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EXPERIMENTAL ARTICLES

Regulation of the Adhesion of *Pseudomonas fluorescens* Cells to Glass by Extracellular Volatile Compounds

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Abstract—The effect of the gaseous metabolites of one *Pseudomonas fluorescens* culture on the attachment of cells of another *P. fluorescens* culture to glass was studied. Gaseous metabolites increased the number of unattached cells by 10–30% and the mean residence time of cells attached to glass by 100%. These effects were presumably due to the yet unidentified compound, which we called volatile antiadhesin. This compound could be adsorbed by activated charcoal and HAYESEP-Q adsorbent.

Key words: adhesion, *Pseudomonas fluorescens*, regulation, volatile compounds

Microorganisms evolve a variety of gaseous metabolites [1, 2], which can serve as trophic substances, interspecies communication signals, and defence agents [1, 3]. Some gaseous metabolites can provide intraspecies cell-to-cell communications [4]; that is, they act as gaseous analogues of homoserine lactones [5]. The biological role of the majority of gaseous microbial metabolites is unclear [1]. In particular, little is known about their effect on the attachment of bacterial cells to various surfaces.

In the present work, which is a continuation of our studies of the mechanism of adhesion of *Pseudomonas fluorescens* cells [6, 7], we concentrated on the ability of their abundant gaseous metabolites [1] to affect bacterial adhesion to glass.

MATERIALS AND METHODS

Experiments were carried out with the strain *Pseudomonas fluorescens* NCIMB 9046 obtained from the National Collection of Industrial and Marine Bacteria (NCIMB) in Aberdeen (United Kingdom). The strain was grown at 30°C in 100- or 250-ml flasks with 10 and 50 ml of medium shaken at a rotation speed of 100 rpm (Lab-line orbital incubator shaker). The growth medium was M9 [8] supplemented with 0.2% glucose and the following microelements (mg/l): $MgSO_4 \cdot 7H_2O$, 247; $CaCl_2$, 14.7; $MnSO_4 \cdot 4H_2O$, 0.4; $ZnSO_4 \cdot 7H_2O$, 0.4; $FeCl_3 \cdot 6H_2O$, 1 (pH 7.0) [9]. The flasks were inoculated with 16-h stationary-phase cultures added in amounts of 2–5%. To obtain cells for adsorption experiments, the culture grown to the exponential phase (3–5 h of growth; $OD_{600} = 0.05–0.1$) was filtered through a 0.45-μm-pore-size Millipore filter, and the residue cells were washed and resuspended in fresh nutrient medium. The culture which served as the donor of culture air (CA) was grown to the mid-exponential phase ($OD_{600} = 0.6–0.7$). Culture growth was monitored by measuring culture turbidity at $\lambda = 600$ nm on a Pye-Unicam SP-450 UV/VIS spectrophotometer.

The effect of the CA on cell attachment was studied in experiments designed as follows. Flask 2 (250 ml in volume) with the mid-exponential donor culture was connected to 100-ml flask *1* with sterile nutrient medium via silicone tubing *3* ($d = 8$ mm; $l = 20$ cm) passing through the foam bung stoppers of the flasks (Fig. 1a). This system was incubated for 30–60 min, after which the medium in flask 1 was inoculated with washed cells prepared as described above (the resultant culture was referred to as a recipient culture). In some experiments, to enhance the effect of the culture air, it was pumped through the recipient culture. For this purpose, filter-sterilized atmospheric air was bubbled through a donor culture (50 ml) in flask *2* to displace the gas phase from this flask into flask *1* with sterile nutrient medium (Fig. 1b). After pumping the air at a rate of 0.1 flask volume per min for 1–2 h, the medium in flask *1* was inoculated with washed cells, and the air pumping was resumed. In the control experiment, flask *2* contained sterile medium. In a number of experiments, the gas phase from flask *2* was passed through trap *7* with activated charcoal (BDH, United Kingdom) or HAYESEP-Q adsorbent (Hayes Separation Inc., the United States).

