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Control of AtaA-mediated bacterial immobilization by casein hydrolysates

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***Acinetobacter* sp. Tol 5 exhibits an autoagglutinating nature and high adhesiveness to various abiotic surfaces through its bacterionanofiber protein AtaA. We have developed new bacterial immobilization methods utilizing the high adhesiveness of AtaA. We previously reported that salt is essential for the adhesiveness of AtaA. In the current study, we unexpectedly found that Tol 5 cells were not immobilized onto polyurethane foam support during growth in LB medium although AtaA was properly expressed and displayed onto the cell surface. The adhesion of Tol 5 resting cells was not affected by sugars but drastically inhibited by yeast extract and casein hydrolysates such as tryptone and casamino acids technical grade (CA-T). Some amino acids, which are major components of CA-T, partially inhibited the adhesion of Tol 5 cells. Experimental results suggested that oligopeptides might effectively inhibit the cell adhesion. Immobilized cells onto the support through AtaA were detached in CA-T solution. Also, the detached cells could be re-immobilized onto the support without impairing of their adhesiveness by replacing CA-T solution to a basal salt medium. Microscopic observation revealed that breaking of AtaA-mediated cell–cell interaction is important for the detachment of Tol 5 cells from the support. CA-T also inhibited AtaA-mediated autoagglutination and dispersed cell clumps through AtaA. This is the first report on adhesion inhibitors against AtaA and suggests that casein hydrolysates like CA-T would be a powerful tool for controlling AtaA-mediated bacterial immobilization.**

[**Key words:** Adhesion; Autoagglutination; Immobilization; Trimeric autotransporter adhesin; *Acinetobacter*; Adhesion inhibitor; Casein hydrolysate; Dispersion]

Microbial adhesion is the first step of colonization, biofilm formation, and pathogen infection. In a medical field, resistant surfaces and inhibitors against microbial adhesion and biofilm formation have been extensively studied to avoid infectious disease (1,2). On the other hand, microbial adhesion is recognized to be beneficial in waste treatment and bioprocesses for chemical production (3,4). Controlling microbial adhesion is important for both opposite goals: its prevention and inhibition; its enhancement and promotion (5).

Acinetobacter sp. Tol 5 is a nonpathogenic and toluene-degrading bacterium isolated from a biofiltration system (6), and exhibits an autoagglutinating nature and high adhesiveness to various abiotic surfaces including hydrophobic plastics, hydrophilic glasses, and metals (7). These adhesive characteristics are mediated by the cell surface protein AtaA, which is a large fibrous protein consisting of 3630 amino acids and belongs to the trimeric autotransporter adhesin (TAA) family (8). TAAs are outer membrane proteins widely distributed in Gram-negative bacteria, and they form homotrimeric structures with a common N-terminus–head–stalk–membrane anchor–C-terminus architecture (9,10). Most of reported TAAs derived from pathogenic bacteria specifically adhere to host cells and biotic molecules such as fibronectin and collagen (10,11). Although AtaA shares the common architecture of TAAs, it uniquely mediates tenacious and nonspecific adhesion of bacterial cells to both biotic and abiotic surfaces.

Bacterial immobilization is an important and common method for the efficient use of whole-cell biocatalysts, and various immobilization methods, such as physical adsorption, gel entrapment, and biofilm immobilization, have been developed and improved (3,12,13). The characteristically adhesive feature of AtaA can be conferred to other non-adhesive and non-agglutinating Gram-negative bacteria by transformation with *ataA* gene (8). Thus, we have developed methods for bacterial cell immobilization using AtaA and demonstrated their advantages over conventional methods (14–16). Bacterial cells displaying AtaA on their surface can be quickly immobilized onto various types of supports made from desirable materials for application, such as polyurethane (PU) foam, glass wool, cellulose fiber, and steel wool (16).

Recently, we reported that salt is essential for the high adhesiveness of AtaA and even cells expressing *ataA* cannot adhere in pure water. Using this phenomenon, we demonstrated that bacterial cells expressing *ataA* can be immobilized and detached repeatedly only by rinsing the immobilized cells with pure water, and that both bacterial cells and supports can be reused for another reaction (17). On the other hand, compounds that affect the adhesiveness of AtaA have never been reported. In this study, we accidently found that LB medium inhibits the AtaA-mediated bacterial immobilization. We subsequently investigated the specific compounds that are responsible for the inhibition and their application to the control of bacterial immobilization.

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MATERIALS AND METHODS

Materials L-threonine and glycine were purchased from Tokyo Chemical Industry (Tokyo, Japan) and MP Biomedicals (Santa Ana, CA, USA), respectively. L-phenylalanine, L-proline, L-isoleucine, L-tyrosine, L-valine, L-glutamine, L-histidine, L-serine, L-asparagine, and L-tryptophan were purchased from Kishida Chemical (Osaka, Japan). Other amino acids and sugars were purchased from Fujifilm Wako Pure Chemical (Osaka, Japan). Casamino acids technical grade (CA-T), casamino acid (CA), yeast extract, and tryptone were purchased from Becton, Dickinson and company (Franklin Lakes, NJ, USA). Basal salt (BS) medium and BS-N medium were prepared as described previously (18,19). Luria–Bertani (LB) Lennox medium was purchased from Nacalai Tesque (Kyoto, Japan). Two kinds of polyurethane foam support (1-cm^3 cube), CFH-40 with a specific surface area of 50 cm^2/cm^3 and CFH-30 with a specific surface area of 37.5 cm^2/cm^3, were given by Inoac Corporation (Nagoya, Japan) and used for bacterial cell immobilization.

