

# Growth of Streptonigrin-Resistant *Staphylococcus epidermidis* with Defective Siderophore-Mediated Iron-Uptake System in Human Peritoneal Dialysate Solution

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시데로포아 매개성 철흡수기전 활성이 결여된  
피부 포도알균의 복막투석액에서의 증식

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**목 적 :** 피부 포도알균은 복막투석과 관련한 복막염의 가장 흔한 원인균이다. 철흡수기전 활성이 복막투석액내 피부 포도알균 증식에 중요한 역할을 담당하고 있다고 알려져 있으나 돌연변이 균주를 이용하여 철흡수기전의 중요성을 밝힌 보고는 아직까지 없다. 그래서 본 연구에서는 철흡수기전의 활성이 결여된 돌연변이 균주 (streptonigrin-resistant *S. epidermidis*; SRSE) 를 분리하였고 이 SRSE 균주의 특성을 모균주와 비교하였다. **재료 및 방법 :** *S. epidermidis* KCTC1917 균주를 streptonigrin에 반복적으로 노출시킴으로써 내성균주를 분리하였다. 배지와 복막투석액에서 세균의 증식은 파장 600 nm에서 배양액의 흡광도를 측정함으로써 관찰하였다. 시데로포아 생산능력을 비교하기 위해서는 CAS 한천배지 확산법을, 트랜스페린으로부터 철을 획득하는 능력을 비교하기 위해서는 6 M urea-gel 전기영동법을, 철과 결합한 시데로포아의 이용능력을 비교하기 위해서는 bioassay를 이용하였다.

**결 과 :** 두 균주 모두 철결핍배지에서는 증식하지 못하였지만  $\text{FeCl}_3$ 를 첨가한 경우 활발히 증식하였다. SRSE 균주는 철포화도가 낮은 트랜스페린으로부터 철을 획득할 수 없었고, 모균주에 비해 상대적으로 더 많은 시데로포아를 생산하였음에도 불구하고 철포화도가 높은 트랜스페린으로부터 철을 획득하는 능력도 결여되어 있었다. 두 균주 각각으로부터 시데로포아를 얻어 각각의 균주가 접종된 배지에 추가로 공급하였을 경우, 모균주는 트랜스페린에 결합된 철을 이용하여 증식이 촉진되었지만 SRSE 균주의 증식은 촉진되지 않았다. 복막투석액에서 두 균주의 증식을 관찰한 결과, 모균주에 비해 SRSE 균주의 증식이 저하되었다.

**결 론 :** 이로서 SRSE 균주는 시데로포아 생산능력이 결여되었기보다는 철과 결합한 시데로포아를 이용하는 능력이 결여되어 있음을 알 수 있고 시데로포아 매개성 철흡수기전이 복막투석액내 피부 포도알균 증식에 중요한 역할을 담당하고 있음을 알 수 있다.

**핵심용어 :** 피부 포도알균, 복막투석액, 철, 시데로포아

## INTRODUCTION

Staphylococci are known to be one of the most

common causative agents of nosocomial and community-acquired infections (1,2). Of the various staphylococcal infections, continuous ambulatory peritoneal dialysis (CAPD) peritonitis has been at issues because the number of patients undergoing CAPD of increasing. 50-80% of CAPD peritonitis is caused by staphylococci, particularly *S. epidermidis* (3-5).

Generally, commercially available unused CAPD solution fails to stimulate staphylococcal growth. However,

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the CAPD solution is enriched by plasma ultrafiltrate during dialysis. Although this human peritoneal dialysate solution (HPD solution) is highly nutritious that it stimulates staphylococcal growth to some extent, HPD solution maintains iron-restricted conditions (6-8) as the free iron in the human body fluids is only  $10^{-18}$  M, which is much less than the minimal iron concentration (0.4-4.0  $\mu$ M) required by most pathogenic bacteria for the initiation of growth (9). Most iron in the human body is bound to iron-binding glycoproteins, such as transferrin and lactoferrin, or sequestered intracellularly. Iron is virtually the only nutrient which limits bacterial growth inside the human host, and is known to be an important 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 a sparse environment (9). For this reason, most pathogenic bacteria have developed their own specific high-affinity iron-uptake systems (IUS).