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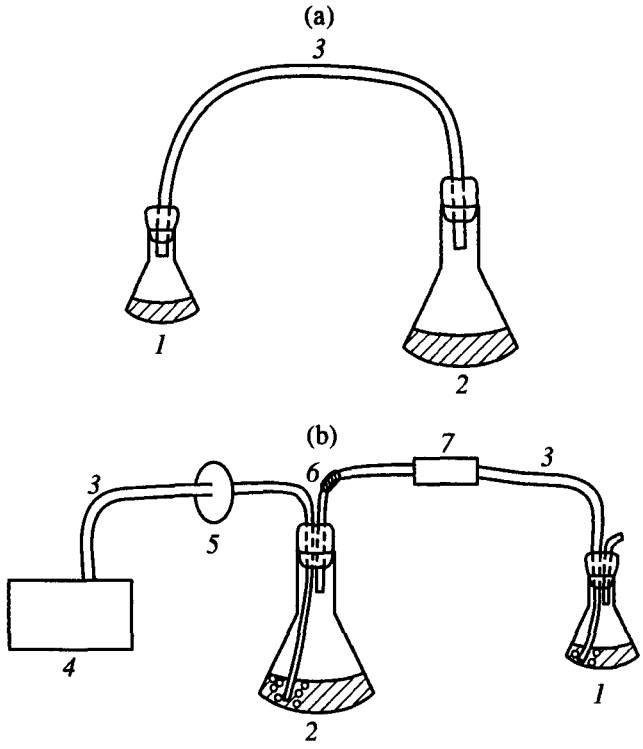

Fig. 1. Experimental setups for studying the effect of culture air on the adsorption of *P. fluorescens* cells: (a) passive diffusion of culture air via silicone tubing; (b) pumping of culture air; (*1*) recipient culture; (*2*) donor culture or sterile medium (control); (*3*) silicone tubings; (*4*) pump; (*5*) bacterial filter; (*6*) cotton-wool trap to capture moisture droplets; and (*7*) trap with sorbent (in some experiments).

Cell adhesion was characterized by three parameters: adhesion value, adhesion residence time, and the amount of unattached cells. Adhesion value, defined as the percentage of cells attached to the flask walls, was calculated by the following formula:

$$(OD_{ini} - OD_{min})/OD_{ini}, \quad (1)$$

where OD_{ini} is the optical density (turbidity) of the culture at the moment of inoculation and OD_{min} is the minimum culture turbidity corresponding to the maximum level of adhesion.

Adhesion residence time was defined as the time elapsing between the moment of cell inoculation and the moment of extensive detachment of cells from the flask walls (the latter moment was determined by a drastic increase in the culture turbidity).

The amount of unattached cells was calculated by the formula:

$$(OD_{min\ exp}/OD_{min\ contr}), \quad (2)$$

where $OD_{min\ exp}$ and $OD_{min\ contr}$ are the minimum turbidities of the experimental (treated with CA) and control (untreated with CA) cultures, respectively.

Experiments were performed no fewer than three times. The results presented are the means of 2–3 replicated measurements in the typical experiments. Statistical parameters (arithmetic mean and standard deviation) were calculated using the Statgraphics software package.

RESULTS

Due to the high adhesiveness of *P. fluorescens* cells, their growth curves exhibited a characteristic trough within the first hour after inoculation (Figs. 2 and 3). Such a decrease in the culture turbidity has been recognized as the result of the attachment of cells to the flask walls [6, 7]. The phase of attachment was followed by the phase of rapid cell detachment.

When the recipient and donor cultures were merely connected via a silicone tubing (Fig. 1a), the adhesion of recipient cells in the presence of the CA of the donor culture decreased by as little as 6%, and the amount of recipient cells remaining unattached increased by 10%, presumably due to a low flow of the CA through the connecting tubing. To enhance the effect of the CA of the donor culture, it was pumped through the recipient culture (Fig. 1b). Such a procedure reduced the cell adhesion value by 10–50% (on the average, by 20%) and increased the amount of unattached cells by 30%. Furthermore, adhesion residence time increased by 1.5 h, or twofold (Fig. 2).

If the CA from flask 2 was passed through trap 7 with HAYESEP-Q sorbent, the effect of the CA substantially diminished, so that the pattern of cell adhesion was analogous to that in the control: within one hour after inoculation, cells tended to attach to the glass but then detached from it and resumed their exponential growth (Fig. 3). A similar effect was observed if trap 7 was packed with activated charcoal (data not shown).

DISCUSSION

The data obtained suggest that the exponentially grown *P. fluorescens* culture produces a volatile compound, arbitrarily called volatile antiadhesin (VAA), that diminishes the number and increases the residence time of adsorbed cells. It should be noted that the latter effect of VAA was much stronger than the former effect: VAA induced a severalfold increase in the adhesion residence time and only a several-percent decrease in the adhesion value.

