Why do isolates of eubacterial species have different growth rates under the same conditions?

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Introduction

Microbial ecology and microbial taxonomy have many related concerns in understanding the diversity of prokaryotic organisms. In examining the literature however it is clear that the issue of growth rate is of secondary interest to microbial taxonomists. Many of the studies of bacterial isolates and their taxonomic organization examine relatedness using genetic and phenotypic markers. The genetic markers employed vary from DNA-DNA hybridization, 16S rRNA sequencing, tRNA profiling, etc. A DNA-DNA relatedness of 70% or greater is the current guideline for eubacterial species definition [20], but a 98% relatedness based on 16S rRNA and other definitions are worth debating [19]. Typically, the phenotypic markers are scored as presence/absence of growth on various carbon sources, for example [11]. This type of analysis of relatedness is understandably limited when faced with some 100 or more substrates, but clearly the measured growth rate of different strains on a given substrate varies and is of interest.

In contrast microbial ecology is very focused on growth rate, although usually in the absence of taxonomic information. Bulk growth rates are estimated using the incorporation of thymidine [6] or leucine [9]. In culture work RNA content is related to growth rate [8], and thus the level of RNA or RNA:DNA ratios in cells from natural samples are possible measures of growth rate [10]. The interpretation of field data is much less clear than that of lab data on isolates, however. The technique has not yet reached the level of measuring growth rates of particular species at the individual cell level.

Some recent pioneering efforts examine growth rates of specific taxonomic entities such as species in the field by following the labelling of DNA of specific taxa with 3H-thymidine [12] or bromodeoxyuridine [18]. In one thymidine method, the bulk labelled DNA is isolated and hybridized to query DNA from a specific organism. The change in activity of the captured DNA is proportional to the growth rate of the organism [12]. In the bromodeoxyuridine method, the labelled DNA is captured using an antibody. Its species affinity is probed with 16S rRNA specific PCR reactions and subsequent analysis of the phylogenetic position of the captured sequences [18].

One assumption behind these approaches, especially the thymidine method, is that the growth rates of individual cell representatives of a species are essentially the same under the same environmental conditions. Thus the average rate that is measured will be of use in understanding the population. Is this true? My belief, (this is an opinion article), is that it will be dangerous to take these rates at face value. As shown below, isolates of the same species can have very different growth rates. The measured species growth rate will be an average of widely varying rates or possibly bimodal distributions. The reasons for clonal growth rate differences have been poorly studied because the topic falls somewhere between the fields of taxonomy and ecology.

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Examples of isolate growth rate divergence

Several reviews describe and discuss the ubiquitous clonal variation of growth rates and other properties in eukaryotic phytoplankton species [2, 21]. Since many of these species are thought to reproduce asexually, the same issues discussed hold for prokaryotic organisms. In looking at the primary literature, there are several examples in which isolates of a bacterial species can have different growth rates under otherwise identical conditions. In a study by Ruby and Morin [13], the growth rates of 4 to 7 isolates for each of four *Vibrio* species at four temperatures are compared. For some groups, *Photobacterium phosphoreum*, for example, the growth rates are very similar at each temperature. In contrast, for the other three species there are some large differences in growth rate (all in minimal medium). At 35°C, for example, some isolates will not grow while others will. One problem with the interpretation of this particular data set is simply the species definition. It would be useful to have a molecular confirmation of the species definitions that would now be possible. For example, do the outlying isolates represent related but different "species".

Much of the variation occurs at the temperature extremes where there is a greater possibility of artifacts. The cultures are transferred from 20°C and so may experience some temperature shock which may have a variable probability of killing the cells, giving zero growth rates. Because of the number of growth rate measurements, there appears to be no replication of the growth measurement of individual isolates which would address this issue. It is also possible that, because of the temperature transition, not all measurements provided a real acclimated growth rate, although this is less likely. In summary, this paper provides a clear demonstration of growth rate variation in several species, but leaves open questions of its extent and mechanism.

