Wickerhamomyces Yeast Killer Toxins

Subjects: Toxicology | Infectious Diseases
Contributor: Laura Giovati

Bacteriocins are ribosomally synthesized antimicrobial peptides produced from a wide variety of bacteria that inhibit the growth of similar or closely related bacterial strains. A similar phenomenon of competition is present in yeasts, based on the production of killer toxins (KTs, or mycocins) that are secreted proteins or glycoproteins capable of killing susceptible microorganisms with various mechanisms of action, through interaction with specific superficial receptors. Possible implications and applications of the yeast killer phenomenon in the fight against infectious diseases are reviewed in this work, with particular reference to some wide-spectrum killer toxins (KTs) produced by *Wickerhamomyces anomalus* and other related species.

Keywords: killer yeasts ; killer toxins ; antimicrobial activity ; medical applications ; Wickerhamomyces anomalus

1. Introduction

Competition for space, nutrients and other resources in the environment is a rather common mechanism through which different species of microorganisms can interact with or prevail over others, determining the composition of microbial communities within different ecological niches. The production and excretion of molecules with toxic activity, such as bacteriocins and yeast killer toxins, can be included among the mechanisms by which some microorganisms can harm or kill their competitors, contributing to natural selection.

Bacteriocins are ribosomally synthesized antimicrobial peptides produced from a wide variety of bacteria that inhibit the growth of similar or closely related bacterial strains. Broad-spectrum bacteriocins have also been described. Based on their structure, mode of action, mechanism of biosynthesis and self-immunity, bacteriocins could deserve serious consideration as potential alternatives to traditional antimicrobials for use in agriculture, food storage, veterinary and even human medicine [1][2][3].

A similar phenomenon of competition is present in yeasts, based on the production of killer toxins (KTs, or mycocins) that are secreted proteins or glycoproteins capable of killing susceptible microorganisms with various mechanisms of action, through interaction with specific superficial receptors. Interestingly, killer yeasts are immune to their own KTs. Since the first description of the “killer phenomenon” in Saccharomyces cerevisiae, by Bevan and Makower in 1963 [4], more than 100 different species of yeasts belonging to more than forty genera, including basidiomycetes and ascomycetes, have been described as killer yeasts, thus attesting to the widespread diffusion of the phenomenon and its ecological relevance [5][6]. Numerous studies over the years have greatly contributed to clarifying the molecular characteristics of various KTs, their physiology and mode of action and the genetic determinants encoding for their production. As relatively simple eukaryotic cells, killer yeasts have also represented a valid model to study relevant aspects of eukaryotic cell biology, such as mechanisms of processing and extracellular secretion of proteins. Comprehensive reviews have been published on these subjects to which the reader is referred [5][6][7][8][9][10][11][12].

Although the spectrum of antimicrobial activity of KTs was initially considered limited to susceptible cells belonging to the same species as the producing yeast or to closely related species, it is now known that some KTs may be active against a great variety of eukaryotic and prokaryotic microorganisms. Taxonomically unrelated fungi, bacteria, protozoa and even achlorophyllous saprophytic algae are among the described KT-susceptible strains, including spoilage and pathogenic microorganisms involved in relevant plant, animal and human infections [6]. The mode of action, which still needs to be clarified for many KTs, is variable and includes cell wall or membrane damages as well as arrest of the cell cycle [10].

2. Wickerhamomyces anomalus Killer Strains and WaKTs

In the last two decades, there has been a continuous reclassification of yeasts, including KT-producing strains, into new genera and species. For example, based on nuclear DNA reassociation studies and phylogenetic analysis of gene sequences, species of the genus Hansenula have been reassigned to the genus Pichia and then to the genus