Cultivation, preparation, and immobilization of bacterial cells *Acinetobacter* sp. Tol 5 (6) and its Δ*ataA* mutant strain 4140 (20) were grown at 28°C in LB medium or BS medium supplemented with 0.5 μl/mL toluene as a carbon source as previously described (7,19). Production of AtaA and its cell-surface display were analyzed by immunoblotting and flow cytometry using anti-$AtaA_{699\text{-}1014}$ antiserum as previously described (8,21).

For cell immobilization during growth, cells were grown in 30 mL of LB or BS medium at 28°C for 18 h with shaking at 115 rpm in the presence of three pieces of PU foam support (CFH-40) in a 100 mL Erlenmeyer flask. For quantification of cell mass, dry cell weights of the immobilized and planktonic cells were measured as described previously (16).

To prepare detachment cells for repetitive immobilization, 100 mL of cell suspension in BS medium was transferred to a 500-mL Erlenmeyer flask containing 12 pieces of PU foam support (CFH-30). The flask was shaken at 115 rpm at 25°C to immobilize the cells. After a 2-h shaking, the supernatant was discarded and the PU foam support with immobilized cells was rinsed with BS-N medium to release cells adhering loosely. Then, 100 mL of BS-N medium supplemented with 1% CA-T was transferred to the flask and shaken at 115 rpm for 60 s to detach the cells. After removing the support, the planktonic cells were harvested by centrifugation at 2500 ×*g* for 10 min. The harvested cells were washed with deionized water (dH_2O) and resuspended in BS-N medium at an OD_{660} of 1.0.

Adherence, autoagglutination, and dispersion assays Adherence assays were performed as described previously (7), with the following modifications. Bacterial cells were suspended in BS-N medium supplemented with a compound to examine its inhibitory effect on cell adhesion. The cell suspension was placed into a 96-well polystyrene (PS) microplate (Becton, Dickinson and Company) and incubated for 2 h, at 4°C, avoiding cell growth.

Autoagglutination assays were performed by a tube-settling assay as previously described (7,8), with slight modifications. Glass test tubes containing cell suspension were left to stand for 6 h at 4°C, avoiding cell growth. The autoagglutination ratio was calculated from the decrease in the optical density at 660 nm (OD_{660}) of the cell suspension.

For dispersion assay, 15-mL centrifuge tubes (Proteosave SS; Sumitomo Bakelite, Tokyo, Japan) containing 8 mL of cell suspension in BS-N medium at an OD_{660} of 1.0 (OD_{660}-initial cells) were left to stand for 3 h at 28°C to settle cell clumps generated by autoagglutination. Subsequently, a compound was added to the tube at the final concentration of 1% (wt/vol) to examine its ability to disperse the cell clumps. The tube was gently inverted three times and left to stand for 5 min at 28°C. From the top of the tube, 1 mL of cell suspension was sampled and the OD_{660} was measured (OD_{660}-dispersed cells). The dispersion ratio was calculated using the following equation:

$$\text{Dispersion ratio (\%)} = \frac{OD_{660}\text{-dispersed cells}}{OD_{660}\text{-initial cells}} \times 100 \quad (1)$$

Quantification of immobilization of resting cells onto polyurethane foam support and detachment from the support Bacterial cells were suspended in BS-N medium at an OD_{660} of 1.0. Thirty milliliters of the cell suspension was transferred to a 100-mL Erlenmeyer flask containing three pieces of PU foam support (CFH-30) and shaken at 115 rpm at 25°C for 2 h. The immobilization ratio was determined from the decrease in the OD_{660} of the cell suspension as described previously (16).

For the quantification of detachment of cells, bacterial cells were suspended in BS-N medium at an OD_{660} of 1.0. Thirty milliliters of the cell suspension was placed into a 100-mL Erlenmeyer flask and shaken with three pieces of PU foam support (CFH-30) at 115 rpm at 25°C. After a 2-h shaking, the supernatant was discarded. The PU foam support with immobilized cells was rinsed with BS-N medium to detach cells adhering loosely, placed into a 100-mL Erlenmeyer flask containing 30 mL of BS-N medium supplemented with 1% (wt/vol) CA-T, and shaken at 115 rpm. After the 60-sec shaking, the OD_{660} of the suspension containing detached cells was measured (OD_{660}-detached cells). To quantify cells that remained attached, three pieces of the support were collected and shaken vigorously in dH_2O to force to detach the cells, and the OD_{660} of the cell suspension was measured (OD_{660}-attached cells). The detachment ratio was calculated using the following equation:

$$\text{Detachment ratio (\%)} = \frac{OD_{660}\text{-detached cells}}{(OD_{660}\text{-detached cells} + OD_{660}\text{-attached cells})} \times 100 \quad (2)$$

