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Changes of Cell Surface Hydrophobicity of a *Serratia marcescens* with Cultivation Time and Temperatures

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배양온도와 시간에 따른 *Serratia marcescens* 표면의 소수성 성질변화

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***S. marcescens* cultured at 30°C with vigorous shaking was shown to produce red-pigment, prodigiosin, in the senescent phase of growth. Also, it showed many hydrophobic characteristrics, which were tested by the adherence to noncharged surfaces of polystyrene dishes, a typical agent for the binding of hydrophobic cells and molecules. However, when the cell was cultured at 37°C, it no longer produced either red pigment or hydrophobic materials. Therefore, the bacteria cultured at 37°C was completely washed-out from the polystyrene dishes at the copious washing step with tap water, in contrast to the cell cultured at 30°C which was sticked onto the polystyrene dishes very tightly. The lipid compositions extracted from the *S. marcescens* cultured at 30°C or 37°C were very different from each other; the phospholipids, glycolipids and unidentified lipids were produced from the cell cultured at 30°C, whereas large amounts of serratamolide, amphipathic compound, were produced from the cell cultured at 37°C. The data suggest that the pronounced cell surface hydrophobicity of the *S. marcescens* is mediated by a combination of several surface factors that were affected by cultivation time and temperatures.**

Cell surface hydrophobicity is currently recognized as an important factor in the adhesion and proliferation of microorganisms on a wide variety of solid surfaces, including marine sediments (1), oils (2,3), nonwettable plastics (4), phagocytes (5), teech (6) and one another (7).

In natural ecosystems,in which adhesion to inert surfaces is nonspecific, in contrast to biological surfaces, where specific receptors and lectins play a major role (8,9). Hydrophobicity seems to be the most important factor in the nonspecific adhesion of bacteria to interfaces. The hydrophobic surface properties of *S. marcescens* and other *Serratia* species are of considerable interest, both from an ecological and medical point of view. *S. marcescens* is a common environmental microorganism of remarkable historical interest (10) which has been increasingly implicated as a primary pathogen in numerous infections (11,12). Bacterial hydrophobicity plays an important role in determining the ability of *Serratia* cells to adhere at and clonize the air-water interfaces (13, 14), as well as in scavenging of organic materials at solid-liquid interfaces (15)

In this study, we examined the hydrophobic characteristics of *S. marcescens* and the changes of

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lipid compositions in cell with the cultivation time and temperatures.

Materials and Methods

Bacterial strains and growth conditions

S. marcescens KCTC2172 was used in all experimensts. Cells were maintained on nutrient agar at 4°C and were subcultured every month. For measuring the cell surface hydrophobicity, 0.1 m*l* of bacteria from an overnight culture was inoculated into 50 m*l* of LB broth and incubated in shaking incubater at 150 rpm at 30°C or 37°C.

Cell surface hydrophobicity

Cell surface hydrophobicity was determined by measuring the adherence of cells to noncharged surfaces of polystyrence dishes, essentially as described by Rosenberg(4). Bacteria was washed twice and suspended in PUM buffer (22.2 g of $K_2HPO_4 \cdot 3H_2O$, 7.26 g of KH_2PO_4, 1.8 g of urea, 0.2 g of $MgSO_4 \cdot 7H_2O$, distilled water to 1000 m*l* [pH 7.1]) to an optical density of 1.4 at 400 nm. Samples (50 μl) of each cell suspension were added to a flat-bottomed 80 mm diameter dishes plate (nontreated polystyrene: NUNC, Rockilde, Denmark). After 20 min of incubation at room temperature, the dishes were washed copiously wiht tap water. In order to facilitate visualization, the dishes were fixed by dipping in methanol and stained with Gentain violet, and allowed to dry. The adherent cell spots were observed with Reichert microscope (Microstar IV).

Visible spectra

The occurrence of red pigment, prodigiosin, in the cell of *S. marcescens* or its supernatent was measured. The cell supernatent was obtained from the cells cultured in LB broht at 30°C or 37°C by centrifugation at 8600 x g for 20 min with various culture times. And the precipitated cells were washed twice with PUM buffer and resuspended in washing buffer to an optical density of 1.4 at 400 nm. The changes of absorption pectra of these solution were scanned by a Du-65 visible spectrophotometer (Beckman) at 400-700 nm.

