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SHORT COMMUNICATIONS

Adhesive and Growth Properties of the R and S Variants of *Pseudomonas fluorescens*

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Abstract—A *P. fluorescens* NCIMB 9046 culture resuscitated from the lyophilized state on LB agar and stored in a mineral medium with glucose for 15 days comprised 90% of the S variant and 10% of the R variant. The R variant was more adhesive to glass and grew faster than the S variant.

Dissociation of bacterial populations is a well-known phenomenon [1, 2], lying in the formation of different morpho-physiological types of cells (typically, R, S, and M variants or dissociants) which spontaneously transform into each other at a frequency of 10^{-2} to 10^{-4} per one cell division as a result of genome rearrangement [1, 3]. Dissociation is described for both gram-negative and gram-positive bacteria. In spite of the fact that dissociation is widespread among bacteria, some researchers believe, as did one of the authors of this publication, that they are dealing with mutants. For instance, Pringle *et al.* have reported the isolation of attachment mutants [4], although they obviously were dealing with dissociants.

In the accompanying paper, we presented the first results of the investigation of the adhesive properties of *P. fluorescens* NCIMB 9046 [5]. This strain, which was obtained from the National Collection of Industrial and Marine Bacteria (NCIMB) in Aberdeen (Scotland) in a lyophilized state, was first resuscitated on LB agar [6] and then grown in mineral M9 medium [6] with glucose and microelements [7] to the stationary phase (16 h of growth). The adhesive and growth characteristics of this culture, which was used as material for inoculation, were described earlier [5]. Storing this culture at 4°C for 2 weeks led to a loss of its adhesive ability. The reasons for the effect observed were investigated in this work.

Material for inoculation was stored at 4°C for 2 weeks and then analyzed by plating its tenfold serial dilutions onto LB–wort (1 : 1) agar. About 90% of the cells of this material produced small colonies (1.2 mm in diameter after growth at 30°C for 24 h and then at 24°C for the next 24 h) which were whitish, viscous, raised, and even-edged. Such colonies, as they are described, could be produced by S-type cells. The other colonies (10%) were larger (2.2 mm in diameter after 2 days of growth under the same conditions), cream-colored, dryish, and flat (upon senescence) or cone-shaped (when young). Such colonies were obviously produced by R variant cells. The M variant was not revealed at all.

The subculturing of not very old cultures (up to one month old) of R and S variants on LB–wort agar did not cause their dissociation. However, in older cultures, the R variant transformed into the S variant. For instance, 3-month-old cultures of R and S variants resuscitated on agar media and then cultivated in M9 medium to the late stationary phase (2 days of growth) contained only S-type cells. No reverse transformation of the S variant into the R variant was observed. Taking into account that we examined more than 400 colonies, the inference can be drawn that the possible content of the R variant in the senescent S variant is less than 0.25%.

After the inoculation of 16-h-old cells into fresh M9 medium in an amount of 5%, both R-type and S-type cells were attached to the flask walls, as was judged from a transient decrease in the optical culture density (see figure and publication [5]). The percentage of attached cells was about 2.5-fold higher in the case of the R variant than in the case of the S variant (65 and up to 25%, respectively). The maximum specific growth rates of the R and S variants were 0.64 and 0.38 h^{-1}, respectively.

This apparent contradiction (the R variant grows faster and is more stable during storage than the S variant [8], but, on the other hand, the R variant is gradually displaced by the S variant upon storage) can be explained by the fact that the R variant is more adhesive to glass than the S variant. As a result, R-type cells were concentrated on the flask walls, whereas the liquid phase (from which cells were plated onto solid media for enumeration) contained mainly S-type cells. Furthermore, the high frequency of the transition of the R variant into the S variant could also contribute to the prevalence of S-type cells.

Our data on the adhesiveness of *P. fluorescens* dissociants agree with the findings of Pringle *et al.* [4]. At the same time, the R variant of *Rhodococcus rubropertinctus* is less adhesive than the S variant of this bacte-

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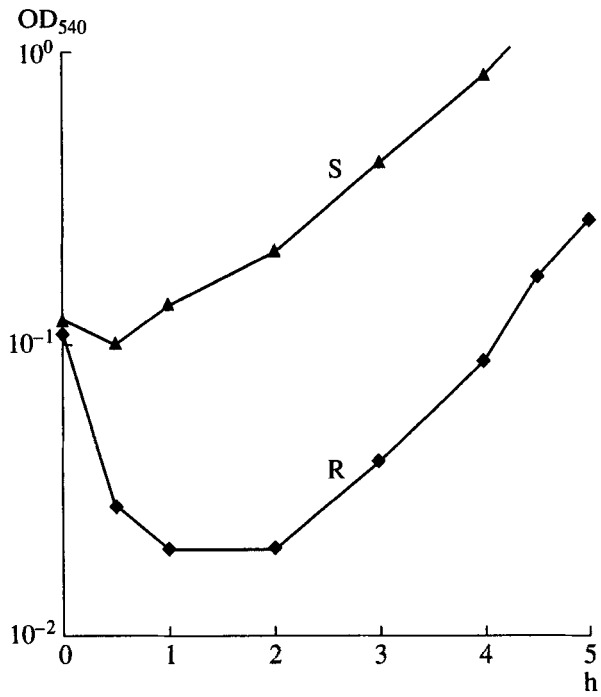

Growth of the R and S variants of *P. fluorescens* in M9 medium.

rium [9]. In any case, the possibility of dissociation of bacterial cultures should always be taken into account by researchers, especially when they investigate the responses of microorganisms to various stresses, since it is known that R, S, and M variants substantially differ in resistance [10].

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