Killer phenomenon in yeast: An Overview

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Abstract: n challenging for limited resources, microorganisms have improved advanced strategies for winning selective characteristics over their competitors. One of these is the secretion of toxic compounds that findings in killing or growth stopping of other microorganisms. The initial discovery of killer toxin-secreting strains of *Saccharomyces cerevisiae* (*S. cerevisiae*) in the mid-sixties represents the beginning of intensive research in the yeast virology field. Some yeast strains are secreted killer toxins as the proteins which kill sensitive cells of the same yeast genera. In addition, it could be found more new yeast species have been estimated produce killer toxins versus the pathogenic yeasts, especially *Candida albicans.* The killer phenomenon is widespread between yeasts, therefore, Cryptococcus, Debaryomyces, Hanseniaspora, Hansenula, Kluyveromyces, Metschnikowia, Pichia, Ustilago, Torulopsis, Williopsis, and Zygosaccharomyces have been toxin-production and killer sensitive yeast. Some secretion killer toxins from many yeasts have been purified and described; and also the genes encoding the killer toxins have been cloned and described. Many various components of the in the sensitive cells for the killer toxin action have been identified. In addition, killer yeasts, killer toxins, and killer viruses have taken more attention to their possible applications in biomedicine and gene technology. The importance of yeast killer toxins and have been involved in various areas implicated food fermentations/yeast-based bakery product. Yeastskiller toxins may have potential applications as bio-preservatives, bio-control particularly.
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Introduction

A killer phenomenon in yeast, which is the ability to secrete a toxic agent by some strains of a defined species that is toxic to sensitive individuals of the same and related species or genra, was initially reported in *Saccharomyces cerevisiae*. A similar phenomenon was earlier reported in bacteria and the secreted agents were referred to as colicins[1]**.** Following the first noticing, showed that the activity of killer toxins to limited species of yeast. The results observed that the killer activity could be displayed versus a large differents of unrelated eukaryotic and prokaryotic microorganisms? This finding led to the re-estimation of the yeast killer phenomenon, with particular confirmation on the sudden sensitivity of microorganisms of clinical benefit likes *Candida albicans*, *Pneumocystis carinii* and *Mycobacterium tuberculosis*[2]**.** Certain yeast species have the chance of the possibility to produce killer toxins (mycotoxins), which may prevent the growth of microorganesmes and namely, these yeasts are called killer yeasts. The production of yeast killer toxins supplies a characteristic to such yeast species to successfully compete with their co-inhabitants. Consequently, killer yeast is actions selfishly to succeed at the expense of others as they have improved a technique (killer toxin) to successfully compete or kill other yeast and microbes[4]. The information of the structure and role of the yeast cell wall by the searches of the yeast killer toxin receptors to microorganisms and resistant mutant strains have been contributed enormously. This wall is no longer considered a mere cover understanding that only physical and osmotic protection to the cell but to some extent is now recognized to be a dynamic cell constituent with absolutely necessary roles. It is a complex structure that supplies selective permeability, enzyme support, cell–to–cell recognition and adhesion that plays a role in the protein secretary pathway[5]**.** After the first studies on the nature of the killer factors, genetic studieson killer yeastsshowed the involvement of cytoplasmic non-Mendelian genetic determinants, double-stranded RNA (dsRNA) also known as virus-like particles (*mycoviruses*)[6]. Besides S. cerevisiae, identified killer toxin-producing yeasts have been in *Candida, Cryptococcus, Debaryomyces, Hanseniaspora, Hansenula, Kluyveromyces,*

Metschnikowia, Pichia, Torulopsis, Ustilago, Williopsis and *Zygosaccharomyces,* indicating that the killer phenomenon is indeed widespread among yeasts. More possible usages for the killer phenomenon have been reported. In the bakery industries, the killer character can be utilized to combat wild, polluting *Saccharomyces* strains[7-8]. Furthermore, the killer toxin of *Kluyveromyces phaffii* was used as a bio-preservative agent to control peculates wine yeasts[9]. In the food industries, killer yeasts have been used to control spoilage yeasts in the preservation of food [10]. In the medical field, killer character can be used in bio-typing of pathogenic yeasts[11]. In addition, the killer toxin produced by *Pichia anomala*and *Williopsis markii* **Walker *et al.*[12]** has been proposed as an anti-mycotic agent. Although of all mentioned potential applications of killer yeasts or their killer toxins, killer yeast strains can be trouble in commercial treatments may be able they can kill desirable strains. It also can be a big problem if the phenomenon found in clinically important strains. The genetic fundamental for killer phenotype expression can be quite changing; in the little issues where killer have clearly been identified, they are cytoplasmically patrimonial double-stranded RNA (dsRNA) viruses, linear dsDNA plasmids or chromosomal genes. Until now, the occurrence of the phenomenon among industrial and clinically important yeast strains and species and their responsible genes are not clear[13].