Confocal laser scanning microscopy Bacterial cells were suspended in BS-N medium at an OD_{660} of 1.0 and placed on a 4-well glass plate (Matsunami Glass, Osaka, Japan). After a 2-h incubation at 28°C, the cell suspension on the plate was removed and rinsed with BS-N medium or BS-N medium supplemented with 1% (wt/vol) CA-T solution twice. The remained cells on the glass plate were stained with 5 μM SYTO9 (LIVE/DEAD *Bac*Light Bacterial Viability Kit; Thermo Fisher Scientific, Waltham, MA, USA) for 10 min. The stained cells were rinsed with BS-N medium twice and observed by a confocal laser scanning microscope (CLSM) (FV1000D; Olympus, Tokyo, Japan).

RESULTS

Bacterial immobilization via AtaA during growth in LB or BS medium We previously reported that AtaA-producing cells can be immobilized onto PU foam support during growth in BS medium in a shaken flask (16). However, we found an unexpected phenomenon, that is, Tol 5 cells hardly adhere to PU foam support in LB medium during growth. Fig. 1A shows the appearances of culture broth (30 mL) of Tol 5 wild type (WT) and Δ*ataA* cells after growth in 100 mL flasks shaken at 115 rpm in the presence of PU foam support. BS medium was transparent after the cell growth of Tol 5 WT because the cells were not suspended in the medium but adhered to the support. Of course, Δ*ataA* cells could not adhere to the support and their suspended cells made BS medium opaque. By contrast, LB medium became opaque although Tol 5 WT cells were grown in the presence of the support. In fact, although the total dry weight of Tol 5 WT cells grown in LB medium was fourfold of that in BS medium, most of the cells were planktonic in LB medium but immobilized onto the support in BS medium (Table 1).

Therefore, we suspected whether the expression of the *ataA* gene was suppressed in LB medium. However, immunoblotting of the whole cell lysate showed that the production of AtaA was not decreased in LB medium compared with BS medium (Fig. 1B). Flow cytometry showed that the amounts of AtaA molecules displayed on the cell surface were similar, independent of culture media (Fig. 1C). These results showed that the expression of the *ataA* gene is not suppressed in LB medium and suggested that LB medium contains some inhibitors against the AtaA-meditated cell adhesion.

Effects of sugars and protein hydrolysates on the AtaA-mediated cell adhesion Subsequently, we investigated the inhibitory effect of yeast extract and tryptone, major components of LB medium, and glucose and arabinose, sugars commonly used in bacterial culture media as a carbon source or an inducer, on the adhesion of Tol 5 cells without cell growth. Adherence assays using a well-plate were performed at 4°C with the addition of one of these medium components from 0.01% to 5%, a concentration range of their general use. As a result, glucose and arabinose showed no inhibitory effect on Tol 5 cell adhesion even in the highest concentration tested (5%) (Fig. 2). By contrast, the adhesion ratio of Tol 5 cells decreased as the concentrations of yeast extract and tryptone increased, showing an inhibitory effect on the cell adhesion. Therefore, we hypothesized that protein hydrolysates inhibited the AtaA-meditated cell adhesion. To verify this hypothesis, we evaluated the inhibitory effect of CA-T, which was an acid hydrolysate of casein and contained amino acids and oligopeptides. In CA-T solution, the cell adhesion ratio gradually decreased as the concentration of CA-T increased like tryptone and yeast extract. Thus, it was confirmed that Tol 5's AtaA-mediated cell adhesion is inhibited by protein hydrolysates.

