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Killer Yeast as a Novel Tool in Biotechnology: Advances in Targeted Antimicrobial Strategies

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Abstract

Killer yeasts, a distinct group of yeast strains capable of secreting extracellular proteinaceous toxins lethal to susceptible microorganisms, have emerged as promising biotechnological tools for developing targeted antimicrobial strategies. These killer toxins act through specific mechanisms—such as inhibition of β-glucan synthesis, disruption of membrane integrity, or interference with vital metabolic pathways—thereby enabling the selective elimination of pathogenic fungi and certain bacteria while preserving beneficial microbiota. These toxins, often referred to as killer toxins, act through highly specific mechanisms-such as disrupting cell wall synthesis, impairing membrane integrity, or inhibiting essential metabolic pathways-allowing for the selective elimination of pathogenic fungi and certain bacteria. Engineered killer yeasts have demonstrated promising applications across multiple sectors, including food preservation, where they can prevent spoilage caused by fungal contamination; agriculture, where they can function as biological control agents against plant pathogens; and clinical settings, where they may provide alternative or adjunct therapies for antifungal-resistant infections. Additionally, killer yeast strains can be integrated into biofilm management strategies, disrupting microbial communities that are otherwise resistant to chemical disinfectants. Despite their potential, several challenges remain in translating killer yeast technology to large-scale and real-world applications. These include ensuring consistent toxin production under industrial conditions, assessing long-term ecological impacts, navigating regulatory frameworks, and addressing potential resistance development in target populations. Continued interdisciplinary research combining microbiology, biotechnology, and systems biology is essential to fully harness the potential of killer yeast in targeted antimicrobial interventions. This high specificity offers an advantage over conventional broad-spectrum antimicrobials, which often induce resistance and disrupt microbial balance. This review consolidates current progress and emerging insights, emphasizing both the opportunities and the constraints of killer yeast technology as a next-generation, precision-oriented antimicrobial platform aligned with sustainable biotechnology and the One Health approach.

Keywords: Killer Yeasts, Killer Toxins, Antifungal Resistance, Biocontrol, Biotechnology, Food Safety

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INTRODUCTION

Yeasts are ubiquitous eukaryotic microorganisms that inhabit a wide range of ecological niches, including the human body, foods, and various natural environments. Their adaptability enables them to thrive under diverse environmental conditions, contributing significantly to biological and industrial processes. In the food industry, yeasts play a pivotal role in the fermentation of numerous products such as bread, wine, beer, kefir, kumis, and cheese (Zhong et al., 2024; Chan et al., 2025; Unver et al., 2025). The increasing prevalence of antimicrobial resistance (AMR) poses a serious global threat to human health, agriculture, and food security, necessitating the development of innovative and sustainable antimicrobial strategies. Through their metabolic activities, these organisms facilitate the development of desirable sensory and nutritional properties in fermented foods. However, despite their beneficial contributions, certain yeast strains can act as spoilage agents, causing undesirable changes in food products and leading to substantial economic losses (Boynton, 2019; Dhillon et al., 2025).Among the diverse functional attributes of yeasts, the production of antimicrobial compounds is of particular interest.

