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Bacteria on Leaf Surfaces and in Intercellular Leaf Spaces

Abstract. *Ultraviolet irradiation kills bacteria on the leaf surface but not those in the intercellular leaf spaces.*

Plant leaves provide habitats for saprophytic microorganisms and infection courts for various plant pathogens. This ecologically neglected environment has been called "the phyllosphere" (*1*). One of the problems in a study of this environment is the determination of which microbes occur on the surface of the leaf and which occur in the intercellular spaces, such as the substomatal chambers.

The number of viable cells of *Xanthomonas phaseoli* var. *sojensis* recovered from intact soybean leaves steadily decreased for 12 hours after inoculation and washing (Fig. 1*A*). How many of the original number of viable cells were on the surface, and how many were in the intercellular spaces of the leaf? Perhaps the decrease in number for the first 12 hours was a reflection of the death rate of those bacteria on the surface, particularly since *X. phaseoli* var. *sojensis* has a rapid death rate on drying and exposure to air (Fig. 1*C*). When leaves were treated with ultraviolet light (UV) for 15 minutes, there was an initial decrease in the number of viable cells that was followed by a continuous increase (Fig. 1*B*).

Cultures of *X. phaseoli* var. *sojensis*, a pathogen of soybean, and *Serratia marcescens*, a saprophyte in soil and water, were cultured on nutrient agar. Soybean plants (*Glycine max* var. Blackhawk) were grown in a greenhouse. Leaves were inoculated by spraying a suspension (10^8 cells/ml) on the undersurface which had first been sprayed with water until the intercellular spaces were filled (water-soaked). After 5 minutes the inoculated leaves were thoroughly rinsed with running water.

Numbers of viable bacteria were determined by grinding a disk (12 mm) of leaf tissue against the side of a test tube with a stirring rod and making culture plates at appropriate dilutions. Colonies representing the number of viable cells were well separated from fragments of leaf disks.

Ultraviolet light was applied with a General Electric G30T8 germicidal lamp at a distance of 46 cm (a dosage of approximately 3000 ergs sec^{-1} cm^{-1}).

Ultraviolet light has little penetrating capacity and should kill microorganisms on the surface of the leaf before killing those in the intercellular spaces. With increasing exposure to UV the total number of viable bacteria should decrease for a time (indicating the death rate for those on the surface) and then remain constant with prolonged exposure (indicating the number of bacteria in intercellular spaces and protected from the UV). The number of viable cells of *X. phaseoli* var. *sojensis* and *S. marcescens* applied by inoculation to soybean leaves decreased rapidly with exposure to UV for 15 minutes and then remained constant with prolonged exposure (Fig. 2, *B* and *C*). Viable cells of the naturally occurring bacterial flora in uninoculated but water-soaked leaves responded in a like manner (Fig. 2*E*).

The cells surviving prolonged treatment do not represent bacteria resistant to UV, since cells of *X. phaseoli* var. *sojensis* suspended in water to a depth of 1 mm are rapidly killed by exposure to UV (Fig. 2*D*). And that the leaf does provide protection from UV is indicated by the survival of *X. phaseoli* var. *sojensis* when the side of the leaf opposite to that which has been inocu-

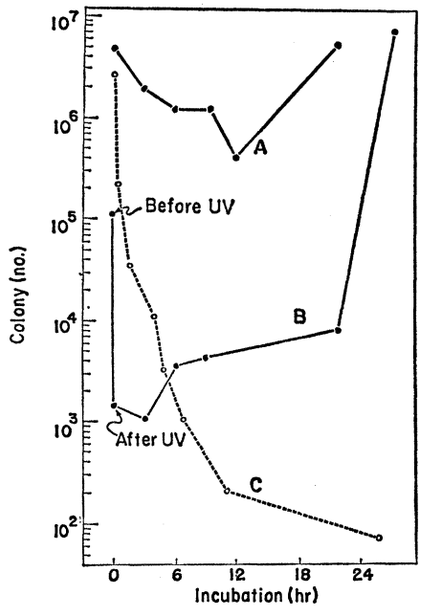

Fig. 1. Number of colonies of *X. phaseoli* var. *sojensis* from intact soybean leaves after inoculation and incubation. *A*, Without exposure to UV; *B*, with 15 minutes exposure to UV immediately prior to counting; *C*, the death rate of the organism after drying and exposure to air on disks on aluminum foil.

