Chapter

# Developments in Probiotic Use in the Aquaculture of *Salmo* Spp.

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## Abstract

While interest in probiotic use in aquaculture is not a new phenomenon, the past few years have seen great developments in probiotic research in *Salmo* spp.. This review examines the corpus of literature surrounding the use of probiotics in some of the species of *Salmo* most important to modern aquaculture, including *Salmo salar*, *S. coruhensis*, *S. trutta,* and *S. trutta caspius,* with a particular emphasis on the most recent research. The use of many of these probiotics is associated with such host benefits as enhanced growth, nutrition, and immunity. These benefits and the potential applicability of these probiotics to the modern aquaculture of *Salmo* are reviewed herein.

**Keywords:** Salmo, Atlantic salmon, brown trout, Coruh River trout, Caspian brown trout, *S. salar*, *S. trutta caspius*, *S. coruhensis*, *S. trutta*, fish probiotics, aquaculture probiotics

## 1. Introduction

As in other animals, the gut microbiome of fish is a dynamic, complex, organ-like system that may contain trillions of microorganisms. It is implicated in a number of functions critical to the animal's health, including digestion, immunity, and nutrient absorption, which may in turn contribute to such things as development, growth and metabolism [1].

There are a few ways by which these salubrious phenomena are known to occur. In terms of immunity, some beneficial bacteria may produce such things as bacteriocins, peroxides, and acids in order to inhibit the growth of pathogenic microorganisms, and have further been observed to mitigate damage caused to the intestines by pathogens [2, 3]. Furthermore, some bacteria have been known to promote the host fish's immunity through such means as the enhanced infiltration of epithelial leukocytes, the modulation of cytokine and chemokine expression, enhanced leukocyte activity, and enhanced lysozyme activity in the cells of the mucosa [4–8]. On the other hand, some bacteria have been known to enhance fish digestion and nutrient absorption through such things as increasing the length of microvilli, enhancing the fold length of the mucosa, secreting various digestive enzymes, and by fermenting non-digestible compounds (e.g., complex carbohydrates) to make them usable [4, 9–11].

In order to take advantage of some of these beneficial health effects, it is critically important that fish reared in aquaculture maintain healthy gut microbiota. This may help ensure the success of aquaculture operations by preventing the spread of disease and promoting the healthy growth of fish to market size. To this


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end, there has been a considerable amount of research on the fish microbiome and the use of probiotics in fish significant to the field of aquaculture. In general, the term "probiotic" typically refers to a sample of live microorganisms that is used in order to confer some kind of health benefit [12]. In many cases, probiotics are consumed in order to allow the microorganisms to enter the organism's gut, from which point they can exert their salubrious effects. In some cases, probiotics are combined with prebiotics, which are compounds designed to bolster the growth of beneficial bacteria. In many cases, these oligosaccharides can be digested by the bacteria, but not the host. The combination of probiotics and prebiotics is sometimes referred to as synbiotics [12].

Of the aquaculturally significant fish in which probiotics have been studied, genus *Salmo* is perhaps among the most important. This genus contains a number of species relevant in modern aquaculture, including *Salmo salar* (the Atlantic salmon), *Salmo trutta* (the brown trout), *S. trutta caspius* (the Caspian trout/salmon) and *S. coruhensis* (the Coruh River trout), *inter alias*. Of these species, *S. salar* is perhaps the most important to global aquaculture. According to the Food and Agriculture Organization of the United Nations, farmed *S. salar* comprises greater than 90% of all farmed salmon and greater than half of all salmon production worldwide [13].

Underscoring the importance of *Salmo* in modern aquaculture, there is a rapidly growing corpus of literature surrounding the use of probiotics in this genus. The benefits to fish immunity and growth that stem from this research may have the potential to greatly benefit the salmon aquaculture industry, thus necessitating further review of the recent pertinent literature.