Some yeast strains secrete proteinaceous toxins, commonly referred to as "killer toxins," which are typically low-molecular-weight proteins, glycoproteins, or exopolysaccharides. These compounds selectively inhibit or kill susceptible yeast strains while leaving resistant strains unaffected (Billerbeck et al., 2024). Conventional broad-spectrum antibiotics and fungicides, though effective in the short term, often disrupt beneficial microbiota and accelerate the emergence of resistant microbial strains. In this context, biological control systems based on microorganisms and their metabolites have gained significant attention as environmentally friendly alternatives. Among these, killer yeasts—yeast strains capable of secreting extracellular proteinaceous toxins that selectively eliminate competing microorganisms—represent a promising yet underexploited resource in modern biotechnology. The killer phenomenon was first reported in *Saccharomyces cerevisiae* and has since been identified in more than 90 yeast species belonging to multiple genera, including *Pichia*, *Candida*, *Kluyveromyces*, *Torulaspora, Wickerhamomyces* and *Zygosaccharomyces* (Cambaza et al., 2019; Chan et al., 2025; Vepštaitė-Monstavičė et al., 2025). Yeast strains exhibit four distinct toxin-related phenotypes: killer (K), sensitive (S), neutral (N), and killer-sensitive (K-S). Killer toxins are heat-labile, losing their structural integrity and activity even at body temperature. Interestingly, they exhibit selective lethality towards eukaryotic cells without adversely affecting human or probiotic cells, making them particularly attractive as biological control agents. In fermentation systems, killer yeasts can suppress the growth of wild yeast contaminants that compete with starter cultures for nutrients, thereby ensuring the proper progression of fermentation and improving final product quality.Industrial applications of killer yeasts are well documented in beer, wine, and bread fermentations (Billerbeck et al., 2024; Molina-Veraet al., 2024; Chan et al., 2025; Unver et al., 2025). Beyond these traditional uses, killer yeast strains have been isolated from diverse natural sources, including lakes, rivers, fruits, and vegetables, indicating their ecological versatility. Given their unique antimicrobial capabilities and selective activity, killer yeasts hold considerable promise as bio-control tools in food biotechnology, offering both quality enhancement and microbial safety in fermentation-based industries. Recent advances in molecular biology, *omics* technologies, and synthetic biology have transformed the potential of killer yeast systems. Genetic engineering tools, particularly CRISPR/Cas-based genome editing, have enabled the precise manipulation of toxin genes, optimization of secretion pathways, and enhancement of toxin stability under industrial and clinical conditions. Moreover, transcriptomic and proteomic studies have deepened the understanding of host–toxin interactions, opening new avenues for tailoring killer yeast strains with improved performance and broader biotechnological applications (de Ullivarri et al., 2024; Chan et al., 2025). Despite these advances, challenges remain in the translation of killer yeast systems into large-scale applications. Key issues include maintaining consistent toxin production, assessing ecological and biosafety impacts, understanding resistance development in target organisms, and complying with evolving regulatory frameworks. Addressing these challenges will require interdisciplinary collaboration across microbiology, bioengineering, and systems biology. In summary, killer yeasts embody a new frontier in targeted antimicrobial biotechnology, offering a precision-oriented and sustainable approach aligned with the principles of the One Health concept. Continued exploration of their genetic, biochemical, and ecological potential will be critical for harnessing their role in next-generation antimicrobial strategies (Chan et al., 2025; Vepštaitė-Monstavičė et al., 2025).

TECHNOLOGICAL SIGNIFICANCE OF KILLER YEASTS

Competition for nutrients among bacteria, yeasts, and molds complicates food fermentation processes. Organic acids, hydrolytic enzymes, and aroma compounds produced by these microorganisms are essential for safe food fermentation and nutritional quality (Satora et al., 2014; Alturki et al., 2019).

Killer yeasts are widely present in the natural microflora of products such as fruits and decaying vegetables. They significantly influence the composition and development of the surrounding flora, excluding their own cells. Consequently, killer yeasts are employed in the preservation of various foods, including beer and wine, by inhibiting undesirable microorganisms. Killer yeasts exhibit lethal, inhibitory, and growth-suppressing effects against both strains of their own species and sensitive strains of other species (Lim and Tay, 2011; de Ullivarri et al., 2014; Cambaza et al., 2019; de Ullivarri et al., 2024).

Extensive research has explored the industrial applications of killer yeasts. While they are most commonly observed in fermentation processes within the food industry, they also play active roles in medical research and the pharmaceutical sector. Their use is particularly suitable for controlling undesirable microorganisms in various fermented products. In recent years, applications of killer yeasts in the biomedical field have also been reported (Ci et al., 2010; Cambaza et al., 2019; Mannazzu et al., 2019; Giometto et al., 2021; Unver et al., 2025).

In the production of alcoholic beverages such as beer and wine, wild yeasts can develop alongside starter yeasts, leading to product contamination and altering the course of fermentation. Studies have reported that wild strains can be eliminated through killer yeast activity. Today, toxin-producing yeasts are also utilized in the wine industry. Due to their antagonistic effects against wild and even some starter yeasts, killer yeasts have been shown to be beneficial, and in later stages, they have been introduced as starter cultures. In a study by Benda (Benda, 1985), a toxin-producing *Saccharomyces* strain isolated from grape must was used in wine fermentation and compared with fermentations carried out by spontaneous flora. The results indicated that fermentations using the killer yeast strain were inhibited. The study emphasized that transferring this trait to wine yeast and obtaining such strains as pure cultures could improve fermentation quality (Satora et al., 2014; Mannazzu et al., 2019).

