Probiotic mechanisms and practical considerations for monogastric livestock

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Abstract

The intestinal microbiota and its functions are regarded as critical for host health and disease. Probiotics can influence the gut microbiome and its interactions with the host, and are currently defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Probiotics have become common components of strategies to promote livestock health, welfare and productivity, not least due to restrictions on the use of antimicrobial drugs. Common probiotic organisms are considered commensals and are 'generally recognized as safe' (GRAS) via oral administration. This review outlines potential probiotic mechanisms, including recent findings. These mechanisms include those interactions primarily occurring between the supplemented probiotic microorganisms and the indigenous intestinal microbiota, perhaps within the gut lumen, as well as more direct interactions with the host via mucosal receptors or more distally following absorption of microbial components. There is good evidence that the gut microbiome is relatively stable in 'healthy' individuals and resistant to 'colonisation' by exogenous microbes, which helps exclude pathogens, but has implications for the establishment of probiotics, and could increase the importance of microbe-microbe interactions. However, such microbiomes may be receptive to novel microbes or functions, while supplemented probiotics may dominate luminal populations, particularly in less populated regions of the intestine. Moreover, host-adapted microbes or microbiomes may elicit different host responses and/or be more effective. Some considerations for the interpretation of study results, including extrapolation from different models or microbial strains, are also included. In addition, notable mechanistic and/or pathogen challenge studies from pigs and poultry are highlighted to underline the recognised potential of probiotics in these species, particularly as the appropriate selection of microorganisms and their application continues to be better understood and improve.

Keywords: probiotic, pig, poultry, microbiota, microbiome, intestine

1. Introduction

Microbial colonisation of the intestine is primarily considered to begin during birth as microbes are acquired from the immediate environment. In humans, the gut microbiota is highly dynamic until around 3 years of age, when the composition and function of the microbiota are reported to become relatively stable and more adult-like (Derrien et al., 2019). Similarly, the gut microbiota is highly variable in young swine and poultry and stabilises over the first weeks and months of life. Importantly, the developmental trajectory of the gut microbiota is profoundly shaped by early husbandry practices. For example, chicks raised with adult hen contact can acquire an adult-type microbiota composition during the first week of life, which confers greater resistance against pathogens (Kubasova et al., 2019). Excellent reviews on the gut microbiota of poultry and swine have been published recently (Rychlik, 2020; Aluthge et al., 2019). Across species, compositional and functional diversity increases with age and inter-individual variations decline. Studies suggest that different gut segments (e.g. ileal vs. caecal) or sites (mucosal vs. luminal) provide niches for dissimilar microbial populations. *Lactobacillus* spp. are relatively acid tolerant and dominate proximal GIT segments, with more diverse (composition and function) microbial populations in distal parts. Although the 'healthy' adult-like gut microbiota is considered fairly stable, it is constantly influenced by host and external factors, notably diet, medication (e.g. antibiotic) use, and pathogens.

The gut microbiota and its functions are considered crucial for host health and disease (Dogra et al., 2020). Therefore, seeking to modify the composition of the gut microbiota and/or its functions are regarded as opportune ways to influence host health. For many years, sub-therapeutic antibiotics have been used to promote health, welfare and productivity of livestock, presumed to be through modulation of microbiome-host interactions, although their precise mode of action has not been confirmed (Broom, 2017). Concerns about antibiotic resistance have led to societal and legislative action to reduce the use of antibiotics in animal production (Grant et al., 2018), and the evaluation of alternative strategies such as probiotics.

Many associate Elie Metchnikoff, a Nobel Prize winning, Ukrainian born, zoologist and developmental biologist, as the founder of the probiotic concept (Gordon, 2008). Probiotics are currently defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014) and often include *Lactobacillus* spp., *Bifidobacterium* spp. or *Bacillus* spp., which are 'generally recognized as safe' (GRAS) per os and/or are members of a 'healthy' gut microbiota (Rainard and Foucras, 2018). Spore-forming microbes, such as *Bacillus* spp., have encouraged a particular interest as the spores resist harsh environmental conditions (e.g. gastric environment, feed processing, etc.) (Grant et al., 2018). Probiotics have attracted considerable research interest and have become key components of animal production strategies to promote health and welfare in an era of more restrictive, or judicious, use of antibiotics.