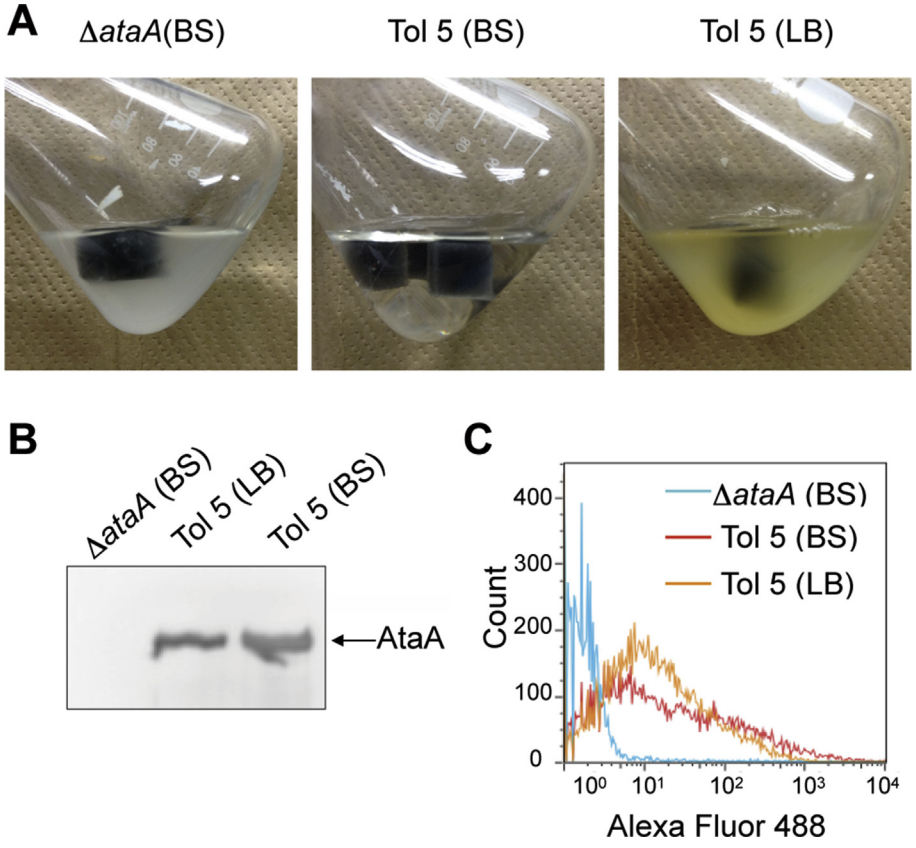

FIG. 1. Effect of the growth medium on the cell immobilization of *Acinetobacter* sp. Tol 5 and the production and cell surface display of AtaA. (A) AtaA-mediated bacterial immobilization during growth. Each photo shows the cell suspension after 18-h cultivation of Tol 5 Δ*ataA* in BS medium, Tol 5 in BS medium, or Tol 5 in LB medium. (B) Immunodetection of produced AtaA using anti-AtaA antiserum against whole cell lysates. (C) Flow cytometry using anti-AtaA antiserum for confirming the cell surface display of AtaA. Cyan, red, and orange lines represent Tol 5 Δ*ataA* cultured in BS medium, Tol 5 cultured in BS medium, and Tol 5 cultured in LB medium, respectively.

Effects of amino acids on the AtaA-meditated cell adhesion Because major components of CA-T were amino acids, we examined the ability of each amino acid to inhibit Tol 5 cell adhesion by the adherence assay in the presence of 1% each amino acid or CA-T. It was virtually impossible to dissolve tyrosine, tryptophan, asparagine, or leucine to 1% into the test solution (BS-N medium) at the test temperature (4°C). Among these four amino acids, only leucine showed the significant inhibitory effect even at 0.5%; the adhesion ratio of Tol 5 cells decreased by over 60% (Fig. 3A). Among other 16 amino acids, basic amino acids (lysine, arginine, and histidine) did not affect Tol 5 cell adhesion at 1%. By contrast, the adhesion ratio of Tol 5 cells decreased in the presence of 1% non-charged amino acids except proline; phenylalanine, leucine, isoleucine, methionine, valine, alanine, serine, threonine, glutamine, and cysteine reduced the adhesion ratio to about 25–60%, regardless their hydrophobicity. Likewise, acidic amino acids, glutamic acid and aspartic acid reduced the adhesion ratio to about 50%. However, these amino acids showed less inhibitory effect on Tol 5 cell adhesion than CA-T (Fig. 3A). In addition, the content of methionine, which showed the highest inhibitory effect among amino acids, was relatively low in CA-T. Therefore, the drastic inhibitory effect of CA-T was assumed to be caused by a synergistic effect of several amino acids (first hypothesis). Then, an amino acid mixture solution corresponding to 1% CA-T composition was prepared as shown in Table S1 and used for the adherence assay as an additive. Although the amino acid mixture partially inhibited Tol 5 cell adhesion, its inhibitory effect (by less than 40% reduction) was much less than that of CA-T (by near 90%) (Fig. 3B). Thus, the first hypothesis was denied.

Second hypothesis to explain the high ability of CA-T to inhibit Tol 5 cell adhesion was the large contribution of some oligopeptides contained in CA-T to the inhibition. We examined the inhibitory effect of another grade of regents of acid hydrolysis products of casein, casamino acids (CA), which contained more amino acids and less undegraded oligopeptides than CA-T, in the cell adherence assay. As shown in Fig. 3B, the effect of CA on Tol 5 cell adhesion was clearly less than that of CA-T. This result supported the second hypothesis.

To clarify that the acid hydrolysis products of casein inhibit AtaA from mediating bacterial cell adhesion, ADP1/pAtaA was subjected to the cell adherence assay in the presence of CA-T, the amino acid mixture, or CA. As a result, the inhibitory effects of these additives on ADP1/pAtaA cell adhesion were in similar levels with those on Tol 5 cells (Fig. 3B), suggesting that CA-T directly interacts with AtaA to inhibit bacterial cell adhesion.

Detachment of cells immobilized through AtaA by CA-T The finding that the AtaA-mediated cell adhesion is drastically inhibited by CA-T prompted us to examine the ability of CA-T to detach bacterial cells immobilized onto a material

TABLE 1. Dry cell weights of the immobilized cells onto PU foam support and the planktonic cells.