*S. epidermidis* also possesses a well-developed high-affinity IUS in order to thrive in condition being very low iron-availability, such as the HPD solution (10). Although some controversy exists regarding the subject, *S. epidermidis* is known to express iron-repressible transferrin-binding protein (designated as Tpn) on its cell wall (11-15). Siderophore-mediated IUS is a basic and essential IUS in most bacteria, with the notable exceptions of the highly human-adaptive *Haemophilus* and *Neisseria* species (9). *S. epidermidis* also possesses a highly developed siderophore-mediated IUS. It is well known that *S. epidermidis* produces several types of siderophores for iron uptake (10,16). Recently, an ATP-binding cassette (ABC) transporter for uptake of the iron-siderophore complex was also identified in *S. epidermidis* (17). Recent mutational studies have demonstrated that *S. aureus* strains with higher levels of IUS activity were able to grow better *in vivo* as well as *in vitro* than strains with lower levels of IUS activity (18-21). However, there is no report elucidating the effect of the IUS activity on the *in vivo* and *in vitro* growth of *S. epidermidis* using mutants with defective IUS.

In the present study, we isolated a streptonigrin-resistant *S. epidermidis* (SRSE) strain with defective

IUS from the *S. epidermidis* KCTC 1917 strain by exposing it repeatedly to streptonigrin (22), which kills bacterial cells with higher IUS activity and higher intracellular iron-storage capabilities. Using this SRSE strain, we investigated the effects of the IUS activity on the growth of *S. epidermidis* on transferrin-bound iron and in HPD solution, which can be an *ex vivo* experimental model which mimics the *in vivo* state.

## MATERIALS AND METHODS

### 1. Laboratory ware, reagents and media

Laboratory wares were prepared as described in our previous studies (23,24). Unless otherwise noted, all reagents were purchased from Sigma (St. Louis, U.S.A.). Normal Brain Heart Infusion (BHI) broth (Difco, Becton and Dickinson Co., France) was deferrated as described in the previous studies (23,24), and the residual iron concentration of the deferrated iron-deficient (ID) BHI broth was less than 1.0  $\mu$ M. When necessary, 0.5 mg/mL of human-partially iron-saturated transferrin (PT) and holotransferrin (HT), and 10  $\mu$ M of ferric chloride (FC) were supplemented into the ID-BHI. The same volume of phosphate-buffered saline (PBS, pH 7.2) was supplemented as the control. PT and HT contained iron of 300-600 and 1,200-1,600  $\mu$ g per 1 g of protein, respectively. For bioassay, MM9 minimal medium (25) with 0.5 mg/mL of HT was used. Deferoxamine, a well-known hydroxamate-type siderophore, was purchased from Ciba Geigy Pharmaceutical Company (Switzerland).

### 2. Isolation of streptonigrin-resistant *S. epidermidis*

A streptonigrin-resistant *S. epidermidis* (SRSE) strain was isolated from *S. epidermidis* KCTC1917 (ATCC12228) strain using the same method as described previously (23,24). In brief, *S. epidermidis* KCTC1917 strain was exposed to the paper disc containing 125 ng of streptonigrin on BHI agar. The nearest colony from the discs was picked up to culture in BHI broth. This procedure was repeated eight times until growth-inhibition zone around the disc disappeared completely (Figure 1). Subcultured in the absence of streptonigrin, this SRSE strain did not

reverse back to streptonigrin-susceptible one.

### 3. Preparation of the HPD solution

The HPD solutions (Daniel PD-2 Peritoneal Dialysis Solution with 1.5% Dextrose, Daxter Healthcare PTE LTD, Singapore) were obtained after performing dialysis on patients with chronic renal failure, who were concurrently undergoing CAPD in the nephrology department of Chosun University Hospital. The HPD solutions were immediately stored at  $-25^{\circ}\text{C}$  until use. Immediately prior to use, five samples of the HPD solutions were pooled and filter-sterilized with disposable membrane filters ( $0.45\ \mu\text{m}$  pore-sized, Millipore), and inactivated at  $65^{\circ}\text{C}$  for 30 min. Iron concentration of the HPD solution was very low, to the extent that they were undetectable by Ferrozine assay (28). Protein levels were higher and glucose levels were lower in the HPD solution than in the unused CAPD solution (23,24).

### 4. Preconditioning, growth conditions, and monitoring

The two bacterial strains grown in the normal BHI were inoculated into the normal BHI containing  $200\ \mu\text{M}$  of dipyrityl, an iron-chelator, and cultured with vigorous shaking (220 rpm) at  $37^{\circ}\text{C}$  overnight in order to adapt the bacterial strains to the iron-restricted conditions and to lower intracellular iron-storage. About  $1 \times 10^6$  cfu/mL of these two preconditioned strains were inoculated into the 15 mL of the test media and the HPD solution, and cultured with vigorous shaking (220 rpm) at  $37^{\circ}\text{C}$  for 24 hours. During culturing, the culture fluids were obtained at 0, 3, 6, 9, 12 and 24 hours for monitoring bacterial growth; the culture supernatants obtained at the same times were used for other assays applied in this study.

