PROGRESS REPORT

Microorganisms Study
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Microorganisms Colonizing Straws
Buried in Chile Desert Soil Samples

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ABSTRACT

Sterilized plant parts were buried in three Chile Atacama Desert soil samples. These organic traps were removed from the soil samples, washed free of adhering soil particles, and plated onto the surface of agar contained in petri dishes. Two genera of fungi and four bacteria were isolated from the organic traps. Dilution techniques yielded information different from that obtained from the trap burial technique.

Terrestrial desert environments appear to limit the activity of living processes. In many deserts where macroorganisms do not exist for prolonged periods of time, microorganisms are found which indicates that these organisms may have greater abilities to survive a wider variety of environmental conditions. Microbial populations in soil are commonly represented by many diverse forms of microorganisms including fungi, bacteria, Actinomycetes, algae, protozoa and Myxomycetes. The existence of a specific organism in a given locality usually develops through the ability of that organism to grow and multiply on the available substrates or to be transmitted to the locality from elsewhere. Information included herein is primarily involved with the microorganisms that grow and multiply on the organic substrates buried in the Chile desert soil. Interrelationships between microorganisms are dependent on available substrate, and the distribution of microorganisms within the soil is then related to both the present and past occurrence of

substrates (Garrett, 1963). Microorganisms in the assimilative stages decompose existing substrates, and as a substrate is exhausted the microorganisms die or reproductive or resting structures are formed. While some resting cells eventually perish, others may come into contact with a fresh substrate and initiate a new cycle of vegetative development.

Harder(1948), Remy(1949), Sörgel(1941), Sadasivan(1939), Walker(1941), Staffeldt(1951), and Calderon and Staffeldt(1965) buried organic materials in soils to trap the microorganisms capable of invading and digesting the buried materials. These traps included sterilized and non-sterilized stems of wheat, oats and clover, grass fragments, pollen grains, hemp seeds, houseflies and ant larvae. Sadasivan(1939) and Walker(1941) using the same straw colonization technique and surface sterilization with silver nitrate, recognized a limited number of genera of fungi. Staffeldt(1951), employing the same colonization technique under aerobic conditions and without surface sterilization, recognized 38 genera of fungi and representatives of the Actinomycetes, Myxomycetes and Basidiomycetes.

Continued use of this technique to determine the types of soil inhabitants in Chile desert soils appeared quite feasible.

Soil samples were collected aseptically by Dr. Roy Cameron, Senior Biologist, Jet Propulsion Laboratory, from the surface—1cm (#290), 1-4cm (#291), and from the 4-12cm depth (#292) and it was placed in sterile plastic-lined sacks. At the time of the collection, it was noted that all of the soils were sandy with some white, crusty material evident and this material increased in quantity with depth of the soil (Cameron, et al, 1965).

All samples were air dry at time of collection. Temperature and humidity measurements were made at that time. The soil temperature was

22°C (at 5cm), air temperature was 18°C, and the relative humidity was 56%. Sterilized plastic-lined canvas sacks were used to transport these samples. Samples were then sent to the Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California and were later sent to the microbiological laboratory at New Mexico State University for microbial analysis.

The microorganisms inhabiting these soils were trapped by allowing them to grow into sterile organic plant parts. Healthy, unbroken straws were selected from baled alfalfa (Medicago sativa) hay obtained from New Mexico State University Agronomy Farm. The straws were cut into pieces 3 inches in length, moistened and steam sterilized for thirty minutes at 15 pounds pressure.

Fifteen sterile straws were placed aseptically into a 3-inch sterilized screw capped jar with sterile forceps and labeled, soil (#290). The extra straws added were in case of incidental mishap in transferring straws. Straws were added to each of two other jars in this manner and labeled, soil (#291) and soil (#292) respectively. Soil samples were then poured into the respective jars, agitated and placed at room temperature.