	Immobilized cell (mg)	Planktonic cell (mg)	Total cell (mg)
Δ*ataA* (BS)	0	6.6 ± 0.0	6.6 ± 0.0
Tol 5 (BS)	5.7 ± 0.5	0	5.7 ± 0.5
Tol 5 (LB)	0.1 ± 0.1	22.2 ± 0.4	22.3 ± 0.4

Data are expressed as mean ± SEM (n = 3).

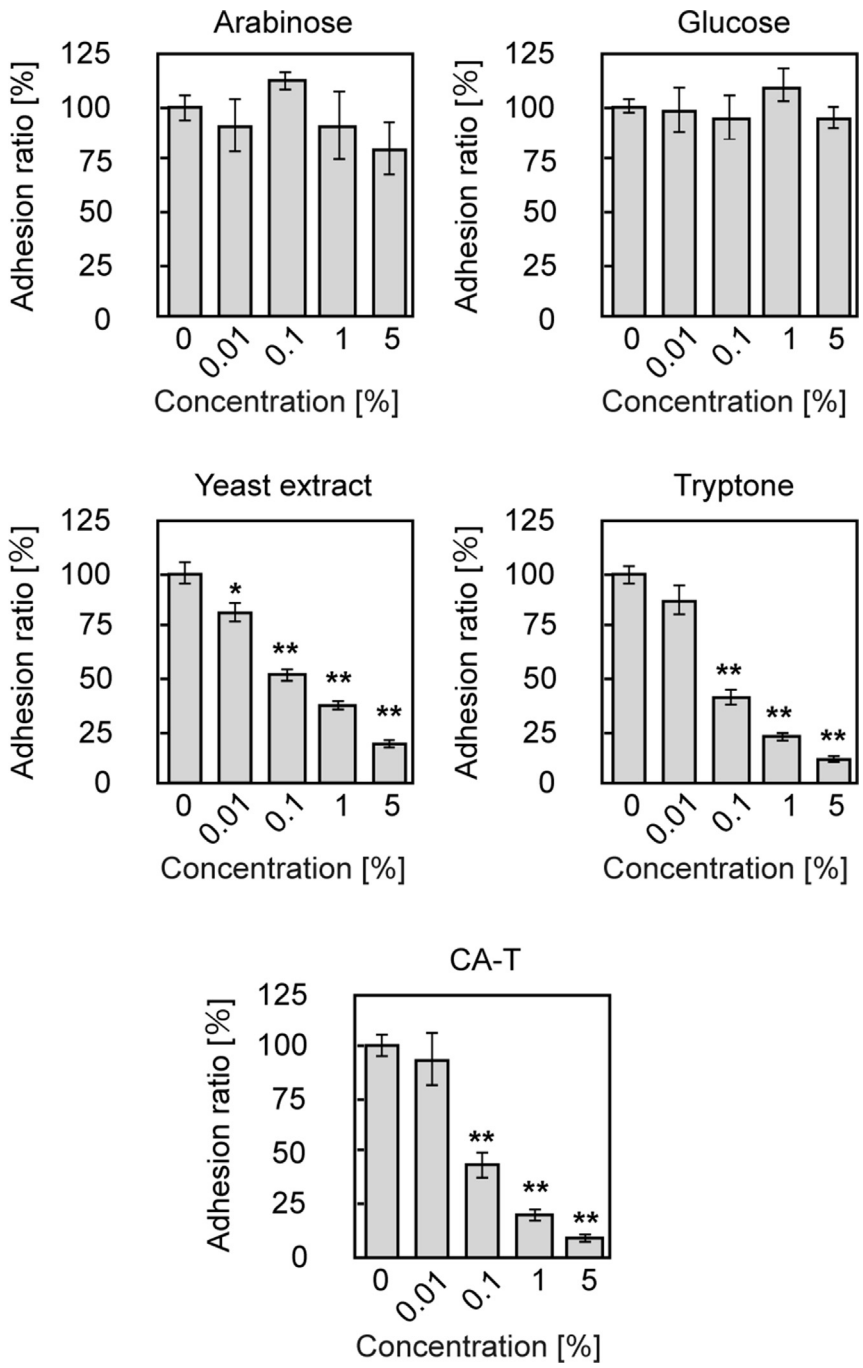

FIG. 2. Inhibitory effect of medium components on AtaA-mediated bacterial adhesion. Data are expressed as mean ± SEM (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$, compared with each 0% sample by *t*-test.

surface beforehand. A piece of PU foam support onto which Tol 5 cells were immobilized was placed in a 30 mL vial containing a stirring bar and 10-mL BS-N medium with or without 1% CA-T. During stirring for 60 s, in the presence of 1% CA-T, the immobilized cells were rapidly detached from the support and dispersed into the medium; a mark printed on a sheet of paper that was placed behind the vial containing CA-T became invisible by the dispersed cells (Fig. 4A). By contrast, the cells remained to be immobilized by just stirring without CA-T. For quantification of the cell detachment, detachment tests in Erlenmeyer flasks were also performed. In the presence of 1% CA-T, approximately 80% of immobilized cells were detached from PU foam support (Fig. 4B). Thus, it was revealed that CA-T has the ability to not only inhibit cell adhesion but also detach cells immobilized onto material surfaces through AtaA beforehand.