### 5. Chrome azurol S (CAS) agar diffusion assay

*S. epidermidis* has been known to produce several types of siderophores. Therefore, in order to semi-quantitatively analyze the production of total siderophores, a CAS agar diffusion assay was performed according to the method described by Shin et al. (29). Briefly, a CAS agar plate was punched with a gel puncher in order to make small holes. Each hole was

filled with  $100\ \mu\text{L}$  of culture supernatant, and the plates were incubated at  $37^{\circ}\text{C}$  overnight. The size of yellow or orange haloes which formed around holes indicated the total siderophore activity, regardless of the type of siderophores.

### 6. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and 6 M urea-gel electrophoresis

In order to visualize the destruction of transferrin by staphylococcal proteases and the binding of transferrin to the whole cells, equal volumes ( $20\ \mu\text{L}$ ) of the culture supernatants were mixed with sample buffer and heated at  $100^{\circ}\text{C}$  for 5 minutes, and then electrophoresed on 5% stacking gel and 10% running gel at a constant 100 V for 2 hours. In order to directly observe the acquisition of iron from transferrin, equal volumes of the culture supernatants were mixed with sample buffer containing 8 M urea, but not SDS and mercaptoethanol, and allowed to react at  $37^{\circ}\text{C}$  for 30 minutes, and then electrophoresed on 5% stacking gel and 6% running gel containing 6 M urea at a constant 100 V for 2 hours (8,11,31). Proteins were visualized by Coomassie blue staining.

### 7. Crude low-molecular-siderophore fraction and growth-stimulating activity of the siderophore fraction

The culture supernatants obtained from the 24 hour-culture of the two strains in ID-BHI broth were ultrafiltrated (Vivaspin, MWCO 10,000, Sartorius, Germany) in order to obtain the crude fraction of low-molecular-weight-siderophores. The siderophore activity levels of the culture ultrafiltrates from the two strains were compared and equalized with de-ionized water on a CAS agar plate. In order to compare the growth-stimulating ability of these siderophores or the ability of the two strains to utilize the iron-siderophore complexes, about  $1 \times 10^4$  cfu/mL of the two strains were spread on MM9 agar supplemented with  $0.5\ \text{mg/mL}$  of HT. At that time, the discs containing  $30\ \mu\text{L}$  of each culture ultrafiltrate were placed onto the surface of the agar, and the plates were incubated at  $37^{\circ}\text{C}$  for 48 hours.

All experiments performed in this study were repeated three times, and representative ones of each

experiment are shown.