This review seeks to summarise probiotic mechanisms and to consider practical implications for their use in monogastric production animals to help achieve the benefits more consistently.

2. Overview of proposed probiotic mechanisms

Microorganisms ingested either naturally or via oral supplementation arrive in the relatively hostile environment of the proximal gastrointestinal tract (GIT), which includes enzymatic, acidic and specifically antimicrobial secretions. For these reasons, the anterior compartments of the GIT invariably harbour a less densely populated and diverse microbiota than more posterior segments, and may thus be more amenable to modification by newly arriving microbes, even in relatively low numbers. To remain viable, indigenous or ingested microbes must resist these natural defences. In addition, it is also important to consider what we mean by the term colonisation. Colonisation is proposed to refer to a stably replicating microbial population within a GIT compartment, whereas the presence of metabolically-active microbes could be more short-term and transient, persisting for only a few days to a week or so (Marco, 2019). It could, therefore, be proposed that microbes within the gut lumen are more likely to encompass more transient populations than those associating more closely with the mucosa. These aspects are important factors when considering the dynamics of microbes within the GIT, and interactions with the host, and their modification through exogenous microbial supplementation.

2.1 Direct microbe-microbe interactions

Table 1. summarises the potential mechanisms for probiotics. Upon arrival into the GIT, exogenous microbes, as well as being exposed to host secretions, will interact with the indigenous microbiota. Microbial cells that remain metabolically active will consume nutrients and produce metabolites. This competition for nutrients can deprive the native microbiota, including unfavourable members, of necessary growth substrates and can thus constrain their numbers. In addition, some nutrients utilised by the supplemented microbes will also be unavailable to the host. However, exogenous microbes often produce enzymes (e.g. carbohydrate-active enzymes) that help digest indigestible food components and thus liberate more digestible substrates or metabolites for the resident microbiota or the host. For example, butyrate-producing bacteria may utilise simpler breakdown products from more complex substrates or end products of fermentation (e.g. lactate and acetate) (Belenguer et al., 2006). Alternatively, metabolites or compounds produced by the incoming microbes can be inhibitory to other members of the community. These include short-chain fatty acids (SCFAs), hydrogen peroxide, nitric oxide, and antimicrobial peptides (AMP) (e.g. bacteriocins) (Rainard and Foucras, 2018). Microbes producing such compounds tend to have mechanisms that prevent self-inhibitory/destructive effects. SCFAs have well established inhibitory effects against various microorganisms through dissociation and acidification of the external environment, or the passage of undissociated molecules into the cytoplasm of the microbial cell where they dissociate, acidifying the internal cellular environment and disrupting normal functioning (Theron and Rykers Lues, 2011). In recent insightful work, SCFA production, notably acetate, was proposed to be critical for eliciting colonisation resistance in mice (Sorbara et al., 2019). Similar studies in poultry and swine would be very informative and should be encouraged.

Antimicrobial peptides are a heterogeneous group of compounds that can have narrow or broad-spectrum antimicrobial activity through membrane permeabilization or affecting cellular protein production, or they can interact directly with the host (O'Connor et al., 2020). Probiotics producing AMP have garnered much interest as they may help these strains achieve a competitive advantage over their susceptible microbial neighbours, which may help with establishment within the GIT or

inhibit less favourable community members. Studies have suggested that AMP-producing bacteria have subtle effects on gut microbiota composition (O'Connor et al., 2020), which might actually be desirable, but the extent to which they beneficially influence the composition and activity of the GIT microbiota, or specific pathogen populations, *in-vivo* needs further investigation.