Subsequently, we tried to re-immobilize the bacterial cells detached by CA-T. Fresh and detached Tol 5 cells were prepared and shaken in Erlenmeyer flasks in the presence of PU foam support to examine the time profile of cell immobilization. The detached cells were re-immobilized onto the support as rapidly as the initial immobilization of the fresh cells and the re-immobilization ratio reached 90% within 30 min (Fig. 4C), suggesting that AtaA was not impaired by the detachment by CA-T and that immobilizing and detaching bacterial cells could be repeated using CA-T.

Next, we investigated how CA-T detached immobilized Tol 5 cells from the surface. Tol 5 cell suspension in BS-N medium was

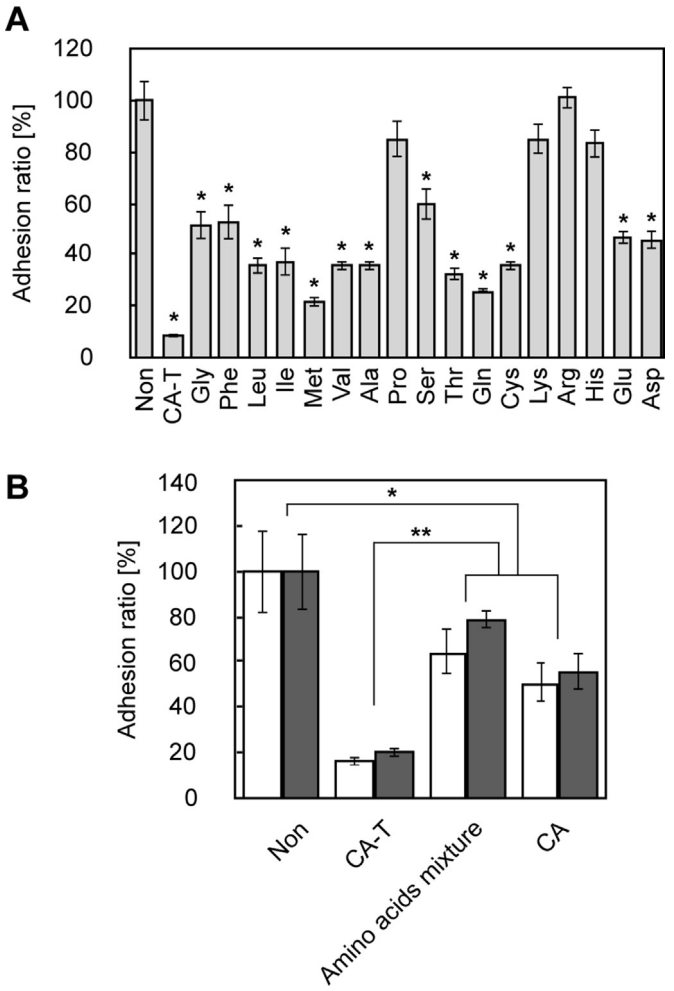

FIG. 3. Effect of additives on AtaA-mediated bacterial adhesion. (A) Inhibitory effect of amino acids on AtaA-mediated bacterial adhesion. Non means amino acid-free solution. Data are expressed as mean ± SEM (n = 5). $^{*}P < 0.01$, compared with Non by *t*-test. (B) Inhibition of AtaA-mediated bacterial adhesion by CA-T, amino acids mixture, and CA. Non means amino acid-free solution. Open and closed bars indicate Tol 5 cells and ADP1/pAtaA cells, respectively. Data are expressed as mean ± SEM (n = 5). Statistical significance, $^{*}P < 0.05$, $^{**}P < 0.01$.

placed onto a glass well-plate and incubated for 2 h at 28°C for cell adhesion. After the incubation, the cell suspension was removed and the well was rinsed with just BS-N medium or BS-N medium supplemented with 1% CA-T. The remained cells on the glass plate were observed by CLSM after fluorescent staining with SYTO9. On the well rinsed with BS-N medium, the cells remained to be immobilized on the glass surface, forming a thick and dense bacterial cell mat (Fig. 5). In contrast, the rinse with CA-T completely removed the aggregating cells and scatteredly left the cells directly adhering to the glass surface.

Inhibition and dispersion effects of CA-T on the cell–cell interaction through AtaA Because the results shown in Fig. 5 suggested that CA-T especially breaks the cell–cell interaction through AtaA, we investigated the ability of CA-T to inhibit autoagglutination and disperse cell clumps through AtaA. Tol 5 cells were subjected to the autoagglutination assay in BS-N medium supplemented with glucose or CA-T. The autoagglutination of Tol 5 cells was inhibited drastically by 1% CA-T but slightly by 1% glucose (Fig. 6A). Next, glucose or CA-T was added to Tol 5 cell clumps settled at the bottom of BS-N medium in a centrifuge tube for the dispersion assay. After inverting the tube three times, 1% CA-T dispersed the cell clumps and the dispersion ratio exceeded 80% while 1% glucose showed no effect on the cell clumps (Fig. 6B).