Thin layer chromatography

Bacteria were grown at 30°C or 37°C on LB broth for 3days. To examine the changes of lipid compositions in cells according to culture temperatures, bacterial cells were harvested and washed twice with phosphate buffered salt saline (8.0 g of NaCl, 0.2 g KCl, 1.15 g of K_2HPO_4, 0.2 g of KH_2PO_4, distilled water to 1,000 m*l* [pH7.1]). Following a 15 min centrifugation (8,600 × g), the pellet was extracted with 10 volumes of ethanol. After removal of sediments by centrifugation as above and removal of the ethanol by evaporation, the dry material was further extracted with chloroform-methanol (2:1, v/v). Then, the supernatent was washed three times with 2 M-KCl and separated the lower organic phase. After concentration of this solution by rotary evaporator, the extracts were examined on a thin layer chromatography plate of silica gel (Kiesel gel 60 F254, E. Merck AG, Germany) in a solvent system of a chloroform-methanol-acetone-acetic acid (90:10:6:1 by volume). For detection of components (16), the plate was sprayed with 50% H_2SO_4 in ethanol and heated briefly at Ca. 200°C.

Results

S. marcescens grown on LB broth at 30°C with vigorous shaking produced cell associated red pigment, prodigiosin. However, when the cell was grown at 37°C, it no longer produced the red pigment as shown in Fig 1. That is, when the cell of *S. marcescens* was cultured with vigorous shaking at 30°C, it produced pinkish-red pigment at the stationary phase of cell growth. However, the pigment of cell cultured at 37°C showed a turbid-yellow color of media itself. The production of red pigment in the washed cell grown either at 30°C or 37°C was monitered by UV-visible spectrophotometer. The visible spectra of these samples were shown in Fig. 2, where the two absorption peaks, 500 and 540 nm, were observed only in the cell cultured at 30°C but not in 37°C. The same absorption spectra were obtained at the supernatents of the cell, of which the bacterial cells were completely removed. This showed that the pigment produced from the cell cultured at 30°C was gradually excreted into the extracellular space. Therefore, the production of red pigment from the cell can be measured by the absorbance changes at 500 nm from the cell supernatents. Fig. 3 showed that the production of red pigment with the growth curve of cell cultured at 30°C. The red pigment production showed a lag similar to that for growing cells, but the maximal amount of red pigment was produced at later stationary phase (Fig. 3). From these

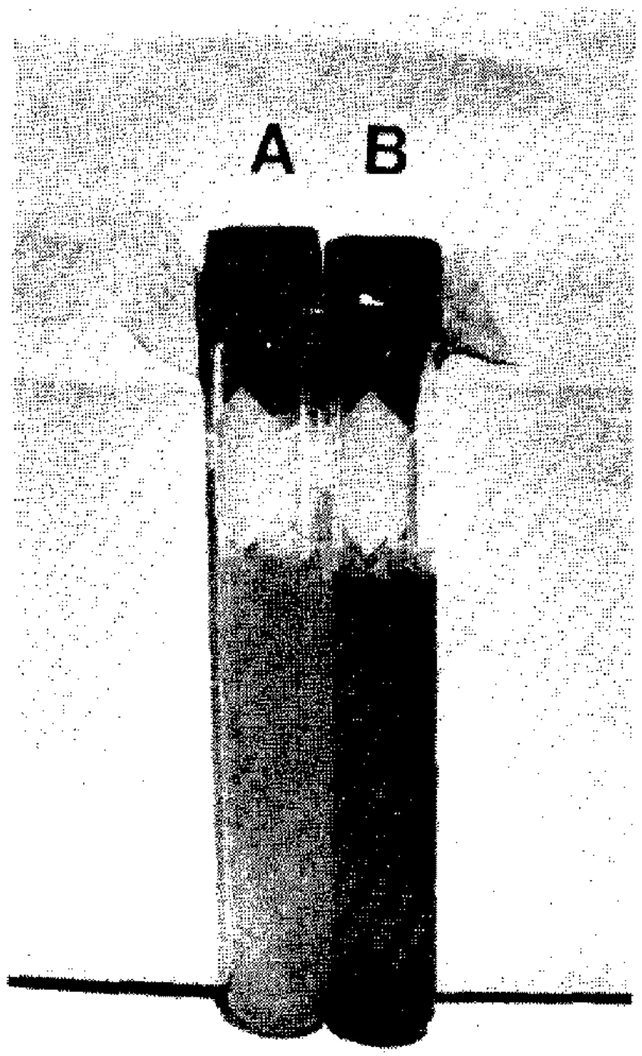

Fig. 1. Effect of culture temperatures on the production red pigment.
S. marcescens was cultured at 37°C (A) or 37°C (B) for 3days with vigorous shaking.