Quorum sensing (QS) molecules are another group of compounds produced by bacteria that accumulate in the local environment in a bacterial cell density-dependent manner (Coquant et al., 2020). These molecules can be detected by both the microbial community and the host. With regards to the microbial community, once a specific threshold of QS molecules is reached, certain microbial genes are induced, notably those related to virulence factors (e.g. biofilm formation) (Coquant et al., 2020). Biofilms, an aggregate of microbes that adhere to a surface and are protected by a self-produced matrix, are a predominant/natural microbial growth mode (Penesyan et al., 2020). Probiotic-type bacteria have been shown to inhibit QS molecules and reduce the expression of virulence factors by pathogens (Suez et al., 2019). Typically, these studies have been conducted *in-vitro* and, given commensals produce and respond to QS signals (Miller and Bassler, 2001), further clarification is needed to confirm how these dynamics play out *in-vivo*.

Additionally, probiotics have been demonstrated to bind pathogens into co-aggregates, which inhibits biofilm formation and pathogen growth (Monteagudo-Mera et al., 2019). Inhibiting biofilm formation is particularly important as this protective structure confers greater resistance on the encased microbes to antimicrobial compounds, although disrupting biofilms can also lead to their dissemination, which can complicate and spread infections (Penesyan et al., 2020).

Table 1. Summary of potential probiotic mechanisms

Direct microbe-microbe interactions	
• Competition for nutrients	• Constrain the numbers of unfavourable microbes
• Production of simpler substrates, metabolites, antimicrobial compounds or quorum sensing molecules	• Nutrients for resident microbiota (or host) • Help establish in the GIT or inhibit less favourable microbes • Affect quorum sensing related (virulence) gene expression
• Co-aggregating pathogens	• Inhibit formation of biofilms and growth
• Metabolise toxins in the GIT	• Potential to modify toxigenicity to gut microbial community (and host pathology)
Microbe-host interactions	
• Production of metabolites, antimicrobial compounds, quorum sensing molecules or neurotransmitters	• Sensed by, and influence, host cell signalling and function
• Engage host cell receptors	• Shape cell function and communication - modulate barrier function, immune regulation and cell processes • Reduce availability to less desirable microbes

• Metabolise host secretions	• Potential to modify their function and/or effectiveness

2.2 Microbe-host interactions

The mechanisms outlined in the previous section could be orchestrated by probiotic microbes from within the gut lumen. The mechanisms we will now go on to describe require more intimate interaction with the intestinal mucosa, although it is possible that all proposed mechanisms are more effective when occurring closer to the epithelium to, for example, achieve optimum concentrations. As alluded to previously, SCFAs, AMP, QS molecules and neurotransmitters can directly interact with the host via epithelial receptors or, following absorption, at more distant sites, and thus influence host cell signalling and function. SCFAs are detected by G protein-coupled/free fatty acid receptors (Garrett, 2020), while receptors for QS molecules remain unclear but both G protein-coupled and peroxisome proliferator activated receptors (PPAR) have been proposed as candidates (Coquant et al., 2020). In recent eloquent work by Hu et al. (2018), a AMP (gassericin A) produced by two *Lactobacillus* strains was found to bind to Keratin 19, an intestinal epithelial cell plasma membrane protein, which increased the expression of proteins associated with intestinal fluid absorption and decreased those associated with fluid secretion, and protected against diarrhoea in weaned piglets. Additionally, various bacteria have been reported to be able to produce a range of mammalian neurotransmitters (e.g. dopamine, noradrenaline, serotonin, gamma-aminobutyric acid) or metabolites with the potential to interact with, and influence, the enteric, peripheral and central nervous systems (Strandwitz, 2018). The effects of SCFAs on host physiology (e.g. gut motility and hormone secretion, chromatin regulation, immunological function, etc.) are well accepted (Krautkramer et al., 2020), while the impact of other microbial products are becoming better understood. However, compounds produced by probiotics have the potential to act both locally and more remotely in the host.

