The role of intracellular pH in environmental adaptation of yeasts
Ullah, A.

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Perspectives
The research presented in this thesis contributes to the understanding of modes of growth inhibition of weak acid preservatives and intracellular pH dynamics in different environmental conditions.

Approximately 40% of the food produced for human consumption is spoiled. Only a limited number of compounds is allowed for use as food preservative to inhibit spoilage caused by microbes, because of safety reasons. Among these few, weak organic acids (WOA) are naturally occurring food preservatives that are used in food industry to prevent or delay spoilage and to help extending the shelf life. With the passage of time, spoilage microbes are becoming resistant to preservatives. In yeast, even innate resistance is a major problem. Zygosaccharomyces bailii, for example, can grow in the presence of legally allowed preservative concentrations [1]. Still, weak acid preservatives are considered comparatively effective against yeasts. The exact antifungal mechanism of these preservatives was not clear at the start of this investigation. Understanding of antifungal modes of WOA and mechanisms yeast uses to adapt and resist are required to design new preservatives.

At the start of this study many mechanisms were known to at least partially contribute to WOA growth inhibition. We set out to dissect the contributions of these various factors, and the study allowed us to distinguish major from minor contributions in a WOA-type specific manner. Toxicity of WOA depends on their lipophilic nature, as their fast diffusional entry can not be easily restricted. We confirmed that the main inhibitory mechanism of less lipophilic acids such as acetic acid is cellular acidification. This aspect is additionally dependent on the specific monovalent cation environment of the cell (Chapters 2 and 4). Acidification does not contribute much to growth inhibition by lipophilic acids. Still, a mutant (pdr12Δ) defective in anion efflux with increase lipophilic WOA susceptibility failed to restore pHi. This suggested an interaction between the anion and proton efflux, which was confirmed for the polar WOAs.

The adaptation of yeast to WOA includes a remodeling of plasma membrane and cell wall to reduce the entry and by active extrusion of acids [1]. Thus, new antimicrobials could be designed to target membrane pumps. The proton and anions efflux pumps and their transcriptional regulators (H+-ATPase, V-ATPase, Pdr12p, Tpo2/3p, Haa1p) could be good molecular targets to inhibit the yeast growth or prolong their lag phase. Growth and pHi also were strongly related [2]:
In almost all stress conditions; we observed that growth could only be resumed if cells could restore the pH\textsubscript{i} to above 5.5. Thus, the pH\textsubscript{i} homeostasis machinery itself is a potential antifungal target [3]. In our lab, we have previously performed genome-wide analyses of pH\textsubscript{i}. This could be extended to assess acidification upon WOA exposure to identify novel genes as targets. Also, intracellular acidification could be used as a quick readout in high-throughput assessment of the growth inhibitory potential of combinations of conditions or compounds.

The success of pathogenic yeasts such as *Candida albicans* is their phenotypic plasticity. Phenotypic switching of *Candida albicans* from the unicellular yeast form to hyphal growth is considered a major strategy in adaptation to host environment as well as an essential aspect of virulence. It has been shown that pH\textsubscript{i} is associated with morphological switching of *C. albicans* [4]. Moreover in chapter 5 we showed that the two important environmental cues (ambient temperature and pH\textsubscript{ex}), which are required for phenotypic switching in *C. albicans*, affect pH\textsubscript{i} in *S. cerevisiae* and *C. glabrata*. Recent literature has also suggested the role of pH\textsubscript{i} in virulence and antifungal drug resistance. Therefore, understanding of pH\textsubscript{i} homeostasis in pathogenic yeasts could lead to the identification of new targets to reduce their fitness and virulence properties.

Stress tolerance in yeast is a key to their success in virtually all industrial processes [5]. Although the exact mechanism is not clear there is evidence that pH\textsubscript{i} contributes to stress tolerance by inducing resistance. For instance, it has been shown that if cells are exposed to different stresses (e.g. WOA, ethanol) that reduce pH\textsubscript{i}, cells acquire thermotolerance [6, 7]. Additionally, reduction of growth rate was shown to lead to increased tolerance. As pH\textsubscript{i} was also shown to control growth rate [8] manipulation of pH\textsubscript{i} could affect resistance. The identification of the underlying connections, both in signal transduction and physiology, would therefore benefit the industrial process.

Another area of research where understanding of the pH\textsubscript{i} is required is cancer. Dysregulation of pH\textsubscript{i} is hallmark in a variety of human cancers [9]. In contrast to normal cells, lactate fermentation in cancer cells reduces the extracellular pH (~6.7–7.1) [9]. Increased expression of pH\textsubscript{i} homeostatic mechanisms in the transformed cells themselves leads to a high intracellular pH (>7.4). This
dysregulation of pH_i may well be coupled to the transformation of normal cellular growth into uncontrolled cellular proliferation. Cancer cells increase their proton extrusion capacity by increasing the activity of H^+ ATPases and Na^+-H^+ exchanger to maintain high pH_i. Therefore, it has been proposed that pharmacological inhibitors of plasma membrane pumps can be used as a therapeutic tool for cancer treatment. Along similar lines, it has recently been shown that antitumor agent 3-bromopyruvate (3-BP) enter yeast cells through the Jen1p monocarboxylate/H^+ symporter of the plasma membrane in glucose repressed conditions [10]. The uptake rate of the compound through Jen1p is dependent on the pH gradient across the membrane. While the entry route of 3-BP in cancer cells is still unknown, S. cerevisiae pH_i studies contribute to these research lines.

References