The effect of VAA greatly varied (Figs. 2 and 3), so that even small changes in the pumping rate or culture

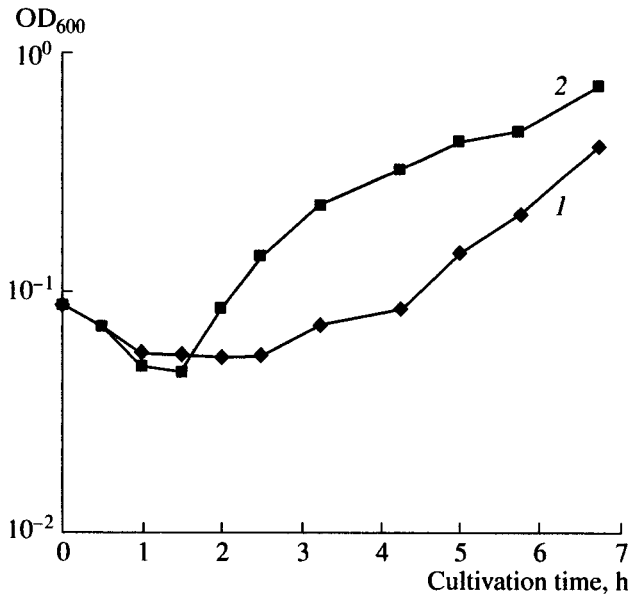

Fig. 2. Growth of *P. fluorescens* in the presence of (*1*) culture air and (*2*) atmospheric air bubbled through sterile medium.

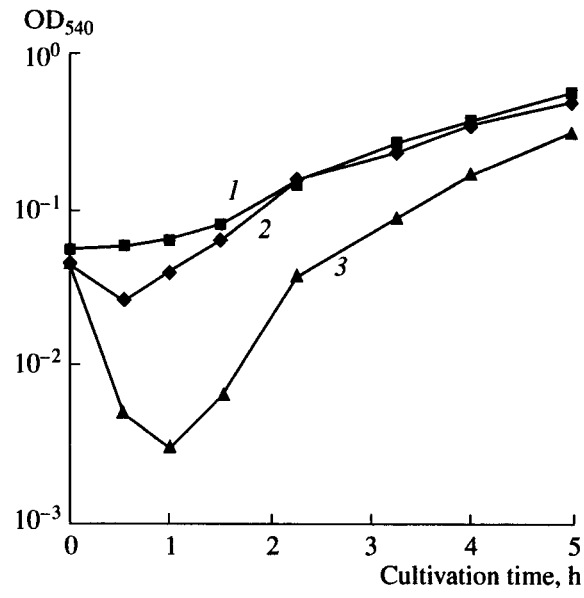

Fig. 3. Growth of *P. fluorescens* in the presence of (*1*) culture air, (*2*) atmospheric air bubbled through sterile medium, and (*3*) culture air passed through sorbent.

density led to great changes in the adhesion value and residence time.

The VAA-induced lag in bacterial growth was not genuine, since *P. fluorescens* cells continue to grow in the attached state [6], as was proven by the equal turbidities of experimental and control cultures by the stationary growth phase (7–9 h after inoculation).

It is known that bacteria evolve a variety of volatile metabolites [1, 2]. Some such metabolites can suppress the growth of other microorganisms, inhibit the formation and germination of spores, stimulate the production of fruiting bodies [1, 3], and act as metabolic autoregulators [4], but the biological role of the majority of volatile metabolites remains unknown [1]. In our opinion, VAA may serve as a volatile space sensor, which is responsible for the balance between adsorbed and free cells in a bacterial population. When the life space available to bacteria is large, so that the concentration of VAA is small, the bacterial population disseminates through intense cell desorption. But when the life space is small and the concentration of VAA is high, microbial cells "prefer to divide" on a surface, being reversibly attached to it. The high variability of the experimental data is consistent with the anticipated role of VAA in the life of *P. fluorescens*, since such a role implies a high sensitivity of cell adhesion to environmental conditions.

In biological activity (in particular, the ability to decrease the adhesion of bacterial cells and to extend their residence time in the adsorbed state), VAA resembles the nonvolatile AA described earlier [7]. Therefore, these two factors may be of the same chemical nature. The adsorption of VAA on activated carbon or HAYESEP-Q sorbent makes it possible to separate VAA and to study its properties. Work along this line is in progress.

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