Probiotics can bind to the gut mucosa via various receptors, with host pattern recognition receptors (PRR), expressed on the surface of intestinal epithelial cells (IEC), recognising conserved microbe-associated molecular patterns (MAMPs). Occupation of binding sites and space at the interface between the host and the gut microbiota deprives pathogens, toxins or other molecules that would engage host receptors from the opportunity to do so. This aspect has been a key feature of the probiotic concept. Through engagement of host receptors, probiotics can help to shape cell function and communication in a microbe-specific manner via even subtle differences in the composition, structure and cell surface presentation of their MAMPs (Lebeer et al., 2010). As indicated, access to host surfaces and receptors could be dependent on the microbial population density at a particular site, which might suggest different probiotic mechanisms or influence in different segments of the GIT. Engagement of host PRRs activates cell signalling pathways that culminate in the expression of a variety of genes that are cell- and stimulus-specific (Brignall et al., 2019). In IEC, these responses have mostly been investigated in the context of barrier function, immune regulation and cell processes. Studies have reported that probiotics may strengthen barrier function through increased expression of cytoplasmic and transmembrane proteins for the formation of tight junction complexes between IEC to regulate paracellular permeability and enhanced secretion of host antimicrobial defences (e.g. mucus, IgA, HDP), shape immune responses through production of cytokines and chemokines, and influence epithelial cell turnover (Grant et al., 2018). It is also possible that probiotics or their

components can directly engage and shape immune cell populations and function in the lamina propria beneath the IEC monolayer via antigen presenting cell activity/transport (e.g. dendritic cell dendrites sampling luminal contents) or translocation. Translocation of probiotics, their components or metabolites from the intestine to the lamina propria, or more systemically. could influence cell function and host physiology at more distant sites and/or globally (Dang and Marsland, 2019).

Host secretions can also be modified by probiotics. Microbes expressing bile salt hydrolases can remove taurine or glycine conjugated with liver-produced primary bile acids entering the intestine via the bile duct. These unconjugated bile acids can be further dehydroxylated by gut microbes to form secondary bile acids. For a review of bile acid transformations, signalling and roles in disease, please see Krautkramer et al. (2020). Similarly, some microbes can metabolise and utilise components of mucus, a key host secretion helping to control gut microbes, thus altering its composition and structure (Juge, 2019). These modifications can change the function of these secretions and thus influence intestinal dynamics and host physiology.

As outlined, there are various proposed mechanisms for probiotics to influence the composition and activity of the intestinal microbiota and interactions with the host. Adherence to host intestinal epithelial cells is often the first step of pathogenesis and so direct microbe-to-microbe inhibition mechanisms, to prevent unfavourable microbes contacting or colonising, are probably favoured over more indirect ones (e.g. host responses), as microbes can thus be controlled without engaging host defences. The net effect of different mechanism(s) employed by a probiotic strain within an individual will likely govern any (measurable) outcome of their deployment, for example, modulation of disease dynamics and/or digestive or metabolic efficiency. In the next section, we will consider the practical implications and application of probiotics in monogastrics

3. Practical considerations for probiotic application in monogastric livestock

Understanding their dynamics within the gut is key to the successful deployment of oral probiotics and Table 2. outlines key points and related implications. Various factors (e.g. feed processing), which are important, but will not be covered further here, could affect the viability of probiotics prior to ingestion. Studies report that, following ingestion, numerous probiotic strains survive intestinal transit in various species (Suez et al., 2019). However, it is fair to say that many researchers working in the area of probiotics do not consider (most of) them to colonise the intestine (i.e. to form a stably replicating population). Instead, they are proposed to have a short-term, transient presence in the gut. Lines of evidence that support this notion are that 1) detection of the probiotic strain(s) in faecal samples generally ceases shortly after oral supplementation ends (Suez et al., 2019; Pisula, 2018), and 2) the presence of the supplemented probiotic strain(s) in faeces probably doesn't correlate well with mucosa-association (Zmora et al., 2018). Individuals have been classified as those “permissive” or “resistant” to mucosal probiotic colonization, with “permissive” subjects appearing to be those lacking intestinal microbes or functions that are similar to those provided by the probiotic (Zmora et al., 2018). Care should be exercised when interpreting studies where the techniques used are not sensitive enough to properly distinguish between the supplemented strains and phenotypically or genetically similar strains already present in, or voided from, the intestine (or even viable vs. non-viable microbes) and/or where faecal or even luminal samples are used as a proxy for ‘colonisation’ assessment. It is proposed that stable microbiomes are relatively resistant to the establishment of exogenous

