‘Fossil’ DNA – How many observed soil bacteria are actually alive?

In the first half of the 20th century, researchers studying soil bacteria had a problem: the number of organisms they could grow in the lab only accounted for ~1% of the total population that they could see under a microscope. Partly this is because soils are more heterogeneous than a petri dish. Advancements in molecular biology created an opportunity to indirectly study microbes in soil, not by growing them, but by identifying them through their DNA. Although this allows us to identify microbes, it does not offer us much information on their growth in soil. More recently RNA has been extracted from soil, in an attempt to better understand microbial growth strategies. For example, it has been proposed that some microbes use many nutrients quickly and reproduce rapidly (copiotrophs), but others use tiny amounts of nutrients and reproduce very slowly (oligotrophs). We designed an experiment to test the validity of rRNA to uncover the copiotroph/oligotroph paradigm. We applied these methods to two different soils, each of which had been incubated in microcosms for one year. In some samples, we added ground corn stalk to provide extra nutrients; others were not amended.

To measure the activity of individual ‘species’ of bacteria (defined as 97+% genetically similar), we calculated the ratio of the number of ribosomal RNA sequences for each species divided by the number of genes that code for the ribosomal sequence. Ribosomal RNA (rRNA) is often studied to show cell activity, because 1) ribosomes produce protein in cells (e.g. more active cells usually have more ribosomes), and 2) RNA degrades very quickly, so you can assume that bacteria identified through ribosomes were active at the time of sampling.

We hypothesized that some bacteria (copiotrophic groups) would have variable rRNA : rDNA ratios between the corn and no corn treatments. Other bacteria (oligotrophic groups) would have similar rRNA : rDNA ratios across all samples. In contrast to this hypothesis, we observed that all groups (regardless of theorized trophic strategy) had highly variable activity ratios, up to five orders of magnitude in a single group. Furthermore, activity ratios were lower than we expected; on average 82% of species had rRNA : rDNA ratios below 1. In a living cell, this is impossible – all cells, active or dormant, require at least one ribosome to function. The logarithmic mean of rRNA : rDNA for all species was 0.24, implying that at least 76% of all DNA we extracted and identified was remnant, ‘fossil’ DNA. Had we performed this study with only rDNA, using it to identify bacteria and explain why certain species are more common in different habitats or make predictions about their functions, our results would have been based mostly dead organisms. There are many studies showing that DNA could be preserved in soil and sediment, especially in marine sediments. Our

results raise a question, often ignored: how many soil bacteria identified through DNA extraction alone are actually alive?

Publication

Microbial rRNA:rDNA gene ratios may be unexpectedly low due to extracellular DNA preservation in soils.
Dlott G, Maul JE, Buyer J, Yarwood S.
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