The effect of Biolex® MB40 on aquaculture-relevant gut microbial communities:
in vitro experiment on the prebiotic effect and *in vivo* experiment in rainbow trout *(Oncorhynchus mykiss)*

after D. Merrifield et al., Plymouth University, UK, 2014 & 2015

Brewers' yeast cell walls, rich in β-glucans and mannan oligosaccharides, have been successfully used in practical animal nutrition for many years. Being supplemented to the diet they are considered beneficial for gut health by establishing a physiological microbial environment in the gut. This is mainly induced by a direct prebiotic effect on beneficial bacteria and direct (agglutination) or indirect suppression of a range of pathogenic bacteria. Gut health is of utmost importance as the gastrointestinal tract has been suggested as one of the major routes of infection in fish (Ringo *et al.*, 2016). The two experiments described hereafter assessed the prebiotic effect of Biolex® MB40 (a brewers' yeast cell wall product) *in vitro*, and subsequently its *in vivo* effect on gut microbial communities in rainbow trout (*Oncorhynchus mykiss*).

Experiment 1: utilization of Biolex® MB40 by probiotic bacteria *in vitro*

Experimental design:

Cultures of *Bacillus subtilis, Lactobacillus plantarum, Pediococcus acidilactici* and *Leuconostoc lactis* were added to glucose-free culture media (Dulbecco's modified eagle medium, DMEM). In addition, glucose or Leiber Biolex® MB40 was added at 5% w/v as the sole carbon source. The flasks ($n = 3$) were then incubated over 24 hours at 37° C and bacterial growth was recorded at regular intervals.

Results and Discussion:

B. subtilis, L. plantarum, P. acidilactici and *Leuc. lactis* grew well in DMEM + glucose (data not shown) acting as the positive control. With Biolex® MB40 acting as the carbon source also excellent growth of all four species was observed (Fig. 1). This highlights the prebiotic properties of Biolex® MB40 and the ability of these strains to utilize it as a nutrient source. As members of these potentially probiotic species are often indigenous to the fish gut (except *P. acidilactici*), this indicates that dietary supplementation of Biolex® MB40 may support the growth of beneficial microbes in the fish intestine.

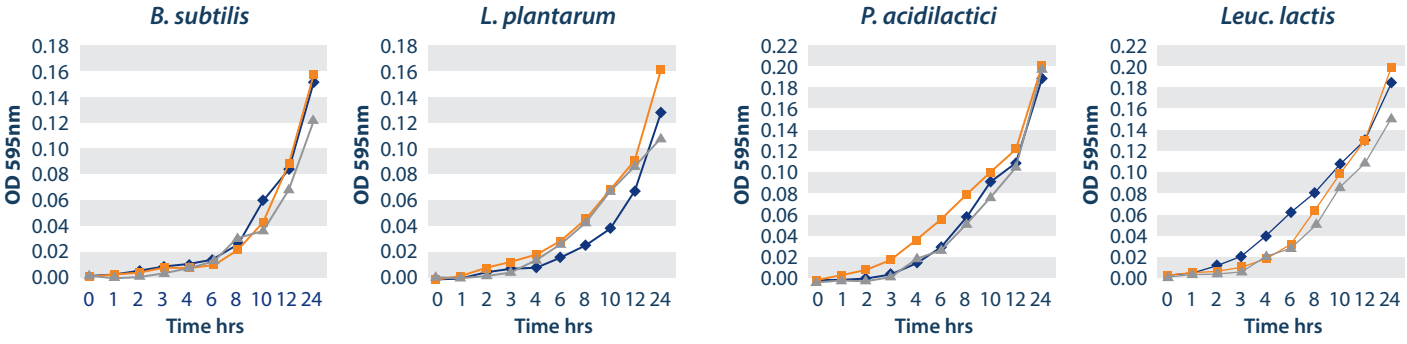

Figure 1: *B. subtilis, L. plantarum, P. acidilactici* and *Leuc. lactis* growth in DMEM minimal media with Leiber Biolex® MB40 as the carbon source. Each assay was conducted in triplicate.