microbes, a feature that is clearly advantageous for protection against pathogens. This suggests that administration during periods of instability or dysbiosis (e.g. earlier in life, following antibiotic administration, etc.) could better establish probiotics, or mucosa-associate them, in the GIT, and thus increase their influence. Whilst some individuals may benefit more than others, probiotic administration could, potentially, equalise microbiome functions and health and growth performance in a population.

A consensus therefore exists that an appropriate probiotic can at least viably transit through the intestine but direct interactions with the host at the mucosal interface seem less likely, particularly in 'healthy' individuals with stable microbiomes. A recent study in healthy humans did, however, report that the ingested probiotic strains remained within, and could dominate, the ileal microbiota for several hours (Hori et al., 2020), although microbiome modulation beyond a transient increase in the supplemented probiotic is debated in humans (Suez et al., 2019). Moreover, diversity is often proposed as a favourable characteristic of the gut microbiome (Dogra et al., 2020), although some oral animal growth or health promoters purportedly reduce diversity (Broom, 2017), which warrants further understanding. Microbiome modification has been associated with probiotic provision in both pigs and poultry (Wang and Ganzle, 2019; Redweik et al., 2020a) but should be carefully interpreted due to direct effects of the probiotic on relative abundances and genes, and indirectly from changes in host physiology. Given the regular feeding or drinking patterns of monogastric farm animals, it is conceivable that probiotics administered in this way could achieve a near continuous presence in at least parts of the GIT during the supplementation period. It is also worth noting that even though their characteristics should be quite different to probiotics, very low numbers of oral pathogens (e.g. E. coli O157:H7; <100 colony forming units) can profoundly affect intestinal physiology and cause disease (Saunders, 2017). Collectively, this indicates sufficient opportunity for probiotics to engage in direct microbe-microbe interactions and contribute to microbial products interacting with the intestinal epithelium or absorbed by the host to help shape their physiology and health. However, where particular microbes locate in the GIT and exert influence could be important as, for example, the presence of 'lactobacilli' in different gut segments has been associated with better or worse growth performance (Mota de Carvalho et al., 2020).

Table 2. Summary of current evidence and implications for probiotic use

Key points	Implications
Unlikely to colonise in many individuals with stable microbiome but likely dominate less populated GIT regions for a period after ingestion	• Direct microbe-microbe interactions and or diffusion of microbial products from the gut lumen likely to be important. • Influence (+/-) perhaps greater in more proximal, less populated GIT segments. Implications of reduced diversity? • Regular dosing (e.g. feeding and/or drinking) patterns likely maintain a 'pool' of the probiotic in the GIT • Target periods of microbiome instability • Benefit of non-viable microbial supplementation?

Microbiomes lacking certain microbes or functions could be more amenable to their supplementation	• Potentially reduces variation in microbiomes and phenotype between individuals
Low numbers of ingested microbes can cause profound effects in GIT	• Probiotic dose(s) based on demonstrable efficacy even if seemingly dwarfed by indigenous microbial populations
Aerotolerant or spore-forming bacteria have survival advantage in aerobic farm environment	• These types of microbes more likely to persist in the environment and colonise early, oxygenated GIT • Consider oxygen sensitivity of probiotic product, administration method and oxygen availability in targeted GIT segment
Some probiotic traits may extend across species or genera, but others strain specific	• Depending on desired characteristics, either multiple taxa or only specific strain(s) could be most suitable
Indications that host-related or adapted probiotics are more effective for certain applications (e.g. resist *Salmonella* colonisation)	• Selecting host-adapted microorganisms or microbiome transplant material for a particular host species may enhance effectiveness
In-vitro, unconventional animal models, studies in non-target species, etc., may not translate *in-vivo* to conventional target population	• Mechanistic or non-mechanistic studies in target species most informative
Some good studies in target species demonstrating efficacy for specific applications	• Some confidence that findings could translate to other settings with same species and application

