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EXPERIMENTAL ARTICLES

Role of Distant Interactions in the Regulation of the Adhesion of *Pseudomonas fluorescens* Cells

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Abstract—The effects of distant interactions (LRI) and culture air on the adhesion of *Pseudomonas fluorescens* cells were studied. One *P. fluorescens* culture was found to diminish the adhesion of cells of another, glass-screened, *P. fluorescens* culture by 30% (in the absence o air exchange between cultures). This effect was interpreted to be due to penetrating LRI. Under the combined action of LRI and culture air (the latter alone reduced cell adhesion by only several percent), the amount of unattached cells increased 2- to 30-fold (on the average, by a factor of nine). Such a great reduction of cell adhesion indicated the synergistic action of LRI and culture air.

Key words: distant interactions, bacteria, adhesion, regulation

Communications of organisms with each other and the ability to acquire the information about the environment have been recognized as one of the major attributes of their life [1]. Organisms can communicate through chemical [2, 3], mechanical, and physical [4] signals. Investigation of the latter signals, whose transmission is mediated by physical fields (in other words, distant interactions), was initiated by A.G. Gurvich in the 1920s [5]. It was shown that the weak ultraviolet radiation emitted by cells of plants, animals, and microorganisms is involved in the regulation of mitosis, and they named it mitogenetic radiation. In the 1920–1930s, mitogenetic radiation was shown to be able to affect the growth, morphology, and the lag phase duration of various bacteria [21]. Recent reports have demonstrated the involvement of distant interactions (LRI) in the regulation of liquid bacterial cultures [6, 7], spore germination, and bacterial adaptation to stresses [8, 9]. To the best of our knowledge, the effect of LRI on the adhesion of bacterial cells has not yet been described.

The adhesion of bacterial cells to solid surfaces is a means of bacterial adaptation to different ecotopes and varying environmental conditions [10]; therefore, bacteria must possess mechanisms for the regulation of their adhesion.

The aim of the present work was to search for physical factors regulating the adhesion of *Pseudomonas fluorescens* cells to glass.

MATERIALS AND METHODS

The strain *Pseudomonas fluorescens* NCIMB 9046 used in this study was obtained from the National Collection of Industrial and Marine Bacteria (NCIMB) in Aberdeen, the United Kingdom. The strain was grown in M9 medium [11] supplemented with glucose (0.2%) and trace elements (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; and $FeCl_3 \cdot 6H_2O$, 1 (pH 7.0) [12]. The bacterium was cultivated in 100- or 250-ml flasks containing 15 or 50 ml of medium on a Lab-line orbital incubator shaker (180 rpm) at 30°C. Material for inoculation was grown to the stationary phase (16 h of growth). The inoculum concentration ranged from 2 to 5%. The culture was grown to the exponential phase (3–5 h of growth), after which it was used either directly as a donor culture or was processed to obtain recipient cells. In the latter case, the culture was passed through a 0.45-μm-pore-size Millipore filter. The residue cells were washed with fresh nutrient medium on the same filter and resuspended in the medium to a turbidity of 0.05 to 0.1 optical density (OD) unit. Culture growth and cell adhesion were followed by measuring culture turbidity at 600 nm on a Pye-Unicam SP-450 UV/VIS spectrophotometer.

Hereinafter, I shall use the terminology of Kaznacheev [4], who proposed to name the culture that serves as the source of culture air or LRI the "donor culture" or the "source culture." Correspondingly, the test culture is referred to as a "recipient culture" or a "sensor culture."