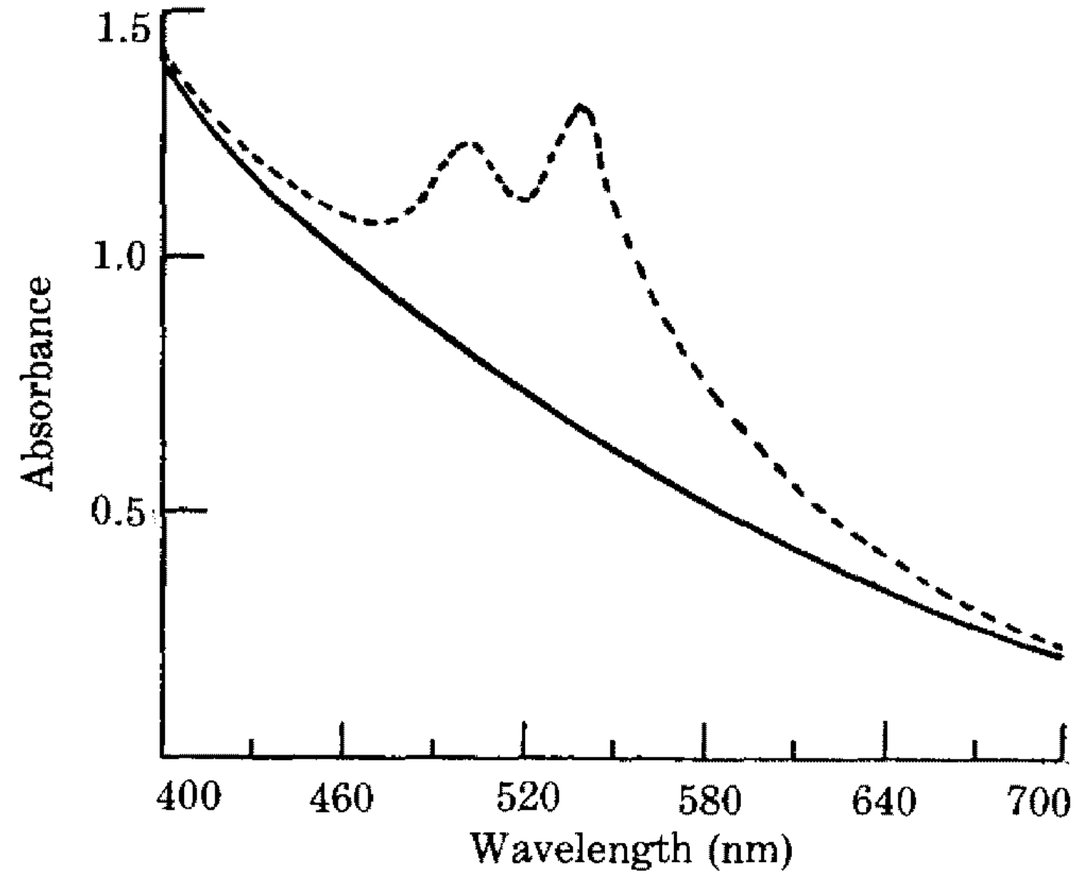

Fig. 2. Absorption spectra of red pigment produced from the bacterial cell of *S. marcescens*.
For scanning the absorbance changes for the pigment of cells at 400-700 nm, the cells were harvested, washed twice with PUM buffer and resuspended in this buffer to an optical density of 1.4 at 400 nm. The absorbance changes of this resuspended cell cultured at 30°C (...) or 37°C (–) were measured with a reference of PUM buffer.

data, it can be known that the red pigment produced from the bacterial cell of *S. marcescens* is proportionally secreted to the growing media with time and temperature dependent manner.

