

**Extremophilic filamentous fungi – source of biomolecules  
with potential industrial applications**

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## Abstract

Extremophilic filamentous fungi represent an important source of biomolecules with potential industrial applications. Due to adaptive mechanisms allowing them to thrive in harsh environmental conditions, they are able to produce molecules with improved stability, bioactivity, and novel properties. Despite their advantages, biomolecules from extremophiles are still mostly unexplored, especially when it comes to extremophilic fungi.

The aim of this doctoral dissertation was to characterize filamentous fungi obtained from extreme environments and assess the industrial applicability of their biomolecules, including hydrolytic enzymes, pigments, and biosurfactants, with particular emphasis on the food industry.

This study tested 21 strains of halophilic and halotolerant filamentous fungi isolated from saline soils in Poland and Italy, as well as a psychrophilic fungus *Aureobasidium pullulans* P8 isolated from Antarctic soil. Halophiles were tested for salt tolerance, production of extracellular hydrolytic enzymes (enzymatic index evaluation), the ability to produce biosurfactants (screening for hemolytic activity followed by assessment of emulsifying abilities for selected strain), and their biosafety by screening for selected mycotoxins (HPLC-based method). Following observations made during halotolerance assays, the halophilic *Aspergillus tubingensis* FF14 was selected for pigment production and detailed identification (UV-Vis and UHPLC-DAD-ESI-MS/MS), assessment of antioxidant properties (DPPH and FRAP assays), and optimization of production conditions using a statistical approach (Taguchi method). The psychrophilic *A. pullulans* P8 was investigated for biosurfactant production at low temperatures, which included determination of emulsifying properties of crude extracts (emulsifying activity, emulsion index, and oil dispersion abilities) and chemical characterization (FTIR). Whole genome sequencing was conducted for selected strains; genomes were assembled, deposited, and analyzed to identify gene and biosynthesis pathways involved in the production of identified compounds.

The obtained results provide a comprehensive characterization of extremophilic filamentous fungi and their capacity to produce bioactive biomolecules under environmental stress conditions. Screening of halophilic and halotolerant fungi demonstrated that all strains exhibited tolerance to elevated salinity. All of the strains tolerated up to 10% NaCl with seven of them were able to grow even at 17.5% NaCl. The most common optimal growth concentration was 5% NaCl, classifying those strains as moderately halophilic. Additionally, all of the tested halophilic strains exhibited the ability to produce at least one extracellular hydrolytic enzyme under saline conditions. Among them, *Ramularia mali* FF1 was recognized as the most efficient producer of multiple enzymes, exhibiting the highest enzymatic index of 10.67 for pectinolytic activity. Biosurfactant screening revealed hemolytic activity for 14 strains at 5% NaCl, with just 4 showing positive results at 10% NaCl. *Acremonium sclerotigenum* FF3 demonstrated the highest action at higher salinities, and further analyses confirmed its emulsifying properties. Mycotoxin screening has shown that the production

of the analyzed mycotoxins was limited, with ochratoxin A detected only for *Penicillium canescens* S10.

Based on observations from the screening, pigment production was further investigated in the selected halophilic strain, *A. tubingensis* FF14, which demonstrated intracellular pigmentation under saline conditions. The isolated intracellular pigments were further identified as neoxanthin, violaxanthin and antheraxanthin. The antioxidant activity of the pigment mixture was confirmed, though it was found to be lower than that of the  $\beta$ -carotene standard. Production conditions were successfully optimized using a statistical approach, leading to increased pigment yield. Genome annotation and comparative analysis showed the presence of biosynthesis gene clusters related to carotenoid biosynthesis, supporting pigment identification. However, no direct xanthophylls-related genes and biosynthetic pathways were identified highlighting the need for further transcriptome analyses.

Alongside the halophilic fungi, a psychrophilic fungus was also investigated as another group of extremophiles capable of producing surface-active compounds. The psychrophilic *A. pullulans* P8 demonstrated the ability to produce cold-active biosurfactants. The production and emulsifying properties of crude biosurfactants were confirmed at low temperatures, showing an emulsion index for rapeseed oil up to 34.11% at 10°C. Moreover, improved oil-displacement and drop-collapse activity were also observed for crude biosurfactants obtained from cultures cultivated at lower temperatures. The characterization of the produced biosurfactants revealed their glycolipid nature and structural similarity to liamocins and polyol lipids. In addition, genome analysis revealed genes and biosynthesis gene clusters associated with biosynthesis of liamocins, which supported the experimental findings.

In conclusion, this doctoral dissertation demonstrates that extremophilic filamentous fungi represent a highly diverse and promising source of industrially relevant biomolecules. This research expand current knowledge of the metabolic potential, adaptability, and biotechnological importance of these microorganisms. Moreover, this research provides a strong scientific foundation for the future exploration and application of extremophilic filamentous fungi in innovative industrial and biotechnological processes.

**Keywords:** extremophiles, filamentous fungi, biomolecules, enzymes