Experiments were performed using a setup (Fig. 1) similar to that described earlier [6]. Flask 2 (100 ml in volume), containing 10 ml of a recipient culture, was fixed, using plastic foam disk *3*, in 500-ml plastic beaker *4* with 50 ml of donor culture *1*. The initial turbidities of recipient and donor cultures were 0.05–0.1 and 0.6–0.8 units, respectively. The donor culture was placed in beaker *4* 20–30 min prior to the addition of the recipient culture. In this setup, the donor culture could affect the recipient culture either through LRI alone or through LRI and volatile compounds contained in the culture air (CA). When it was necessary to

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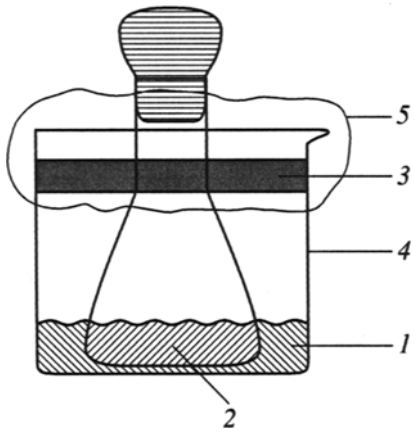

Fig. 1. Experimental setup to study the effect of donor culture on the adhesion of *P. fluorescens* cells: (*1*) donor culture, (2) recipient culture, (*3*) plastic foam disk, (*4*) 500-ml plastic beaker with donor culture, (*5*) air-proofing silicone rubber membrane (absent when the culture air exchange between the cultures is necessary).

prevent the CA transfer from the donor culture to the recipient culture, the cultures were separated from each other by an air-proofing silicone rubber membrane tightly embracing the flask and beaker necks.

Cell adhesion was characterized by two parameters: the adhesion level and the amount of unattached cells. The adhesion level, defined as the percentage of cells attached to the flask walls, was calculated by the following formula:

$$(OD_{ini} - OD_{min})/OD_{ini} \times 100\%, \quad (1)$$

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.

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 experimental (treated with CA, or LRI, or both) and control (untreated) cultures, respectively.

The data obtained were linearized by taking the logarithms and the significance of the difference between the experimental and control data was determined using the parametric Student's *t*-test or the nonparametric sign test and Wilcoxon signed-rank test [13, 14]. Relevant calculations were carried out using the Statgraphics software package. The necessity of applying nonparametric statistical tests was due to a considerable scatter of experimental data, so that parametric tests (Student's and some others) were inapplicable in some of the experiments.

All the experiments were repeated at least five times. The results presented are the means of 2–3 replicated measurements.

RESULTS

The transfer of exponential-phase *P. fluorescens* cells to fresh nutrient medium resulted in a 20–80% decrease in the culture turbidity in 30–60 min (Fig. 2). The degree and the rate of the turbidity drop depended on the initial culture density, the culture-to-flask volume ratio, as well as the concentration and the age of the inoculum. As was shown earlier, the decrease in the culture density is due to the reversible attachment of cells to the flask walls [15].

When the rubber membrane 5 (Fig. 1) was absent, the growing donor culture decreased the adsorption of the recipient cells by 10–20% (on the average, by 13%) (see table and Fig. 2); in this case, the number of unattached cells increased 2- to 30-fold (on the average, by a factor of 9). No changes in the maximal specific growth rate, the time of cell detachment, or in the biomass yield were observed.

The effect of the volatile adhesion-modifying compound present in the culture air on the adhesion of *P. fluorescens* cells to glass was investigated earlier [16]. Under experimental conditions close to those used in the present work, this compound, which was named volatile antiadhesin (VAA), diminished cell adhesion by 6%, while the percentage of unattached cells increased by 10% (see table).

When the rubber membrane 5 (Fig. 1b) was present, thus preventing the exchange of culture air between donor and recipient cultures, the percentage of unattached cells increased by 50%. It should be noted that the results of these experiments exhibited a somewhat higher variability and a lower reliability than is required for biological experiments.

When the cultivation flask was wrapped in aluminum foil, the percentage of unattached cells at the moment of maximum adhesion was 10–20% (on the average, by 13 ± 5%) higher than in the control.

