THE MEASUREMENT OF BACTERIAL ATTACHMENT TO SURFACES IN STATIC SYSTEMS

MADILYN FLETCHER
Center of Marine Biotechnology
Maryland Biotechnology Institute
University of Maryland System
600 E. Lombard St.
Baltimore, Maryland 21202
USA

1. Introduction

There have been many laboratory studies that have attempted to measure the adhesive properties of bacteria or their attachment to solid surfaces in laboratory systems. Many of these investigations have used "static" systems, which were still or gently agitated and where there was no directional flow. (Flow cell systems for evaluating adhesion are dealt with elsewhere in this volume.) These static system studies have employed a variety of designs, using numerous types of bacteria, substrata, and environmental conditions. They have usually been carried out in order to evaluate (a) the adhesiveness of specific bacteria, (b) the suitability of particular materials as attachment substrata, or (c) the influence of environmental factors on the attachment process. Some laboratory procedures can also be applied to studies in natural environments (e.g. water, soil, water delivery systems, cooling towers), but they must first be validated in the laboratory. Such methods include microcosopy, analysis of biochemical markers, and nucleic acid hybridization.

2. Experimental Design

2.1 INTRODUCTION

The numbers of bacteria that attach to surfaces and the rate of deposition depend on the species and strains of bacteria and on their nutritional status (e.g. growth phase, whether they are starved for specific nutrients). Attachment will also be influenced by environmental conditions, including nutrient sources and concentration and flux (Molin et al., 1982; Knox et al., 1985; Mc Eldowney and Fletcher, 1986), electrolyte concentration (Marshall et al., 1971b; Ørstavik, 1977; Knox et al., 1985), pH (Gordon et al., 1981; Harber et al., 1983), and temperature (Fletcher, 1977; Harber et al., 1983).

The characteristics of the solid surfaces are also extremely important, as can be the procedures used to rinse surfaces after attachment and before enumeration of attached cells. The results will depend heavily on the experimental design. Consequently, experimental variables must be evaluated and selected carefully, so that the design will address the specific question of interest. If the ultimate goal is to understand a natural process, it is extremely important that the experimental design is consistent with the natural system being modelled.

2.2 SELECTION OF ORGANISMS

The adhesive properties of bacteria depend upon their genetic capabilities and upon the modulation of those characteristics by their metabolic state. Also, laboratory culture can result in significant changes in adhesiveness of natural isolates with time, possibly because of selection of less adhesive strains by repeated transfer from liquid suspension. Recently, it has been clear that some bacteria are able to undergo genetic changes, termed phase variation, so that progeny express one of two, or more, adhesive phenotypes. For example, *Pseudomonas atlantica* (Bartlett et al., 1988) and *P. fluorescens* (Pringle et al., 1983) can both produce progeny of three separate colony types (with corresponding differences in adhesion ability), i.e., mucoid, smooth, and crenated colonies. With *P. fluorescens* H2, the crenated form is the most adhesive, whereas the smooth form is moderately adhesive, and the mucoid form, which produces an alginate exopolymer, is poorly adherent (Pringle et al., 1983; Pringle and Fletcher, 1983).

The polymers produced at the bacterial surface, not surprisingly, have a considerable influence on attachment. Bacterial species differ in their abilities to produce extracellular polymers, such as polysaccharide and protein fibrils (i.e. pili, fimbriae). There are also considerable differences in the compositions of extracellular polysaccharide, surface proteins, and lipopolysaccharide produced by different species and strains. The importance of the presence and compositions of these various surface polymers in the adhesion process can be demonstrated by experiments with mutants that are altered with respect to specific cell structures. Mutations in bacteria, such as *Escherichia coli* (cf. Isaacson, 1985) and *Streptococcus salivarius* (Weerkamp et al., 1986), that alter specific cell surface components can result in altered adhesiveness. For example, the attachment of *S. salivarius* to hydrophobic surfaces varied depending upon the density of a fibrillar layer on the cell surface and the properties and surface exposure of specific types of fibril (Weerkamp et al., 1987).

Growth conditions of bacteria can affect their adhesiveness, but there is no consistent pattern in such alterations (Marshall et al., 1971b; Molin et al., 1982; McEldowney and Fletcher, 1986). For example, in batch culture adhesiveness can vary with growth phase; however, there is no general rule as to whether cells are more adherent in exponential phase or in stationary phase (Harber et al., 1983; Rosenberg and Rosenberg, 1985).

Interactions among different species can also influence the outcome of attachment (McEldowney and Fletcher, 1987). This could be due to the production of metabolites that are released and subsequently influence the adhesion process through