Similarly, Petering et al. (1991) employed *Saccharomyces* wine yeast to determine killer activity in grape juice. They examined yeast isolates obtained from specific vineyards and various regions worldwide to identify strains with killer activity. Measurements conducted at different pH levels and temperatures revealed the optimal pH and temperature for maximum killer activity. They reported that killer toxins eliminated sensitive yeast strains at a ratio of 2:1, with the highest

activity observed at pH 3.1 and 18 °C. It was further suggested that other killer yeast strains would exhibit peak activity under similar conditions. In beer and wine production, inoculation with selected killer yeast starter cultures in the early stages of fermentation has been proposed as a strategy to control contaminating and spoilage yeasts. Magliani et al. (2008) reported studies on the use of natural or genetically modified killer yeast strains as starter cultures in the wine industry to meet quality requirements. Although the positive attributes of killer yeasts make them promising for wine fermentation, their ability to slow or halt fermentation can result in high residual sugar content in dry wines. To prevent such undesirable quality characteristics, it is essential to identify and develop killer yeast strains that do not cause these negative effects (Özçelik et al., 1996; Comitini et al., 2004; Parveen and Begum, 2010; Parafati et al., 2022; Cambaza et al., 2019; Unver et al., 2025). Using such strains as pre-cultures in fermentation could allow killer yeasts to dominate the environment, complete fermentation, and ultimately yield higher-quality wines (Marquina et al., 2002; Altuntaş and Ozcelik, 2007; Buzdar et al., 2011; Mehlomakulu et al., 2014; Zhong et al., 2024).

While the use of killer yeasts in fermentation helps control undesirable yeasts that can negatively impact quality and cause spoilage, it can also lead to the inhibition of desirable yeasts necessary for complete fermentation. The presence of killer yeasts in even low concentrations, such as 1%, can inhibit the starter strain responsible for fermentation (Billerbeck et al., 2024).

In addition to alcoholic fermentations like wine and beer, recent studies have shown that killer yeasts may also be utilized in olive production. Due to the killer activity of certain yeast species in olives, their use as biocontrol agents has been proposed, which could reduce the need for chemical preservatives and support the growth of lactic acid bacteria (Mehlomakulu et al., 2014; Parafati et al., 2022). In a study by Kara and Özbaş (2013), it was suggested that these benefits could be achieved. Hernandez et al. (2007), in their research on green olives in Portugal, examined killer activity in yeast strains isolated from olives obtained from different regions. The study found that under high pH (8.5) and high salt concentration (10%) conditions, killer strains effectively inactivated sensitive yeasts. The authors emphasized that future studies could focus on using killer strains as biocontrol agents against wild yeast contaminants, thereby eliminating the need for chemical preservatives.

Beyond olives, Lim and Tay (2011) conducted a study in Malaysia in which they isolated 252 yeast strains from fermented foods such as tapai, fermented fruits and vegetables, tempeh, miso, other fermented beans, yogurt, soy sauce, rice wine, and vinegar. Nineteen of these isolates exhibited killer activity. Their findings indicated that killer yeasts could inhibit pathogenic conditions caused by *Candida* species responsible for candidiasis in fermented products. This biological inhibition could reduce the need for pharmaceutical interventions to treat such infections.

Studies have demonstrated that the degree of killer activity can vary depending on whether fermentation is continuous or batch-operated (Yehia et al., 2022; Billerbeck et al., 2024). In a study conducted by Ramon-Portugal et al. (1998), two strains-sensitive (522D) and killer (K1)-were isolated from a mixed culture population of S. cerevisiae. Under batch fermentation conditions, it was observed that, at specific concentrations, killer toxin eliminated 5% and 10% of the total sensitive yeast population, respectively. The authors reported that different concentrations of killer yeast altered the proportion of the sensitive cell population that was eliminated. In continuous fermentation systems, the toxin was found to act on the entire sensitive cell population.

Killer yeasts secrete protein-based toxins that can be applied in both medical and biotechnological fields (Giometto et al., 2021; Giovati, 2021; Chan et al., 2025). These yeasts are of particular interest in industrial and clinical applications and exhibit significant potential within their own species. Their toxins have been used for the biological control of microorganisms classified as contaminants in fermentation and medical applications (Buzzini et al., 2004; Bajaj et al., 2013; Giometto et al., 2021; Giovati, 2021; Chan et al., 2025).

Humans and animals are exposed to yeasts from birth. Although most yeast species are non-pathogenic to mammals, approximately 200 species are known to cause disease in these hosts. In recent years, fungal pathogens have increasingly caused severe diseases in immune compromised individuals, particularly those undergoing intensive chemotherapy, receiving immunosuppressive drugs, or suffering from HIV-related immune deficiency. To combat these conditions, the efficacy of killer yeasts has been enhanced. Recent studies have adopted biological control strategies, yielding promising results (Yener, 2006; Ochigava et al., 2011; Kast et al., 2014; Giovati, 2021; Chan et al., 2025; Unver et al., 2025).