### 1.1 Overview of the gut microbiota of *Salmo spp.*

To understand the use of probiotics in *Salmo*, it is important to first critically examine this genus' gut microbiota. The intestinal mucosa of *S. salar* can harbor trillions of microorganisms, the majority of which are bacteria. Typically, the identity of these microorganisms may vary considerably depending on several variables, including diet, the bacteria present, biogeography and environmental factors, stress, captivity, disease, the host's species, and life cycle stage [14–19]. To further complicate matters, not all microorganisms in the salmon gut are permanent residents. While many bacteria are indeed capable of colonizing the intestinal mucosa in the long-term, the presence of others may only be transient [20]. Such transience may be due to a number of factors, including the inability to compete with bacteria that have already colonized the gut for such things as nutrients or space, direct inhibition by pre-existing bacteria (e.g., via the secretion of antimicrobial peptides), or even just the general inability to colonize the intestinal tract [2, 21].

Regardless of this dynamism and complexity, researchers have identified a diverse array of bacteria that may inhabit the digestive system of *Salmo*. In one study, the mid and distal intestinal mucosa of *S. salar* kept in seawater were found to contain bacteria from a number of phyla, including Proteobacteria (which comprised ~90% of all mucosal bacteria), Actinobacteria, Armatimonadetes, Spirochaetes, Bacteroidetes, and Firmicutes [20]. Furthermore, in the digesta of *S. salar*, Proteobacteria, Firmicutes, Fusobacteria, and Bacteroidetes (among other less abundant bacteria) were documented [20]. In another study, the digesta of *S. salar* parr reared in a freshwater loch or a recirculating aquaculture system (RAS) were also found to contain representatives of other additional phyla, including Tenericutes and Acidobacteria, among others [22]. In a different study featuring wild *S. salar* at different life cycle stages, Nitrospirae were also found to be abundant in the digestive tracts of parrs, smolts, and adults. This study also found that

Firmicutes, Actinobacteria, and Bacteroidetes occur at far lower levels in marine adults than in freshwater life stages [18]. Overall, these phyla are represented by dozens of genera in the *S. salar* microbiome, notably including *Carnobacterium*, *Lactobacillus*, *Pediococcus, Lactococcus*, *Vibrio*, *Pseudomonas*, *Aeromonas*, *Yersinia*, and *Mycoplasma*, many of which will be discussed further in this review [18, 20].

## 2. Probiotic use in *Salmo*

In general, colonization is critically important to the establishment of probiotic bacteria within a fish's digestive tract. There are a few main considerations behind this probiotic colonization. Firstly, the bacteria must not pose any danger to the host fish or be otherwise pathogenic. Instead, they should exhibit salubrious effects as described previously. Secondly, it is critical that the bacteria are able to reproduce successfully within the fish's gut, such that their rate of multiplication exceeds the rate at which they are expelled [2]. Thirdly, the bacteria must be able to adhere to the intestinal mucosa. There are a few additional considerations to this third point. For example, the bacteria must be able to successfully compete with other bacteria for adhesion space. Some bacteria (including some lactic acid bacteria) are known to have specific adhesion sites on the intestinal epithelium, while others are known to adhere non-specifically [2, 23, 24]. These considerations are critically important to all potential fish probiotics.

**Table 1** provides an overview of the bacterial genera that have been investigated as probiotics in genus *Salmo*:

| ***Salmo* species** | **Bacterial genus** | **Bacterial species** | **Citation** |
|---|---|---|---|
| *Salmo trutta caspius* | *Bacillus* | *B. subtilis & B. licheniformis** | [25–27] |
| | *Pediococcus* | *P. acidilactici*** | [28–30] |
| | *Lactobacillus* | *L. plantarum* | [31] |
| *Salmo salar* | *Carnobacterium* | *Carnobacterium* spp. | [9] |
| | | *C. divergens* | [3, 32] |
| | *Pediococcus* | *P. acidilactici*** | [11, 33] |
| | *Lactobacillus* | *L. delbrueckii* | [34] |