Wickerhamomyces [13][14][15][16]. Among the species belonging to this genus, W. anomalus has aroused particular interest in microbiology and biotechnology fields, food production and biopreservation, as well as the development of innovative therapeutics, due to its specific characteristics, adaptation properties, frequent detection in natural environments (plants, soil, fruits, animals) and involvement in various fermentation processes [17]. Another key feature of W. anomalus is its capability to produce and secrete KTs, characterized by a broad spectrum of activity, comprising relevant plant, animal and human pathogens [18][19]. Many KTs exert their optimal activity at acidic pH and temperatures below 30 °C, which could be a problem for biomedical application [5]. Over the years, various KTs produced by W. anomalus (named WaKTs or, previously, PaKTs and HaKTs) have been described [20][21][22][23]. Notably, some killer strains can produce more than one toxin with different characteristics [24]. The most attractive features of some of these chromosomally encoded KTs [25] are the broad spectrum of activity and their mechanism of action, mediated by the interaction with specific cell wall receptors. In most cases, 1,3 or 1,6 β-glucans are the potential receptors and/or targets of KTs, sometimes characterized by exo-β-glucanase activity [20][23][26][27][28].

3. Killer Toxins' Medical Applications

Three possible medical applications of W. anomalus and related species killer strains or their KTs have been tested and suggested: 1. direct use of killer yeasts as biological competitors; 2. direct use of KTs as potential antimicrobial molecules with broad activity; 3. production and use of immunological derivatives of KTs. In the following paragraphs, killer strains and KTs for these applications are described (summarized in **Table 1**).

Table 1. Killer strains and KTs for applications in the medical field.

Killer Toxin (KT)	KT Producer	MM (kDa) [1]	Target	Function	Application	References
WaF17.12	Wickerhamomyces anomalus F17.12	140	Plasmodium berghei	β-glucanase activity	Killer yeast	[29][30][31][32]
Wa96603KT	W. anomalus ATCC 96603	220	Bacteria, fungi, protozoa, viruses	Various/nd	KT and derivatives	[33][34][35][36][37][38][39]
HM-1	W. saturnus var. mrakii IFO 0895	10.7	Yeasts	Inhibition of β-1,3-glucan synthase	KT and derivatives	[40][41][42][43][44][45][46][47]
Panomycocin	W. anomalus NCYC 434	49	Human dermatophytes, Candida spp.	β-glucanase activity	KT	[20][27][48][49][50][51]
Wa1F1-KT	W. anomalus 1F1	160–170	Candida spp.	β-glucanase activity	KT	[23]
WA40M1, WA45M2 and WA92M3	W. anomalus WA40, WA45 and WA92	nd	C. albicans, Acinetobacter baumannii	nd	KT	[52][53]
KT	W. anomalus LMA-827	nd	Listeria sp.	Pore formation	KT	[54]
KT	W. anomalus YF07b	67	Candida spp.	Membrane permeabilization	KT	[55]

Killer Toxin (KT)	KT Producer	MM (kDa) [1]	Target	Function	Application	References
Mycocin	W. anomalus tp2-15	45, 50	Candida mesorugosa	β-glucanase activity	KT	[56]

[1] MM, molecular mass (kDa); nd, not determined.

4. Conclusions

A direct use of killer yeasts or their KTs as competitors against microbial pathogens is limited by several problems, except perhaps in the interesting option of their exploitation in vector insects for the symbiotic control of arthropod-borne diseases. KT-derived peptides or immunological derivatives of KTs, such as KT-mimicking Abs and their fragments, have been extensively described. KT- and Ab-derived peptides, once selected and tested, can be easily managed in terms of synthesis, quantitative production and sequence modification, in order to improve their activity and delivery systems, in view of a possible therapeutic use.

In the 21st century, infectious diseases still represent an important challenge for human health, despite the improvements in hygiene, healthcare and socioeconomic status and the extraordinary success of preventative and therapeutic approaches. Globalization and climate changes are favoring the emergence and re-emergence of new and old etiologic agents, often characterized by intrinsic or acquired resistance to anti-infective agents. The growing crowd of immunocompromised or otherwise debilitated individuals represent a further, dramatic challenge for the treatment of infectious diseases. All this severely limits the available therapeutic armamentarium, strongly highlighting the need to develop new therapeutic tools and approaches. In this scenario, KT- and Ab-derived peptides can provide leading structures for the rational design of novel, target-directed compounds applicable, by their own or in synergy with existing agents, in the field of human, animal and even plant infectious diseases.

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