1. Yeast Killer Toxins and Killer Phenomenon:

The antibiotics are the bioactive materials that are produced by any organism and it has to activity versus fungi, bacteria, viruses, and cancer cells. Also, it has been recognized that under competitive cases, the killer phenomenon shows a significant characteristic to these yeast strains varsus other sensitive microbial cells in their environmental niches[14]. Killer toxins have been collected into three kinds, killer toxin 1 (K1), (K2) and (K28), based on their killing profiles and a shortage of cross-immunity [15]. However, wine *S. cerevisiae* strain has been found to produce a new killer toxin (Klus) that kill all the before recognized *S. cerevisiae* killer strains, moreover to other yeast species, involved *Kluyveromyces lactis* and *C. albicans.* The killer phenotype is a grant by a medium-size double-stranded RNA (dsRNA) virus in S. cerevisiae[16]. After the killer toxin-sensitive *S. cerevisiae* HAU-1 was fused with the killer toxin-producing *S. cerevisiae* MTCC 475, the averaged incorporation could stably produce both ethanol and killer toxin[17]. The majority of pathogenic kinds of yeast have been found to reason disease in marine animals and killer yeasts and their killer toxins may have possibility utilize in the control of these pathogens[18]. Moreover, findings on the killer phenomenon in yeasts have supplied worthy insights into a number of basic parts of eukaryotic cell biology and interactions of various eukaryotic cells[19-20-21]. Meanwhile, clarification of molecular techniques of their action will be beneficial to improve the strategies and design synthetic chemicals to fight the harmful fungi in human, animals, and plants[22]. It should be exhausting that killer yeasts between natural yeast isolates have great biodiversity, in terms of their biochemical properties, genetic determinants, their spectra and techniques of their killer toxin actions. Majority novel killer toxins have been purified and described, and their genes have been cloned[13-14-19-20].

2. Extra chromosomally Encoded Toxins:

A. dsRNA Viral Toxins:

A quantity of well-described yeast killer toxins is encoded by killer genes with unusual cellular localization. The firstly discovered *S. cerevisiae* killer strains Woods and Bevan[23] who observed to harbor dsRNA viruses, which belong to the *Totiviridae* family and exist in pairs of separately encapsulated virus-like particles in the cytoplasm. Carefully wanted for the system is the presence of the 4.6 kb L-A helper virus, which encodes the great capsid protein (Gag) and an RNA-dependent RNA polymerase (Pol). The Gag-encoding ORF1 and Pole coding ORF2 of L-A overlap in the -1 reading frame, and a programmed -1 ribosomal frameshift findings in the formation of a Gag-Pol fusion protein, which is demanded the explicative cycle of the virus[24]. The yeasts *Totiviridae* viruses shortage the extracellular path of transportation and are thus termed virus-like particles (to distinguish from viruses with an infectious cycle). Well-differentiated and functionally distinct *S. cerevisiae* toxins encoded by dsRNA viruses are K1 (encoded by M1 virus), K2 (encoded by M2 virus) and K28 (encoded by M28 virus). Klus, the dsRNA-encoded toxin was determined and considerable progress made in the properties of the Klus-encoding M and connected assistant viruses[25].

B. Yeast Cell-Virus Interaction:

Most of killer phenotypes of yeast are determined by extra chromosomal elements that are dependent on nuclear genes for its maintenance and expression. **Magliani *et al.*** [22] showed that beside *KEX1* and *KEX2* genes, which control the expression of killer phenotype by coding proteases needful for treatment the killer protein and also other yeast proteins. Moreover, other yeast genes (SEC genes) are included in the secretion of the mature toxin. **Matsumoto *et al.*[26]** described that, there are two groups of host genes can influence the propagation of L-A and M1 virus-like particles. They are super killer (SKI) genes and the conservation of killer (MAK) genes. The products of the SKI2 to SKI8 genes, named for the phenotype of the mutants, suppress the copy

