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This Week's Citation Classic®

Rosenberg M, Gutnick D & Rosenberg E. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol. Lett.* 9:29-33, 1980.
[Dept. Microbiology, George S. Wise Fac. Life Sciences, Tel Aviv Univ., Ramat Aviv, Israel]

A simple method is proposed for measurement of cell-surface hydrophobicity of microorganisms. Turbid, washed cell suspension (1.2 ml) is vortexed for two minutes in the presence of 0.01-0.2 ml of test hydrocarbon (*n*-hexadecane, *n*-octane, and *p*-xylene). Adhesion is calculated from the decrease in turbidity of the lower aqueous phase. [The *SCI*® indicates that this paper has been cited in more than 250 publications.]

Mixing Water, Oil, and Microorganisms

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In 1971, Eugene Rosenberg and David Gutnick initiated a highly successful collaboration in petroleum microbiology at Tel Aviv University that would last for some 15 years. When I joined Eugene's lab as a PhD candidate in the fall of 1978, the "oil club" was at its zenith, with some 15 postdoctoral fellows, graduate students, and technicians.

My initial interest was in studying adhesion of oral microorganisms. E. Rosenberg managed to persuade me that unraveling the secrets of how hydrocarbon-degrading bacteria adhered to oil droplets would eventually have some bearing on the oral cavity. I was subsequently assigned to study the mechanism responsible for the observed adhesion of *Acinetobacter calcoaceticus* RAG-1 to hydrocarbon and its role in enabling growth on this water-insoluble substrate.

The technique described in this *Citation Classic* came about via a circuitous route. I had been trying to generate uniform coatings of solid paraffin on various surfaces and subsequently measure adhesion. David, having read a recent article by M. Fletcher and G.I. Loeb,[1] suggested that polystyrene might be a suitable surrogate attachment surface. A strain of *Serratia marcescens* adhered so magnificently to polystyrene that we were tempted to test its (heretofore unreported) ability to degrade hydrocarbons. I prepared a minimal growth medium containing phosphate, urea, and magnesium sulphate (so-called PUM buffer), overlaid with n-hexadecane. Anxious to see any net increase in cell growth, I added a particularly turbid inoculum of cells and aerated overnight with vigorous shaking. The next morning I was perplexed to find that not only had the cells *not* grown, but they appeared to have disappeared altogether! They turned up after a brief search, bound to the surface of the hexadecane droplets.

It soon became evident that a short duration of strong vortexing was sufficient to distinguish between cell suspensions that adhered and those that did not. The extent of adhesion to increasing volumes of several test hydrocarbons could be simply ascertained by measuring the drop in turbidity in the lower aqueous phase following phase separation.

Initially disappointed that adhesion to hydrocarbon was not unique to oil-degrading microorganisms, it slowly dawned on us that we had discovered a much more general phenomenon. As it turned out, we were not the first to link microbial adhesion to oil with cell-surface hydrophobicity.[2,3] However, a fortuitous choice of adherent and nonadherent strains, together with the demonstration that surfactant reversed the adhesion, supported the premise that the hydrophobic surface properties of a wide variety of microbial cells could be measured in this extremely simple manner.

Since other hydrophobicity techniques were already available for studying bacterial surfaces, we did not initially anticipate that the assay would generate much interest. However, soon after the paper appeared, the technique was successfully used by several leading adhesion researchers, including I. Ofek, E. Beachey, R. Doyle, and R.J. Gibbons. This probably was an important factor in establishing the assay.

The bacterial adhesion to hydrocarbons (BATH) test, in various modifications, is still routinely employed (since it is also used to study fungi, it has been recently renamed the "microbial adhesion to hydrocarbons," or MATH, test).

1. **Fletcher M & Loeb G I.** Influence of substratum characteristics on the attachment of a marine pseudomonad to solid surfaces. *Appl. Environ. Microbiol.* 37:67-72, 1979. (Cited 100 times.)
2. **Mudd S & Mudd E B H.** The penetration of bacteria through capillary spaces. IV. A kinetic mechanism in interfaces. *J. Exp. Med.* 40:633-45, 1924. (Cited 35 times since 1945.)
3. **Marshall K C & Cruickshank R H.** Cell-surface hydrophobicity and the orientation of certain bacteria at interfaces. *Arch. Mikrobiol.* 91:29-40, 1973. (Cited 90 times.)

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