The adherence of *S. marcescens* to polystyrence dishes was represented in Fig. 4. Whereas the cell grown at 30°C was tightly adhered to polystyrene dishes (Fig. 4-A), the cell grown at 37°C was completely removed at the washing procedures (Fig. 4-B). This fact showed that the cell surface hydrophobicity of the cell cultured at 37°C was lost after growth at this temperature. From the above results, it was known that the *S. marcescens* cultured at 37°C don't produce the red pigment, prodigiosin, as well as no hydrophobic substances. Most laboratory strains cultured either at 30°C or 37°C did not adhere to polystyrene dishes and were removed from the dishes after the vigorous washing step with tap water. These included colonies of *Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus subtillis* (data not presented). Adherence of *S. marcescens* to polystyrene dishes was also found to depend on the age of colonies. The red pigment colonies grown at 30°C more than 2 days were sticked onto the dishes very tightly (Fig. 5-A, B) in proportion to the growth time of cells, but the nonpigmented

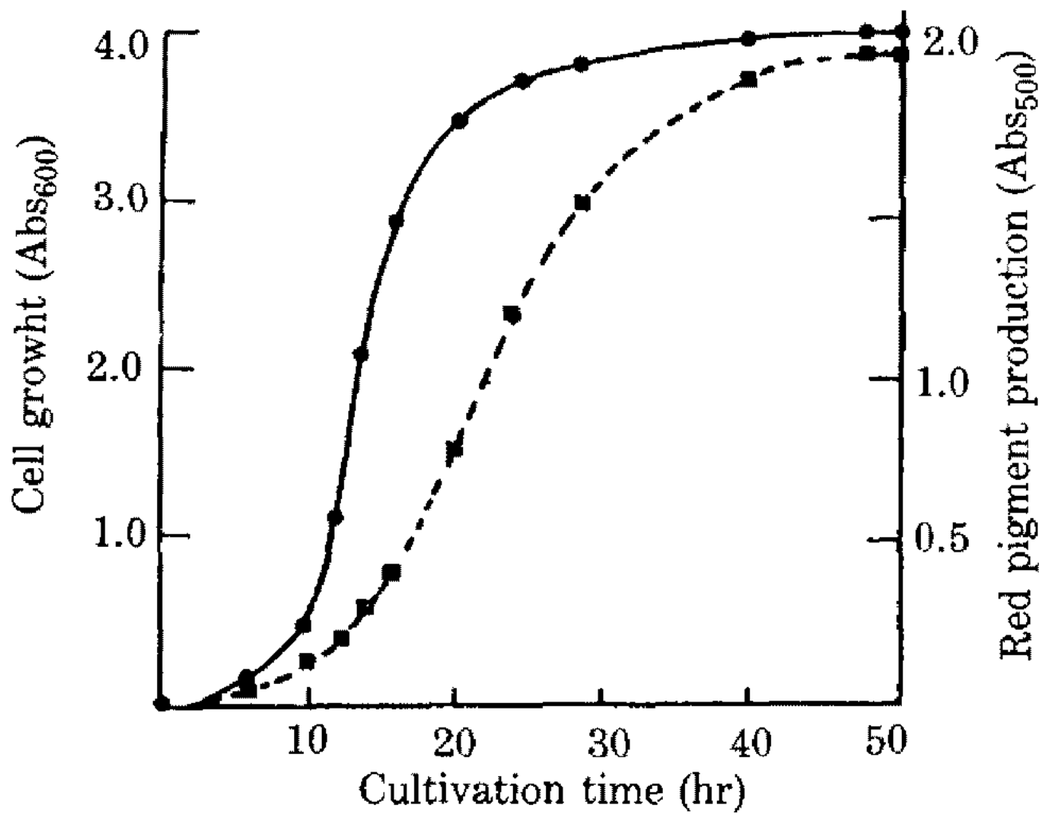

Fig. 3. Growth curve of *S. Marcescens* cultured at 30°C and the production of red pigment.
The cell growth was measured at Abs_{620}(–): The production of red pigment from the supernatents of *S. marcescens* was monitered by changes of Abs_{500} (...) with culture time.