Two straws were removed aseptically from each jar after 2, 4, 8, 16, and 32 days. These straws were washed free of adhering soil particles with running tap water. In addition they were rinsed 5 times with tap water which was followed by 5 rinses in sterile distilled water. The straws were then placed in sterile paper towels to absorb the free moisture. Each straw was aseptically cut into three equal parts and planted at three points on the surface of carrot-decoction agar, contained in 9cm petri dishes. This made a total of two plates for each soil sample or 6 plates per examination

date. With three pieces of the cut straw on each plate this would allow a microbe to appear 18 times per examination date.

The petri dishes containing carrot-decoction agar and the plated straws were examined within 24 to 48 hours for growth of fungi, bacteria and Actinomycetes. Representatives of unidentified microbes were transferred to agar slants in test tubes for further study and identification. The dishes were also examined at frequent intervals thereafter, to note the presence of additional microbes which may have a slower growing rate.

Dilution plates were then prepared to determine the number of microbial colonies present. Dilutions of 1:10, 1:100, and 1:1000 were made in the following manner: 1.5gms of each soil was added to 13.5mls of Tryptose agar, agitated and poured into petri plates for the 1:10 dilution. This same procedure was followed and aseptically handled to obtain the remaining dilutions.

Bacteria isolated from soil samples were tested to determine if they possessed any antogonistic effects to other organisms. The isolated organisms were streaked on the agar surface and the test organisms, Bacillus megaterium and Pseudomonas aeruginosa were streaked perpendicular to and across the soil isolates. Tryptose agar was employed during this phase of the investigation.

The above experiments were duplicated at a later date and similar results were obtained.

Microorganisms colonized the sterile, organic traps throughout the 32-day burial in three Chile desert soils. The occurrence of these organisms on straws removed during 5 predetermined examination periods are expressed in percent occurrence in Figure 1. Bacteria were found much more abundantly