In cyanobacteria, Karsten [7] compares the growth rates at different salinities of ten *Microcoleus chthonoplastes* isolates. There are growth rate differences at low and high salinities. Interestingly, the maximum growth rates for the isolates varies from 0.2 d^{-1} to 0.54 d^{-1}, approximately a factor of two. The strains seem to show a bimodal distribution of growth rates.

In our own work we are studying the growth rates of isolates of *Synechococcus* spp. from the California current. In particular we are concentrating on three isolates which form a loose clade based on *rpo*C1 sequence data; CC9317 and CC9305-3 are 92% identical over 612bp and both are 89% identical to CC9318 [16]. The strains also have similar pigment characteristics (PUB:PEB ratio). It is clear however that strain CC9305-3 will grow significantly faster than the two other strains (See Figure 1). This difference is equivalent to the CC9305-3 cells doubling every other day while the other two isolates will double every three days on nitrate. The growth rate of CC9305-3 is also faster than the other two strains when ammonia is the nitrogen source, suggesting it somehow has an intrinsically faster growth rate. An unrelated strain isolated from the same environment, CC9311, is capable of doubling every day under the same conditions, so we believe the media conditions are generally conducive to *Synechococcus* growth.

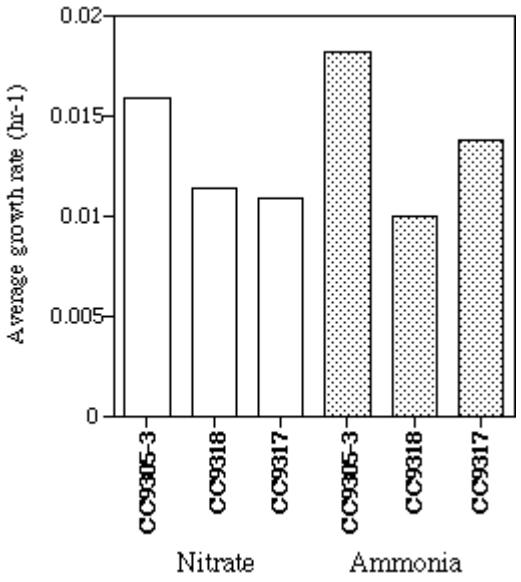

Fig. 1. Three *Synechococcus* isolates from the California Current were grown in autoclaved seawater enriched with 200μM nitrate or ammonia and the same levels of phosphate, vitamins, and trace metals. See Toledo and Palenik [16] for culturing details. The growth rates (k) of the strain CC9305-3 were always greater than those of CC9317 and CC9318. Growth rate was calculated with the equation $A=A_0e^{-kt}$. The coefficient of variation of measurements of k was about 11%.

Mechanisms of isolate growth rate divergence

The absolute growth rate of an organism in the field is dependent on the characteristics of the organism and its environment. Intrinsic cell characteristics determining growth rate are transport (the rate a cell can accumulate nutrients such as C, N, P, etc.), metabolism (the rate a cell can transform these nutrients into basic cellular constituents such as protein, lipid, DNA, RNA), and energy metabolism (the rate a cell can obtain and expend energy). The absolute growth rate of the cell is also dependent on characteristics of its environment, including the levels of temperature, light (as an energy source), nutrients, and toxins. Biological factors such as disease (viral predation), grazing, etc affect the fate of individual cells, and the **net** growth rate of strains or species. These biological factors are missing from our laboratory experiments, yet the fate of a species in the field is dependent on its net growth rate not its absolute growth rate.

There are several possible hypotheses for why isolates of a species have differences in absolute growth rates under identical laboratory conditions: 1)microspeciation; 2) circadian effects 3)deleterious mutations; 4) balancing selection.