Experiments were also performed to compare the effects of live bacterial culture (OD = 0.5–0.6) and dead culture (OD = 1.5–2.0) killed by autoclaving. The dead culture diminished cell adhesion in 14 of the 20 experiments performed. The confidence level of these results determined by the nonparametric methods of the sign test, and the Wilcoxon signed-rank test was 90%. The observed decrease in cell adhesion can be explained by the effect of LRI, whose source is biomass irrespective of whether it is alive or dead.

DISCUSSION

The effect of the adhesion-modifying factors studied (VAA and LRI) is more profound if estimated with respect to the number of unattached cells remaining in the liquid phase rather than to the number of cells attached to the flask walls (see the table). Unattached cells allow a microbial population to disseminate, although such cells are more susceptible to unfavorable factors than the adsorbed cells [10]. Therefore, a bal-

ance between the number of attached and unattached cells is very important for the survival, development, and dissemination of bacterial populations. VAA and DI are probably involved in the regulation of this balance.

It should be noted that the confidence level of the results presented is rather low (from 75 to 95%). For this reason, the inference on the adhesion-modifying activity of LRI was drawn based on the following four groups of arguments.

(1) In spite of the fact that the confidence level of the results obtained was lower than 95%, it was still much higher than 50% (the confidence level typical of completely random experimental data).

(2) Evidence for the adhesion-modifying activity of LRI was obtained in two independent sets of experiments with (i) live culture relative to sterile nutrient medium and (ii) dense dead culture relative to low-density live culture.

(3) The data obtained (see the table and Fig. 3) cannot be explained merely by the effect of VAA without considering the effect of LRI. Although the effect of LRI alone on cell adhesion is weak (of the order of several percent), LRI and VAA acting together raise the number of unattached cells 90-fold. Such a profound combined effect of VAA and LRI on cell adhesion suggests the synergistic action of these adhesion-modifying factors.

(4) The existence of distant interactions between bacterial cells is still the subject of debate and the data on this subject are so far scarce. The observations of LRI between *Vibrio costicola* and another bacterium [6] and between the bacterium *Pseudomonas corrugata* and the fungus *Gaeumannomyces graminis* [7] are not very convincing. More reliable evidence for the existence of distant interactions between bacterial cells was obtained by Matsuhashi *et al.*, who showed that *Bacillus carbonifillus* cells responded to growth-promoting physical signals from cells of homologous and heterologous bacteria [8, 9] and that spore germination was promoted by remote carbon materials (graphite and coal, but not diamond) [17]. Experimental data presented in this paper also indicate that remote dead carbon-containing material, namely, autoclaved biomass, may affect cell adhesion. Strictly speaking, A.G. Gurvich also observed the biological activity of physical factors in lifeless organic solutions [5].

When comparing the results presented here and those reported by Matsuhashi *et al.*, one more interesting fact stands out. In our experiments, VAA and LRI increased the number of unattached cells by 10 and 50% when acting separately and by 900% when acting together. On the other hand, Matsuhashi *et al.* emphasized that the effect of LRI was much stronger if donor and recipient cultures were placed in a closed box made of acrylic plastic. We suppose that the culture air accumulated in the closed box might promote the adhesion-modifying effect of LRI. Thus, it is reasonable to suggest that the biological role of distant interactions is to help cells with choosing the survival strategy under supraoptimal growth conditions in the presence of the required nutrients. Physical and chemical signals transmitted from a growing culture located nearby seem to "inform" another culture that the conditions are appropriate for growth. The synergistic action of such signals is necessary when they are weak, as in the case under discussion.