number of M1 virus-like characters and the translation of its mRNA. SKI2, a great protein of helicase family, has also been observed to suppress L-A, L-BC, and the 20S ssRNA replicas. The SKI genes show to shape, a host antiviral system that is fundamental to the cell only for repressing viral propagation[27]. The host defends itself from viruses by recognizing their transcripts as viral or non-self by the absence of the 5' cap or 3' poly A tail structures and limiting their translation[24]. **Schmitt and Neuhausen**[28] investigated that, *observed that, SKI2, SKI3, and SKI8 genes have been reported to act by considerably suppressing the translation of non-poly (A), 5'-uncapped mRNAs, such as those of L-A and M1.* There are additional than 30 chromosomal MAK genes, fundamental for cell growth, are indispensable for propagation and conservation of the killer phenotype. Only three of them (MAK3, MAK10, and PET18) are required for the propagation of L-A dsRNA. The MAK3 gene encodes N-acetyl transferees, which is perhaps included in N-acetylation of mitochondrial proteins and is responsible for the acetylation of N-terminus of the great coat protein needed by L-A and M1 for viral assembly. The MAK10 product is a protein required for the best growth of the fungus on non-fermentable carbon sources, is comparable to α subunit of T-cell receptors, is needed by both L-A and M for their propagation and perhaps establishes the full viral particle[29]. The product of one of the petite genes (PET18), which is required for replication of mitochondrial DNA and for cell growth, is perhaps particle connected and contributes the stability of the viruses, as the *MAK10* productdoes[30]. **Carroll and Wickner**[31] investigated that, in an increase to the L-A encoded proteins, numerous more MAK genes are indispensable for the propagation of the killer-encoding M satellite dsRNA. These gene products involved various 60S ribosomal subunit proteins like (MAK1); an indispensable membrane-connected protein with β-transduction repeats (MAK11); and a nuclear protein needed to transit G1 (MAK 16).

C. K1, K2 and K28 Modes of Action (Figure 1):

The killer toxins have various modes of action even nevertheless; they do have one thing in combined: All viral toxins (K1, K2, and K28) kill a sensitive yeast cell in a receptor-mediated two-step process). The first step includes a rapid and energy autonomous binding to a toxin receptor within the cell wall of a sensitive target cell. In the case of K1 and K2, this essential receptor has been specified as β-1, 6-D-glucan, forasmuch the cell wall receptor for K28 is a high molecular mass α-1,3-mannoprotein. The second step was energy-determined by included toxin translocation to the cytoplasmic membrane and influence with a secondary membrane receptor. After having arrived the cytoplasm membrane, the K1 toxin exerts its lethal influence by ion channel formation and disruption of cytoplasm membrane role. The lethal influence of K1 toxin included the disturbance of electrochemical ion inclination towards the plasma membrane, which findings from the augmentation permeability for H and an uncontrolled seepage of K ions and which is followed by seepage of small molecules from the cell such as amino acids and glucose. The difference to the monophonic method of action in which K1 is a thing done from outgoing the cell, K28 appears the first viral killer toxin, which enters a sensitive target (yeast) cell by endocytosis. Next receptor- go between entry into the cell, the toxin disconnect the secretion pathway in reverse (via Golgi and ER), thereafter enters the cytosol and finally transducers its toxic signal into the yeast cell nucleus where the lethal news occur. Killer toxin K28 reasons objection of both DNA synthesis and gemmate cycle, consequently happing a loss of cell viability[21].

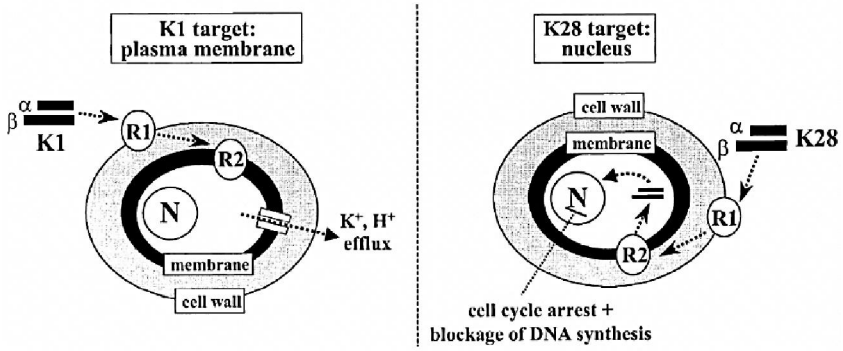

Fig. 1: Receptor-mediated mode of action of the yeast K1 and K28 viral toxins. Killing of a sensitive yeast cell is envisaged in a two-step process involving initial toxin binding to receptors at the level of the cell wall (R1) and the cytoplasmic membrane (R2). After interaction with the plasma membrane, K1 is acting from outside the cell and disrupts cytoplasmic membrane function, while K28 enters the cell by endocytosis in order to reach its final target, the yeast cell nucleus
(Source : Schmitt&Breinig, 2002)