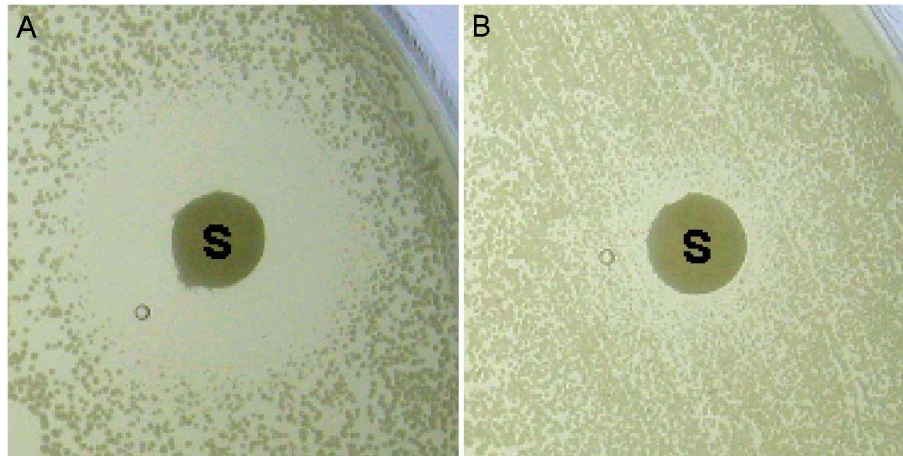
## RESULTS

### 1. Isolation and functional characterization of the SRSE strain

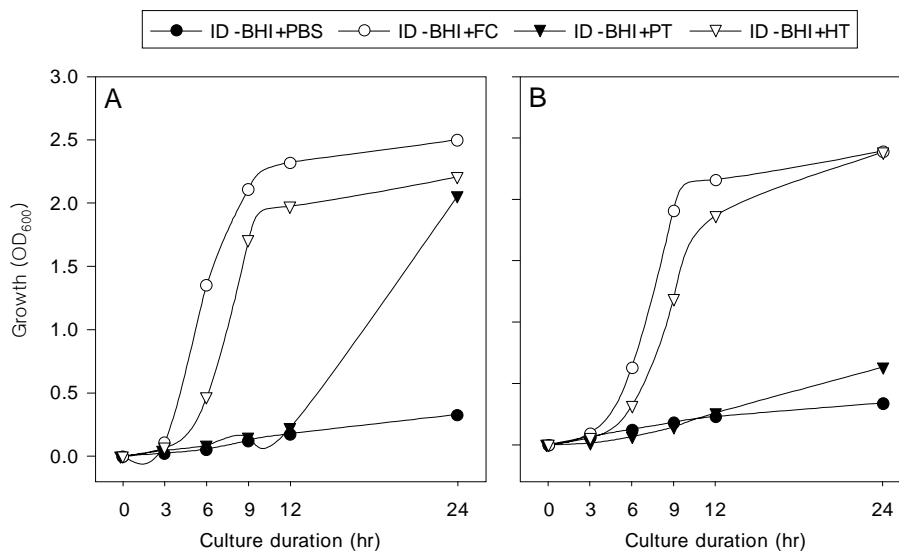
*S. epidermidis* KCTC1917 was exposed repeatedly to the disc containing 125 ng of streptonigrin until the

growth inhibition zone around the disc completely disappeared on the MM9 agar (Figure 1). When sub-cultured subsequently in the absence of streptonigrin, this SRSE strain did not reverse to streptonigrin-susceptible one.

In order to functionally characterize the IUS of the SRSE strain, the SRSE strain and its parental strain were cultured in the ID-BHI with PBS, FC and



**Figure 1.** Susceptibility against streptonigrin. Streptonigrin-resistant *S. epidermidis* (SRSE; B) was isolated from *S. epidermidis* KCTC1917 (A), as described in Materials and Methods. About  $1 \times 10^6$  cfu/mL of the two strains were spread on the BHI agar with cotton swab, and then paper discs containing 125 ng of streptonigrin (S) were placed onto the agar surface. The plates were incubated at 37°C overnight.



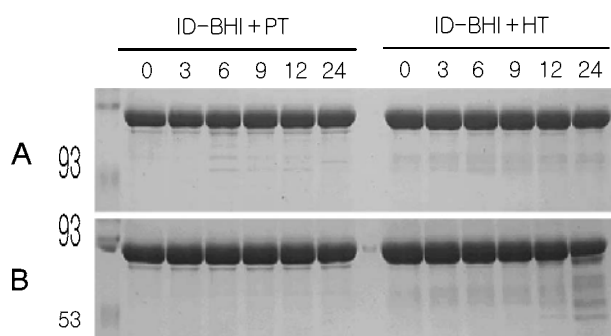
**Figure 2.** Comparison of growth between the SRSE (B) strain and its parental strain (A) in the iron-deficient (ID-) BHI containing phosphate-buffered saline (PBS), 10  $\mu$ M of  $\text{FeCl}_3$  (FC), 0.5 mg/mL of partially iron-saturated transferrin (PT) and holotransferrin (HT). About  $1 \times 10^6$  cfu/mL of the two strains were inoculated and cultured with vigorous shaking (220 rpm) at 37°C for 24 hours. Bacterial growth was monitored by measuring the optical density (OD) of the culture fluids obtained at the indicated times at a wavelength of 600 nm. Of the triplicate experiments with similar results, a representative result is shown.

transferrin-bound iron (Figure 2). The growth of the SRSE strain in the ID-BHI with  $\text{FeCl}_3$  and HT was slightly delayed compared with that of its parental strain, not showing a noticeable difference in the growth rate between the two. The parental strain was able to grow even in the ID-BHI containing PT, but was not the SRSE strain. Both strains did not grow in the ID-BHI containing only PBS.

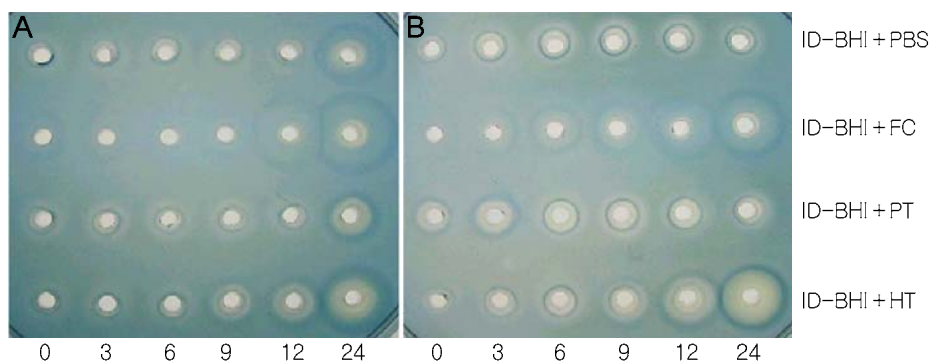
In order to dismiss a possibility that the differences in the ability to utilize transferrin-bound iron resulted from the destruction of transferrin by the proteases of *S. epidermidis*, or the binding of transferrin to the putative transferrin receptor on the cell wall of *S. epider-*

