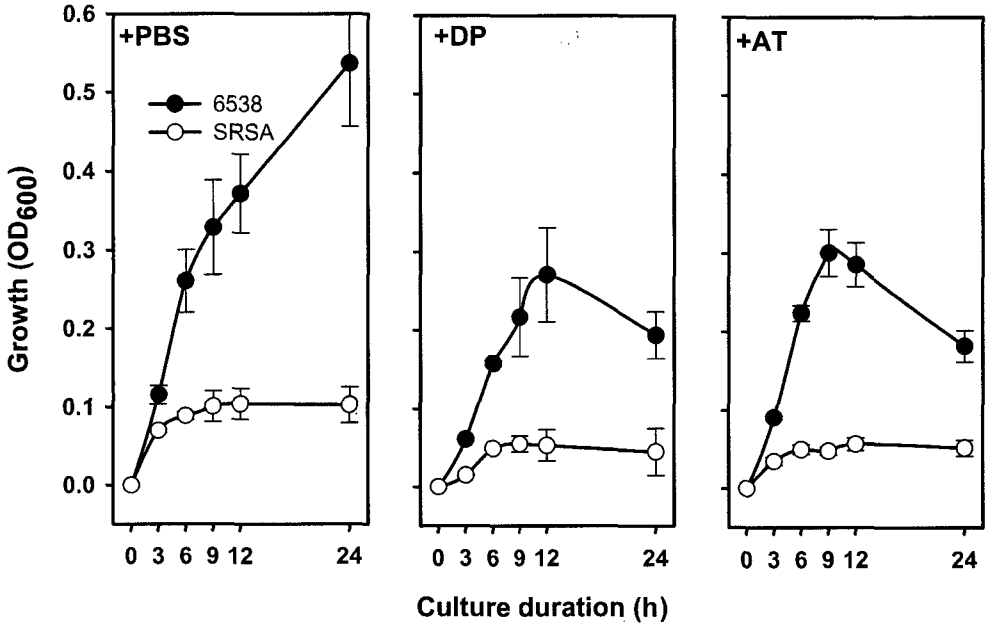

Fig. 5. Inhibition of growth in the streptonigrin-resistant strain (SRSA) *and its parental strain* (6538) in human peritoneal dialysate solution supplemented with 0.5 mM of dipyridyl (DP) and 0.5 mg/ml of apotransferrin (AT). An equal volume of phosphate-buffered saline (PBS) was added as the control. Bacterial growth was monitored by measuring the optical density (O.D.) at a wavelength of 600 nm of the culture fluids obtained at the indicated times.

dialysis (Modun *et al.*, 1998; Chung *et al.*, 2003). Also, in the present study, both strains failed to grow in the CAPD solution, regardless of the supplementation of exogenous iron (data not shown). Conversely, the growth of both strains was stimulated in HPD solution, and was stimulated to a greater extent in HPD solution which had been supplemented with FC and HT (Fig. 4). When HPD solution was supplemented with PT, the growth of both strains remained unaffected (data not shown). The growth of the SRSA strain was less robust than that of its parental strain. The retarded growth of the SRSA strain recovered to a level commensurate with that of its parental strain, when deferoxamine was exogenously added to the HPD solution (data not shown). However, when both strains were cultured in HPD solution with dipyridyl and AT, both of which are iron-chelating agents, the growth of both strains was inhibited (Fig. 5). Similar results were observed when dipyridyl and AT were added to the iron-supplemented HPD solution (data not shown). These results demonstrated that iron availability in the HPD solution functioned as a growth-limiting factor, the activity of the siderophore-mediated IUS contributed to *S. aureus* growth in HPD solution, and moreover, that iron-chelators could be employed in the prevention and treatment of staphylococcal infections.

