Fish feed production from pure culture PHA-rich microbial proteins obtained from agricultural residues

G. Pesante and N. Frison

Department of Biotechnology, University of Verona, Verona, 37134, Italy

Keywords: microbial proteins, fish-feed, polyhydroxyalkanoates, agricultural residues, acidogenic fermentation

Presenting author email: giovanna.pesante@univr.it

Microbial proteins (MP), also called single cell proteins (SCP), are dried monocellular microorganisms such as bacteria, yeasts, fungi or algae, which are used for food or animal feed (Litchfield, 1983), and constitute an emerging market with a growing number of industries applying for patents in the field (Ritala et al., 2017). Microorganisms are easier and cheaper to grow than crops or livestock, their cultivation is independent from seasons and climate, and their production is more environmentally sustainable because it has a lower carbon footprint (Vermeulen et al., 2012), it doesn't need fertilisers and it uses less water and arable land (Mekonnen and Hoekstra, 2014). MP are utilised in light of the presence of numerous nutritive components in the microbial cells, which are mostly proteins, but also carbohydrates, lipids, vitamins, minerals and even nutraceuticals (Ravindra, 2000). MP containing polyhydroxyalkanoates (PHAs), a biobased, biodegradable and biocompatible type of polyester produced by some microorganisms (Sabapathy et al., 2020), give an advantage when used as fish feed, due to the probiotic and prebiotic effects of PHAs, determining a boost of the fish's immune system and a consequent increase in the animals' growth and survival rates (Suguna et al., 2014).

MP can be grown using different types of wastes as feedstock (e.g. food, agricultural residues, wastewaters), making the whole production cheaper compared to the use of fit-for-purpose feedstock.(Wang et al., 2021) The use of pure cultures or mixed microbial cultures (MMC) can result in advantages or disadvantages: MMC are easier and cheaper to handle because the production doesn't require sterilisation, and are more resistance and resilient to changes (Vethathirri et al., 2021). Pure cultures however result in MP with a more predictable nutritional composition, and the presence of toxic substances or unwanted by-products can be avoided, making the final product safe for the end-users (Sharif et al., 2021).

The work presented here originates from previous research published by the authors, where agro-waste was bioconverted via acidogenic fermentation into volatile fatty acids (VFA)-rich fermentate, and was used to produce PHA-rich MP, whose nutritional value was tested via feeding trials with the fish species *Danio rerio* (zebrafish) (Pesante et al., 2022). While in the published work the MP was composed of MMC, this experimentation focused on axenic culture of *Thauera* sp. Sel9, a known PHAs accumulator, often found in MMC selected for the PHAs production (Andreolli et al., 2022). The fermentation fluid obtained from agro-waste was used in fed-batch reactors to produce bacterial biomass, which was analysed for its nutritional value, with a focus on the protein content and amino acid composition. The obtained MP was used for feeding trials with zebrafish, with the aim of assessing its suitability as as partial protein substitute in aquaculture, but also to determine the extra value given by the presence of PHAs. The experimental set-up is illustrated in Figure 1.

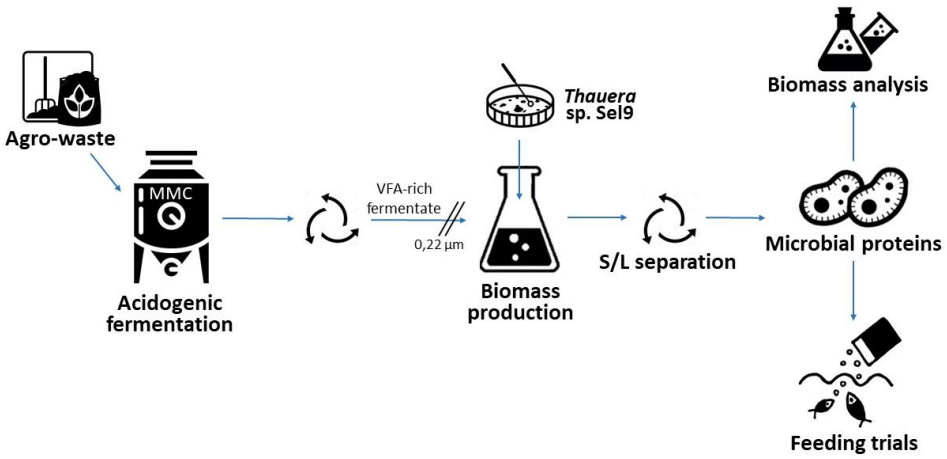

Figure 1. Experimental set-up.

The obtained biomass, analysed for its microbial community via 16S rRNA sequencing, resulted to be pure *Thauera* sp. Sel9. The protein content reached 74.0% of the total solids (TS) (dry weight) and 65.3% of volatile

solids (VS), a consistent enrichment compared to the protein found in the fermentation fluid used as nutrient for the bacterial cultures (Figure 2). The amino acid composition showed that *Thauera*'s proteins are of excellent value, including all the ten essential amino acids for fish, as well as all the18 amino acids whose presence and quantity were tested.