3. Chromosomally Encoded Killer Toxins:

A. *Cyberlindnera*:

The genus *Cyberlindnera* comprises several species with well-characterized chromosomally encoded killer toxins[32]**.** Two extensively studied toxins from this genus are HM-1 and WmKT from *C. mrakii* (formerly known as *Williopsis saturnusvar, mrakii* and *Hansenula mrakii*)[33]**.** HM-1 (also known as HMK) is a 10.7 kDa basic protein, which is encoded by the *HMK* gene and exhibits remarkable thermo- and pH-stability that is probably achieved by five intermolecular disulfide bonds. HM-1 resists therapy at 100C for 10 min and stays active in among pH 2 and 1134. Previously work particularly that HM-1 binds to and prevents b-1,3-glucan synthase, a key enzyme included in cell wall synthesis35. This restrained action toward b-1,3-glucan synthesis was thought to damage cell wall resynthesize in zones of bud formation and to reasons follow pore formation and cell lysis. It was moreover explained that osmotic stabilization restrained the toxic influences of HM-1 toxin[36]**.**

B. Pichia:

Several species of the genus Pichia are known as toxin producers. Some well described Pichia killer species have been moved to various genera like *Millerozyma* or *Wicker hamomyces* produces a 19 kDa killer toxin which encourages toxic influences by forming ion-permeable channels[37]. Such ion channels were observed to finding in cell retraction accompanied by leakage of ions, adenosine-triphosphate, and a lowering of intracellular pH. A similar toxic principle was afterward assigned to a number of supplementary toxins from diverse sources. The P. kluyveri toxin is active at acidic conditions pH (2.5 and 4.7) and at temperatures below 40 oC[38.] Another species of the genus, *halotolerant* yeast *P. membranifaciens*, secretes a toxin termed PMKT (*P. membranifaciens* killer toxin), which presentations a similar toxic principle as for the P. kluyveri toxin. Meanwhile, PMKT activity is enhanced by the presence of salt. PMKT is similar to the P. kluyveri toxin in size (18 kDa) and was reported to be active against sensitive yeast cells at temperatures below 20 ºC and at acidic pH (below 4.8). It is presumed that PMKT first binds to b-1, 6-glucan as the primary receptor and subsequently reacts with Cwp2, a cell wall manno-protein39. Interestingly, the mature form of Cwp2 is covalently linked to b-1, 6-glucan, while its precursor is attached to the plasma membrane via a glycosyl phosphatidyl inositol (GPI) anchor. Consequently, it is given that interactions among PMKT and Cwp2 may assist the transportation of the toxin from its primary cell wall receptor to the cell membrane, where lethal ion channel formation occurs[40]. Transcriptional profiling of S. cerevisiae cells exposed to PMKT detected the induction of genes of the greatest glycerol (HOG) pathway, liking later monitoring substantive above for the mechanistically unrelated HM-141. Moreover, mutants' shortage in Hog1 is hypersensitive to both HM-1 and PMKT. Hence, PMKT and HM-1 both induce a coordinated transcriptional response in cells resembling the response to osmotic stress, which clearly counteracts the toxic influences of both toxins. Furthermore, studies are required to research whether both toxins have yeast killer toxins essentials and implementations, additional mechanistic similarities not yet known. Another strain of the same species (P.membranifaciens CYC1086) is known to produce a toxin (PMKT2) with diverse properties[42]. PMKT2 is greater than PMKT and exhibits various mode of action. Instead of utilizing b-glucan as the primary receptor, PMKT2 binds to mannoproteins and stops the growth of target cells by inducing an early S-phase cell cycle arrest. At low doses, PMKT2 encourage apoptotic cell death, similar to a number of mechanistically unrelated killer toxins[43.]

C. Wickerhamomyces* and *Millerozyma:

A variety of killer toxins have been characterized in various strains of *Wickerhamomyces anomalous* (formerly *Pichia anomaly)* several of which were isolated from agricultural or food sources[44]. Production of killer toxins or other growth inhibitory compounds is quite common in this species as a systematic screening detected antagonistic activities in more than 70% of *W.anomalous* strains examined from the Russian Collection of Microorganisms (VKM). **D. *Kluyveromyces, Lachancea* and *Tetrapisispora.***

Several *Kluyveromyces* species secrete toxins with various properties. *K. lactis* produces the well-described toxin zymocin, which is encoded by a cytoplasmic plasmid system. Chromosomally encoded toxins are recognized in *K.wickerhamii* (KwKt) and *K. marxianus* (K6)45. The species *K. waltii* and K. phaffii are also toxin producers and were reclassified as *Lachancea waltii* and The rapists *port phaffii*, respectively. The *T. phaffii* toxin recognized as KpKt (*Kluyveromyves phaffii* killer toxin) is a 33 kDa protein encoded by the TpBGL2 gene and prevents glucanase activity, similar to several other toxins from *W. anomalous*. KwKt and K6 were purified moreover as proteins of 72 and 42 kDa; moe, their mode of action stays unknown so far[46]**.**

