

## Survival of *Azotobacter* in Dry Soil

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Detection of viable *Azotobacter* in soils stored in the laboratory for more than 10 years suggests that these bacteria can remain dormant in nature for prolonged periods of time. Studies of dried cultures show that cysts of *A. vinelandii* 12837 remain viable for at least 10 years, whereas vegetative cells do not survive for even 1 year under the same conditions.

A few bacteria (including *Azotobacter*, *Myxococcus*, *Nitrosocystis*, and *Nitrocystis*) have the capacity to exist in a state of cryptobiosis by forming cysts that are resistant to adverse environmental factors such as desiccation (4) and radiation (7). These dormancy structures were first associated with prolonged life spans by Winogradsky (9) and, in 1948, Pochon and Tchan (2) wrote . . . "Ils peuvent être conservés pendant des mois et des années . . .," but it was not until 1961 that a period of cyst survival was accurately documented. Socolofsky and Wyss (5) observed viable cysts of *A. vinelandii* strain 12837 in cultures that were allowed to dry slowly and kept for 1 year in the laboratory. The same cultures were tested subsequently (4), and the cysts were shown to be still viable after 2 years of storage.

This is a report of the survival of *Azotobacter* for periods of 10 to 13 years in dry soils and the survival of cysts of *A. vinelandii* 12837 for a period of 10 years in dry cultures stored at room temperature in the laboratory.

### MATERIALS AND METHODS

**Soils.** Thirty-nine soils obtained between 1961 and 1963 (7) were kept in tightly sealed screw-cap vials. They were stored at room temperature in a box protected from dust, chemical fumes, and excessive heat. These chernozem and sandy soils were collected throughout south and central Texas and shown to contain viable cells of *Azotobacter* at the time of collection.

**Dried cultures.** Cultures of *A. vinelandii* 12837 were grown in plates of Burk medium (8) at room temperature. These cultures consisted entirely of vegetative cells when examined in 1963. Cysts were produced by growing *A. vinelandii* 12837 in plates of Burk medium containing 0.3% (vol/vol) *n*-butanol instead of glucose. The majority (90 to 95%) of the cells in these cultures were in the cyst form when last examined in 1963. Both vegetative cell and cyst

culture plates were wrapped in aluminum foil and allowed to dry slowly at ambient laboratory conditions.

**Viability tests.** Soil containers were shaken vigorously to mix soil particles well, and small amounts of soil were sprinkled directly onto the surface of Burk agar plates. Soils were also cultured in modified Augier medium (7). Approximately 0.1 g of soil was placed in 5 ml of liquid medium in large test tubes. Dried plate cultures were tested by breaking the agar film with sterile forceps and glass rods in a hood previously disinfected by a germicidal ultraviolet lamp. Flakes (ca. 1 mm<sup>2</sup>) of dried agar were placed on the surfaces of Burk agar plates and in tubes of Augier medium, as for soils.

All plate cultures were incubated at 26 C and examined at 2 to 5 days. Tubes of modified Augier medium were incubated in a reclining position to enhance aeration and were examined at the end of 10 days. This medium yields thick, viscid growth characteristic of *Azotobacter*. If growth was not positively identified as *Azotobacter*, subcultures were made onto plates of Burk agar and organisms were identified by growth characteristics and morphology.

### RESULTS

The data in Table 1 show the results of periodic examinations of stored soils, and the data in Table 2 show the results obtained from soils which had been irradiated (gamma radiation, <sup>60</sup>Co) (7) prior to storage. No comparison is intended; the irradiated soils are included simply to increase the number of soils examined.

Examination of 36 samples kept at room temperature shows that cysts of *A. vinelandii* 12837 remain viable in dried cultures stored for 10 years, whereas vegetative cells stored under the same conditions were inactivated in less than 2 years.