*midis*, the culture supernatants obtained from the ID-BHI containing PT and HT were electrophoresed on SDS-gel (Figure 3). Minor destruction of transferrin was sometimes observed, but bore no correlation to its ability to utilize transferrin-bound iron. In addition, the transferrin band taperings were not observed on SDS-PAGE. This indicated that neither the proteases nor the putative transferrin receptor were involved in the utilization of transferrin-bound iron under our experimental conditions.

The production of total siderophores between the two strains was compared on CAS agar plates (Figure 4). Owing to the sensitivity of CAS agar diffusion assay and the iron-chelating activity of ID-BHI medium itself (10,29), the activity of small amounts of siderophores in the early growth phase was masked. The activity of siderophores was not detected in the ID-BHI with PBS because both strains failed to grow in that medium. However, although both strains grew actively in the ID-BHI with FC, the production of siderophores from them was suppressed. Both strains produced the largest amount of siderophores in the ID-BHI with HT. The parental strain produced siderophores to some extent in the ID-BHI with PT, but the SRSE strain did not. This indicates that the production of siderophores is regulated via a ferric uptake repressor (Fur) by iron concentration or iron availability, and that the ability to grow actively in iron-restricted conditions is required for the effective production of siderophores, and vice versa.



**Figure 3.** Comparison of the proteolytic cleavage of transferrin and the binding of transferrin to whole cells between the SRSE strain (B) and its parental strain (A) in the iron-deficient (ID-) BHI containing 0.5 mg/mL of partially iron-saturated transferrin (PT) and holotransferrin (HT). Bacterial growth is shown in Figure 2. SDS-PAGE was performed using the culture supernatants (20  $\mu\text{L}$ ) obtained at the indicated times (top). Standard molecular size markers are shown on the left. Of the triplicate experiments with similar results, a representative result is shown.



**Figure 4.** Comparison of the production of total siderophores between the SRSE strain (B) and its parental strain (A) in the iron-deficient (ID-) BHI containing PBS, 10  $\mu\text{M}$  of  $\text{FeCl}_3$  (FC), 0.5 mg/mL of partially iron-saturated transferrin (PT) and holotransferrin (HT). Bacterial growth is shown in Figure 2. For the measurement of total siderophores, CAS agar diffusion assay was performed using the culture supernatants obtained at the indicated times (top). Clear yellow or orange halos indicate the activity of total siderophores. Of the triplicate experiments with similar results, a representative result is shown.

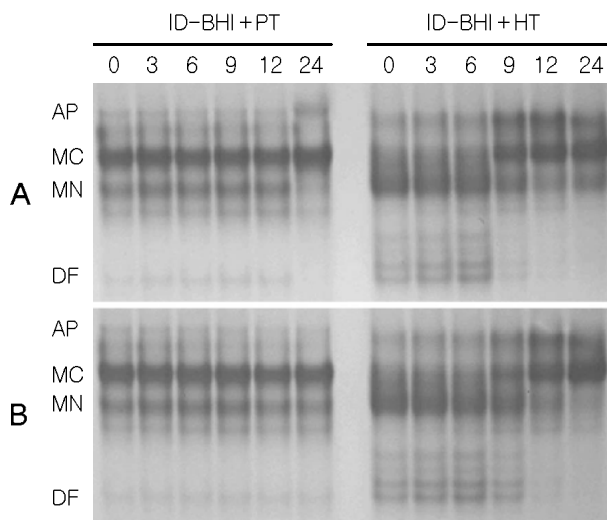
However, a noticeable thing was that the SRSE strain produced more siderophores than the parental strain in the ID-BHI with HT. Therefore, the ability to capture iron from PT and HT between the two strains

was compared on 6 M urea-gel. Although the SRSE strain produced considerably larger amount of siderophores in the ID-BHI with HT than its parental strain (Figure 4), it captured iron from HT less effectively than its parental strain (Figure 5). These implied that the SRSE strain was unable to uptake its own siderophores complexed with iron captured from HT.