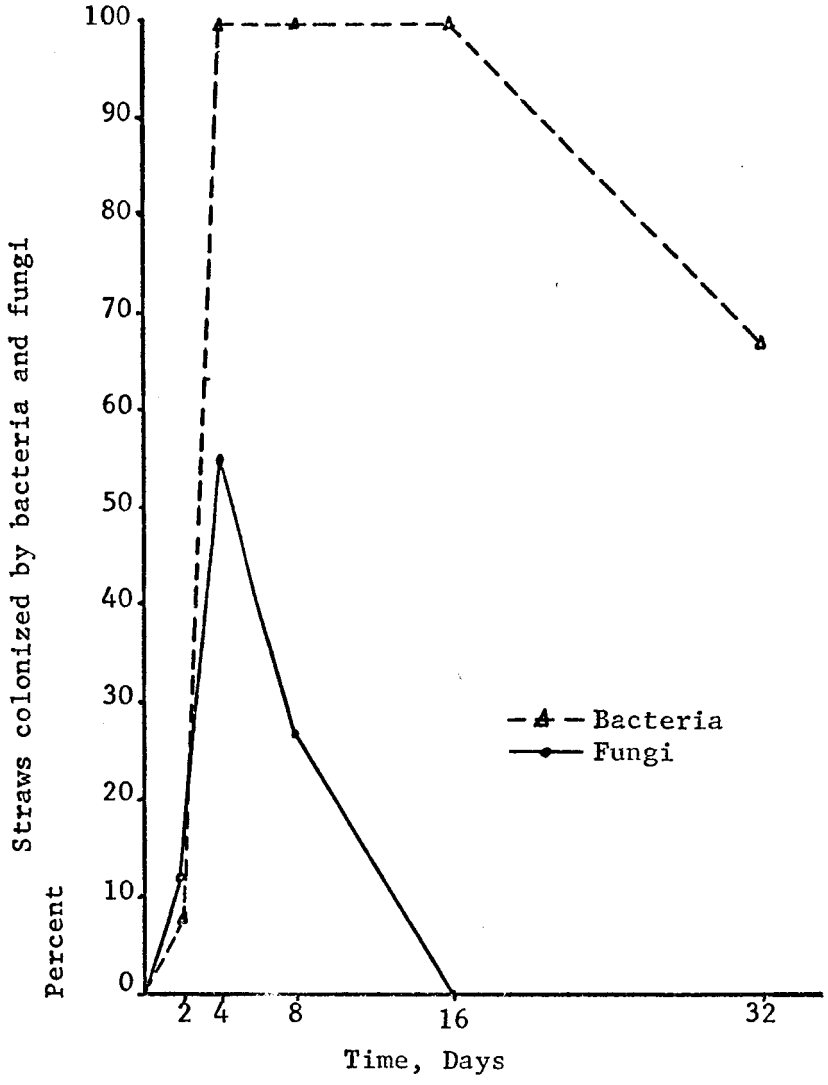

Fig. 1. Bacterial and Fungal colonization of straws buried in 3 Chile soil samples for a 32 day period.

than were fungi. All straws removed after 4, 8, and 16 days of burial were colonized by bacteria. Fungi were observed during the early examinations and were not found growing from straws after 16 and 32 days of burial.

The predominant organisms invading straws were members of the genus Bacillus which colonized 74% of all the sterile, organic traps. Four species or different forms of the genus Bacillus were isolated from the sterile straws. The percent of straws colonized by bacteria is shown in Figure 2. Examinations of organic traps revealed that bacteria colonized the straw substrates buried in soil of the 4-12cm depth after two days of burial. All straws were invaded by bacteria when buried in soils removed from the surface (#290), 1-4cm (#291), and 4-12cm (#292) depths after 4, 8, and 16 days of burial. Total colonization of straws continued to be observed from traps buried in soils #291 and #292 while no bacteria were found in traps buried in soil removed from the surface after the 32-day burial period.

Two species of fungi, Fusarium chlamydosporum and Aspergillus nidulans, were found colonizing a number of the above mentioned traps (Fig. 3). Fusarium chlamydosporum was found colonizing traps buried in soil sample #290 during one examination of straws which occurred on the fourth day. Some of the straws removed from soil samples #291 and #292 were invaded by A. nidulans. In sample #292 A. nidulans appeared on traps removed after the first examination, increased in numbers on the straws removed after the fourth day, decreased after the eighth day, and did not appear after the sixteenth day. Aspergillus nidulans was found colonizing straws buried in soil sample #291 only on the eighth day examination.