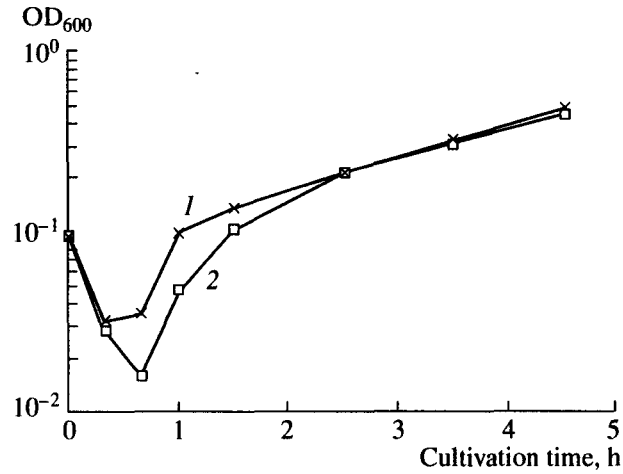

Fig. 2. Growth of the recipient *P. fluorescens* culture when culture air exchange between the beaker and the flask is possible: (*1*) beaker contains live *P. fluorescens* culture, and (*2*) beaker contains nutrient medium.

These speculations are confirmed by the results of experiments with aluminum foil, since the electromagnetic radiation reflected from the foil is perceived by a culture as a signal from another culture to diminish adhesion.

The exact physical nature of LRI remains to be understood. The mitogenetic radiation described by A.G. Gurvich was shown to be weak ultraviolet radiation [5]. On the other hand, Kaznacheev considered the signals between the tissue culture cells that caused a "specular cytopathic effect" to be infrared radiation [4], and Matsuhashi *et al.* believed that the physical signals, which they described, were ultrasonic.

Effect of culture air and distant interactions on the adhesion value and the amount of unattached cells remaining in the liquid phase. Data presented in the first column (Culture air (VAA) alone) are taken from the publication [16]

	Culture air (VAA) alone	LRI alone	Culture air and LRI
Decrease in cell adhesion, %	6	4	13
Increase in the amount of unattached cells, %	10	50	900

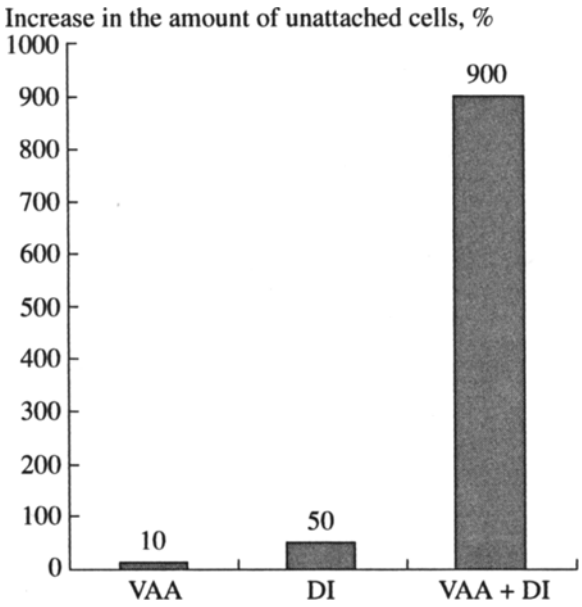

Fig. 3. Effect of VAA and DI acting alone and together on the amount of unattached *P. fluorescens* cells.

As for the physical signals revealed in the present work, they obviously were not ultraviolet, since the culture flasks used were made of ordinary glass, which is opaque to ultraviolet radiation. Taking into account the facts that bacterial cells bear a negative net surface charge [18, 19] and that the adhesion of bacterial cells on a surface depends on its charge [19], one more mechanism of action of distant interactions can be proposed. As soon as bacterial cells attach to the outer surface of a flask, they change not only the charge of this surface but also the charge of the inner surface of the flask, thereby influencing the adhesion of bacterial cells on the inner surface.

The target of LRI in bacterial cells that is responsible for changes in cell adhesion is still unclear, although the involvement of exopolysaccharides, lipopolysaccharides, glycoproteins, and proteins [19], as well as cell motility [20], cannot be excluded.

Thus, the present work provides further evidence for the existence and importance of DI in bacterial life. Distant interactions between bacterial cells decrease cell adhesion to surfaces. A volatile compound produced by cells can greatly enhance the effect of LRI. Both live and dead bacterial cells are capable of distant interactions.

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