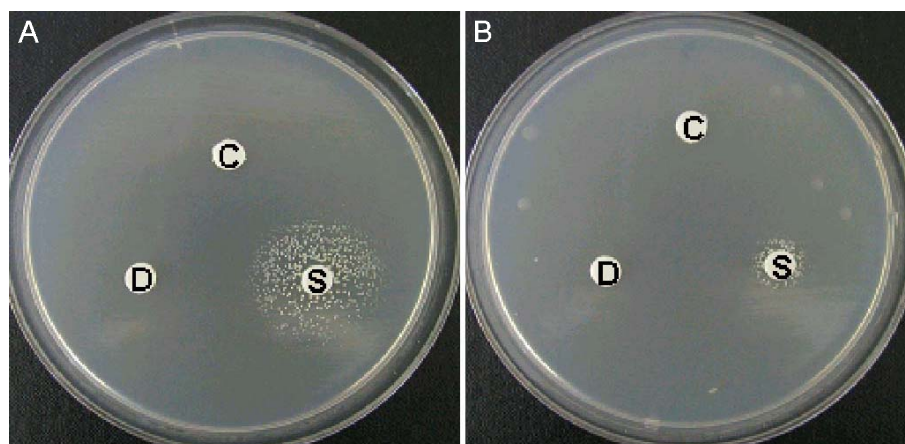
In order to compare the ability of the two strains to utilize the iron-siderophore complexes, their growth was compared. When the discs containing their own siderophores were placed on MM9 agar with HT, the growth of the parental strain, but not the SRSE strain, was stimulated around the discs (Figure 6). The growth of SRSE strain was not stimulated even when the disc containing the siderophores from the parental strain was applied (data not shown).

## 2. The growth of SRSE strain in the HPD solution

*S. epidermidis* is known to be one of the most common pathogen of CAPD peritonitis. Moreover, HPD solution can be a useful *ex vivo* experimental model for studying staphylococcal infections (6, 8, 23). Therefore, in this study, we used the HPD solution to assess the effect of siderophore-mediated IUS activity on the growth of *S. epidermidis* and to evaluate the potential of siderophore-mediated IUS as a new target for development of therapeutic or preventive agents

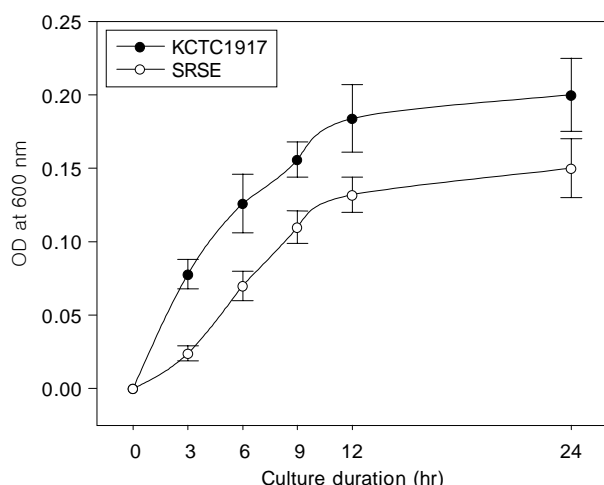


**Figure 5.** Comparison of the ability to remove iron from transferrin between the SRSE strain (B) and its parental strain (A) in the iron-deficient (ID-) BHI containing 0.5 mg/mL of partially iron-saturated transferrin (PT) and holotransferrin (HT). Bacterial growth is shown in Figure 2. 6 M urea-gel electrophoresis was performed using the culture supernatants (20  $\mu$ L) obtained at the indicated times (top). Transferrin is separated into four forms according to the iron-saturated level of transferrin: apo (AP), C-terminal monoferric (MC), N-terminal monoferric (MN) and diferric (DF) forms. Of the triplicate experiments with similar results, a representative result is shown.



**Figure 6.** Comparison of the ability to utilize the iron-siderophore complexes between the SRSE strain (B) and its parental strain (A) in the MM9 agar containing 0.5 mg/mL of holotransferrin (HT). The crude siderophore fractions were obtained by ultrafiltration, as described in Materials and methods. About  $1 \times 10^4$  cfu/mL of the two strains were spread on the MM9 agar, and then the paper discs (S) containing 30  $\mu$ L of the siderophore fractions were placed on the agar surface. Discs containing 30  $\mu$ L of deferoxamine (100  $\mu$ M; D) and uninoculated broth (C) were placed as negative controls. The plates were incubated at 37°C for 48 hours. Of the triplicate experiments with similar results, a representative result is shown.