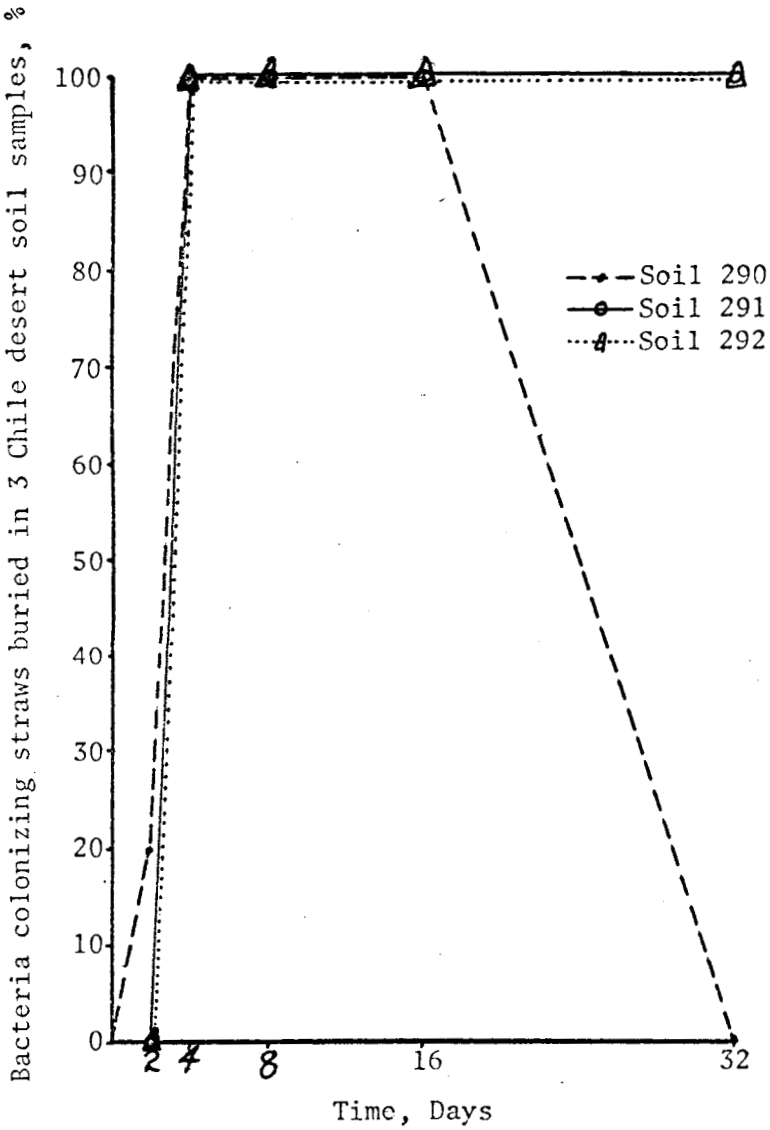

Fig. 2. Bacterial colonization of straws buried in 3 Chile soil samples for a 32 day period.

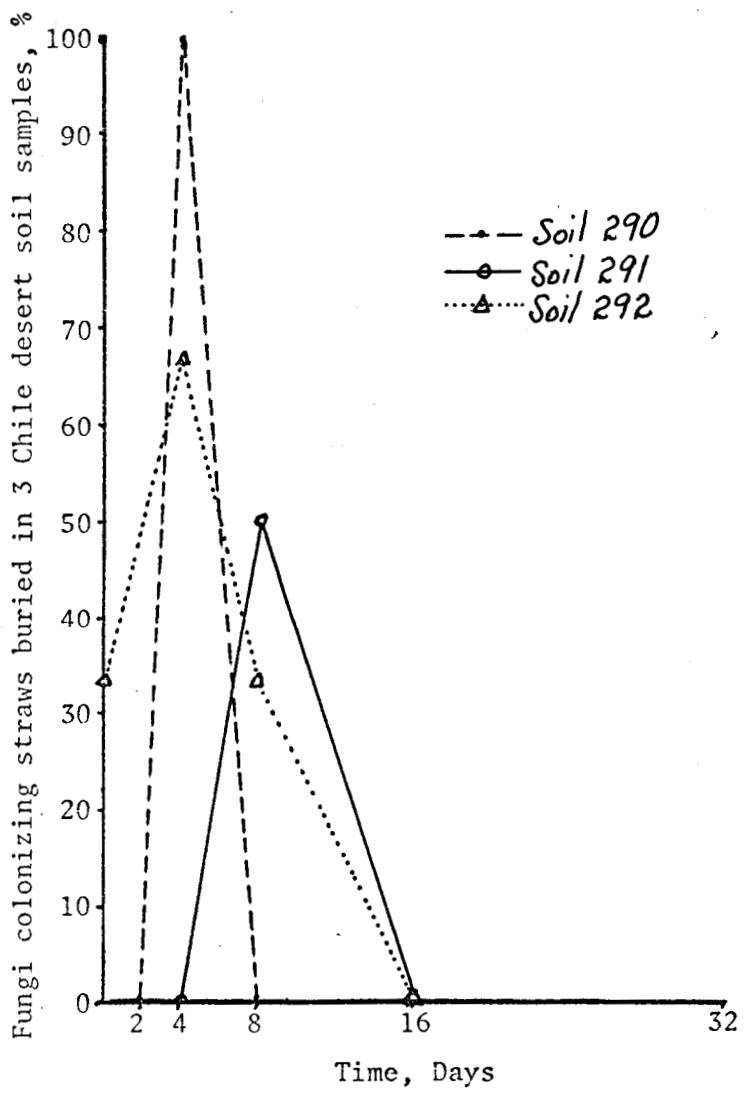

Fig. 3. Fungal colonization of straws buried in 3 Chile soil samples for a 32 day period.