### DISCUSSION

It is inferred from these observations that *Azotobacter* cells remain viable in dry soils for

TABLE 1. *Survival of Azotobacter in soils stored in the laboratory*<sup>a</sup>

Designation	Origin	Soil type	Date of collection	1965	1967	1968	1969	1970	1973
East	San Antonio	Chern	Aug 1961	+	+	+	+	+	+
Southside	San Antonio	Sandy	Aug 1961	+	+	+	+	+	+
Mat West	San Antonio	Chern	Aug 1961	+	+	+	+	+	+
Cadillac	San Antonio	Sandy	Aug 1961	+	+	+	+	+	+
Shadywood	Austin	Chern	Aug 1961	+	+	+	+	+	+
12-700	Austin	Chern	Sept 1961	+	+	+	+	+	+
12-800	Austin	Chern	Sept 1961	+	+	+	+	+	+
12-2,200	Austin	Chern	Sept 1961	+	+	+	+	+	+
	Mission	Sandy	Dec 1961	+	+	+	+	+	+
	Midland	Sandy	Dec 1961	+	+	+	+	+	+
A	Austin	Chern	Sept 1962		+	+	+	+	+
I	Austin	Chern	Sept 1962		+	+	+	+	+
J	Austin	Chern	Sept 1962		+	+	+	+	+
250-Control	Austin	Chern	Oct 1962	+		+	+	+	+
300-Control	Austin	Chern	Oct 1962	+		+	+	+	+
Sandra	Midland	Sandy	Dec 1962	+		+	+	+	+
Control	Austin	Chern	Feb 1963	+		+	+	+	+
150-Control	Austin	Chern	May 1963	+	+	+	+	+	+
3.3-Control	Austin	Chern	May 1963	+	+	+	+	+	+
1-Control	Austin	Chern	Sept 1963	+	+	+	+	+	+
1-1-Control	Austin	Chern	Sept 1963	+	+	+	+	+	+
	Waco	Chern	Oct 1963	+		+	+	+	+
Downtown	Austin	Sandy	Oct 1963	+		+	+	+	+
	Manor	Sandy	Oct 1963	+		+	+	+	+
	San Marcos	Chern	Oct 1963	+		+	+	+	+
	Brooks	Chern	Oct 1963	+		+	+	+	+

<sup>a</sup> Blanks in the columns indicate that the soil was not tested in the year indicated. Chern, Chernozem.

TABLE 2. *Survival of Azotobacter in soils irradiated on the date of collection and then stored in the laboratory*<sup>a</sup>

Designation	Origin	Type	Date of collection	Radiation (krads)	1968	1969	1970	1973
A	Austin	Chern	Sept 1962	100	+	+	+	+
I	Austin	Chern	Sept 1962	100	+	+	+	+
J	Austin	Chern	Sept 1962	100	+	+	+	+
100 KR	Austin	Chern	Oct 1962	100	+		+	+
250 KR	Austin	Chern	Oct 1962	250	+	+	-	-
300 KR	Austin	Chern	Oct 1962	300	+		-	-
100	Austin	Chern	May 1963	100	+			+
3.3	Austin	Chern	May 1963	330	+		+	+
	Waco	Chern	Oct 1963	200	+		+	+
Downtown	Austin	Sandy	Oct 1963	200	+		+	+
	Manor	Sandy	Oct 1963	200	+	+	+	+
	San Marcos	Chern	Oct 1963	200	+		+	+
	Brooks	Chern	Oct 1963	200	+		+	+

<sup>a</sup> Blanks in the columns indicate that the soil was not tested in the year indicated; - indicates no *Azotobacter* detected. Chern, Chernozem.

more than 12 years and that cysts of *A. vinelandii* 12837 survive for extended periods in dried cultures. Although it is fairly well established that seeds are protected by their hard, moisture-resistant outer testa (1) and that the spore coat is also hard and moisture resistant (3), the *Azotobacter* cyst coat is neither (6).

Therefore it appears that the basis for extended survival depends on more than just hard, impervious, protective coats. Although the significance of the survival of dormant forms is not completely understood at this time, the findings presented here indicate that the phenomenon is not limited to seeds and spores.

. These soils and dried cultures will be examined in 1979 and again in 1984, and the result of those observations will be published at the latter date.

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