*Asterisks denote the use of commercial probiotic formulations: one asterisk denotes BetaPlus ® and two asterisks denote Bactocell ®. These products are discussed in greater detail below. See* ***Table 2*** *for an overview of other probiotic formulations.*

**Table 1.**
*An overview of different bacterial species investigated as probiotics in genus Salmo.*

### 2.1 *Carnobacterium*

*Carnobacterium* is a genus of Gram-positive lactic acid bacteria (LAB) within the phylum Firmicutes. They are ubiquitous in nature and can survive low temperatures and anaerobic conditions with elevated concentrations of carbon dioxide [35]. In terms of fish, some *Carnobacteria* (such as some strains of *Carnobacterium maltaromaticum*) have been known to be pathogenic in certain salmonids (e.g., *Oncorhynchus mykiss*), while others have been found to exhibit beneficial effects within the gut microbiome [9, 32, 35, 36]. Perhaps one of the most important benefits of probiotic *Carnobacteria* is their ability to inhibit the growth of pathogenic bacteria within the *Salmo* gut. This ability is most likely due to *Carnobacteria's* ability to produce antimicrobial bacteriocins, which may serve to both inhibit pathogenic bacteria and help the *Carnobacteria* to survive within the competitive environment of the gut microbiome [37]. However, the ability of probiotic *Carnobacterium* to inhibit different pathogenic species is known to vary by strain [32, 37]. As a type of LAB, *Carnobacteria* are also capable of lactic acid production, which may also have an inhibitory effect on pathogens. Further recent research has also suggested that the presence of *Carnobacterium* in the pyloric caeca of *S. salar* is associated with enhanced flesh color. While it is hypothesized that this may be related to the production of carotenoids by the *Carnobacteria* as well as their pro-immune effects, the true reason for this phenomenon remains unclear [38].

In an early study conducted in 2000, it was revealed that *Carnobacterium* strains isolated from the intestine of *S. salar* could inhibit a number of pathogenic bacteria, including *Aeromonas hydrophila, Aeromonas salmonicida, Vibrio ordalli, Vibrio anguillarum, Streptococcus milleri, Photobacterium damselae piscicida,* and *Flavobacterium psychrophilium in vitro* [9]. In this study, it was also found that the administration of these *Carnobacteria* for at least fourteen days was able to promote the survival of *O. mykiss* in the context of infection by *A. salmonicida, Yersinia ruckerii,* and *V. ordalli*. This study also found that it took 28 days of probiotic administration to achieve the maximum intestinal *Carnobacterium* levels, and that the cessation of probiotic administration in fry and fingerlings resulted in the bacteria becoming undetectable in the gut in ten days or less [9]. It was further confirmed in another study the coincubation of *C. divergens* strain 6251 with the pathogenic *A. salmonicida* and *V. anguillarum* was able to prevent (but not alleviate) damage to *S. salar* microvilli [3]. A later study demonstrated that the administration of a commercial prebiotic (namely EWOS prebiosal) to help promote probiotic bacterial growth vastly enhanced the ability of *C. divergens* to adhere to the epithelia and mucosa of the proximal (but not distal) intestine of *S. salar* [32].

Overall, certain strains of genus *Carnobacterium* (such as the aforementioned *C. divergens* strain 6251) may have great potential as a probiotic for *S. salar*, with the ability to bolster the fish's immunity and perhaps even flesh color. While it is possible that the probiotic may need to be frequently readministered, the use of a commercially available prebiotic may be beneficial in enhancing bacterial adhesion to the salmon gut.

### 2.2 *Pediococcus*

*Pediococcus* is a genus of Gram-positive LAB within the phylum Firmicutes. It is an acidophilic, facultative anaerobe with well-established probiotic properties, even in humans. Like *Carnobacterium*, *Pediococcus acidilactici* is known to produce bacteriocins and lactic acid, which are potentially useful in inhibiting the propagation of pathogens within the digestive tract [39].