The microspeciation hypothesis says that different isolates represent strains that have slightly different adaptations to environmental conditions. Some strains may be better adapted to a lower temperature regime while others may be adapted to a higher temperature regime. Because environmental temperature changes, different isolates will do better at different times of year. When we assay these isolates at the same temperature, we see growth rate differences. This argument can be made for any environmental variable. *In situ* evidence suggests that microspeciation is occurring within the cyanobacterium *Prochloroccus* [4], which according to the current definition is a single species, and likely many other bacterial and eukaryotic phytoplankton species.

A second, still unexplored, mechanism for differences in growth rate is related to the microspeciation hypothesis. If there is a strong selective pressure, especially for photoautotrophs, to replicate during a particular part of the light/dark cycle, then cells may evolve maximum growth rates with measurable values near one doubling every day, every other day, etc. When conditions are replete, the strains optimized to one doubling a day may do the best, but when conditions are poor, the cells optimized to double every other day may do the best. Does this explain the kind of growth rate differences of our California Current *Synechococcus* isolates?

A third hypothesis is that strains simply accumulate mildly deleterious mutations that confer a slower growth rate. These strains are continually created and lost from the population depending on the mutation rate of the organism and the growth and loss rates of the cells.

The hypothesis of balancing selection requires more explanation. There is evidence for what I will call eubacterial life history strategies, or ways in which cells try to avoid grazer or viral death. For example, the cell could produce significant quantities of a secondary metabolite whose only purpose is to affect other organisms but that requires significant expenditure of energy or another resource to produce. A potential example of this is in a cyanobacterial sponge symbiont [17]. Alternatively, the cell may change the structure of a cell surface protein involved in nutrient transport or cell wall formation to make it more resistant to phages or grazers, but possibly at the same time reduce its transport rate or increase its cell wall synthesis phase. A hint of this appears in the work of Ernst [3]. It is also clear that viruses are common in aquatic environments and sensitive and resistant strains of bacteria such as *Synechococcus* can be found [15]. The key point here is that these strategies may reduce the cell's absolute growth rate, but confer an advantage by reducing its death rate, thus increase its net growth rate.

Let us accept for the moment that bacteria have such life history strategies. At the same time let us suppose that if this strategy is genetically knocked out through a mutation, the cell will grow faster. Its cell numbers will increase relative to its cousins, until the viruses to which it is now sensitive bring its growth under control. There may be a form of herd immunity in which the resistant population helps protect the sensitive population as well [5]. A state will exist where there is a relatively faster growing population that is sensitive to a virus and a slower growing population that is resistant. Both could have the same net growth rate and thus coexist. This mechanism of balancing selection would generate clones which, when assayed in the lab, will show absolute growth rate differences.

How are we going to figure out the mechanisms for the differences in clonal growth rate? The most promising route is to use molecular genetics. For example, one approach to discovering the reasons why CC9305-3 grows faster than the other two strains is to make a genomic library of CC9305-3 and conjugate [1] it into CC9317 and try to find clones which now have a faster growth rate (If they exist, they should take over if grown in semicontinuous batch culture). One could try to then determine the genetic element that provides this growth rate advantage. One simple hypothesis is that there might be an additional copy of the 16S rRNA gene in CC9305-3 relative to the other strains that provides a growth rate advantage under certain circumstances, but perhaps a disadvantage under slow growth as seen in *E. coli* [14].

Summary

Clear examples exist of single species in which the growth rates of individual eubacterial isolates measured in the lab vary significantly. I think we need to do more than demonstrate these differences; we need to ask why. I have outlined some mechanisms that would lead to growth rate differences, and there probably are others. Molecular genetic studies could help elucidate which of these mechanisms are actually occurring.

If we were able to make a measurement of the absolute growth rates of single cells of a species in its environment, what would we find? Possibly the environmental conditions are different enough from those "ideal" conditions used in the lab that the individual cells would have a much tighter range of growth rates. Alternatively, we could find a large range of growth rates or, at an extreme, “quantized” growth rates. An average (what researchers are currently trying to measure) would likely be meaningless in this case. A technique to measure single cell growth rates in the field would obviously be immensely valuable to microbial ecology.

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