In a study by Bajaj et al. (2013), *Pichia kudriavzevii* RY55 was reported for the first time to exhibit killer activity. This strain, obtained from the culture collection of the Fermentation Biotechnology Laboratory at the School of Biotechnology, was found to inhibit pathogenic bacteria harmful to human health, including *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Pseudomonas alcaligenes*. Given the reported health risks posed by these pathogens, the biological inhibition achieved by *P. kudriavzevii* RY55 suggests its potential use as a biocontrol agent for food preservation in the fermentation industry and as an antimicrobial chemotherapeutic agent in medicine. Furthermore, future research could focus on transferring the gene encoding killer toxin directly, enabling the yeast to simultaneously protect foods and function as a chemotherapeutic agent, thus serving multiple industries and sectors.

Some yeasts also demonstrate potential as biocontrol agents against pathogenic plant molds. In a study by Santos et al. (2004), killer toxin produced by *P. membranifaciens* was shown to act as a potential control agent against Botrytis cinerea, the causative agent of grey mold. Similarly, Waema et al. (2009) tested killer yeast toxins against plant pathogens responsible for wood decay in trees and reported lethal effects.

In recent years, *Candida* infections have risen sharply, particularly among severely immunocompromised patients. The most frequently isolated yeast in clinical samples is *Candida albicans*, the most pathogenic member of the genus. However, the increasing incidence of infections has also been attributed to other species, including *Candida parapsilosis*, *Candida*

tropicalis and *Candida glabrata*. Antimicrobial resistance in *Candida* species has been reported, particularly due to antibiotic and antifungal drug exposure (Serviene and Serva, 2023; Chan et al., 2025).

MECHANISTIC INSIGHTS INTO THE ACTİON OF KILLER YEASTS

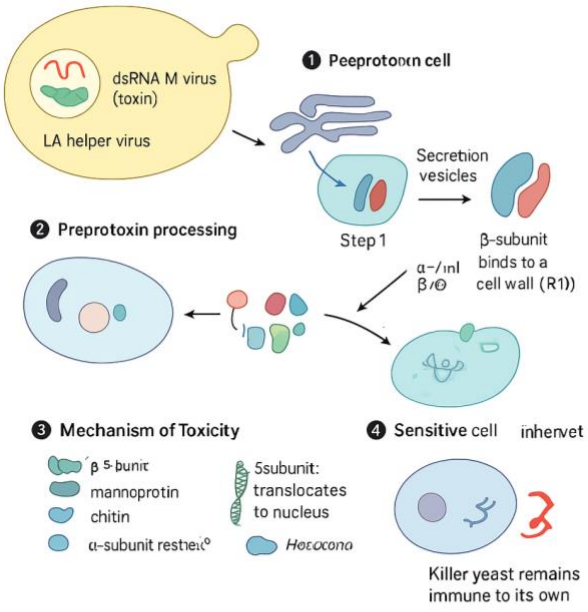
Killer yeasts produce proteinaceous toxins with lethal effects on sensitive cells and exhibit interactions with host viruses (Banjara et al., 2016; Billerbeck et al., 2024). Schmitt and Breining (2006) reported that certain killer yeasts harbor viruses. These toxins act on macromolecules, and sensitive cells in the logarithmic growth phase are particularly vulnerable, with inhibition levels reaching 70-80%, sufficient to induce cell death. Bussey (1972) demonstrated that protein synthesis in sensitive cells ceases upon exposure to killer toxins, leading to cell mortality.

In *Saccharomyces cerevisiae*, four toxin types-K1, K2, K28, and Klus-have been identified, encoded by medium-sized double-stranded RNA (dsRNA) viruses (El-Banna et al., 2011). Killer toxins are generally encoded by dsRNA (e.g.,*S. cerevisiae* M1), linear plasmids (e.g., *Pichia acaciae*, pPac1-1), or chromosomal DNA (e.g., *Williopsis mrakii*, HMK) (El-Banna et al., 2011; Mannazzu et al., 2019; Zhong et al., 2024). The expression of the killer phenotype requires two distinct dsRNA viruses: the LA helper virus and the M killer virus, encapsidated separately. These viruses in *S. cerevisiae* correspond to M1, M2, M28, and Mlus, with molecular sizes of 1.7–2.3 kb (El-Banna et al., 2011; Maqueda et al., 2011), and belong to the Totiviridae family (Schimitt and Breining., 2006; Orentaite et al., 2016; Aday et al., 2021).