*P. acidilactici* has been investigated as potential probiotic in both *S. salar* and *S. trutta caspius.* In both fish, much of this research has been conducted using Bactocell ®, a commercially available strain of *P. acidilactici* (strain MA 18/5 M) that is also used in other animals of agricultural significance [28–30]. It is one of the only aquacultural probiotics approved in the European Union [33]. Overall, Bactocell ® seems to exhibit some promise in promoting the immunity and growth of *S. trutta caspius*. In one study, Bactocell ® was found to significantly decrease the feed conversion ratio in *S. trutta caspius* following five treatments with the probiotic. Furthermore, white blood cell concentrations were noted to have increased, suggesting pro-immune effects related to the presence of the probiotic. Curiously, red blood cell counts were also found to be lowered relative to the control group that did not receive the Bactocell ® treatment [30]. In another study in which Bactocell

® was co-administered with iron, parameters like body weight gain and specific growth rate were also found to be enhanced relative to the control group [28].

This same strain of *P. acidilactici* (MA 18/5 M) has also been investigated as a probiotic in *S. salar.* In one such study, saltwater Atlantic salmon were administered *P. acidilactici* MA 18/5 M and short chain fructooligosaccharides (as a prebiotic) twice a day for 63 days. Among other things, the researchers noted that this synbiotic (a combination of a probiotic and prebiotic) modulated both local and systemic immunity. For example, it was found that synbiotic administration was associated with the increased expression of the pro-inflammatory cytokines interleukin-1β, tumor necrosis factor α, and interleukin in the intestinal tissue, in addition to increased expression of the antiviral molecules toll-like receptor 3 and myxovirus-resistant protein 1. Furthermore, the researchers observed increased epithelial leukocyte infiltration in the intestines and elevated serum lysozyme levels. Notably, the fish that received the synbiotic also exhibited greater villus length relative to the control and decreased but recoverable intestinal bacterial loads (without adverse health consequences) [11]. In another study, it was found that Bactocell ® was capable of modulating several parameters related to distal intestine inflammation, which overall helped to counteract the inflammation [40]. A later study further studied the administration of *P. acidilacti* MA 18/5 M to *S. salar*, in addition to also considering the effects on the gut microbiome that occur with the transition from the freshwater and saltwater stages. Overall, *Pediococcus* was found to significantly impact the Atlantic salmon microbiota, as in the previous study. Notably, among the fish that received the probiotic treatment, *Pediococcus* was present in greater abundance in both the digesta and mucosa in the freshwater salmon than in the saltwater salmon. In spite of this, the effect of the probiotic treatment on the composition of the gut microbiota was found to be the greatest among the saltwater fish [33].

In summary, there is some evidence to suggest that *P. acidilactici* MA 18/5 M may exhibit positive effects on the growth of *Salmo* spp., as well as significant immunomodulatory effects. Furthermore, its availability on the market may make this strain an enticing choice for those interested in using effective probiotics in their aquaculture systems.

### 2.3 *Bacillus*

*Bacillus* is a diverse genus of Gram-positive bacteria within the phylum Firmicutes. There are three main species of *Bacillus* that have been investigated as probiotics for *Salmo*, the two most notable of which are *B. subtilis* and *B. licheniformis*. While *Bacillus* species are not LAB, they have nevertheless been successfully implemented as probiotic agents in aquaculture, exhibiting the ability to enhance growth and immunity in a few species [41, 42].