colonies cultured less than 24 hrs were not adherent (Fig. 5-C). These data suggested that the appearance of red pigment in *S. marcescens* has been closely correlated with increasing cell surface hydrophobicity. However, it might not be concluded that only the red

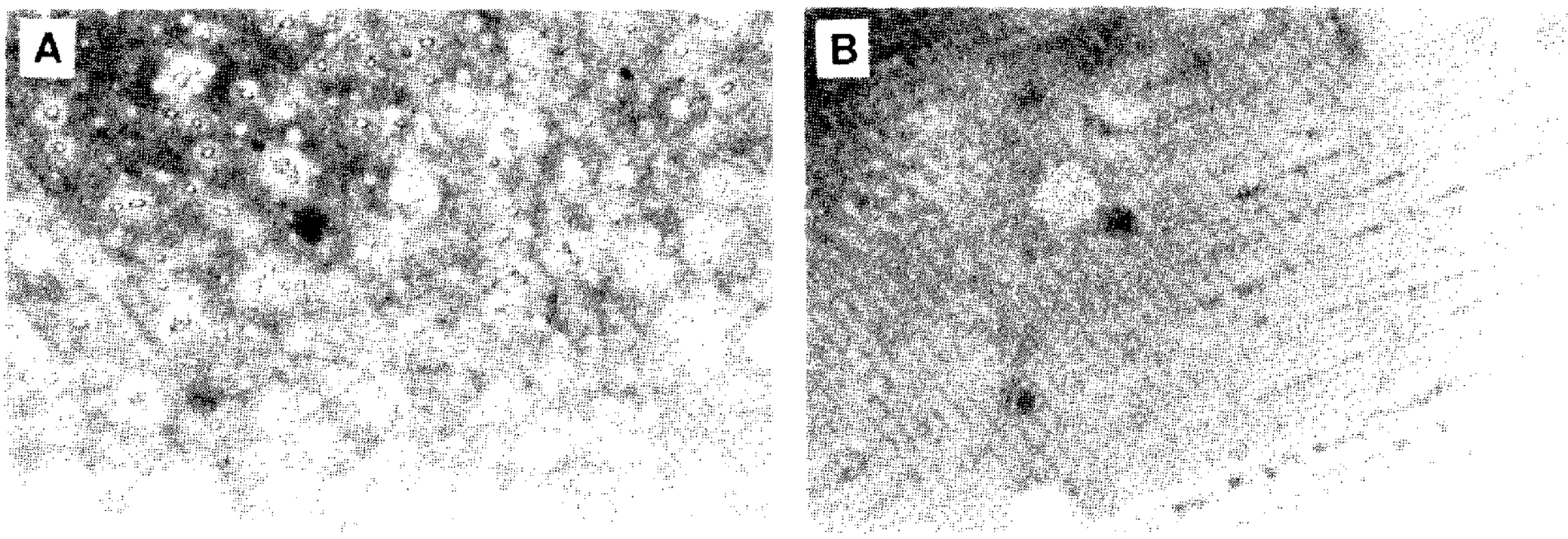

Fig. 4. Microscopic photographs showing the adherence of *S. marcescens* to noncharged polystyrene dishes (1000x).
The adherence of cell cultured at 30°C (A) or 37°C (B) for 3days.