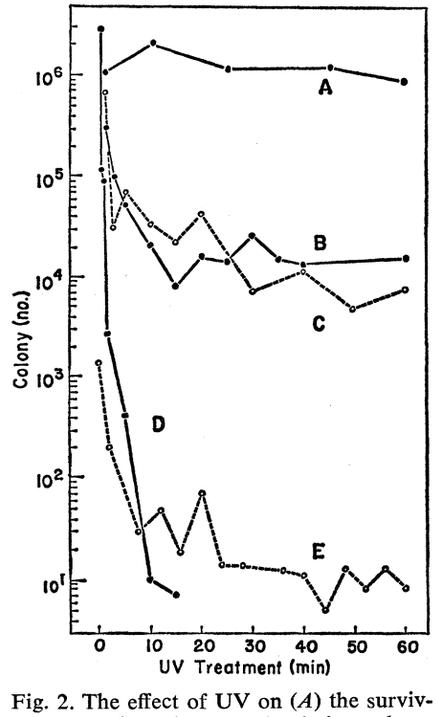

Fig. 2. The effect of UV on (*A*) the survival of *X. phaseoli* var. *sojensis* in soybean leaves when the side opposite to that which is inoculated is irradiated and when (*B*) the inoculated side is irradiated. (*C*) Survival of *S. marcescens* in soybean leaves when the inoculated side is irradiated. (*D*) Survival of cells of *X. phaseoli* var. *sojensis* when suspended in water and irradiated. (*E*) Survival of naturally occurring bacterial flora in soybean leaves when both sides of the leaf are irradiated.

lated is irradiated (Fig. 2*A*). However, bacteria (*S. marcescens*) on the irradiated surface are rapidly killed as indicated by leaf disk prints (*2*) made by pressing the inoculated and irradiated surface against nutrient agar. After 5 to 10 minutes there was a noticeable reduction in the number of colonies, after 15 minutes there were very few, and after 20 minutes there were none.

The use of UV to determine if bacteria are on the leaf surface or in the intercellular leaf spaces is a useful tool in the study of microorganisms in this environment.

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Sedimentation Velocity Experiments: Position and Motion of Schlieren Peaks

Abstract. *There are four possible cases for single solute peaks in ultracentrifuge sedimentation velocity experiments. The peak can be either above or below the baseline and can move toward or away from the cell bottom. With appropriate solvents these cases are demonstrated for a single polymeric solute.*