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Experiment 2: the effect of dietary Biolex® MB40 on the rainbow trout gut microbiome

Experimental design:

Rainbow trout with an average weight of 128g were allocated to triplicate tanks in a closed recirculatory system (20 fish/tank). Two isonitrogenous (43% protein) and isolipidic (20%) diets were formulated to meet the known requirements of rainbow trout (NRC, 2011), one of them containing 0.2% Leiber Biolex® MB40. The fish were fed at a rate of 2% of biomass per day over 28 days. At the end of the experiment mucosa and digesta samples were aseptically obtained from the gut ($n = 6$). Subsequently, sample processing, next-generation sequencing and bioinformatics were conducted according to standard procedures and previously published scientific methodology.

Results and Discussion:

Bioinformatic analysis revealed that a total of 200,386 reads were retained. Good's coverage rarefaction curves for all individual samples reached a plateau close to 1 (i.e. 0.989-0.991), indicating that the microbiomes were fully sampled. The sequence distribution at the genus level is presented in Figure 2, and Table 1 illustrates the most important changes in digesta and mucosal samples.

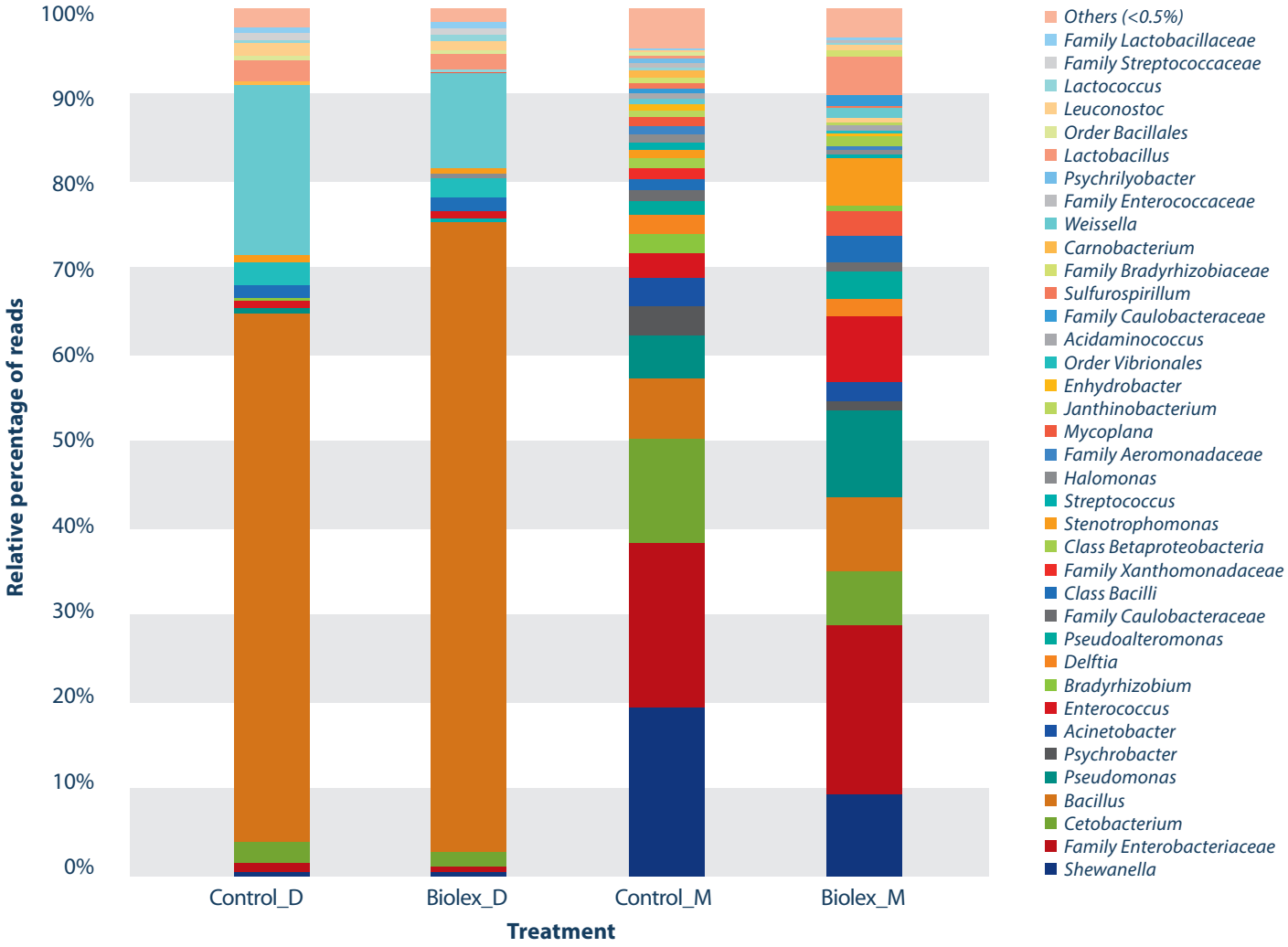

Figure 2: The relative proportion (%) of reads from the intestinal digesta (D) and mucosa (M) by treatment, assigned at the genus level >0.5%.