**Figure 7.** Comparison of growth between the SRSE strain and its parental strain (KCTC1917) in the human peritoneal dialysate solution. About  $1 \times 10^6$  cfu/mL of the two strains were inoculated and cultured with vigorous shaking (220 rpm) at 37°C for 24 hours. Bacterial growth was monitored by measuring the optical density (OD) of the culture fluids obtained at the indicated times at a wavelength of 600 nm. Of the triplicate experiments with similar results, a representative result is shown.

against *S. epidermidis* infections.

Both stains did not grow in the unused CAPD (data not shown). In contrast, the growth of both strains was stimulated in the HPD solution, but the growth of the SRSE strain was retarded compared with that of its parental strain (Figure 7). Similar results were also shown when FC or HT was exogenously added into the HPD solution (data not shown).

## DISCUSSION

It is believed that bacteria exhibiting streptonigrin-resistance also have low IUS activity and intracellular iron-storage due to the fact that streptonigrin kills bacteria with higher IUS activity and intracellular iron-storage (22). Therefore, streptonigrin has been used to select a mutant with defective IUS capacity (32). The SRSE strain isolated in this study also had the defective siderophore-mediated IUS activity, which resulted from its inability to utilize iron-siderophore complexes. Accordingly, the SRSE strain could not effectively uptake iron from transferrin. No further molecular characterization of the SRSE strain was undertaken in the present study due to the fact that the IUS of *S. epidermidis* might be composed of a complex and sophis-

ticated set of interactions between various proteins (17–21,32). It was likely that streptonigrin caused non-specific multiple mutations in this complex IUS via the formation of free radicals (22). In addition, as the growth of SRSE was slightly retarded even in the ID-BHI containing HT and FC when compared with that of the parental strain, streptonigrin might also cause nonspecific multiple mutations in other genes related to bacterial growth.

Although the IUS of *S. aureus* has been extensively studied (18–21,32), data on the IUS of *S. epidermidis* is lacking (17). Although it has been known that the IUS of *S. epidermidis* was less developed than that of *S. aureus* (13,14), *S. epidermidis* also possesses high-affinity IUS. *S. epidermidis* produces various types of siderophores (10) and has an ABC transporter system for the uptake of iron-siderophore complexes, which is not fully identified (17). In addition, *S. epidermidis* has been known to express transferrin-binding protein (or transferrin receptor; named Tpn or StbA) on its cell wall for direct uptake of transferrin-bound iron, but this Tpn-mediated IUS remains controversial (8,11–15). In our recent study (details will be reported elsewhere), *S. aureus* was able to utilize transferrin-bound iron via siderophore-mediated IUS but not via Tpn-mediated IUS. Moreover, several researches have revealed that *S. epidermidis* failed to utilize transferrin-bound iron despite its ability to express larger amount of Tpn on its cell wall than *S. aureus* (11–14). Similar results were observed in this study. *S. aureus* was able to grow actively even in the presence of apotransferrin (data not shown), whereas *S. epidermidis* was able to grow actively only in the presence of holotransferrin, which was over 80% iron-saturated. This indicates that *S. epidermidis* expressing more Tpn was unable to utilize transferrin-bound iron as effectively as *S. aureus*. In other words, the staphylococcal Tpn did not play an important role in the iron-uptake from transferrin. Generally, it has been known that the higher the iron-saturation level of transferrin, the more easily bacteria can uptake iron from transferrin (14). For this reason, the SRSE strain was thought to be able to easily uptake iron from HT.

Some researches have demonstrated that some bacterial proteases, especially *Pseudomonas aeruginosa*

proteases, were able to destroy human transferrins to assist the iron-uptake from transferrin via siderophore-mediated IUS (33). In this study, minor destruction of transferrin was observed, but the level of transferrin destruction was inconsistent with the uptake of iron from transferrin. Therefore, staphylococcal proteases were not thought to be involved in the uptake of iron from transferrin.

The reason why both strains produced the largest amount of siderophores in the ID-BHI with HT could be explained by the results of Lindsay and Riley (25). They reported that *S. aureus* was able to grow similarly and produce siderophores regardless of iron availability, whereas *S. epidermidis* grew poorly in medium without iron supplements, and their growth and siderophore production were enhanced only when iron was supplemented. Similar results were also observed in our recent work (details will be published elsewhere). *S. aureus* produced siderophores and grew in ID-BHI containing apotransferrin and PT, as well as HT, whereas *S. epidermidis* produced siderophores and grew only in ID-BHI containing HT. This indicates that *S. epidermidis* has higher intrinsic iron-requirement for the initiation of growth and the production of siderophores than *S. aureus*.

