AQUA FIELD
TRAKIA UNIVERSITY
NATIONAL DIAGNOSTIC SCIENTIFIC RESEARCH VETERINARY INSTITUTE
"PROF. Dr. G. PAVLOV"
UNIVERSITY OF FORESTRY
THE STEPHAN ANGELOFF INSTITUTE OF MICROBIOLOGY-BAS

Combination of probiotic bacteria and Antarctic yeast as a food supplement for Nile tilapia (*Oreochromis niloticus*) grown in an aquaponic system

N. Terziev[1,2], D. Ivanov[3], M. Krastanova[4], K. Genova[4], S. Rusinova-Videva[5], P. Orozova[3]

[1] Trakia University, Stara Zagora
[2] Aqua Field, Stara Zagora
[3] National Diagnostic Scientific Research Veterinary Institute "Prof. Dr. G. Pavlov“, Sofia
[4] University of Forestry, Sofia
[5] The Stefan Angeloff Institute of Microbiology-BAS, Plovdiv

Aquaponics, the combination of hydroponics and aquaculture in one growing system, is a controlled environment production system that potentially has increased environmental and consumer benefits compared to traditional production methods. There are various ways to configure aquaponic systems, including variations in fish species, water circulation, lighting, plant species/density, etc. Two varieties of *Lactuca sativa*, a commonly cultivated crop in aquaponics, were grown in a system with tilapia (*Oreochromis* spp.) as the fish species. No additional nutrients were added to the system to evaluate the feasibility of growing lettuce with the residual nutrients from tilapia.

The world is facing a number of serious problems of which population rise, climate change, soil degradation, water scarcity and food security are among the most important. Aquaponics, as a closed-loop system consisting of hydroponics and aquaculture elements, could contribute to addressing these problems (Goddek et al., 2015). Aquaponics incorporates hydroponics (soilless plant production) and aquaculture (fish production) into a closed-loop, recirculating system (Rakocy et al., 2006). Waste from the fish production provides the primary nutrients for the crop plants, the root systems of which provide a natural filter for the water recirculated to the fish (Rakocy et al., 2006). Thus, fish food becomes the only nutritional input (nutrients) into any aquaponic system. Nutrient cycling consists of fish waste (ammonia, urea) converted by nitrifying bacteria in a biofilter into a form of N (nitrate N) that can be taken up by plant roots (Diver and Rinehart, 2006).

Although Antarctica is a geographical region that represents the largest polar territory, it is the least explored one. As one of the most extreme environments, the Antarctic desert suggests the presence of some key survival mechanisms in the microorganisms that inhabit it. The presence of specific metabolism in the insufficiently studied Antarctic yeasts suggests the existence of new unexplored biomolecules.

The newly isolated species *Dioszegia* sp. AL_{105} belong taxonomically to the Kingdom Fungi, Division Basidiomycota, class Tremellomycetes, family Bulleribasidiaceae, genus Dioszegia. The Antarctic species was isolated from penguin feathers. During submerged cultivation of the strain, a biomass amount of 4 g/L was accumulated.

penguin feathers

Dioszegia sp. AL105

Submurged cultivation

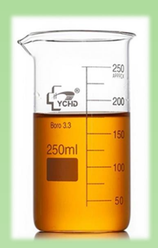
Bacillus subtilis AB1

Bacillus subtilis AB1 has been shown to be an effective probiotic in controlling infections caused by fish-pathogenic Aeromonas sp. in rainbow trout. Oral administration is possible to control diseases in fish (Newaj-Fyzul et al., 2007).

The experiment was conducted in 2024 in the Production System of Aquaponic Center "AQUA FIELD" Ltd. and Trakia University - Stara Zagora in closed recirculating systems for aquaculture and plant crop production. The aim of this study is to evaluate the effect of the probiotic strain AB1 (*Bacillus subtilis*), combined with yeast (*Dioszegia* sp. AL_{105}), isolated from penguin feathers during the 31st Bulgarian Antarctic Expedition, on the vitality of Nile tilapia, cultivated together with black pepper (*Capsicum annuum*) and sweet basil (*Ocimum basilicum*) in an aquaponic system. The Antarctic yeast *Dioszegia* sp. AL_{105} was isolated from penguin feathers from Livingston Island (Antarctica). The yeast is orange in color. A culture cultivated in depth in flasks was used to prepare a nutritional supplement. The well-developed culture of *Dioszegia* sp. was separated from the culture liquid and then washed twice with water. The culture thus obtained was stored at 4ºC. Two experimental units were used under identical conditions: a test group and a control group. Each unit consisted of an aquaponic module with a deep-water culture system (RAFT) including two containers: one for growing vegetables on floating polystyrene boards (n=6) measuring 60 x 120 cm each and another for growing fish with a volume of 378.5 L. Each aquarium contained 30 tilapia individuals (average weight 148 g). For 60 days, the control group received a limited standard feed, while the experimental group was fed the same amount of feed enriched with a 10:1 ratio of probiotics and Antarctic yeast. The phagocytic activity of leukocytes was studied. The results indicated that the experimental group exhibited increased appetite, faster growth and reduced presence of opportunistic pathogenic bacteria in the gastrointestinal tract. In addition, these fish reached sexual maturity significantly earlier compared to the control group. Small fish were observed exclusively in the experimental unit.

Bacillus subtilis AB1

Dioszegia sp. AL_{105}



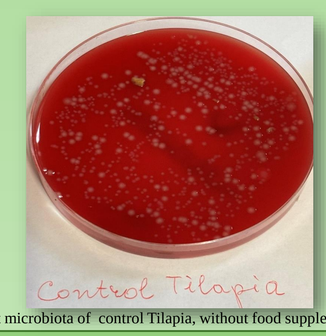
Food supplement

Nile tilapia

Small fish

Gut microbiota of Tilapia, with food supplement

Nile tilapia



Gut microbiota of control Tilapia, without food supplement

In conclusion, in aquaponics, the combination of probiotic bacteria and Antarctic yeast *Dioszegia* sp. AL_{105} applied as food supplement was effective for Nile tilapia production. Future studies will determine the impact of the combination of Antarctic yeast and the probiotic strain AB1 on the immune parameters in Nile tilapia in aquaponics.

ACKNOWLEDGEMENTS

We thank the staff of the Bulgarian Antarctic Expedition for their logistic support. We thank Prof. Brian Austin for kindly providing strain *Bacillus subtilis* AB1. This research was funded by the Bulgarian Ministry of Education and Science through the National Centre for Polar Studies, and Sofia University “St. Kliment Ohridski” in the frames of the National Program for Polar Studies 2022-2025 (grant numbers 70-25-40/23.01.2023).