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Bacterial Adherence to Polystyrene: a Replica Method of Screening for Bacterial Hydrophobicity

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A simple replica method is described for the rapid identification of colonies of bacteria which adhere to polystyrene. A correlation was found between the adherence of bacterial strains to polystyrene and cell surface hydrophobicity, suggesting the use of this technique in screening for cell surface mutants and in the isolation of hydrophobic bacteria from nature.

Hydrophobic interactions play a role in the adherence of microorganisms to a wide variety of surfaces. The hydrophobic nature of the outermost bacterial surface has been cited as a factor in the partitioning of microorganisms at interfaces (2, 8), in the adherence of bacteria to nonwettable plastic surfaces (3, 5, 6), in the attachment of bacteria to phagocytes (15) and other mammalian cells (1, 9, 10a, 12), and in the growth of cells on insoluble hydrophobic substrates, such as hydrocarbons (M. Rosenberg and E. Rosenberg, J. Bacteriol., in press).

We previously described a rapid method for the measurement of bacterial surface hydrophobicity based on the adherence of washed cells to liquid hydrocarbons (10, 11). This technique, as well as other methods for measuring bacterial hydrophobicity (7, 9, 12, 13), is inadequate for the simultaneous screening of large numbers of bacterial samples.

In this report a simple method for the rapid initial identification of colonies of hydrophobic cells is described. This technique is based on differential cell adherence to polystyrene. The adherence of a number of bacterial species to polystyrene has been investigated by several authors and attributed to hydrophobic interactions between cells and the plastic surface (5, 6). In preliminary experiments we found a direct correlation between bacterial strains which showed a high affinity for liquid hydrocarbons and the ability of these strains to adhere to polystyrene. Similarly, bacterial strains which did not adhere to hydrocarbons could easily be displaced from the polystyrene surface by washing. These results suggested that a simple replica plate method could be used as a rapid test for the screening of bacterial colonies for cell surface hydrophobicity.

In the proposed technique, a flat polystyrene disk is pressed onto the surface of an agar plate containing the colonies to be screened. Two types of sterile disks were employed: (i) 25-mm-diameter, untreated, round polystyrene cover slips supplied by Lux Scientific Corp., Newbury Park, Calif.; (ii) 80-mm-diameter disks cut from standard polystyrene petri dishes (Miniplast, Ein Shemer, Israel; and NUNC, Roskilde, Denmark). The replica of the colonies obtained on the polystyrene surface was then washed for 2 min under a vigorous stream of tap water to remove all cells which were not firmly bound. At this stage, translucent areas corresponding to colonies of adherent cells could be observed on the polystyrene surface; microscopic observation of these spots revealed a dense layer of attached cells. To facilitate visualization and comparison with the original colonies, the replica was fixed by dipping in methanol and stained with gentian violet.

Results obtained by this technique are shown in Fig. 1 to 4. In all of the experiments, cells were grown on nutrient agar plates supplemented with 0.5% NaCl and examined after 48 h of incubation. In Fig. 1, colonies of *Acinetobacter calcoaceticus* RAG-1 (ATCC 31012), a strain which adheres avidly to hydrocarbons (10, 11), were compared with colonies of MR-481, a mutant derived from RAG-1 which is deficient in cell surface hydrophobicity and unable to adhere to hydrocarbons (10a; Rosenberg and Rosenberg, J. Bacteriol., in press). Mutant MR-481 cells were completely removed from the polystyrene replica in the washing procedure, whereas a clear replica of RAG-1 colonies was obtained. Mixtures of RAG-1 and MR-481 colonies which were not distinguishable by colony morphology could be easily resolved by this method (Fig. 2).

Most laboratory strains tested did not adhere to polystyrene and were removed from the disks after the washing procedure. These included colonies of *Escherichia coli* K-12 DR107, *Micro-*

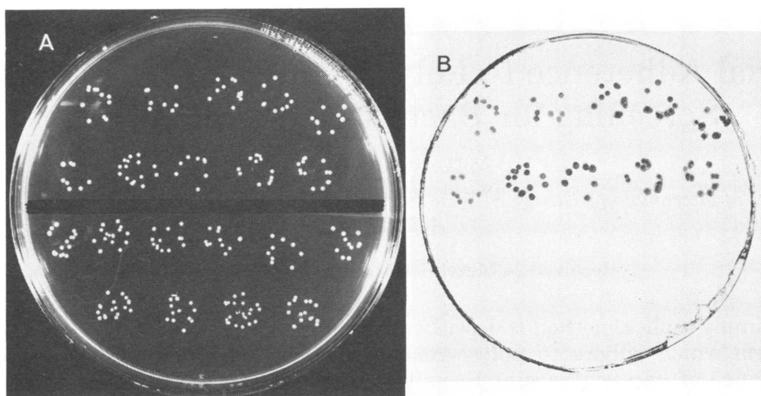

FIG. 1. *Replica of RAG-1 and MR-481 colonies. A nutrient agar plate (A) containing colonies of RAG-1 (upper half) and MR-481 (lower half) was replicated onto polystyrene, washed, and stained as described in the text. Only RAG-1 cells were adherent (B).*