M viruses encode preprotoxins, which are processed into mature killer toxins. They rely on the larger 4.6 kb dsRNA LA helper virus for replication, transcription, and encapsidation, while also conferring functional immunity (El-Banna et al., 2011; Billerbeck et al., 2024). In killer yeasts, single-stranded RNA transcripts of toxins are translated in the cytoplasm into preprotoxins. Secretion involves the endoplasmic reticulum, Golgi apparatus, vacuoles, endosomes, and lysosomes, culminating in the release of mature toxins (Magliani et al., 2008; Alturki et al., 2019).

Killer toxins inhibit sensitive cells via receptor-mediated interactions with cell wall and plasma membrane components. All known killer toxins are proteins or glycoproteins and act in a two-step process (Table 1) (Billerbeck et al., 2024; Vepštaitė-Monstavičė et al., 2025). The first step involves binding to primary receptors (R1) on cell wall mannoproteins or β-1,6-glucans, which is rapid and energy-independent. The second step involves energy-dependent interaction with secondary receptors (R2) at the plasma membrane, resulting in inhibition. Toxins either disrupt ion channels, particularly potassium flux, or directly target the nucleus to halt DNA synthesis, causing cell death (Gier et al., 2020; Billerbeck et al., 2024).

Studies identified the TOK1 potassium channel as a target for the K1 killer toxin, with toxin binding activating the channel independently of additional cellular components, while gene regulation in sensitive cells influences resistance (Sesti et al., 2001; Baeza et al., 2008; Gier et al., 2020). Mature prototoxins are secreted as heterodimeric α- and β-subunits; the β-subunit binds to sensitive cell wall receptors, while the α-subunit enters the nucleus to inhibit DNA synthesis and induce irreversible cell death (Figure 1) (Rodriguez-Cousiño et al., 2022; Molina-Vera et al., 2024).



Killer yeast species	Toxin	Target receptor on sensitive cell
S. cerevisiae	K1. K2	β–ŧ,ɒ,ĩð glucaṅn, mannoprotein, oŕ chitin
Kluyveromyces lactis	K28	DNA sünet'slag anolɑ̈ɑ̈olion
	Chitin	Apoptosis
Pichia membranfaciens	HMK	Membrane pore formation – apopotsis
Williopsis mrakii	K5	Ion flux disruption – apopotsis
Pichia anamola	K5	Membrane integrity loss

Figure 1. Mechanism of Killer Toxins (Rodriguez-Cousiño et al., 2022; Molina-Vera et al., 2024)

Receptor specificity varies among killer yeasts. For instance, K1 and K2 toxins of *S. cerevisiae* target β-1,6-D-glucan receptors (Sertkaya, 2005; Rodriguez-Cousino et al., 2022), *K. lactis* and *P. membranifaciens* bind chitin (14, 53), while *Williopsis mrakii*, *Debaryomyces hansenii*, and *Hanseniaspora uvarum* recognize β-1,6-D-glucan. Other toxins, such as KT28 and *Zygosaccharomyces bailii*, target mannoproteins, and K5 from *P. anamola* binds β-1,3-D-glucan (Table 1) (Billerbeck et al., 2024).

The killer activity mechanism progresses through β-1,3-glucanase activity, interaction with cell wall glucans, membrane pore formation, ion flux disruption, DNA damage induction, apoptosis, cell cycle arrest, and eventual cell death (de Ullivardi et al., 2023; Chan et al., 2025). Notably, protoplasts lacking cell walls are resistant to killer toxins, confirming receptor dependency (Molina-Vera et al., 2024). Despite exhibiting immunity to their own toxins, the exact mechanisms by which killer yeasts resist self-toxins remain unresolved (Figure 1) (Banjara et al., 2016; Molina-Vera et al., 2024).

Table 1. Killer Toxin–Receptor Specificity (Billerbeck et al., 2024).