Thus, it was revealed that CA-T has abilities to inhibit AtaA's function of autoagglutination of bacterial cells and also to break the cell–cell interaction through AtaA forming cell crumps.

DISCUSSION

Inhibitors for bacterial adhesion have been abundantly studied in terms of antibacterial agents and sterilization (1,2). For example, cranberry juice inhibits the adhesion of uropathogenic *Escherichia coli*, and fucosylated oligosaccharides from human milk inhibit the adhesion of *Campylobacter jejuni* (1,22,23). The mechanisms of inhibitions are mainly divided into regulation of gene expression, degradation of adhesins, and competitive inhibition (1,2). A-type proanthocyanidins derived from cranberry juice, one of the most studied bacterial adhesion inhibitors, have been reported as competitive inhibitors against type P fimbriae of *E. coli* (24,25). Casein hydrolysates are also thought to inhibit the AtaA-mediated adhesion in a competitive manner because detached cells by CA-T solution can be re-immobilized onto support in the absence of CA-T, denying degradation or irreversible denaturation of AtaA.

While various amino acids and their mixture inhibit Tol 5 cell adhesion, their inhibiting effects are weaker than CA-T (Fig. 3). Previously, some glycopeptides from casein hydrolysates were shown to inhibit bacterial adhesion (26), suggesting that some

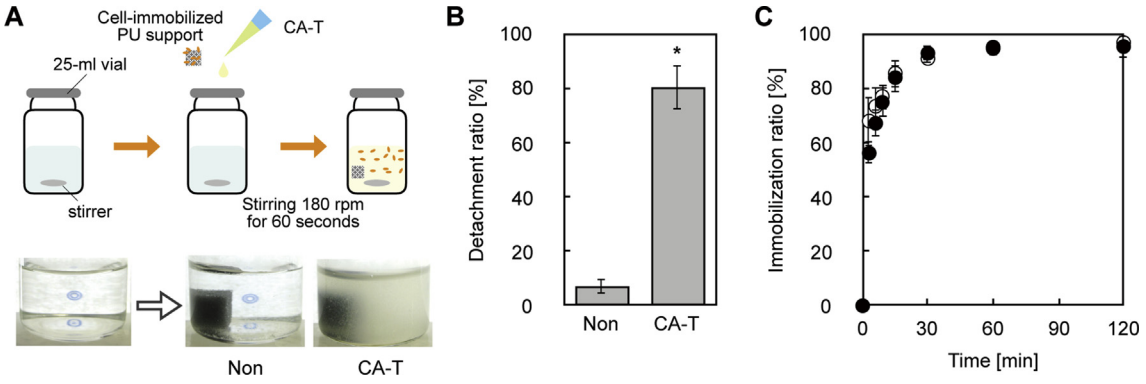

FIG. 4. Detachment and re-immobilization of Tol 5 cells. (A) Upper illustration shows the scheme of the observation for detachment, and lower pictures show the solution after stirring PU foam support with immobilized Tol 5 cells in inhibitor-free solution (Non) or 1% CA-T solution. Note that the turbidity of the solution is caused by dispersion of detached cells from the support. (B) Quantification of the detachment by CA-T. The amount of immobilized cells beforehand is taken as 100%. Data are expressed as mean ± SEM (n = 3). $^{*}P < 0.01$, compared with Non by *t*-test. (C) Time course of immobilization of Tol 5 cells onto PU foam support. The filled circles and the open circles show the immobilization of fresh cells and detached cells from PU foam support by CA-T, respectively. Data are expressed as mean ± SEM (n = 3).

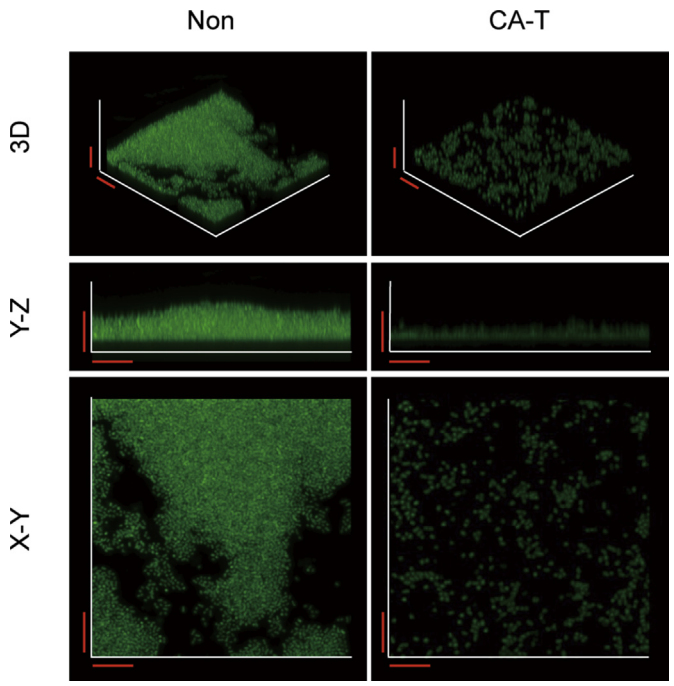

FIG. 5. Tol 5 cells remained attached on a glass plate after a rinse with inhibitor-free (Non) solution or 1% CA-T solution. The cells were stained with SYTO9 (green) and observed by CLSM. Upper, middle, and lower images show 3D visual, Y-Z plane and X-Y plane, respectively. Red scale bars indicate 20 μm.

