PRODUCTION OF CAROTENOIDS FROM AQUACULTURE SIDESTREAMS AS FEEDSTOCK USING THE BACTERIUM *Corynebacterium glutamicum*.

Ina Schmitt[1], Florian Meyer[1], Nadja A. Henke[1], Sinem Zeytin[2], Hilke Alberts-Hubatsch[2], Katerina Kousoulaki[3], Volker F. Wendisch[1], Petra Peters-Wendisch[1]*

[1] Genetics of Prokaryotes, Faculty of Biology and Center for Biotechnology, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany, Email: petra.peters-wendisch@uni-bielefeld.de
[2] Alfred Wegener Institute Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany,
[3] Department of Nutrition and Feed Technology, Nofima, Norway

Introduction

Carotenoids are valuable secondary metabolites with important physiological functions as for instance being precursors in vitamin biosynthesis and high antioxidant potential. Especially astaxanthin is a high value fish feed supplement, already used in salmonid farming, mainly in chemical but also in natural form. Aquaculture is one of the fastest-growing food production systems, enabling to meet the rising consumer demand for healthy seafood, thus the expansion of the demand for natural astaxanthin alternatives is expected. To establish a flexible feedstock concept using aquaculture sidestreams (Wendisch *et al*. 2022; Fig. 1), the GRAS bacterium *Corynebacterium glutamicum* that naturally synthesizes the yellow C50 carotenoid decaprenoxanthin was metabolically engineered to produce a variety of different carotenoids including astaxanthin (Cankar et al. 2023; Henke et al. 2022). Preprocessed aquaculture sidestream (AQ) from a Norwegian aquaculture facility was tested for growth and production of these carotenoids. In a step towards a circular economy in aquaculture, the applicability of astaxanthin produced by *C. glutamicum* was demonstrated in a first trout feeding trial (Zeytin et al. 2022).

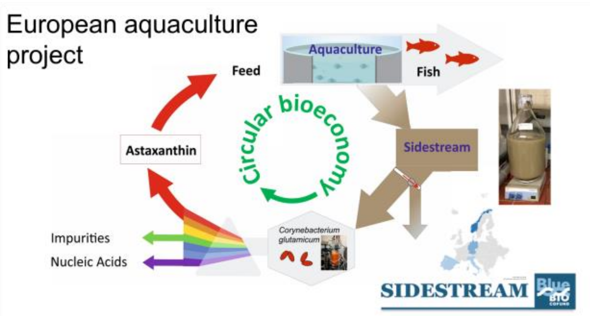

Figure 1. Example of a circular economy in aquaculture using Astaxanthin producing bacterial biomass which is cultivated on preprocessed aquaculture sidestream (AQ).

Material and Methods

The construction of metabolically engineered *C. glutamicum* was described elsewhere (Cankar et al. 2023; Henke et al. 2022). The aquaculture sidestream was collected from the sump of a post-smolt RAS for salmon operated by Lumarine AS (Tjeldbergodden, Norway) outside of Trondheim (Norway). Preprocessing of the liquid aquaculture sidestream in order to use it as a growth medium component was implemented by centrifugation and subsequent sterile filtration of the supernatant. The resulting AQ was supplemented in different amounts to a defined minimal salt medium and used in growth experiments performed with the different *C. glutamicum* strains at 30°C and 180 rpm in shake flasks. Fermentation of the astaxanthin producing *C. glutamicum* strain was performed in either 2 L glass or 19 L steel bioreactors using the same medium.
Preparation of astaxanthin containing biomass for the trout feed trial was performed as shown in Fig. 2.

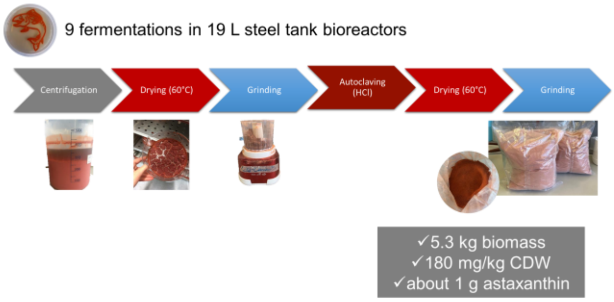

Figure 2. Example of a circular economy in aquaculture using Astaxanthin producing bacterial biomass which is cultivated on preprocessed aquaculture sidestream (AQ).

Results and Discussion

Astaxanthin production by an engineered *C. glutamicum* producer strain was notably enhanced by addition of 20 % AQ in a mineral salts medium (Schmitt *et al.* 2023). The use of AQ as medium supplement was shown to be transferable to production of ß-carotene, lycopene as well as zeaxanthin, canthaxanthin, sarcinaxanthin, BABR and C.P.4504. Astaxanthin production was scaled up to the 2 L bioreactors. Fermentation of the astaxanthin producing *C. glutamicum* strain yielded 5.3 g biomass, containing 1 g natural glycosylated astaxanthin. This biomass was formulated into fish feed and this feed was compared in a trout feed trial to commercially available synthetic and natural astaxanthin sources resulting in a very promising pigmentation although lower growth of the trouts.

Here we could show for the first time a fully circular bioeconomy concept in aquaculture.

References

Wendisch VF, Nampoothiri KM, Lee J-H (2022) *Frontiers in Microbiology* 13: 835131

Cankar K, Henke NA, Wendisch VF (2023) *Systems Microbiology and Biomanufacturing* 3: 110-121

Henke NA, Göttl V, Schmitt I, Peters-Wendisch P, Wendisch VF (2022) *Methods in Enzymology*, 671, 383-419.

Schmitt I, Meyer F, Krahn I, Henke NA, Peters-Wendisch P, Wendisch VF (2023) *Molecules* 28(4):1996.

Zeytin S, Alberts-Hubatsch H, Kousoulaki K, Schmitt I, Meyer F, Peters-Wendisch P, Henke NA, Wendisch VF, Slater MJ (2022) Aquaculture Europe Conference Abstract Book.

This research was supported by the ERA-NET BlueBio COFUND Project SIDESTREAM [Gran ID 68].