Killer Yeast Species	Toxin Type	Target Receptor on Sensitive Cell	Mechanism
S. cerevisiae	K1, K2	β-1,6-D-glucan	α-subunit nuclear entry, β-subunit cell wall binding
S. cerevisiae	K28	Mannoprotein	Ion channel disruption, DNA synthesis inhibition
K. lactis	Various	Chitin	Membrane pore formation → apoptosis
P. membranifaciens	Various	Chitin	Nuclear and membrane targeting
W. mrakii	HMK	β-1,6-D-glucan	Ion flux disruption, apoptosis
P. anamola	K5	β-1,3-D-glucan	Membrane integrity loss → cell death

PARAMETERS AFFECTING KILLER ACTIVITY

Temperature and pH

The optimum pH and temperature for killer toxin activities vary among yeast species. Studies have demonstrated that yeasts exhibit killer activity at different temperature and pH ranges. In a study conducted by Bajaj et al., the optimal temperature ranges for the activity of various killer yeast cells were determined. They reported that *P. anamola* and *P. membranafaciens* CYC 1086 killer toxins exhibited optimal killer activity at 20 °C and 15 °C, respectively (Bajaj et al., 2013; Belda et al., 2017; Chan et al., 2025). Furthermore, it was observed that the killer toxins of *P. anamola* and *S. cerevisiae* were active at 22 °C and 30 °C, but not at 37 °C. In the same study, *S. cerevisiae* HAU-1 killer toxins were found to display maximum activity between 20–35 °C.

Regarding pH, striking results have been reported. Ramon-Portugal et al. (1998) observed that the K2 killer toxin exhibited maximum activity within a pH range of 2.8-4.8. In another study, it was discussed that K2 killer toxin may be more suitable than K1 toxin for application in the wine industry. The research indicated that the K1 killer toxin produced by *S. cerevisiae* exhibited an optimum pH range of 4.6-4.8, whereas the K2 killer toxin produced by the same yeast species was active between pH 2.9-4.9. This broader range makes K2 toxin more hazardous in winemaking, where it plays an inhibitory role in fermentation, as reported by Marquina et al. (2002). More recently, Wang et al. (2007a, 2007b) and Belda et al. (2017) reported that killer yeast toxins exhibit an optimum pH of 4.5 and an optimum temperature range of 20-35 °C (Zhong et al., 2024).

Effect of Metal Ions

It has been reported that certain metal ions act as activators, while others function as inhibitors of killer yeast toxins. Studies have shown that Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, and Co^{2+} ions activate killer yeast toxins. In contrast, Fe^{2+}, Fe^{3+}, Hg^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, and Ag^{+} ions have been identified as substances that reduce or inhibit the activity of killer yeast toxins. In addition to these metals, chemicals such as phenylmethylsulfonyl fluoride (PMSF), iodoacetic acid, ethylenediaminetetraacetic acid (EDTA), and 1,10-phenanthroline have also been reported to negatively affect killer activity, as demonstrated in the studies of Wang et al. (Wang et al., 2007a; Wang et al., 2007b;).

In relation to this, Bajaj et al. (2013) reported that the activity of the *P. anamola* NCYC 434 killer toxin decreased in the presence of Hg^{2+}, and after a certain period, the yeast itself was inhibited. Conversely, in environments containing Pb^{2+}, the toxin activity was enhanced. Another study further demonstrated that the toxin produced by *Williopsis saturnus* was influenced by Li^{+}, Ni^{+}, and Ba^{2+} ions, which exhibited both inhibitory and activating effects depending on the conditions.

Salt Effect

In the study conducted by Aguigar et al. (2008), it was observed that killer activity increased particularly in environments with high salt concentrations. In this research, killer yeasts with high halotolerance and sensitive strains with low tolerance were distinguished, and the interactions between these groups were evaluated. A total of 58 different yeast strains were exposed to environments containing 1, 2, 3, and 4 M NaCl to assess their response to salt stress. The study reported that the main objective was to determine the strains' reactions to salt stress. Among the tested strains, *Candida nodaensis* was identified as the most tolerant to salt stress while also exhibiting strong killer activity.

Similarly, the studies of Suzuki (1999) and Llorente et al. (1997) reported that salt–food isolates in halotolerant yeast strains enhanced killer activity, suggesting that higher salt concentrations increase killer activity. It was emphasized that in the presence of salt, both killer yeast activity increased and the mortality risk of sensitive yeast cells was elevated.

High salt concentrations were found to influence ion transport channels in plasma membranes, thereby allowing killer yeast toxins to inhibit sensitive cells more effectively. The killer toxin produced by *P. farinosa* was also reported to disrupt the translation mechanism in sensitive cells via salt-mediated mechanisms, as described by Suzuki (1999) and Llorente et al. (1997).