The restricted numbers and diversity of microorganisms carrying on the decomposition of organic substrates buried in desert soils necessitated a more critical determination of quantitative information. The number of organisms per gram in the three desert soil samples was determined by using the soil-dilution technique. Soil removed from the surface (#290) contained 6.0 bacteria and 1.1 fungi per gram (Table 1). The number of organisms increased in the soil removed from the 1-4cm depth (#291) to 48.0 bacteria and 6.6 fungi. The soil removed from the 4-12cm depth (#292) contained approximately the same number of bacteria as sample #291, 44.0 and 48.0 respectively. The number of fungi in sample (292) was equal to the number found in the surface soil. Streptomyces spp. appeared when using the dilution technique and were totally absent during the decomposition examination. This factor requires further investigation.

It was assumed that the restricted colonization of organic traps could have been due to the antagonistic effects produced by the colonizers. Interrelationship studies were conducted by pitting one organism against the other. At no time were any antagonistic activities observed between organisms (Table 2). Therefore, further investigation is required to critically explain the restrictive activity of microorganisms observed during this investigation.

Table 1. Determination of numbers of bacteria and fungi per gram of soil in three samples of Chile desert soil

Soil Sample	Depth of Sample	Bacteria	Fungi
290	0-1cm	6.0	1.1
291	1-4cm	48.0	6.6
292	4-12cm	44.6	1.1

Table 2. Antagonistic effect of bacteria isolated from Chile soil when tested against two test bacteria and two fungi isolated during this investigation

	Bacteria			
Organism	1	2	3	4
Bacillus megaterium	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-
Fusarium chlamydosporium	-	-	-	-
Aspergillus nidulans	-	-	-	-

The activities of microorganisms colonizing sterile straws buried in Chile soil were much different than those reported for other soils examined by using the trap burial technique. Relatively few microorganisms were found growing from invaded traps in this experiment while other authors reported the growth of 10-15 organisms from a single organic segment (Staffeldt, 1951). In most instances a single organism was found on the plant part, yet the dilution plating of the soil revealed that a number of organisms were located in the area of the trap. Garrett(1963) partially

explained this situation when he indicated that competition will be restricted to those species that are metabolically and physiologically capable and are present in the immediate vicinity of the substrate at the time it becomes available. Reasons such as the reduced amount of free water available, the employment of ambient laboratory temperature, and others could be given but each would have to be proved by regulating one parameter at a time.

Aspergillus nidulans and Fusarium chlamydosporum constituted the entire fungal population during the trap burial studies. These two fungi did not make up any portion of the population obtained during the dilution investigation. During the dilution studies the most prominant fungi were Hormodendrum hordei and Aspergillus humicola. An understanding of the precise role of each of the above mentioned fungi is necessary to determine if they are truely inhabitants of this desert soil or if they make up a portion of the transient forms that are deposited in this location.

Colonization of the plant stems by the two fungi followed by their disappearance is a little more difficult to explain. Again, Garrett(1963) lists four capabilities of microorganisms that lead to their success in competition. These include: (1) rapid growth rate of hyphae and germination of spores, (2) broad enzyme producing systems, (3) ability to produce toxins and/or (4) tolerance of toxins produced by other microorganisms. These capabilities express the initial success of the microorganisms but may not necessarily relate to the continued activities. Broad enzyme systems and tolerances of toxins produced by other organisms would influence continued success of these fungi. The latter aspect was tested but was not found to be involved. The broadness of the enzyme systems has not been tested nor

has the inhibitory effect of the accumulation of the fungus waste products been measured.

Finally, the ecological importance of microorganisms of desert soils will have to be examined and evaluated on a basis that may differ from those pertaining to other soils. For an organism to be ecologically significant it must be capable of utilizing the substrates available, potentially opportunistic, and present in sufficient numbers to alter the environment. The number of cells suggested for a critical population density is 10^6 cells per ml for a single bacterial species (Brock 1966). If this statement could be converted to biomass and include biological equivalents, it would then be possible to use this concept over time for desert habitats.

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