3.1 Summary of probiotic use in pigs and poultry

In some previous sections we outlined potential mechanisms supporting probiotic use. We will now briefly consider their application based on notable recent examples from the literature. Probiotic use in chickens evolved from the pioneering work of Nurmi and Rantala (1973), who showed that administering the gut contents of adult birds to 1-2 day old chicks profoundly inhibited the establishment of *S. infantis* in the intestine, notably the caeca. They also reported that bovine rumen fluid and equine faeces were ineffective in preventing *S. infantis* colonising the caeca of chicks, indicating that host-adapted microbiomes confer greater resistance. A meta-analysis in 2013, reported that, generally, undefined competitive exclusion products from a chicken source outperformed better defined, commercial products in reducing *Salmonella* in broilers, again indicating greater suitability of host-adapted microbiomes, and that some protective components were missing from the commercial products (Kerr et al., 2013). The seemingly enhanced protection afforded by more complex microbiome preparations has developed interest in the use of caecal or faecal microbiome transplant (C/FMT). CMT has been demonstrated to reduce *Campylobacter jejuni* transmission in seeder experiments when administered within 4 hrs of hatching to Ross 308 chicks, but had a negligible impact if given at 7 days of age, with seeder birds infected at 21 doa (Wigley,

personal communications), supporting early application as a preferred strategy. Interest in early administration has led to evaluation of *in-ovo* or surface application of eggs to transfer a more 'desirable' microbiome to chicks. Richards-Rios et al. (2020) reported that spore-forming bacteria (e.g. *Lachnospiraceae* & *Ruminococcaceae*) were transferred to young birds following surface application of dilute caecal contents from healthy broilers to eggs, but other core members of the microbiota were not (e.g. *Bacteroidaceae*, *Lactobacillaceae*, *Bifidobacteriaceae*). Similarly, given the aerobic environment that chicks are raised in, strict anaerobes that form spores are more likely to be present and able to colonise their GIT early in life (Rychlik, 2020), and *Bacillus subtilis* spores have been shown to germinate in the intestine of chickens (Cartman et al., 2008). Notable studies with probiotics in chickens have indicated benefit for chickens challenged with *Eimeria* (Park et al., 2020), *E. maxima* and *C. perfringens* (Whelan et al., 2019), avian pathogenic *E. coli* (in combination with recombinant attenuated *Salmonella* vaccine) (Redweik et al., 2020b), or vaccinated with whole inactivated avian influenza virus subtype H9N2 (Yitbarek et al., 2019), with various parameters, including immunological, microbiological, pathological, growth, etc. reportedly modified. Studies with commercially available probiotics for poultry have been reviewed recently (Redweik et al., 2020a).

Probiotics have also received considerable interest for swine. Supplementing piglets with *Bifidobacterium lactis* NCC2818 from the first day of life increased tight junction protein expression and decreased mannitol transfer across the intestinal epithelium, had tissue-specific influence on immunological parameters, and modified urinary host-microbial metabolites, although effects on gut microbiota composition were not detected, suggesting metabolic functions were altered (Lewis et al., 2017). As with chickens, attempts have been made to use the sow as a vehicle to introduce probiotics into the immediate environment of, and colonise, piglets at the earliest opportunity, with varying success (Barba-Vidal et al., 2018). Similarly, FMT is also being investigated in pigs. Transferring the faecal microbiome from healthy weaned pigs to contemporaries was reported to confer resistance to diarrhoea (Hu et al., 2018). Two bacterial strains, *Lactobacillus gasseri* LA39 and *Lactobacillus frumenti*, were identified as conferring this resistance through the secretion of a bacteriocin (gassericin A), which engaged a host IEC membrane protein and modulated the expression of proteins involved in intestinal fluid absorption and secretion. Interestingly, FMT from high health sows to 3-4 week old weaned pigs, prior to coinfection with porcine circovirus type 2 and porcine reproductive and respiratory syndrome virus, reduced the numbers of pigs affected by the co-infection and the severity of clinical signs, which may have resulted from higher virus specific antibodies in FMT pigs (Niederwerder et al., 2018). Probiotics have also been reported to be potentially beneficial in experimental models of, for example, enterotoxigenic *E. coli* (Dubreuil, 2017), *Brachyspira hyodysenteriae* and *B. pilosicoli* (Bernardeau et al., 2009), porcine epidemic diarrhoea virus (Tsukahara et al., 2018) and *Salmonella* (Barba-Vidal et al., 2018). Studies with probiotics in swine of different ages were recently reviewed (Wang and Ganzle, 2019).