It has been reported that strains with more pronounced siderophore-producing ability grew better under iron-restricted conditions and in HPD solution (Trivier *et al.*, 1995; Chung *et al.*, 2003), and was more virulent in the *in vivo* infection models (Rozalska *et al.*, 1998) than were strains with less pronounced siderophore-producing ability. More recently, a gene (designated *sbnE*) which appears to be involved in siderophore production has been initially identified and characterized in *S. aureus* (Dale *et al.*, 2004). Mutation of the *sbnE* gene resulted in the abnegation of the bacterial ability to produce an unidentified siderophore, under iron-deficient conditions. The *sbnE* mutant exhibited severely retarded growth in iron-deficient media, but not in iron-sufficient media, and was determined to have been compromised in a murine kidney abscess model of *S. aureus* infection, thereby clearly indicating the importance of siderophore production with regard to the pathogenicity of *S. aureus*.

It has been well established that most bacteria capture iron less effectively from low iron-saturated transferrin than they do from highly iron-saturated transferrin (Martinaho *et al.*, 2001), and a recent study demonstrated that the intravenous administration of AT effectively captured free iron, thus inhibiting *S. epidermidis* growth in the sera of patients receiving stem cell therapy (Bansdorff *et al.*, 2003). In addition, several iron-uptake ABC transporters were identified in the whole genome sequencing of *S. aureus* (Brown and Holden, 2002). Mutations in one or two of these transporters induced impaired utilization of iron-siderophore complexes. Therefore, in addition to iron-chelators which are able to lower iron-availability, these transporters may constitute new targets for the development of new therapeutic agents or vaccines against *S. aureus*.

In conclusion, the results of this study indicated that the low iron-availability of the HPD constituted an antibacterial factor, and that siderophore-mediated IUS activity, specifically the ability of *S. aureus* to produce siderophores and thus capture iron from low iron-saturated transferrin, might well play an important role in bacterial growth in HPD solution. In addition, our results indicate that the possibility of using iron chelators as therapeutic or preventive agents warrants further evaluation.

This study was supported by grants (R13-2004-009) from the Ministry of Science and Technology, Korea, and from the Korea Science and Engineering Foundation through the Research Center for Resistant Cells. We thank Dr. Huh, Young-Chul (Dr. Huh's Dental Clinic, Gwangju, ROK) for his helpful discussion.

References

Ahn, Y.J., S.K. Park, J.W. Oh, H.Y. Sun, and S.H. Shin. 2004. Bacterial growth in amniotic fluid is dependent on iron-availability and the activity of bacterial iron-uptake system. *J. Korean Med. Sci.* 19, 333-340.

Bonsdorff, L., L. Sahlstedt, F. Ebeling, T. Tuutu, and J. Parkkinen. 2003. Apotransferrin administration prevents growth of *Staphylococcus epidermidis* in the serum of stem cell transplant patients by biding of free iron. *FEMS Immunol. Med. Microbiol.* 37, 45-51.

Brock, J.H., P.H. Williams, J. Liceaga, and K.G. Wooldridge. 1991. Relative availability of transferrin-bound iron and cell-derived iron to aerobactin-producing and enterochelin-producing strains of *Escerichia coli* and to other microorganisms. *Infect. Immun.* 59, 3185-3190.

Brown, J.S. and D.W. Holden. 2002. Iron acquisition by Gram-positive bacterial pathogens. *Microbes Infect.* 4, 1149-1156.

Cabrera, G., A. Xiong, M. Uebel, V.K. Singh, and R.K. Jayaswal. 2001. Molecular characterization of the iron-hydroxamate uptake system in *Staphylococcus aureus*. *Appl. Environ. Microbiol.* 67, 1001-1003.

Chung, J.H., M.H. Park, J.H. Kim, Y. Lim, and S.H. Shin. 2003. Growth and siderophore production of staphylococci in human peritoneal dialysate. *J. Korean Med. Sci.* 18, 158-162.

Clarke, S.R., M.D. Wiltshire, and S.J. Foster. 2004. IsdA of Staphylococcus aureus is a broad spectrum, iron-regulated adhesin. *Mol. Microbiol.* 51, 1509-1519.

Dale, S.E., A. Doherty-Kirby, G. Lajoie, and D.E. Heinrichs. 2004. Role of siderophore biosynthesis in virulence of *Staphylococcus aureus*: Identification and characterization of genes involved in production of a siderophore. *Infect. Immun.* 72, 29-37.