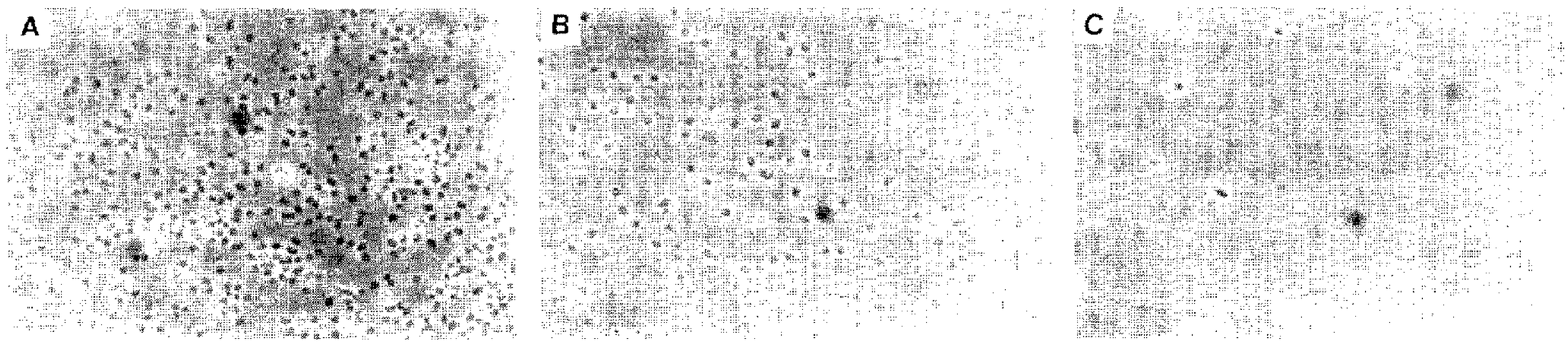

Fig. 5. Adherence of *S. marcescens* to noncharged polystyrene dishes as a function of cell growth.
The photomicrographs (1000×) showed that the adhered cells which were grown at 30°C for 72 hrs (A), 48 hrs (B), and 24 hrs (C).

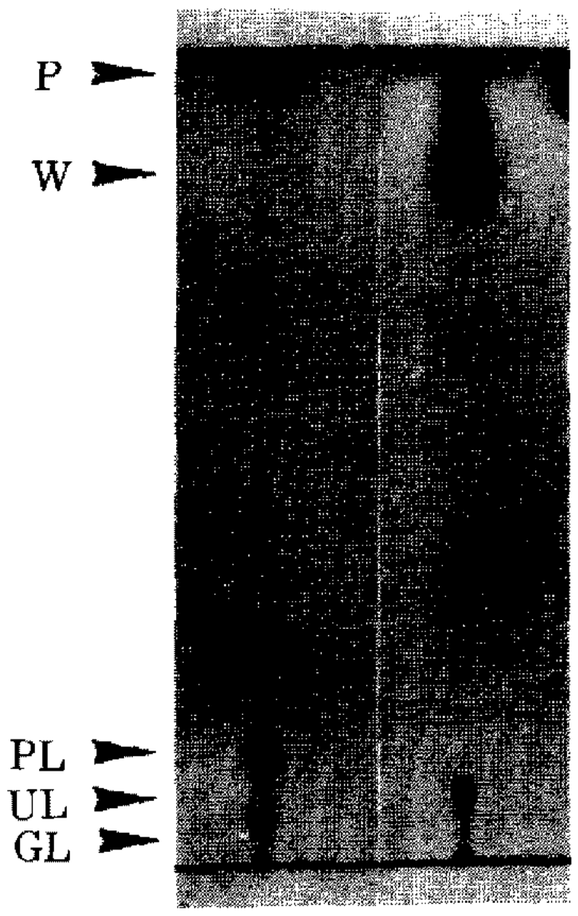

Fig. 6. Thin layer chromatograms of lipids extracted from the cells of *S. marcescens*.
Lipids extracted from the cells cultured at 30°C (lane 1) and cultured at 37°C (lane 2): P, prodigiosin; W, wetting agent (serratamolide); PL, phospholipids; UL, unidentified lipids; GL, glycolipids.

pigment have to be intimately associated with cell surface hydrophobicity, because Roesenberg (17) showed that the sole presence of red pigment did not result in the expression of pronounced hydrophobicity.

Following growth of *S. marcescens* either at 30°C or 37°C, the lipids extracted from the cells were analyzed by thin layer chromatography (Fig. 6). There are much differences for the lipid components extracted from the cells cultured at either temperatures. The cell grown on LB broth at 37°C produced large amounts of cell-associated wetting agent that was proved to be an aminolipid, serratamolide, by Matsuyama *et al* (18). However, when the cells were grown at 30°C, some of phospholipids, glycolipids and unidentified lipids were produced but not the serratamolide, which were identified with authentic samples. It is evident that only the bacterial colony cultured at 37°C produced serratamolide. Due to this material, the cell cultured at 37°C might not be adhered to polystyrence plate and loss of hydrophobicity.

