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Diversity of progeny from a single colony of *Salmonella typhimurium* after 19 months in sealed agar stabs

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Recent studies at the Cancer Research Center revealed numerous mutations in *Salmonella typhimurium* that had been sealed in agar stab vials and stored for over forty years. The bacteria that were conserved in over 20,000 vials were all progeny of the same *S. typhimurium* strain. However, they were not progeny from a single colony (thus, a single parental cell), but from cultures used in genetic studies in several laboratories. To continue the evolutionary and mutational study of *S. typhimurium*, a new set of 100 similar agar stabs were inoculated 19 months ago from a single colony (thus, a single parental cell), and sealed. Cells from this set were assayed to see if mutations had occurred. Through motility tests, colony growth on three media, re-streaking of unique colonies, and phage spot testing, genetic variability was observed after 19 months storage. In this amount of time enough mutation did occur to display diverse phenotypes among progeny of the single strain of *S. typhimurium*. To confront any concerns that the mutations may have been present 19 months ago, a -80°C stock of the parent colony was used as a control. While the phenotypic changes were significantly less than the vials stored for forty years, it is obvious that 19 months was enough time for genetic variability to occur in *S. typhimurium* from a single parent.

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