In terms of *Salmo*, *B. subtilis* DSM 5749 and *B. licheniformis* DSM 5750 have been investigated as probiotics in *S. trutta caspius* in the form of the commercially available product known as BetaPlus ®. In one study, Caspian salmon fingerlings were administered a synbiotic composed of BetaPlus ® and galacto-oligosaccharides. In comparison to the control (which did not receive the synbiotic), the group that received the synbiotic exhibited superior performance in parameters relevant to growth and immunity (among other things). In terms of growth, this includes a lower feed conversion ratio and higher weight gain and protein efficiency ratios. As for immunity, the fish that received the probiotic exhibited increased serum levels of lysozyme, immunoglobulins, bactericidal peptides, agglutinins, lectins, and albumin [42]. In another, similar study with the same species, BetaPlus ® was used in conjunction with isomaltooligosaccharides as the prebiotic. In addition to some similar findings to the previous study, the synbiotic group was found to exhibit greater levels of monocytes, leukocytes, and neutrophils compared to the control, which is further suggestive of enhanced immunity in the context of synbiotic usage [25].

Overall, these findings suggest that the administration of BetaPlus® in conjunction with a prebiotic may be able to significantly enhance both growth and systemic immunity in *S. trutta caspius*. For those involved in the commercial aquaculture of *Salmo* spp., it may be beneficial to consider the use of BetaPlus® or similar probiotic formulations.

### 2.4 *Lactobacillus*

*Lactobacillus* is a genus of Gram-positive LAB within the phylum Firmicutes. Two species in this genus have been investigated as probiotics for species within *Salmo*, namely *Lactobacillus delbrueckii* in *S. salar* and *L. plantarum* in *S. trutta caspius.*

The use of *Lactobacillus delbrueckii lactis* as an aquacultural probiotic has been known to enhance the innate immune response in other fish, such as *Sparus aurata* [43]. In terms of the Atlantic salmon, one study examined this bacterium's ability to remain on the surface of the intestines in an *in vitro* model containing intestinal tissue from an Atlantic salmon, as well as its ability to prevent damage in the context of an *Aeromonas salmoncida salmoncida* infection. Overall, it was found that the *Lactobacillus* was able to persist on the surface of the intestine, and further caused no damage to the tissue (in contrast to the *Aeromonas*). When the *in vitro* intestine model was co-incubated with both the *Lactobacillus* and *Aeromonas*, the former prevented damage to the tissue caused by the latter [34]. Overall, this is likely suggestive of the ability of *L. delbruecki lactis* to contribute to host innate immunity in *S. salar.*

Further work in *S. trutta caspius* provides some evidence for the ability of *L. plantarum*-based synbiotics to promote both host growth and immunity. In one study, Caspian salmon were assigned into eight groups, which featured combinations including the fishes' basal diet, *L. plantarum*, and the prebiotics beta-glucan and mannan oligosaccharide. All groups featuring probiotics and/or prebiotics exhibited decreased feed conversion ratios and feed intake, as well as increased weight gain and protein efficiency ratios. These same groups also exhibited enhanced parameters relevant to immunity, including elevated levels of immunoglobulin M, and lysozyme (*inter alia*). It is also important to note that with the exception of the beta-glucan group, the groups featuring *L. plantarum* exhibited lower cortisol and glucose levels than the other experimental group, suggesting yet another physiological benefit of the use of *L. plantarum* as a probiotic [31].

*In toto*, while there are not many available studies focusing on the use of *Lactobacillus* spp. as a probiotic in *Salmo* on their own, the promising findings related to growth, immunity, and decreased feed intake associated with this bacterial genus may warrant further investigation as a probiotic in *Salmo.*

### 2.5 Other probiotics

In addition to the probiotics discussed above, there are some other probiotics that have been investigated in *Salmo*, including those with multi-genus or variable composition:

#### *2.5.1 Kefir*

There has been some research on the use of kefir as a probiotic for *S. coruhensis*. Kefir is a fermented, dairy-based beverage with origins in the North Caucasus and has been used as a probiotic in humans. Kefir is known to contain a variety of different microorganisms, including representatives of *Lactobacillus, Lactococcus,* and *Leuconostoc*, as well as other LAB, acetic acid bacteria, and even yeasts [44–46]. Overall, the use of kefir as a probiotic in this species seems to offer some promise. In one study, it was demonstrated that groups of Coruh trout administered kefir exhibited decreased activity of catalase, an enzyme with antioxidant activity, as well as decreased levels of the highly reactive compound malondialdehyde in hepatic tissue. This may suggest that the probiotics associated with kefir may have antioxidant activity [44]. In another study with the same species, kefir administration at a dose of ten or twenty grams per kilogram was associated with elevated immunoglobulin, which may suggest that kefir's probiotics may also exhibit immunomodulatory effects in the Coruh trout. However, this same study did not find any difference in growth or survival rates between the control and experimental groups [45]. Finally, in another study, it was found that Coruh trout in the groups administered kefir also exhibited changes in digestive and hepatic enzyme expression and glucose levels relative to the control group. For example, it was found that serum amylase and lipase levels, as well as serum glucose decreased, suggesting that kefir administration may modulate digestion in *S. coruhensis*. Notably, glucose levels were also found to be lower in the kefir groups, which may be suggestive of decreased stress or decreased carbohydrate absorption in the intestines [46].

Overall, kefir may show some promise as a probiotic for *S. coruhensis*, especially considering the relative ease by which it may be acquired and its antioxidant and immunomodulatory properties. However, its inability to affect host growth or survival rates (unlike other previously discussed probiotics) may warrant careful consideration when choosing it as a probiotic for *Salmo* spp..

#### *2.5.2 Other multi-species probiotic formulations*

Other probiotic formulations featuring multiple species of microorganisms have also been investigated. One notable example of such a formulation is Bio-aqua ®, an aquacultural probiotic that is commercially available in Iran. In addition to the species enumerated in **Table 2**, Bio-aqua® further contains yeast extract and fructooligosaccharides as prebiotics. In the sole English language publication investigating this formulation, it was found that Bio-aqua did not produce any significant effects on the growth performance, activity of digestive enzymes, or intestinal histomorphology in juvenile *S. trutta caspius* when administered at a dose of 0.2 grams per kilogram at feeding times [50].

In another study, four experimental groups of juvenile *S. salar* reared in a RAS were administered combinations of *Rhodotorula mucilaginosa* CGMCC 1013 and *Bacillus velezensis* V4 CGMCC 10149 over a 62-day period. Relative to the control group, the experimental group fish exhibited significantly decreased feed conversion ratios and mortality, as well as increased weight gain ratios and specific growth rates. Furthermore, immunological and antioxidant parameters were suggestive of an enhanced immune response and antioxidant activity in the experimental groups in comparison with the control group. Curiously, however, cortisol levels were found to be elevated in the experimental groups relative to the control. Finally, in a challenge trial conducted with *Aeromonas salmonicida*, the experimental group fish exhibited far lower mortality rates than the control group, perhaps due to a combination of the enhanced immune response and the direct inhibition of the pathogen by the probiotic bacteria in the gut [51].

Overall, in spite of the lack of observed benefits in the study featuring Bio-aqua ®, the promising findings of the second study featuring *R. mucilaginosa* and

| *Salmo* **species** | **Probiotic formulation** | **Probiotic microbes** | **Citation** |
|---|---|---|---|
| *S. coruhensis* | Kefir | *Lactobacillus, Lactococcus, Leuconostoc*, other lactic or acetic acid bacteria, and yeasts** | [44–46] |
| *S. salar, S. trutta,* & *S. trutta caspius* | Fermented soymeal*** | Variable, but may include lactic acid bacteria, acetic acid bacteria, and/or yeasts** | [21, 47–49] |
| *S. trutta caspius* | Bio-aqua ® * | *Pediococcus acidilactici, Enterococcus faecium, Bacillus subtilis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus rhamnosus,Bifidobacterium bifidum, Saccharomyces cerevisiae*** | [50] |
| *S. salar* | N/A | *Bacillus velezensis, Rhodotorula mucilaginosa* | [51] |

*One asterisk signifies a commercial probiotic formulation, whereas two asterisks signify a non-bacterial microorganism. Three asterisks denote a hypothetical probiotic source.*

**Table 2.**
*An overview of probiotic formulations featuring multiple bacterial species that have been investigated for use in Salmo.*

*B. velezensis* suggest that the utilization of multiple species of bacteria in probiotics has the potential to be highly productive.

