Abstract

Poland is among the leading fruit producers in the European Union, playing a particularly important role in the cultivation of strawberries and other soft fruits. Due to their excellent taste, rich nutritional value, and wide range of applications in food processing, these fruits constitute a key element of the national agri-food sector. At the same time, their significant susceptibility to microbiological contamination poses a major challenge and a threat to both producers and consumers. Of particular concern in this context are fungi of the genus *Neosartorya*, characterised by exceptional resistance to unfavourable environmental conditions, including high temperatures and the presence of preservatives. Species of this genus which are the teleomorphic forms of *Aspergillus* fungi, are capable of surviving pasteurisation processes, potentially leading to secondary contamination of food products, resulting in both economic losses and a threat to consumer health due to the production of hazardous mycotoxins.

The research presented in this dissertation addresses the characterisation of the morphological, metabolic, and genetic properties of *Neosartorya* spp. fungi, which influence their survival mechanisms, activity, and susceptibility to antimicrobial substances. An important aspect of the study was also the evaluation of the effects of these substances on the microbiome and metabolic potential of strawberries artificially contaminated with these fungi under real storage conditions for plant products. Initially, the effectiveness of both natural plant extracts and synthetic preservatives was evaluated for 20 isolates using disc-diffusion inhibition tests. Then, for 10 isolates selected during the first stage of the study, sensitivity was assessed using Biolog™ MT2 microplates. The results confirmed the diverse efficacy of the tested substances and revealed differences in sensitivity, indicating the existence of five groups of isolates with increasing susceptibility to antimicrobial agents, despite their close genetic relatedness. All tested natural extracts inhibited the growth of *Neosartorya* spp., with lavender, tea tree, and marigold extracts showing the strongest antifungal effects. Sodium metabisulphite, potassium sorbate, sodium bisulphite, and sorbic acid also exhibited inhibitory effects, with sodium metabisulphite being the most effective.

To study the chemical resistance of *Neosartorya* spp., metabolic responses to 120 chemical compounds available in Biolog™ PM 21–25 microplates were analysed. It was demonstrated that the majority of substances stimulated fungal growth, and after 96 hours of incubation, all isolates developed the ability to adapt to stress conditions. The growth plateau phase was reached after approximately 144 hours, indicating effective acclimatization to the presence of toxic compounds. The greatest biomass increase was observed in the presence of antibiotics, anions, and compounds modifying cell membrane function. Sodium cyanate, copper (II) chloride dihydrate, and cisplatin showed pronounced inhibitory properties. Obtained data confirms the significant adaptive potential of *Neosartorya* spp. to various chemical stressors. This ability may be a factor enabling the colonisation of highly processed environments containing preservatives, which has important implications for microbiological control in the industry.

In subsequent stages of the study, the metabolic potential of selected *Neosartorya* spp. isolates was characterised under optimal growth conditions and after exposure to selected plant extracts and food preservatives, using phenotypic microarray tests with Biolog™ FF

microplates. The obtained metabolic profiles demonstrated high metabolic plasticity of the tested isolates and their ability to utilise a wide range of carbon sources, including compounds typical for fruit-based substrates. The fungal response to stress in the presence of marigold extract was characterised by reduced utilisation of amino acids and carbohydrates and increased use of carboxylic acids on FF plates. After exposure to food preservatives, a significant decrease in the utilisation of all tested groups of organic compounds was observed compared to the control. In the study of 10 isolates, differences in resistance were also observed, which allowed the identification of three distinct sensitivity groups, including isolates with high, medium, and low sensitivity to the tested extracts and preservatives.

Microscopic analyses, conducted using scanning electron microscopy (SEM) and confocal fluorescence microscopy, showed that both contact with a preservative and with a plant extract caused deformation of the hyphal surface. The observed changes varied depending on the isolate, indicating a significant role of phenotypic traits in determining fungal resistance to stress factors. Additionally, untargeted metabolomic analysis using LC-QTOF MS was conducted to characterise the metabolic profiles of selected fungal isolates under the influence of antimicrobial substances. The addition of marigold extract or sodium metabisulphite to the growth medium caused distinct metabolomic changes and separation of metabolite spectra depending on the growth conditions. Each isolate had a unique metabolic “fingerprint" and differed in its profile from the other samples, indicating that fungal isolates with varying sensitivity to plant extracts and preservatives had different metabolomic profiles.

Genomic analyses of selected *Neosartorya* spp. isolates were performed using whole genome sequencing (WGS). The obtained data enabled the identification of genes potentially involved in thermotolerance, detoxification of chemical compounds, and the synthesis of secondary metabolites. The presence of numerous genes encoding heat shock proteins and antioxidant enzymes suggests complex adaptive mechanisms of these fungi to environmental and chemical stress.

The final stage of the study included an experiment using strawberries artificially contaminated with selected *Neosartorya* spp. isolates. The fruits were treated with a preservative or a plant extract and subsequently analysed for changes in microbiome composition using next-generation sequencing (NGS) and metabolic activity using EcoPlates (Biolog™). Amplicon sequencing showed that the genus *Pseudomonas* was dominant among the bacterial communities, and the order Burkholderiales increased under the influence of the natural extract, whereas Microbacteriaceae decreased in the presence of the preservative. In fungal communities, both Ascomycota and Basidiomycota were well represented in control samples. The natural extract promoted the development of Basidiomycota, while the preservative maintained a higher share of Ascomycota, including *Aspergillus* and *Botrytis*, which are associated with fruit spoilage. Both antifungal treatments, involving exposure to either a plant extract or a preservative, significantly affected the metabolic profile of microorganisms colonising the fruit, although these effects varied over time and depended on the treatment applied.

The obtained results indicate considerable variation in the response of *Neosartorya* spp. isolates to antimicrobial substances and confirm their ability to survive under adverse

environmental conditions. The collected data point to the important role of metabolic, morphological, and genetic characteristics of *Neosartorya* spp. fungi in shaping their resistance to chemical preservatives and natural plant extracts. These findings may serve as a basis for developing effective strategies to reduce the presence of these fungi in the food production chain and support the development of more sustainable and selective preservation methods for soft fruits.

Keywords: heat-resistant fungi, preservatives, plant extracts, metabolic activity, phenotypic microarrays, electron microscopy, whole genome sequencing, microbiome.