Some of the challenges in the field of probiotics is the translation of results. Firstly, the characteristics of a probiotic in one animal species or experimental model could be quite different to how it behaves in another animal species or model (Rainard and Foucras, 2018). Similarly, while some traits may extend across microbial species or genera, others will be strain specific (Suez et al., 2019; Lee et al., 2010), and so assuming similar results with even two strains of the same species could be incorrect. Various models are used to evaluate the potential benefit of probiotics. *In-vitro* studies may consider, for example, the ability of a probiotic to tolerate conditions within the GIT or adhere to the cell line used. However, currently, these models lack features (e.g. host secretions) typical of *in-vivo* situations

and so cannot capture the full GIT dynamics. Progress continues to be made in this area and we can expect further developments (e.g. organoids). Also, germ-free animal models, etc., do not represent the dynamics in conventional animals and so the activity of a probiotic in these settings is likely to be compromised by a competing, or more complex, microbiome. Finally, for various reasons associated with specific factors related to the experimental conditions, results obtained in more controlled studies might not translate to the field. This is not an exhaustive description of the challenges in interpreting the results of different studies but, hopefully, highlighting them increases awareness of some of the issues.

4.1 Conclusion

As relatively stable or less permissive microbiomes are reportedly more resistant to colonisation by exogenous microbes, non-colonising probiotic mechanisms are probably important for success. Perhaps unsurprisingly, microbiomes are suggested to be more receptive to microorganisms complementing existing community members and functions, although probiotic colonisation and/or extensive replication in the GIT might, in fact, not be desirable as any control of dose, frequency and effect could essentially be lost (Marco, 2019). Regardless, probiotic organisms can persist and dominate less populated regions of the gut, for at least several hours (sufficient to have effects on the microbiota and host), and perhaps exert greater influence here. This, along with specific functions performed by different sections of the GIT, suggest that, for example, a probiotic to support small intestine functions probably requires different attributes to one preventing or reducing caecal colonisation by pathogens. Moreover, host-adapted microbes may be better suited as probiotics or for specific applications. Certainly, host-adapted microorganisms seemingly elicit different host responses to less adapted ones (Wang and Ganzle, 2019). In addition, early colonisation of the GIT with few microbial taxa or probiotic administration after antibiotic treatment can prevent the (re)establishment of a diverse microbial community and negative implications of this need to be fully understood (Wilkinson et al., 2020; Suez et al., 2018), but could explain why more complex microbiome preparations (e.g. FMT) might be more efficacious.

Like all interventions, it is important to appreciate the limitations of probiotics, have realistic expectations, and understand if/when they are most likely to be used successfully for targeting a disease or growth phase in the host species. Good studies with probiotics in target species are providing confidence for certain applications and further mechanistic insight, including interactions with other feed (or water) components or other interventions (e.g. prebiotics, vaccines, etc.) (Redweik et al., 2020a). Avoiding a ‘one size fits all’ approach should improve the consistency and perception of probiotics, as well as appreciating potential pitfalls in extrapolating findings from specific experimental approaches, or across strains or species.

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