These above findings show that the cultivation time

and temperatures play an important role in the habitat of *S. marcescens* cell cycle. However, it cannot be concluded that hydrophobicity is the sole mechanism for the adhesion of *S. marcescens*, because evidence for the role of polymer bridging in the adhesion of bacteria to solid surfaces has also been presented (2).

Discussion

Adsorption of microorganisms to interfaces has become a focus of interest in recent years (19, 20). Adhesion by cell surfaces plays an important role in many biological processes; contact inhibition, cell differentiation, interaction between pathogenic bacteria and various target cells (21). Each of these involves highly specialized mechanisms of recognition mediated by lectins (Sugar and protein carbohydrates) and specific receptors on the cell surfaces. Many microorganisms, such as pathogens and rhizopbia on plant root cells, adhere to surfaces in this specific way. However, this can not apply to all of the organisms, which attach nonspecifically to many different types of interfaces as well as inert surfaces. Among these are the *S. marcescens* bacteria. In addition, there are many microorganisms which depend, in nature, on the degradation of nonsoluble substrates such as cellulose, chitin, elemental sulfur, and petroleumn and so adhere to these substances. The bacterial adherence to noncharged surfaces of polystyrene dishes has been suggested as a good method for measuring the cell surface hydrophobicity (4). By using this method, we found that the *S. marcescens* cultured at 30°C adhered to polystyrene dishes very tightly. On the other hand, cells cultured at 37°C were not attached to hydrophobic polystyrene surfaces. Recent studies of bacterial cell surface hydrophobicity suggest that hydrophobic properties are often determined by an interplay of 'hydrophobins' (hydrophobicity-promoting components) and 'hydrophilins' (hydrophobicity-reducing moieties), which coexist on the cell surface membranes (22). Therefore, Poindexter (23) has shown that the removal of the cell wall or part of it by treatment with lysozyme or mechanical shearing of the cell envelope in omnimixer resulted in the loss of hydrophobicity. This confirms that the hydrophobicity is a surface phenomenon and that the cell envelope is responsible for the hydrophobicity expressed in *S. marcescens*.

The hydrophobicity of *S. marcescens* seems to have a genetic basis, which induces the changes of lipid compositions in cell membranes or controls the production of hydrophobic substances according to the environmental circumstances. In the example of genetic control, Fatton and Shilo (1) isolated a hydrophobic *S. platensis* mutant from the hydrophilic wild type. This mutant have shown to possess hydrophobic cell envelopes.

In summary, the data indicate that the pronounced cell surface hydrophobicity of the cells cultured at 30°C may be due to the simultaneous presence of several components on the outmost bacterial cell surfaces. The results presented here, taken together with other studies of hydrophobicity in *Serratia* strains (14,24), suggest that the factors which affect the hydrophobicity include prodigiosin, as well as the components responsible for the lipid compositions of cell membranes.

요 약

*Serratia marcescens*를 30℃에서 진탕배양했더니, 적색색소인 prodigiosin이 초로기(senescent phase of growth)에서 생성되었다. 그리고 이조건에서 배양한 세포를 polystyrene dish를 사용하여 세포의 hydrophobicity를 측정한 결과 상당한 소수성 성질이 발현되어 대부분의 세포가 비극성 성질의 polystyrene dish에 흡착되었다. 그러나 이 박테리아를 37℃에서 배양했더니, 적색 색소인 prodigiosin도 생성되지 않았을 뿐 아니라 소수성 성질도 발현되지 않음으로서 세포가 polystyrene dish에 흡착되지 않고 pre-washing 단계에서 모두 씻겨져 나갔다. 또한 30℃와 37℃에서 배양한 *serratia marecescens*의 지질성분을 분석한 결과, 30℃에서 배양한 세포의 지질은 phospholipid, glycolipid 및 확인되지 않은 지질 등이 생성되었으나 37℃에서 배양한 세포의 경우는 주로 양쪽성 성질의 aminolipid인 serratamolide가 생성되어, 배양한 온도조건에 따라 뚜렷한 차이를 보였다. 이러한 실험결과로부터, Serratia *marcescens* 세포 표면의 소수성 성질은 세포배양 시간과 배양온도의 조건에 영향을 받는 여러 요인들에 의하여 변화된다는 사실을 알 수 있었다.

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