The ability to produce siderophores under conditions with low iron-availability is very important in the virulence of *S. epidermidis* (35). However, in this study, despite the SRSE strain producing a considerably larger amount of siderophores than its parental strain, it could capture iron from HT less effectively than its parental strain. Our results were similar to those of Trivier et al. (34). They reported that a rapid growing *S. aureus* strain produced earlier but less siderophores than the slow growing one. Both results can be explained if the rapid growing strain (the parental strain in this study) utilized better iron-siderophore complexes via rapid turn-over than the slow growing one (the SRSE strain). Accordingly, these implied that the SRSE strain was defective in its ability to utilize iron-siderophore complexes rather than its ability to produce siderophores.

The ability of bacteria to utilize iron-siderophore complexes for growth, or the growth-stimulating ability of siderophores, depends on the activity of the

iron-uptake ABC transporter systems (16-21). Generally the ABC transporter for the uptake of iron-siderophore complexes consists of three components: a lipoprotein siderophore receptor, membrane permease, and ATPase. Recently, several iron-uptake ABC transporter systems have been identified in *S. aureus* (16). However, only one ABC transporter system (SitABC) has been reported, but not functionally characterized until now (17). Like in *S. aureus*, it is likely that *S. epidermidis* also has more iron-uptake ABC transporter systems. In this study, in order to compare the activity of these ABC transporters between the SRSE and its parental strains, when the discs containing their own siderophores were supplemented, the parental strain was able to grow by using its own siderophores, whereas the SRSE strain failed to grow and utilize its own siderophores (Figure 6), as well as the siderophores derived from its parental strain (data not shown). This clearly demonstrated that the SRSE strain was defective in its ability to uptake iron-siderophores. Accordingly, the SRSE strain has the defective siderophore-mediated IUS, which resulted from the inefficiency of ABC transporters for the uptake of iron-siderophore complexes.

*S. epidermidis* is known to be one of the most common pathogen of CAPD peritonitis. Moreover, HPD solution can be a useful *ex vivo* experimental model for studying staphylococcal infections (6,8,23). Therefore, in this study, we used the HPD solution in order to assess the effect of the siderophore-mediated IUS activity on the growth of *S. epidermidis*, and in order to evaluate the potential of the siderophore-mediated IUS as a new target for the development of therapeutic or preventive agents against *S. epidermidis* infections. The growth of both strains was stimulated in the HPD solution. However, the growth of the SRSE strain in the HPD solution was considerably retarded when compared with that of its parental strain (Figure 7). These results indicated that the siderophore-mediated IUS activity could play an important role in the growth of *S. epidermidis* in the HPD solution as well as other body fluids such as blood and cerebrospinal fluid. Recent studies demonstrated that the intravenous administration of apotransferrin effectively captured free iron and could inhibit the growth of *S. epidermidis* in stem



cell transplant patient sera (36), indicating that iron-chelators, including apotransferrin, can be used for prevention and treatment of *S. epidermidis* infections. Our results also suggest that ABC transporter systems for the uptake of iron-siderophore complexes can be a potential target for the development of therapeutic or preventive agents against *S. epidermidis* infections.

## ABSTRACT

**Background:** *Staphylococcus epidermidis* is the most common pathogen of chronic ambulatory peritoneal dialysis peritonitis. It has been believed that the activity of iron-uptake system (IUS) may play an important role in the growth of *S. epidermidis* in human peritoneal dialysate (HPD) solution, but there is no report using mutants with defective IUS. A streptomycin-resistant *S. epidermidis* (SRSE) strain was isolated from *S. epidermidis* KCTC 1917 and functionally characterized.

**Materials and Methods:** Bacterial growth was monitored by measuring the optical densities of culture fluids obtained at appropriate intervals at a wavelength of 600 nm. CAS agar diffusion assay was used for the comparison of siderophore production, 6 M urea-gel electrophoresis for the comparison of the ability to capture iron from transferrin, and bioassay for the observation of the ability to utilize iron-siderophore complexes.

**Results:** The SRSE strain ineffectively utilized transferrin-bound iron for growth despite its ability to produce considerably larger amount of siderophores than its parental strain. The growth of the parental strain, but not the SRSE strain, was stimulated on transferrin-bound iron by its own siderophores each. The growth of the SRSE strain in the HPD solution was retarded compared to that of the parental strain.

**Conclusion:** These results indicate that the SRSE strain is defective in its ability to utilize the iron-siderophore complexes, rather than its ability to produce siderophores, and that the siderophore-mediated IUS plays an important role in the growth of *S. epidermidis* in HPD solution.

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