Regarding the effect on plasma membranes, Aguigar and Lucas (2008) noted that at intracellular NaCl concentrations of 2 M or higher, K^+ ion levels in ion transport channels decreased, thereby facilitating killer activity by making plasma membranes more susceptible to inhibition.

In another study, Silva et al. (2003) investigated *Z. bailii, P. membranafaciens,* and *Z. rouxii,* which are known as spoilage yeasts in foods. These organisms exhibited killer activity against sensitive cells under high salt concentration conditions. However, it was also reported that despite their high halotolerance, their degree of osmotolerance was not always elevated. In a related study, *S. cerevisiae, P. etchelsii, D. hansenii, P. anamola,* and *P. farinosa* were found not only to display killer activity but also to possess high levels of halotolerance and osmotolerance.

Role of Killer Yeasts in Fermentation

Yeasts have been an integral component of the fermentation industry for centuries, functioning as starter cultures in the production of bread, wine, beer, and a variety of fermented dairy products. Despite their long-standing use, certain yeast strains present challenges due to their ability to produce toxins, which can inhibit or even kill starter strains, thereby slowing down or halting the fermentation process. This toxin-mediated activity has been identified as a potential threat to the stability and efficiency of fermentation.However, subsequent research has provided an alternative perspective, suggesting that killer yeasts may play a beneficial role in fermentation (Belda et al., 2017; Al-Ani and Al-Saaedi, 2023; Al-Obaydi et al., 2023; Chan et al., 2025). Specifically, killer yeasts have been shown to inhibit contaminating wild yeast cells, which are often responsible for undesired fermentation outcomes. By selectively targeting these contaminants, killer yeasts can help sustain fermentation and ensure process consistency. This dual role has shifted the perception of killer yeasts from being problematic organisms to potential allies in controlled fermentation systems.Wine fermentation serves as a prominent example of this paradigm shift. Historically, killer yeasts were considered detrimental to the process; nevertheless, recent studies have explored their application as starter cultures (Aday et al., 2021; de Ullivarda et al., 2024). For instance, Dabhole and Joishy (2005) demonstrated that killer strains of *Saccharomyces cerevisiae* not only initiate fermentation but also enhance wine quality. Their findings highlighted that while some *Saccharomyces* strains remain sensitive to the strong toxicity of *S. cerevisiae* killer toxins, the inhibitory effects are predominantly observed in non-*Saccharomyces* species. This selective activity underscores the feasibility of employing killer yeasts as effective starter cultures.

Moreover, it was reported that wild yeast species such as *Hanseniaspora, Kloeckera, Pichia,* and *Saccharomycodes* were not significantly affected by *S. cerevisiae* killer toxins. This suggests that the use of killer yeasts may not disrupt the entire microbial ecosystem but could instead target specific contaminants, thereby contributing to improved fermentation control and product quality (Alturki et al., 2019).

APPLICATIONS OF KILLER YEASTS AS ANTIMICROBIAL AGENTS

Killer yeasts have emerged as promising antimicrobial agents due to their ability to produce toxins with broad-spectrum activity against yeasts, bacteria, and fungi. While the antimicrobial properties of conventional agents such as antibiotics, bacteriophages, and bacteriocins have long been recognized, the potential of metabolites derived from microorganisms, including killer toxins, has only recently been systematically explored. Research has demonstrated that killer toxins can act as effective antibacterial, antifungal, and antimycotic agents (Aday et al., 2021; Billerbeck et al., 2024). Bajaj et al. (2013) reported that several *Pichia* species exhibit antifungal and antimycotic activity against diverse yeast and fungal species. Specifically, *P. kudriavzevii* RY55 killer toxin showed antimicrobial activity against pathogenic microorganisms such as *Escherichia coli, Enterococcus faecalis,* and *Staphylococcus aureus*, which are clinically relevant in humans.

Similarly, Meneghin et al. (2010) demonstrated that killer yeast strains, including *Candida glabrata, P. anamola,* and other *Candida* species, acted as antibacterial agents against wild contaminants such as *Bacillus subtilis* and *Lactobacillus plantarum* in beverage fermentations. Waema et al. (2009) observed that *Candida krusei* killer yeast, isolated from fermented vegetables, displayed lethal activity against pathogenic species including *E. coli, Salmonella typhimurium,* and multiple *Staphylococcus* species.

