Some are more equal than others

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The microbial communities found in the mammalian large intestine and rumen efficiently degrade many recalcitrant substrates that are resistant to the host’s digestive enzymes. These communities are known from molecular profiling to be highly diverse at the species and strain level, but it may be that only certain specialized organisms (“keystone species”) have the ability to initiate degradation of such substrates, thus releasing energy on which the rest of the community depends. We have recently reported that Ruminococcus bromii has a superior ability to degrade certain forms of particulate resistant starch (RS) when compared with other highly abundant species of amylolytic bacteria found in the human colon and have presented evidence that this bacterium provides an example of a keystone species within the microbial community with respect to RS fermentation. The concept of keystone species can be equally relevant to other activities, e.g., those involved in stabilizing the community.

Introduction

“Some are more equal than others”—this famous quotation from George Orwell’s Animal Farm referred to the extra benefits claimed by the leaders of his supposedly egalitarian (farmyard) societies. It could also be taken, however, to refer to a greater contribution of certain community members compared with others in generating resources on which the whole community depends. The current tendency toward wholly sequence-based descriptions of microbial communities provides little definitive information on the functional roles of the multitude of different phylotypes that make up the community. This can result in a somewhat neutral description of the community in which importance is equated, by default, to relative abundance. On the other hand where functional information is available, typically from cultured representatives, it emerges that some key metabolic or enzymatic capabilities may be limited to a small number of organisms, whose impact on the community may therefore be disproportionately large in relation to their numerical abundance. In some situations such organisms may be said to have a “keystone” role, meaning that their absence would, for example, greatly decrease the degradation and utilization of an important substrate, thus affecting the remainder of the microbial community.

Ruminococcus bromii as a Keystone Species in the Fermentation of Dietary Resistant Starches

A good example of such a keystone species within the human colonic microbiota was reported recently by Ze et al. (2012). Dietary resistant starch is often the single largest source of energy contributing to bacterial growth in the human colon, depending of course on diet composition. Ze et al. (2012) demonstrated an exceptional ability of the human colonic Firmicutes species Ruminococcus bromii to degrade particulate resistant starches (RS). They showed that the amylases of R. bromii strain L2-63 caused extensive degradation of RS even when this strain

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was inoculated into an RS-containing medium that did not support its growth. In contrast strains of three other amylolytic bacteria from the human colon, *Bacteroides thetaiotaomicron* and *Bifidobacterium adolescentis*, although able to grow on the medium containing 30% rumen fluid (Fig. 1 ([b] and [c])), showed a limited ability to utilize boiled RS3 starch. With other added carbon sources, showed a more remarkable increase in growth, monitored by qPCR (Fig. 2). It can be seen that utilization of the boiled RS3 starch was stimulated almost 3 fold when *R. bromii* was present in the consortium (p < 0.001). qPCR analysis demonstrated that although *R. bromii* grew within this defined community, its growth was more limited than in the monoculture (Fig. 1). Other species therefore benefited from the amylolytic action of this primary degrader by competing for the soluble breakdown products, and this competition is assumed to explain the more limited growth of *R. bromii* and reduced overall starch utilization by comparison with the co-culture shown in Figure 1. Butyrate, which is produced by *E. rectale* and *A. hadrua* but not by *R. bromii*, increased in the presence of *R. bromii*, as did the net consumption of acetate which is linked to butyrate production by these species. The mechanisms that allow *R. bromii* to degrade particulate RS with such high efficiency are under investigation. The organization of amylases in Gram-positive bacteria clearly differs from the *rps* paradigm developed for starch utilization by Gram-negative *Bacillus* species which appears more suited to the sequestration of soluble molecules.

Remarkably, the study of Ze et al., (2012) appears to be the first report of cultural work on *R. bromii* since Herbeck and Bryant examined growth requirements in 1974. *R. bromii* is one of the most abundant species in the healthy human colon, and three recent studies showed that its representation in the fecal microbiota was increased in most volunteers when given diets containing RS2 or RS3 resistant starches. In their carefully controlled dietary study, Walker et al., (2011) detected *"Ruminococcus"* in fecal samples from 12 out of 14 obese male volunteers examined. Remarkably, the two exceptions were also the only two people to have residual unfermented starch in their fecal samples. Using a follow-up sample from one of these volunteers, Ze et al., (2012) were able to show that addition of *R. bromii* restored RS3 degradation in vitro, whereas *E. rectale*, *B. adolescentis* and *B. thetaiotaomicron* had little effect.

**Functional Redundancy vs. Niche Specialization: Consequences of Inter-Individual Variation**

At first sight, the concept of keystone species appears to contradict a developing view that functional redundancy is the dominant feature of gut microbial communities. High throughput sequence analyses indicate there is a greater degree of variability within the gut microbial community at the phylogenetic level than...
by Walker et al., (2011). A combination of five-strain consortia comprising: five strains of R. bromii L2-63 (Rb), two consortia comprising: five strains of R. bromii L2-63 (Rb), two consortia comprising: five strains of R. bromii L2-63 (Rb), two consortia comprising: five strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bromii L2-63 (Rb), and four strains of R. bom
1. Akkermansia muciniphila, comes from a different phylum (Firmicutes). In each of these cases newly available genome sequence data will facilitate discovery of the mechanisms used by these intriguing and potentially valuable bacteria to exploit their particular niches.

2. The concept of keystone species can be readily applied to the release of energy from recalcitrant substrates. However, it is likely to prove relevant also to other types of microbial interaction that occur within complex gut communities. Returning to the rumen, bacteria that utilize lactate for example play a key role in stabilizing the community by preventing the drop in pH that results from lactate accumulation. There is evidence that such microbially-mediated buffering applies also to the human colonic microbiota where certain species have the ability to convert lactate into butyrate, acetate or propionate. The key role of such species therefore resides in their stabilizing impact on the gut environment. A case can also be made for other specialist groups such as hydrogen-utilizers whose activities have wide-ranging effects on the rest of the community. In reality it may turn out that we should be talking about “keystone groups” rather than “keystone species” as it would be remarkable if such activities were always limited to a single species. The taxonomic detail is however less important than the insights that can be gained into the functioning and stability of complex gut communities. It is clearly important to identify such keystone species in gut microbial communities and to be able to monitor their populations using metagenomic data and also by more targeted approaches.

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Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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2. Allen-Viras E, Di Gregorio M, White A, Aminov RI. Metabolic activities were always limited to a single representative strain; however it is likely to prove relevant also to other types of microbial interaction that occur within complex gut communities. Returning to the rumen, bacteria that utilize lactate for example play a key role in stabilizing the community by preventing the drop in pH that results from lactate accumulation. There is evidence that such microbially-mediated buffering applies also to the human colonic microbiota where certain species have the ability to convert lactate into butyrate, acetate or propionate. The key role of such species therefore resides in their stabilizing impact on the gut environment. A case can also be made for other specialist groups such as hydrogen-utilizers whose activities have wide-ranging effects on the rest of the community. In reality it may turn out that we should be talking about “keystone groups” rather than “keystone species” as it would be remarkable if such activities were always limited to a single species. The taxonomic detail is however less important than the insights that can be gained into the functioning and stability of complex gut communities. It is clearly important to identify such keystone species in gut microbial communities and to be able to monitor their populations using metagenomic data and also by more targeted approaches.

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