Emori, T.G. and R.P. Gaynes. 1993. An overview of nosocomial infections including the role of the microbiology laboratory. *Clin. Microbiol. Rev.* 6, 428-442.

Lindsay, J.A. and T.V. Riley. 1994. Staphylococcal iron requirements, siderophore production, and iron-regulated protein

expression. *Infect. Immun.* 62, 2309-2314.

Makey, D.G. and U.S. Seal. 1988. The detection of four molecular forms of human transferrin during the iron binding process. *Biochem. Biophys. Acta.* 453, 250-256.

Martinaho, S., L. von Bonsdorff, A. Rouhiainen, M. Lonnroth, and J. Parkkinen. 2001. Dependence of *Staphylococcus epidermidis* on non transferrin-bound iron for growth. *FEMS Microbiol. Lett.* 196, 177-182.

Mazmanian, S.K., E.P.Skaar, A.H. Gaspar, M. Humayun, P. Gornicki, J. Jelenska, A. Joachmiak, D.M. Missiakas, and O. Schneewind. 2003. Passage of heme iron across the envelope of *Staphylococcus aureus*. *Science* 299, 906-909.

Modun, B., A.Cockayne, R. Finch, and P. Williams. 1998. The *Staphylococcus aureus* and *Staphylococcus epidermidis* transferrin-binding proteins are expressed *in vivo* during infection. *Microbiology* 144, 1005-1012.

Modun, B., D. Kendall, and P. Williams. 1994. Staphylococci express a receptor for human transferrin: Identification of a 42-kilodalton cell wall transferrin-binding protein. *Infect. Immun.* 62, 3850-3858.

Ratledge, C. and L.G. Dover. 2000. Iron metabolism in pathogenic bacteria. *Annu. Rev. Microbiol.* 54, 881-941.

Rozalska, B., P. Lisiecki, B. Sadowska, J. Mikucki, and W. Rudnicka. 1998. The virulence of *Staphylococcus aureus* isolates differing by siderophore production. *Acta. Microbiol. Pol.* 47, 185-194.

Sebulsky, M.T. and D.E. Heinrichs. 2001. Identification and characterization of *fhuD1* and *fhuD2*, two genes involved in iron-hydroxamate uptake in *Staphylococcus aureus*. *J. Bacteriol.* 183, 4994-5000.

Sebulsky, M.T., D. Hohnstein, M.D. Hunter, and D.E. Heinrichs. 2000. Identification and characterization of a membrane permease involved in iron-hydroxamate transport in *Staphylococcus aureus*. *J. Bacteriol.* 182, 4394-4400.

Shin, S.H., Y. Lim, S.E. Lee, N.W. Yang, and J.H. Rhee. 2001. CAS agar diffusion assay for the measurement of siderophores in biological fluids. *J. Microbiol. Methods* 44, 89-95.

Sieradzki, K., R.B. Roberts, S.W. Haber, and A. Tomasz. 1999. The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. *N. Engl. J. Med.* 340, 517-523.

Taylor, J.M. and D.E. Heinrichs. 2002. Transferrin binding in *Staphylococcus aureus*: involvement of a cell wall-anchored protein. *Mol. Microbiol.* 43, 1603-1614.

Trivier, D. and R.J. Courcol. 1996. Iron depletion and virulence in *Staphylococcus aureus*. *FEMS Microbiol. Lett.* 141, 117-127.

Trivier, D., M. Devril, N. Houdret, and R.J. Courcol. 1995. Influence of iron depletion on growth kinetics, siderophore production, and protein expression of *Staphylococcus aureus*. *FEMS Microbiol.* Lett. 127, 195-200.

von Graevenitz, A. and D. Amsterdam. 1992. Microbiological aspects of peritonitis associated with continuous ambulatory peritoneal dialysis. *Clin. Microbiol. Rev.* 5, 36-48.

Yeowell, H.N. and J.R. White. 1982. Iron requirement in the bactericidal mechanism of streptonigrin. *Antimicrob. Agents Chemother.* 22, 961-968.