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https://doi.org/10.30970/apmicro.2026.30

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ENGINEERING MICROBIAL PRODUCERS OF ETHANOL AND LACTIC ACID AS ALTERNATIVE FERMENTATION PRODUCTS

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Abstract. Improved pentose alcoholic fermentation in *Ogataea polymorpha* was achieved by positive selection and identification of the genetic determinants *IRA1* and *API1*. Heterologous expression of lactate dehydrogenase genes enabled efficient L-lactic acid production, demonstrating distinct strategies for redirecting yeast metabolism toward alternative fermentation products.
Keywords: *Ogataea polymorpha*, xylose, metabolic engineering, fermentation.

The use of renewable feedstocks for ethanol and L-lactic acid production requires microorganisms capable of efficient conversion of different carbon sources. The thermostolerant yeast *Ogataea polymorpha* utilizes a broad range of substrates, including glucose and xylose, and is capable of alcoholic fermentation at elevated temperatures.

Positive selection combined with whole-genome sequencing and reverse genetics was used to improve alcoholic fermentation of pentoses. The selected *O. polymorpha* strain A107 accumulated 20.91 g/L ethanol from xylose at 45 °C, more than 50-fold higher than the wild-type strain. Mutations affecting *IRA1* and *API1* were identified as important determinants of improved xylose and L-arabinose fermentation.

An alternative direction involved engineering *O. polymorpha* for L-lactic acid production by expression of heterologous lactate dehydrogenase genes. The most efficient recombinant strain accumulated up to 72 g/L L-lactic acid from glucose, whereas production from xylose reached 9.6 g/L.

The obtained results demonstrate that *O. polymorpha* metabolism can be redirected toward either ethanol or L-lactic acid production using complementary strain-development strategies. The identified genetic targets and constructed strains provide a basis for further improvement of both processes.

This work was supported by the National Research Foundation of Ukraine (Grant No. 2025.07/0152).

https://doi.org/10.30970/apmicro.2026.31

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SILVERFISH-ASSOCIATED YEASTS LINK ENVIRONMENTAL PERSISTENCE WITH EPITHELIAL-INTERACTION PHENOTYPES.

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Abstract. Synanthropic insects may represent overlooked reservoirs of viable yeasts in the built environment. Fungal profiling and culture-based characterization of the silverfish *Lepisma saccharinum* revealed a yeast-dominated mycobiota and yielded isolates of *Nakaseomyces glabratus* and *Candida zeylanoides*. The isolates differed markedly in stress tolerance, biofilm formation, and interactions with human keratinocytes. Importantly, environmental robustness did not consistently predict epithelial-interaction phenotypes, indicating distinct and isolate-specific combinations of traits relevant to environmental persistence and potential host interactions.
Keywords: *Lepisma saccharinum, Nakaseomyces glabratus, Candida zeylanoides*, fungal microbiota, epithelial interaction.

Synanthropic insects represent a largely overlooked microbial niche within the built environment, yet little is known about the viable fungi they harbor and their functional properties. We characterized the fungal microbiota associated with the silverfish *Lepisma saccharinum* and examined phenotypic diversity among cultivable yeasts recovered from the insects. ITS1 amplicon profiling of a pooled silverfish sample revealed a strongly yeast-dominated fungal community, with *Candida* accounting for approximately 83 % of assigned reads. Culture-dependent analysis yielded two *Candida glabrata* (currently *Nakaseomyces glabratus*) isolates, LS1 and LS6, and one *Candida zeylanoides* isolate, LS4. The isolates displayed pronounced strain-dependent differences in carbon-source utilization, thermotolerance, halotolerance and biofilm formation. LS1 and LS6 grew at temperatures up to 42 °C, showed greater tolerance to osmotic stress than LS4 and produced more biofilm than the *Saccharomyces cerevisiae* reference strain. Imaging flow cytometry further demonstrated association of all