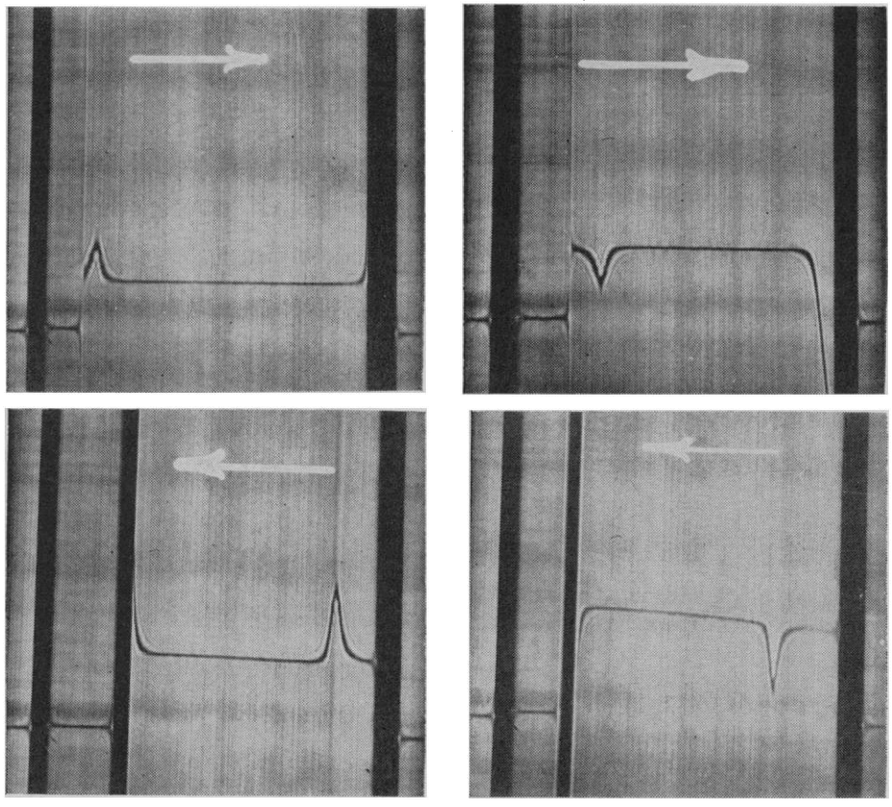
Conventionally, in ultracentrifugation the solute is not only more dense than the solvent, but it also has a higher refractive index. With the usual schlieren optics this leads, in velocity sedimentation, to a peak moving from the meniscus to the bottom and rising above the baseline (Fig. 1). If the solute is lighter than the solvent, the peak must move in the opposite direction—that is, from bottom to meniscus. In such a case (flotation) the refractive index of the solute is often less than that of the solvent, and the peak appears above the baseline (Fig. 3). However, it may not be as obvious that the sedimentation velocity peak can also exist below the baseline in either sedimentation or flotation. Such possibilities are rather infrequent (*1*) and seem to be unfamiliar to many people working with ultracentrifugation. These four distinct cases have not been explicitly described before, and it is our purpose to demonstrate them with a single solute in four appropriate solvents.

The key to the position of sedimentation peaks lies in the fact that the observed schlieren pattern is really a graph of dn/dx plotted against the distance from the center of rotation. We may represent this differential change of refractive index with distance as the product of two factors:

$$\frac{dn}{dx} = \frac{dn}{dc} \quad \frac{dc}{dx}$$

Each of the quantities dn/dc (change of refractive index with concentration) and dc/dx (change of concentration with distance) may be either positive or negative, giving rise to four cases, which are schematically represented as $++$, $+-$, $-+$, and $--$. These possibilities can be experimentally demonstrated with a single solute if one picks appropriate solvents. As an example we have taken polyisodecyl acrylate with density 1.02 and refractive index 1.473 at room temperature. Figure 1 shows its sedimentation in hexanol-1 ($[n]_D^{20} = 1.4135$; density, $\rho = 0.82$). Here both dn/dc and dc/dx are positive. This corresponds, of course, to the most usual case ($++$). In benzene ($[n]_D^{20} = 1.5017$, $\rho = 0.879$) the polymer still sediments but its refractive index is less than the solvent ($-+$), so dn/dc is negative; consequently the peak is just reversed about the baseline from the normal case (Fig. 2).

In chlorobenzene ($[n]_D^{20} = 1.525$, $\rho = 1.107$) both dn/dc and dc/dx are negative ($--$), so the product dn/dx is positive. This means the peak is upright but moves toward the meniscus (Fig. 3). Finally, in *n*-propyl bromide ($[n]_D^{20} = 1.4341$, $\rho = 1.354$) the polymer floats but has a higher refractive index than the solvent, giving the case ($+-$); hence the peak is



Figs. 1–4. Sedimentation peaks for polyisodecyl acrylate. Fig. 1 (upper left). Sedimentation in hexanol-1. Peak moves from the meniscus to the bottom and rises above the baseline. Fig. 2 (upper right). Sedimentation in benzene. The peak is reversed about the baseline from the normal case. Fig. 3 (lower left). Sedimentation in chlorobenzene. The peak is upright but moves toward the meniscus. Fig. 4 (lower right). Sedimentation in *n*-propyl bromide. The peak is downward but moves toward the meniscus.