Additional studies by Polonelli and Morace (1987), Izgu and Altınbay (1997) and Guyard (2002) reported that yeasts such as *Hansenula anomala, Hansenula mrakiii, Kluyveromyces drosophilarum, K. lactis,* and *Candida tropicalis* produce antimicrobial peptide compounds capable of killing Gram-positive and non-pathogenic bacteria. Comparative analyses suggested that toxins, including the K9 toxin from *Hansenula mrakiii*, could serve as growth inhibitors in industrial applications.

Beyond industrial applications, killer yeasts are increasingly investigated for clinical use. Their toxins have demonstrated antimycotic activity against clinically relevant pathogens, particularly *Candida albicans*. While killer toxins are commonly classified as food antimicrobials, their effectiveness in clinical and pharmaceutical contexts has been widely recognized. These findings indicate that killer yeasts represent a versatile biological tool, with potential applications spanning food safety, industrial microbiology, and clinical therapeutics. Continued research is expected to expand their utility and optimize their application as natural antimicrobial agents (Henriques-Normark, 2007; Banjara et al., 2016; Orentaiet et al., 2016; Zhong et al., 2024).

CONCLUSIONS

Killer yeasts are organisms with applications across various industries. Current research indicates their utilization in food, clinical, pharmaceutical, and biomedical sectors. In particular, the toxins produced by killer yeasts during the fermentation stage in the food industry have emerged as a novel technological tool. Initially, killer yeasts were considered undesirable in fermentation processes due to their potential to halt fermentation. However, with the advancement of studies, the use of killer yeast toxins to target wild contaminants during fermentation has become a topic of interest. Killer yeasts

represent a promising and versatile platform for targeted antimicrobial interventions, offering a precision-based alternative to conventional broad-spectrum antifungal and antibacterial therapies. Unlike traditional antifungals, which often exhibit nonspecific cytotoxicity and contribute to the emergence of resistant strains, killer yeasts deliver species-specific toxins capable of selectively eliminating pathogenic microorganisms while preserving beneficial microbiota. This selectivity not only enhances therapeutic efficiency but also aligns with sustainable and ecological principles, particularly in food biotechnology, agriculture, and clinical applications. Subsequent research has proposed the practical application of killer yeast toxins in fermentation, aiming to utilize these yeasts both as starter cultures and as agents that inhibit wild contaminants, thereby safeguarding the fermentation process. Current and future studies are focused on developing organisms that can function as starter strains while simultaneously producing toxins to eliminate unwanted microbial contaminants. Recent advancements in genetic engineering, synthetic biology, and omics technologies have significantly expanded the functional potential of killer yeasts, enabling the optimization of toxin stability, controlled expression, and delivery under diverse environmental and industrial conditions. Engineered strains demonstrate applicability in food preservation, biocontrol of plant pathogens, biofilm management, and adjunctive therapies against multidrug-resistant fungal infections. Compared to conventional antifungals, killer yeasts offer a multi-targeted and adaptive mechanism of action, reducing reliance on chemical agents and potentially mitigating the global challenge of antimicrobial resistance. Globally, including in Turkey, researchers are also exploring the antimicrobial, antifungal, and antimycotic properties of killer yeasts. These properties enable the elimination of pathogenic microorganisms from the environment, reduce the need for chemical agents, and allow for biological control strategies that lessen reliance on conventional drugs. It is anticipated that in the coming years, killer yeast toxins could serve as a natural alternative to chemical interventions. The advancement of this potential relies on continued research and systematic investigations by the scientific community. In the future, killer yeast toxins are expected to find broader applications across diverse fields. Despite these advantages, several critical challenges limit the large-scale adoption of killer yeast technology. Consistent toxin production under industrial and clinical conditions remains a key technical hurdle, while ecological safety, regulatory compliance, and potential resistance development in target populations require thorough assessment. Furthermore, the translation from laboratory studies to real-world applications is constrained by gaps in understanding host–toxin interactions, pharmacodynamics, and long-term ecological impacts. In conclusion, while killer yeasts do not yet replace conventional antifungal therapies, they represent a complementary and innovative approach with the potential to transform antimicrobial strategies across multiple sectors. Future research should focus on integrating systems biology, high-throughput screening, and precision bioengineering to overcome current limitations and fully realize the promise of killer yeasts as sustainable, targeted, and next-generation antimicrobial agents.

Compliance with Ethical Standards

Peer Review

This article has been peer-reviewed by independent experts in the field using a double-blind review process.

Conflict of Interest

The author declares that there is no conflict of interest.

Author Contribution

The author solely conceived, designed, and conducted the study, analyzed the data, and wrote the manuscript.

Ethics Committee Approval

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