oligopeptides or components other than amino acids in CA-T effectively inhibit AtaA-mediated adhesion. Interestingly, the inhibiting effect of CA was weaker than CA-T (Fig. 3). According to BD Bionutrients Technical Manual (27), approximately 60%, 35%, and 5% of the ingredients of CA-T are distributed in compounds with molecular weights under 250 Da, 250–500 Da, and 500–2000 Da, respectively. On the other hand, more than 90% of the ingredients of CA are under 250 Da, and those over 250 Da are less than 10%. The molecular weight of over 250 Da corresponds to that of oligopeptides. In addition, tryptone, another casein hydrolysate that is produced by trypsin digestion and contains various peptides (27), showed the inhibitory effect in a similar level as CA-T (Fig. 2). With considering these, some oligopeptides may inhibit AtaA-mediated adhesion most effectively.

Recently, we showed that cell clumping is important in the AtaA-mediated adhesion process and greatly contribute to the formation of a biofilm-like structure (28). CA-T inhibits AtaA-mediated autoagglutination, disperses cell clumps through AtaA, and detaches cell aggregates immobilized by AtaA from a material surface. Inhibitors in CA-T might interact specifically with AtaA and competitively inhibit the interaction of AtaA with AtaA itself or other cell surface components for autoagglutination. The adhesion of other gram-negative bacteria, such as *Yersinia enterocolitica* and *Bartonella henselae* in which TAAs mediate adherence to their pathogenic targets, autoagglutination, and biofilm formation, could be effectively prevented by casein hydrolysates in the same manner as *Acinetobacter* sp. Tol 5.

Our results taught us that the use of media containing casein hydrolysates should be avoided for their use in bioprocesses employing bacterial cells immobilized through AtaA. From another point of view, compounds which can inhibit cell adhesion and detach immobilized cells give us not only the limitation above but also advantages. Previously, we reported that the cells immobilized by AtaA can be detached by rinsing with dH_2O. However, in a medium containing salts or buffer solution, AtaA-mediated cell adhesion can be prevented and immobilized cells can be detached by just adding CA-T to the solution. This is convenient for bioprocesses, for example, the preparation of whole cell catalysts before immobilization and the recovery of immobilized cells and

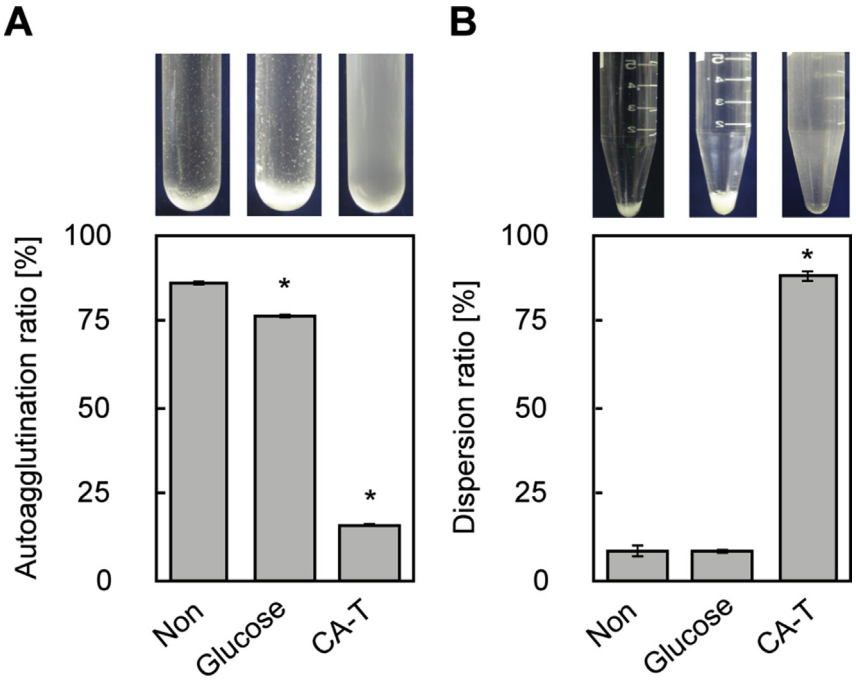

FIG. 6. Inhibition of AtaA-mediated autoagglutination and dispersion of cell clumps by CA-T. Data are expressed as mean ± SEM (n = 3). Non means no addition of glucose or CA-T. $^{*}P < 0.01$, compared with Non by *t*-test. (A) Inhibitory effects of glucose and CA-T on autoagglutination of Tol 5 cells. (B) The ability of glucose and CA-T to disperse Tol 5 cell clumps.

support materials without rinsing with dH_2O or buffer replacement.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2019.04.019.

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