The Exploitation of the Bacillus Subtilis Transforming System in a Bio-Satellite Experiment

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The ease of handling bacteria, the development of astronomical populations from relatively small volumes of material, and their relative toughness to comparatively limiting and minimal environments permits their inclusion with any other biological materials undergoing survey since they do not compete with larger more demanding organisms for space, effort, or money. Most microbial material intended for experiments of this type allow certain standardized tests, such as alterations in viability, metabolic pattern, and the genetic material. The latter is ascertained by screening for mutations (preferably reversions) at standard gene loci. The rapidity of clonal reproduction, and direct expression of novel characters, give the bonus that the results of any genetic alteration would become apparent much within a month after retrieval, in contrast to the higher organisms where several reproductive cycles may be necessary. The aerobic spore-former, Bacillus subtilis is a desirable test material for studying the standard parameters of survival and stability outlined above. Moreover at the same time, an additional and sensitive genetic assay is provided by the DNA transformation system. This system is under exploration at the Stanford University Genetics Department, so the background laboratory machinery is available.

In brief summary, DNA is prepared in a relatively purified form from donor bacteria of one genetic constitution and is then incubated briefly (30 minutes) with competent bacteria of contrasting genotype which served as recipients. This DNA thus represents the fragmented chromosomal material extracted from the organism in a chemical form. After 24 hours the ensuing progeny are ready for classification for the segregating markers (genetic labels). The co-transfer index of markers within one DNA molecule, the relative proportions and other genetic quantities allow certain inferences as to the size and state of the DNA molecules involved. Correlated physico-chemical analyses have demonstrated that the various parts of the chromosomal material can be differentiated and separated by a variety of measurements. This ultimately reflects the guanine-cytosine content of the DNA.

Thus the inclusion of this organism as whole bacteria and DNA extract provides a unique assay material for bio-satellite exploration, as well as the opportunity for learning more about the transformation system itself.

This experiment is intended to test the effects of the free-space environment on the viability and genetic stability of a suitable bacterium. The principal factors of launch and spacecraft environment, exposure to space vacuums, and exposure to solar radiation (penetrating a quartz window) can be treated separately and in combination; comparable controls will be retained without exposing them to the flight.

Materials: Preliminary sketch of requirements

1. Frozen competent vegetative cells: multiply-marked mutant. ca 5 sealed tubes containing 2 ml 10^9 bacteria.
2. Lyophilized sample of DNA from standard donor (quant)?
3. Sealed spore samples.

Controls: Same materials, packed, stored at launch/retrieval center. These materials can be prepared 2- 4 weeks in advance of the experiment.

The volume of material supplied will depend upon estimates of possible reduction on potency, so that despite this probably inevitable hazard, enough survivors for the genetic assay will be forthcoming.

Assay of Retrieved Material:

1. Frozen competent cells: Plate; enumerate; test population for known and unknown markers.
   At the same time transform with standard DNA preparation.
   Examine for change if any in competence; co-transfer index in linkage group.
2. DNA
   Examine transforming power, co-transfer index, and with several testers, translocations (unique re-arrangements of genetic map).
   Physical Measurements: Density, melting-point for various markers, spectra.
3. Spores: Treat as for 1.

The Laboratory Survey:

The control and experimental packets can be quickly surveyed and ascertained not long after the samples are returned to the laboratory.

Viability and genetic stability will be known within a week, and unpredicted genetic changes should they have occurred will begin to be noticed within that period also.

Instrumental Requirements:

The mechanical design and construction of the exposure cans can be accommodated within the Instrumentation Research Laboratories at Stanford University Medical School. From 5 to 10 lbs. of payload weight would accommodate an extensive experiment. The chief special characteristic is allowance for the opening of some cans to allow controlled exposure to free vacuum (harder than obtainable on earth) and the sealing of the cans against contamination at take-off and landing.

Cost Requirements:

About $15,000 of engineering costs are estimated for prototype development. The laboratory experiments can be accommodated within existing funding.

The responsible experimenter is Dr. Esther M. Lederberg, in collaboration with other members of the Genetics Department and the Instrumentation Research Laboratory at Stanford University.