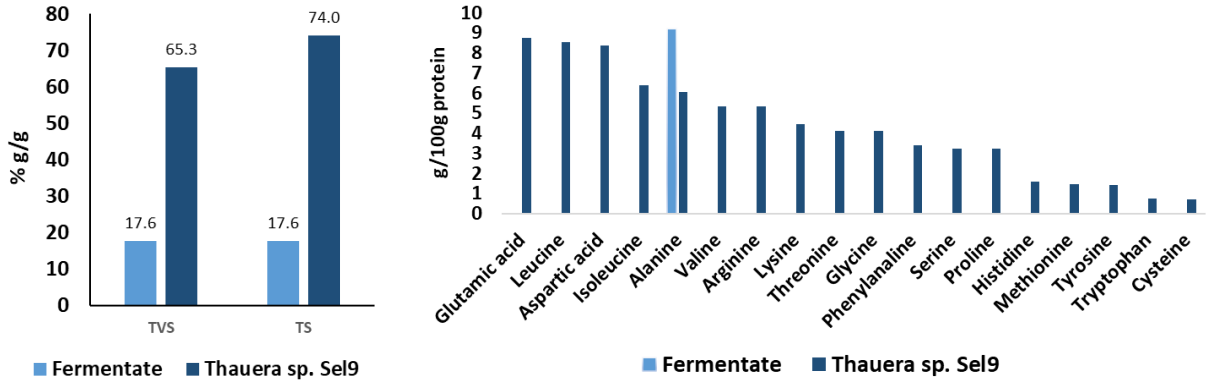

Figure 2. Protein content (left) and amino acid composition of the obtained MP.

Fish feeding trials showed that *Thauera* MP can be used efficiently in replacement of commercial feed, as the fish did not show decreased growth or survival rate when fed with the bacterial biomass. Moreover, the presence of PHAs in the feed improved fish survival and decreased phenotype alterations. This suggests that PHAs producing bacteria could be used directly as fish feed without the need for expensive extraction and purification procedures for the polymer. These preliminary tests with fish suggest that it is possible to biologically convert agricultural residues into feedstuff for pisciculture. However, further feeding trials should be performed on a larger scale and with commercial fish species.

Acknowledgments

We are grateful to Davide Slagenhaufi from the University of Verona for the GC analysis and to Andrea Vettori's group from the Department of Biotechnology of the University of Verona for performing the feeding trials.

References

Andreolli, M., Scerbacov, V., Frison, N., Zaccone, C., Lampis, S. 2022. Thauera sp. Sel9, a new bacterial strain for polyhydroxyalkanoates production from volatile fatty acids. *New Biotechnology*.

Litchfield, J.H. 1983. Single-Cell Proteins. *Science*, **219**, 740-746.

Mekonnen, M.M., Hoekstra, A.Y. 2014. Water footprint benchmarks for crop production: A first global assessment. *Ecological indicators*, **46**, 214-223.

Pesante, G., Zuliani, A., Cannone, E., Greco, F., Tesoriero, C., Vettori, A., Frison, N. 2022. Biological conversion of agricultural residues into microbial proteins for aquaculture using PHA-producing mixed microbial cultures. *Journal of Cleaner Production*, 134554.

Ravindra, A.P. 2000. Value-added food Single cell protein. A review. *Biotechnology Advances*, **18**, 459-479.

Ritala, A., Häkkinen, S.T., Toivari, M., Wiebe, M.G. 2017. Single cell protein—state-of-the-art, industrial landscape and patents 2001–2016. *Frontiers in microbiology*, **8**, 2009.

Sabapathy, P.C., Devaraj, S., Meixner, K., Anburajan, P., Kathirvel, P., Ravikumar, Y., Zabed, H.M., Qi, X. 2020. Recent developments in Polyhydroxyalkanoates (PHAs) production–A review. *Bioresource technology*, **306**, 123132.

Sharif, M., Zafar, M.H., Aqib, A.I., Saeed, M., Farag, M.R., Alagawany, M. 2021. Single cell protein: Sources, mechanism of production, nutritional value and its uses in aquaculture nutrition. *Aquaculture*, **531**.

Suguna, P., Binuramesh, C., Abirami, P., Saranya, V., Poornima, K., Rajeswari, V., Shenbagarathai, R. 2014. Immunostimulation by poly-beta hydroxybutyrate-hydroxyvalerate (PHB-HV) from Bacillus thuringiensis in Oreochromis mossambicus. *Fish and Shellfish Immunology*, **36**(1), 90-7.

Vermeulen, S.J., Campbell, B.M., Ingram, J.S. 2012. Climate change and food systems. *Annual review of environment and resources*, **37**, 195-222.

Vethathirri, R.S., Santillan, E., Wuertz, S. 2021. Microbial community-based protein production from wastewater for animal feed applications. *Bioresource Technology*, **341**, 125723.

Wang, J., Liu, S., Huang, J., Qu, Z. 2021. A review on polyhydroxyalkanoate production from agricultural waste Biomass: Development, Advances, circular Approach, and challenges. *Bioresource Technology*, **342**, 126008.