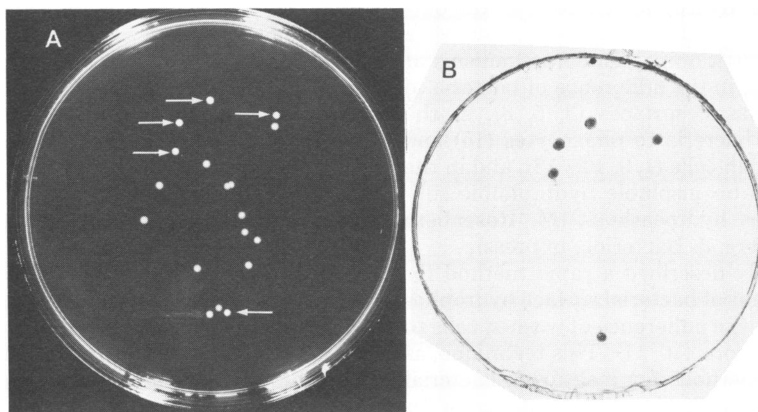

FIG. 2. *Replica of mixed RAG-1 and MR-481 colonies. A mixture of MR-481 and RAG-1 cells was plated onto a nutrient agar plate (A). On the polystyrene replica (B), five colonies of adherent cells, indicated by arrows (A), were identified.*

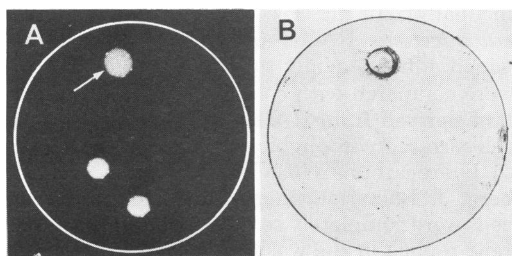

FIG. 3. *Comparison of S. aureus and S. albus colonies. A colony of S. aureus (A, arrow) and two adjacent colonies of S. albus were replicated onto polystyrene (B). A ring of stained cells, corresponding to the S. aureus colony, was observed on the replica, whereas nonadherent S. albus cells were removed during the washing procedure.*

coccus lysodeikticus (ATCC 4698), *Myxococcus xanthus* FB, *Pseudomonas aeruginosa* PAS279, *A. calcoaceticus* (ATCC 17903), and strains of *Photobacterium fischeri*, *Photobacterium leiognathi*, and *Photobacterium phosphoreum*.

Adherence of *Serratia marcescens* and *Serratia liquefaciens* strains to polystyrene was found to depend on the age of the colonies. Whereas pink- and red-pigmented colonies were replicated onto the polystyrene, young nonpigmented colonies were not adherent. The appearance of pigment in *Serratia* cells has been correlated with increasing cell surface hydrophobicity (2, 7, 10).

The stained replica of adjacent colonies of *Staphylococcus albus* and *Staphylococcus aureus* (10) is shown in Fig. 3. A characteristic ring

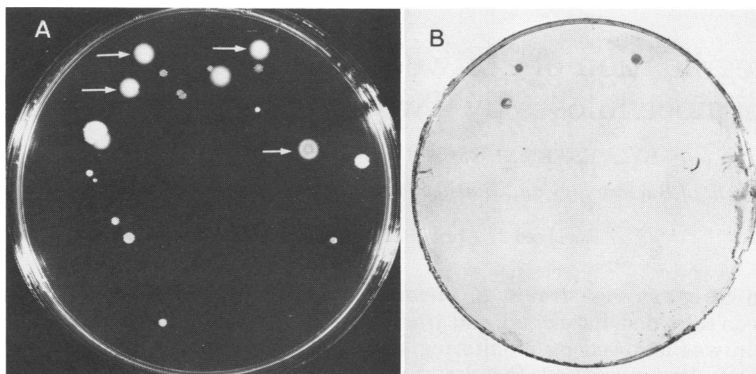

FIG. 4. *Replica of colonies from a soil sample. A sample of soil was diluted and plated onto nutrient agar (A) and incubated at 30°C for 48 h. Several adherent colonies (arrows) were identified on the polystyrene replica (B).*

of stained adherent *S. aureus* cells was obtained, whereas *S. albus* cells were completely washed from the polystyrene surface. The appearance of a ring of adherent cells, rather than a homogeneous spot, might indicate the interference by a cell-free component which coated the polystyrene at the point of contact of the colony and the disk and prevented the adherence of cells to the "conditioned" (4) surface. In a prior study of these two strains, only *S. aureus* cells exhibited affinity toward hydrocarbon (10).

Colonies of adherent cells could readily be identified in isolates from natural sources. For example, the replication of a plate of soil microorganisms yielded several adherent colonies (Fig. 4).

Results presented here further demonstrate the strong correlation between cell surface hydrophobicity and the affinity of bacteria for polystyrene. The main advantage of the described technique is that large numbers of bacterial colonies can be examined simultaneously using inexpensive and readily obtainable materials. The simplicity of this method suggests its implementation in screening for mutants in cell surface hydrophobicity and in the isolation of hydrophobic microorganisms from natural sources.

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