

## (3) Applications of NGS in Evaluating the Efficacy of Feed, Probiotics, and Prebiotics Formulation.

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### Introduction

In 2017, the fisheries sector in Malaysia was valued at RM 14.3 billion and has steadily doubled over the last 10 years (Aziz, 2018). To ensure continual growth in this industry sector, it is necessary for aquaculture producers to ensure a robust, effective, and swift biosecurity system is put in place. In addition to disease screening and prevention, improving fish health through feed additives has also contributed to the steady industry growth.

Feed additives such as probiotics are marketed greatly upon its ability to improve fish health. For example, Carnobacterium has been used as a probiotic as it exhibits an antagonistic effect against pathogenic bacteria such as *Aeromonas* spp. and *Vibrio* spp. by outcompeting these pathogens for space in the fish gut, while also enriching beneficial bacteria (Hoseinifar SH, 2018).

In this whitepaper, we highlight the findings from our latest Next-Generation Sequencing (NGS) run comparing the gut microbiome of fish that was administered with feed additive (referred to as “treated” from here onwards), and fish that was not (referred to as “untreated” from here onwards).

We targeted the bacterial gene that codes for 16S ribosomal RNA (16S rRNA), a section that contains up to 9 variable regions (V1 to V9). The V3 region was chosen for this purpose as it is conserved

among many bacteria, but which contains sufficient variability to enable discrimination of closely related Genus.

### Findings

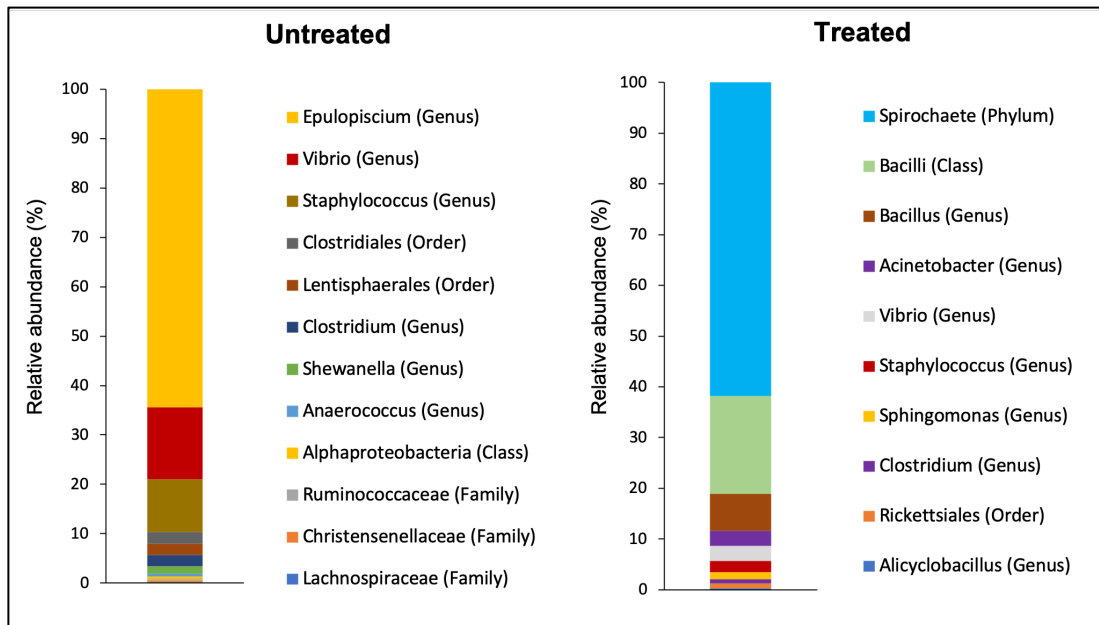
The gut of untreated fish was populated by bacteria in the Genus *Epulopiscium* (44%) and *Vibrio* (34%). When the fish was fed with an over-the-counter feed additive, bacteria in the Genus *Epulopiscium* was no longer detected, and the amount of *Vibrio* dropped to 3%. Conversely, *Spirochaetes* (62%) were enriched in treated fish. It has been shown that *Spirochaetes* are enriched in fish that are fed with *Ulva* spp., a type of algae (Tapia-Paniagua ST, 2019).

Bacteria in the Class Bacilli (19%) and Genus *Bacillus* (7%) were also enriched in the treated fish, which is consistent with an existing study that shows the enrichment of *Bacillus* in the gut of probiotic-fed fish (Mary A. Opiyo, 2019). In this study, the author demonstrated that there was an increase in the levels of antibodies in the probiotic-fed fish, attributing to stronger immune system in this fish compared to the control groups.

### How does this NGS data benefit the livestock industry?

#### *i) Rapid, high-throughput processing*

The strength of NGS comes from two distinct advantages: the ability to process many samples per run (up to 100 samples) and production of comprehensive, deep data from each sample.



In comparison with microbiological assays (which takes up to 3 days to run) or biochemical assays (which do not provide depth of coverage), NGS provides deep raw-data for 100 samples within 36 hours that provides users with data elucidating the composition of the whole microbiome.

*ii) Formulating the “right” type of feed*

It is important to ensure that the feed contains the “right” ingredients as the feed can regulate gut microbiota of the animal. As NGS can process a large number of samples at one time, feed manufacturers can measure the efficacy of different types of feed by elucidating the changes in the gut microbiome composition of their farmed animals.

The use of NGS in assessing the efficacy of feed and feed additives is not limited to the fisheries sector, but also extends to other livestock such as cattle and poultry. Ultimately, livestock farmers will have the ability to make informed decisions when they are formulating their feed products.

**Conclusion**

NGS is a method to elucidate the baseline microbial population within an animal gut. Changes to this baseline population can summarily be measured and determined with NGS as well. This information provides feed manufacturers with a unique method of analyzing and determining the efficacy of their products. Furthermore, this information will indirectly benefit farmers, as they are the end-users of the products produced by feed manufacturer. This will in turn help the farmers to improve or strengthen the health management system of their livestock.

In summary, the potential of NGS can be extended from the aquaculture sector to poultry and even livestock. Aside from the determining the efficacy of the feed additives by measuring the changes in microbiome populations, NGS can also be used to elucidate the constituent items within a product. This is to ensure that feed is not only safe, from a biosecurity perspective, but also complies with the existing legal (and religious) requirements.

## References

1. Aziz A (2018) Minister: Fisheries sector value almost doubled in 10 years. In News, The Malaysian Reserve
2. Hoseinifar SH SY, Wang A, Zhou Z. (2018) Probiotics as Means of Diseases Control in Aquaculture, a Review of Current Knowledge and Future Perspectives. *Frontiers in Microbiology* 9
3. Mary A. Opiyo JJ, Charles C. Ngugi, Harrison Charo-Karisa (2019) Dietary administration of probiotics modulates non-specific immunity and gut microbiota of Nile tilapia (*Oreochromis niloticus*) cultured in low input ponds. *International Journal of Veterinary Science and Medicine*
4. Tapia-Paniagua ST FM, Anguís V, Fernández-Díaz C, Alarcón FJ, Moriñigo MA, Balebona MC. (2019) Modulation of Intestinal Microbiota in *Solea senegalensis* Fed Low Dietary Level of *Ulva ohnoi*. *Frontiers in Microbiology* 10

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