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Virulence or Niche Factors: What's in a Name?

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The increasing interest in the human microbiota raises some interesting questions about the terminology we use to describe some of the structures and strategies employed by commensal and pathogenic microbes to compete in these complex biological ecosystems. For example, all microbes arriving in the alimentary tract face the task of surviving passage through the stomach, coping with bile, interacting with the immune system, competing with the established microbiota, and obtaining sufficient nutrients to gain a foothold in this hostile environment. It is not surprising then that many gastrointestinal microbes (both pathogens and commensals) use similar strategies to overcome the challenges associated with this particular biological niche. Given that many of these structures and strategies were discovered and characterized in pathogens and because they often play important roles in establishing and maintaining an infection, they have often been characterized as virulence factors. It would be misleading to describe the same strategies and structures found in harmless commensals as “virulence factors,” since they represent a *sine qua non* for life in the gastrointestinal tract. It may be time to reconsider and refer to them as “niche factors,” both in terms of providing scientific accuracy but also in light of the growing interest in using gut microbes as probiotics, where the distinction between virulence factors and niche factors is likely to be very important from a regulatory perspective.

Microbiology textbooks typically describe virulence factors as structures or strategies that contribute to the infectious potential of a pathogenic microbe (5). These structures could be capsules, flagella, pili, or type III secretion systems, while strategies could involve the production of exotoxins, iron acquisition, immune evasion (e.g., antigenic variation or phase variation), or translocation of or disruption of host membranes. Usually these structures and strategies can be assigned to two primary categories, those that promote colonization and survival and those that cause damage to the host. However, a case can be made that many of the virulence factors in the former category should be more properly characterized as niche factors, in that they are often shared by harmless commensal organisms occupying the same body site.

One example of a structure that could be used to illustrate this phenomenon is the recently identified type IVb tight adherence (Tad) pili in *Bifidobacterium breve* UCC2003 (7). By using an elegant gene knockout approach, these bifidobacterial pili were shown to be selectively transcribed in the gastrointestinal (GI) tract in murine models, where they promote colonization and aid competition with other members of the gut microbiota. The associated genes are also present in many other *Bifidobacterium* species. However, Tad type IV (TadIV) pili are well-established virulence factors in pathogens; a recent study on similar pili in *Yersinia enterocolitica* starts with the statement “Type IV pili are virulence factors in many bacteria” (9). Are Tad pili virulence factors or is it better to refer to them as niche factors designed to promote competitiveness in the dynamic environment of the GI tract? This is not simply a pedantic or semantic issue, not only because language is important but because a lack of precision can lead to confusion and can affect advances in scientific understanding. An alternative description could be that “Type IV pili are niche factors possessed by many pathogenic bacteria.” This is an equally informative, but perhaps more precise, statement. The same phenomenon applies to a widely commercialized probiotic strain, *Lactobacillus rhamnosus* GG, which possesses proteinaceous pili which are also found

in Gram-positive pathogens, including *Enterococcus faecalis*, where they have been shown to play a role in pathogenesis (4, 6).

This nomenclature has arisen in part because microbiologists working on infectious organisms routinely define any gene product that contributes to the overall virulence potential of a pathogen as a “virulence factor.” This is how many virulence factors have been characterized over years of research. We followed this practice when we identified a bile tolerance system in *Listeria monocytogenes* termed BilE, which is an important strategy for gastrointestinal survival of the pathogen (10). Cells expressing BilE can exclude bile, presumably by efflux, although this remains unproven at this stage. In orally infected mice, the absence of BilE has a significant detrimental effect on the virulence potential of the mutant strain, which is not observed in intraperitoneally infected animals. We hypothesized that BilE was a virulence factor based on the fact that bile is encountered only in the body and also on the fact that the *bilE* genes are under the control of the master virulence regulator in *L. monocytogenes*, PrfA. We concluded that BilE was a gastrointestinal virulence factor, since it is clearly important in the overall pathogenic potential of the organism. However, with hindsight, this is a good example of a “niche factor,” since similar bile tolerance mechanisms (whether they are genotypically similar or not) must exist in commensal organisms which make their home in the bile-rich regions of the GI tract. Perhaps it should no longer be sufficient to define a virulence factor by its overall impact on the infectious dose or virulence potential of a pathogen, but rather it should be mandated that it has to be shown that the particular system both plays a significant role in pathogenesis and is not found in commensal bacteria occupying the same body site or niche. BilE could be better described