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Table 1: Abundance (%) of important OTUs in digesta and mucosa samples

Taxon	Control	Biolex® MB40	P-value	
Digesta				
Staphylococcus	0.4 ± 0.0[a]	0.2 ± 0.1[b]	< 0.01	*Pathogenic potential*
Bacillus	61.2 ± 14.2	72.9 ± 9.9		Probiotic potential (see Exp.1)
Mucosa				
Shewanella	19.6 ± 4.3[a]	9.6 ± 7.7[b]	< 0.02	*Pathogenic potential*
Psychrobacter	3.5 ± 1.7[a]	1.1 ± 0.8[b]	< 0.02	*Pathogenic potential*
Weissella	0.5 ± 0.4[a]	1.2 ± 0.6[b]	< 0.05	Probiotic potential
Bacillus	6.9 ± 2.9	8.5 ± 5.2		Probiotic potential (see Exp. 1)
Lactobacillus	0.4 ± 0.6	4.4 ± 6.9		Probiotic potential (see Exp. 1)
Leuconostoc	0.1 ± 0.1	0.6 ± 0.7		Probiotic potential (see Exp. 1)

[a, b] = *Different superscript letters indicate a significant difference between treatment groups*

Indeed, Biolex® MB40 led to increased presence of the genus *Bacillus* (in both digesta and mucosa samples), and the lactic acid bacteria (LAB) *Lactobacillus* and *Leuconostoc* (mucosa). Members of these genera have proven their probiotic properties in numerous studies, e.g. *B. subtilis* in rainbow trout (Brunt *et al.*, 2007) and Olive flounder (Cha *et al.*, 2013), *L. plantarum* in tilapia (Ren *et al.*, 2013) and rainbow trout (Perez-Sanchez *et al.*, 2011), and *Leuc. lactis* in black porgy (Zhang *et al.*, 2013). Furthermore, the LAB *Weissella* was significantly increased in the mucosa of fish fed Leiber Biolex® MB40. *Weissella* spp. from aquatic origin have been reported to exhibit antimicrobial activity against fish pathogens (Muñoz-Atienza *et al.*, 2013), and Mouriño *et al.* (2012) revealed the probiotic effects of *Weissella cibaria* in hybrid surubins (*Pseudoplatystoma* sp.).

In addition, also a reduction of potentially pathogenic bacteria has been observed. Fish fed the Biolex® MB40 treatment exhibited a significant reduction of *Staphylococcus* in the digesta. This genus contains a number of fish (e.g. *S. aureus*, Kusuda & Sugiyama, 1981; *S. epidermis*, Shah & Tyagi, 1986) and human pathogens (e.g. *S. aureus*). In mucosal samples, Biolex® MB40 caused significantly lower levels of *Psychrobacter* and *Shewanella*. Some species from these genera have been reported to be potentially pathogenic to fish. For example, a study by Hisar *et al.* (2002) reported *Psychrobacter immobilis* to be an opportunistic pathogen, causing infection in rainbow trout. *Shewanella putrefaciens* and *Shewanella baltica* have been associated with spoilage of fish (Vogel *et al.*, 2005; Beaz-Hidalgo *et al.*, 2015). Furthermore, *S. putrefaciens* is also reported to be a potential fish pathogen causing mortalities in marine (rabbitfish, Saeed *et al.*, 1987) and freshwater fish (common carp and rainbow trout, Kozińska & Pękala, 2004).

Conclusions Biolex ® MB40:

- Prebiotic effect on potentially probiotic bacteria (*in vitro* exp. 1)
- Increased presence of potentially probiotic *Bacillus* and LAB's (*in vivo* exp. 2)
- Reduction of potentially opportunistic fish pathogens (*in vivo* exp. 2)

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