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 phyotypes 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. 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).1 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...
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, Eubacterium rectale, Bifidobacterium adolescentis, or E. rectale, was inoculated into an RS-containing medium that did not support its growth. In all three cases, however, co-inoculation with other added carbon sources, showed a surprising ability to utilize boiled RS3 starch.

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Ruminococcus bromii led to greatly increased starch utilization. In Ze et al., (2012),1 good growth of strains of three other amylolytic bacteria from the human colon, Eubacterium rectale, Bifidobacterium adolescentis, or E. rectale, was inoculated into an RS-containing medium that did not support its growth. In all three cases, however, co-inoculation with other added carbon sources, showed a surprising ability to utilize boiled RS3 starch.

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...
at the level of gene categories identified from metagenome sequencing. This leads to the proposition that the phyllo-
genetic diversity can be treated mainly as “noise”, with core functions being per-
formed by a large number of alternative phylotypes. To take one possible example, many Firmicute species within the human gut microbiota utilize the same pathway for butyrate formation. Several ecologi-
cally and nutritionally distinct groups of butyrate producers can be identified but different species within these functional groups, although known to vary between individuals, might be considered essen-
tially interchangeable as agents in the delivery of butyrate to the gut epithelium. On the other hand, we know that these species are not precisely equivalent; varia-
tions in substrate utilization and meta-
Fibrobacter variformis. Interestingly these include two species of Ruminococcus, R. flave-
cetius and R. albus, along with the fibro-
lytic Gram-negative species Fibrobacter and certain anaerobic eukaryotes (fungi and protozoa). Although rumen cellulose breakdown is therefore not attributable to any single species, it has been shown that the bulk of the community depends criti-
cally on these primary cellulolytic organ-
isms for the release of soluble growth substrates. Interestingly the only human colonic bacterium so far shown to be able to degrade crystalline cellulose is another species of Ruminococcus, R. champane-
lensis. It has been proposed that subjects whose colonic microbiota are capable of degrading this type of cellulose are charac-
terized by possession of this species. It is intriguing, but probably not coinciden-
tal, that the same family of Gram-positive bacteria (Ruminococcaceae) includes potential primary degraders of two very different substrates, lignocellulose and resistant starch. Ruminococcus spp were found to represent a 4-fold higher propor-
tion of bacterial 16S rRNA sequences associated with particular material from human fecal samples than in the liquid phase suggesting that their niche involves tight adherence to particles. Specialized cell surface structures and enzyme com-
plexes involved in adhesion and degrada-
tion are the key to microbial degradation of such particular substrates, as has been established for the cellulosytic rumino-
cocci found in the rumen.

 Keystone Species in General

Classic work on the rumen, where the major source of energy for microbial growth typically comes from lignocellulosic plant cell walls, revealed only a small number of microbial species with the ability to degrade cellulose. Interestingly these include three species of Ruminococcus, R. flave-
cetius and R. albus, along with the fibro-

Figure 2. Stimulation of R35 degradation in a five-membered bacterial consortium by R. bromii L2-63 (Rb). Two consortia comprising five strains (R. thermautotrophicum 5482 (Rt), R. adolescentis L2-32 (Ba), E. rectale A1–86 (Er), A. hadrus SS2/1 (Ah) and R. bromii L2-63 (“with Rb”); or four strains (the same mix without R. bromii (“with Rt”)) were incubated in anaerobic medium containing 0.2% boiled R35 and incubated for 48 h at 37°C. (A) Total sugar utilization and reducing sugar accumulation (as glucose equivalents) within cultures (compared with zero time controls). (B) Bacterial 16S rRNA gene copies, estimated by qPCR using specific primer combinations, expressed as doublings (see Figure 1 legend). (C) Acidic fermentation products (mM). Data are means of triplicate cultures.
Akkermansia muciniphila, comes from a different phylum (Verrucomicrobia). In each of these cases newly available genome sequence data will facilitate discovery of the mechanisms used by these intriguing and complex organisms to exploit their particular niches.

The concept of keystone species can be readily applied to the release of energy from complex 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 microbiologically-mediated buffering applies also to the human colonic microbiota where only certain species have the ability to convert lactate into butyrate, acetate or propionate. The keystone role of such species therefore resides in their stabilizing impact on the gut environment. A keystone species in gut microbial communities and to be able to monitor their 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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