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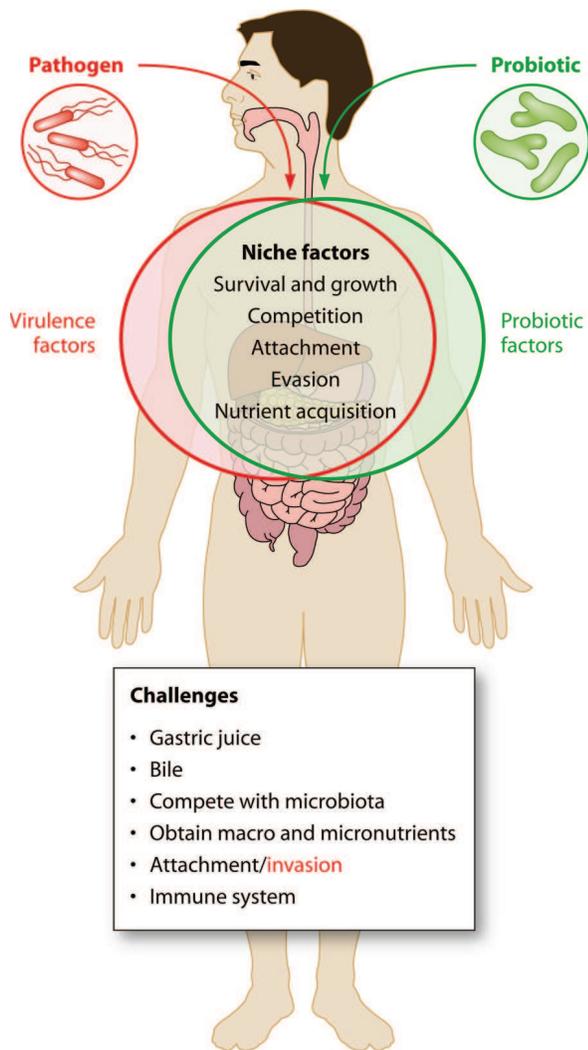


FIG 1 Challenges faced by probiotic and pathogenic organisms.

as a niche factor required for gastrointestinal survival of *Listeria*, which plays an important role in the infectious lifestyle of the pathogen. Another related example is the presence of bile salt hydrolases in gut commensals and in pathogens. One elegant study stated clearly that “*Listeria monocytogenes* bile salt hydrolase is a PrfA-regulated virulence factor” (3). However, we have shown that deleting *bsh* genes in *L. monocytogenes* reduces the ability of the organism to colonize by reducing its ability to cope with bile in the gut (1), and bile salt hydrolase (BSH) activity is also present in many commensals marketed as probiotics (2). This would be another example of a niche factor rather than a virulence factor. In general, we should avoid using the term virulence factor to describe gene products and strategies that are widely disseminated in cohabiting commensal microbes.

The need for more-precise language is especially relevant in the specific case of probiotics. Probiotics are microbes that when consumed in adequate amounts confer a beneficial effect on the host. Probiotics and gastrointestinal pathogens share a similar set of challenges upon ingestion (Fig. 1), and thus, it is not surprising that they share similar strategies for coping with these challenges. This is especially true given that the original source of most pro-

biotics and many pathogens is the gastrointestinal tract itself, where most of these challenges will be continuously encountered. This could have significant implications for probiotic science, in that regulatory agencies are likely to demand complete genome sequences and evidence that “virulence factors” are absent in any novel commensal proposed for use as a probiotic. Indeed, European Food Safety Authority (EFSA) guidelines on microbes that attain the Qualified Presumption of Safety (QPS) status use the terms virulence factor and virulence determinants, but without definition (8). The regulatory intent is clear, in that we should avoid using organisms that could potentially inflict damage to the host under the circumstances of their delivery in high numbers to the GI tract. However, most microbes proposed for use as probiotics will possess many of the colonization and survival strategies also used by pathogens, which may well have been established in the literature as bona fide virulence factors. This could inadvertently lead to regulatory confusion, since a literal interpretation would prohibit the use of *Bifidobacterium breve* and many other bifidobacteria as probiotics on the basis of the presence of genes encoding TadIV pili, which surely is not the intention of the regulatory agencies and is not to the benefit of the consumer.

For the purpose of generating discussion on this topic, it could be proposed that a true virulence factor would be defined as a product, structure, or strategy that helps a microbe gain access to or survive in normally noncolonized body sites or cellular compartments (e.g., internalins and invasins), cause damage to the body (e.g., cytolytic or hemolytic toxins), cause dysregulation of the immune system to the extent of creating disease symptoms (e.g., superantigens), or cause a neurological response that again leads to disease symptoms (e.g., neurotoxins). Niche factors would include products or strategies that promote motility, bile tolerance, immune evasion in nonsterile body sites, macro- and micronutrient acquisition, attachment mechanisms, and various other colonization and microbe-host communication strategies.

There will be instances where colonization factors such as adhesins that promote attachment to normally uncolonized sites, such as Pap pili in uropathogenic *Escherichia coli*, would retain their status as virulence factors, since commensal counterparts are not abundant and colonization of a normally uncolonized site is largely responsible for the disease symptoms. Equally, factors for evading complement and serum antibodies would also continue to be defined as virulence factors, while factors that promote detachment from mucin in the gut would become niche factors. While this proposal is not an attempt to be prescriptive or complete, ideally it would provoke a consideration of whether a given system is a niche or virulence factor on a case-by-case basis. While all might not agree with each conclusion or proposal, it would seem preferable to a default characterization of every system that affects overall virulence as a virulence factor.

We have a complicated relationship with the microbes that inhabit all of our exposed body sites, from commensals that can become opportunistic pathogens in compromised hosts to pathogens that can exist in a carrier state as commensals in healthy subjects. In order to more precisely describe these interactions, we require precise language that informs rather than complicates our understanding. The term niche factors could apply to the gut, oral cavity, skin, and urogenital tract—or any site that is normally colonized in the human superorganism. Of course, niche factors could also apply to other environmental sites outside the body,

and it is important in all cases that the term niche factor be used only in the context of a stated environmental niche.

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