### *2.5.3 Fermented soybean meal*

There has also been considerable research on the use of fermented plant meals (such as soybean meal) in the aquaculture of *Salmo,* including *S. salar, S. trutta,* and *S. trutta caspius* [47–49]. However, it is important to note that this usage of plant meal fermentation is often implemented to enhance the bioavailability of nutrients and energy in plant products that are not natural components of the salmonid diet [52]. Regardless, it is possible that some fermented plant meal products may exhibit probiotic or prebiotic effects in *Salmo.* In one study, the provision of fermented soybean meal to *S. salar* was found to be associated with an increase in the abundance of intestinal LAB (including *Lactobacillus, Pediococcus,* and *Lactococcus*) when compared with a group that was fed a fishmeal-based diet and another that received non-fermented soybean meal [21]. While it is unclear from the results of the study whether this increase in LAB abundance was due to prebiotic or probiotic effects, it is well-established that certain fermented foods (such as miso, which is also soybean-based) are associated with probiotic LAB populations [53, 54]. Therefore, it is plausible that at least some fermented soybean feeds for *Salmo* may exhibit probiotic effects. However, further research may be needed in order to investigate this hypothesis.

## 3. Conclusions

In general, the use of probiotics in the aquaculture of species within the genus *Salmo* has the potential to be highly productive, with the ability to promote fish immunity, nutrition, and growth as well as to decrease the likelihood of mortality. These beneficial effects are more important now than ever, with the increasing popularity of intensive aquaculture systems like RAS. In such crowded conditions, fish are constantly exposed to pathogens, underscoring the need for enhanced immunity. Furthermore, the enhanced growth and decreased mortality from certain diseases associated with the use of some probiotics may help the operators of salmon aquaculture systems to increase their profits and minimize the unnecessary loss of fish. The current market availability of such tested probiotic formulations as BetaPlus ® and Bactocell ® may also be instrumental in helping salmon aquaculturalists to achieve these outcomes.

In spite of the benefits that can be understood from the current corpus of literature pertaining to the use of probiotics in *Salmo*, many questions remain unanswered. Some of these questions are quite simple, and could consider such things as which probiotic species and strains (or perhaps combinations thereof) are the best at achieving particular outcomes in particular species of *Salmo* (e.g., which species and strains result in optimal growth rates in *S. salar*). The answers to such questions would likely necessitate the conduction of relatively large studies featuring multiple experimental groups that each receive different probiotics. Other questions concern the biochemical activity of probiotic bacteria within the host, including such things as how or why certain probiotics influence the host's immune system (e.g., how does BetaPlus® cause elevated white blood cell levels in *S. salar* or, why does the administration of certain probiotics result in elevated immunoglobulin levels). It further remains unclear as to why some probiotic species and strains differ from others in certain effects on the host (e.g., why were host cortisol levels decreased relative to the control when *Lactobacillus plantarum* was used in *S. trutta caspius,* but elevated when *Bacillus velezensis* and *Rhodotorula mucilaginosa* were used in *S. salar*). The elucidation of answers to these (and related) questions may help us better understand the relationship between the salmon gut microbiota and fish health, as well as potentially inform future efforts to optimize the salmon gut microbiome with biotechnology.

Overall, while much remains to be explored in the use of probiotics in *Salmo*, recent findings have strongly indicated that they are associated with remarkable potential benefits that should warrant any *Salmo* aquaculturalist to consider their use.

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