4. Killer Yeast Phenomenon Applications:

Several potential applications for the killer phenomenon have been suggested, in the medical field, in plant and post-harvest diseases protection, in bio typing and in recombinant DNA technology[20]**.**

A. In Food and Fermentation Industries:

The food and beverage industries were between the first to research the implementation of the killer–toxin producing yeasts to kill spoilage microorganisms **(Lowes et al., 2000)**[34]. Yeast strains often achieve competitive characteristic by producing killer toxins, which kill off competing for species sensitive cells relationship to either the same or various species. The use of killer toxins to control yeast population during fermentation has been postulated for beer, wine and bread[47]**.** In food industry, the use of killer yeasts as starter culture could protect against spoilage yeasts[48]**.** Genetically engineered specific killer strain of *S. cerevisiae* could be used as commercial starter cultures in wine fermentation to exhibit the growth of wild strains of *S. cerevisiae* and other carefully concerning *Saccharomyces* through and next alcoholic fermentation, thereby protecting the final product from fermentation and production of a bio-film Moreover, of the possibility of industrial benefit is the result of *Osmophilic* killer yeasts, whose toxic activity was confirmed only in the appearance of a great concentration of salts such as NaCl or KCl. For example, *Osmophilic Kluyveromyces* strains with killer activity against *Zygosaccharomyces rouxii* are beneficial in improving natural keeper to prohibit re-fermentation of salted fermented foods [49]**. Santos *et al.*[50]** reported that, PMKT2, a new killer toxin from *Pichiamembranifaciiens* could be used in wine fermentation to avoid the development of spoilage yeasts. PMKT2 was able to inhibit *Brettanomyces bruxellensis* while *S. cerevisiae*, the fermentation strain, was full resistant. On the other hand, food spoilage caused by microorganisms is a serious problem for the food industry. The exploration of killer yeasts as producers of mycocins active against these undesired microorganisms seems to be promising. Hence, the use of selected killer yeasts as a bio-control method may be related to the improvement of the food industry by reducing the use of chemical preservatives.

B. In Medicine:

The results that the killer activity could be displayed versus a high variety of *eukaryotic* and *prokaryotic* microorganisms led to a re-evaluation of the yeast killer phenomena, with special emphasis on the surprising sensitivity of microorganisms of clinical benefit like *Candida albicans, Pneumocystis carinii and Mycobacterium tuberculosis*[51]. As an antifungal agent, antifungal research is presently focusing on the potential utility of yeast killer toxins as an effective antifungal agent. In the future, killer toxins might find application in the treatment of fungal infection**51.** Within this group, secreted killer toxins fundamentally produced by non-*Saccharomyces* yeasts show a broad spectrum of killing activity versus a high number of human and plant pathogens[52]**.** The killer toxin producing yeasts have a clinical significance due to the search for new anti-mycotic agents against medically important strains that cause human and animal fungal infections[52]**.** Killer toxin activity of *Pichia anomala* was reported to be fungi static for Candida albicans[53]**.** The use of selected toxins against pathogenic yeasts that cause systemic mycoses has also been suggested by **Walker *et al.*[12]**. Meanwhile, great yeast killer proteins prevent their cytotoxic activity only within a tight pH range and temperature among 20ºC and 30ºC and, subsequently, yeast toxins are perhaps not appropriate for oral and /or intravenous management, but implementations in the therapy of superficial lesions might well be possible[21]**.**

As antibacterial agent, Killer activity of yeast might operate over bacteria and could be used for the bio-control of contaminating bacteria for alcoholic fermentation[54-55]**.** It was reported that toxins from *C. glabrata, P. anomala and T. figueirae* were found to be active *against Lactobacillus plantarum* and *Bacillus subtilis*[54]**.** In addition, killer toxin of *Candidakruseii*, isolated from fermented vegetables, exhibited growth inhibition against *E.coli, S. aureus, S.typhimurum* and *B. cereus*[59]**.** The killer activity of *Saccharomyces cerevisiae* against bacterial strains was reported by **Meneghin *et al.*[55]**. Also, **Polonelli & Morace.**[54] mentioned that, *S. cerevisiae* was only capable to inhibit Gram-negative bacteria. However, the inability of K9 killer toxin from *Williopsis saturnus var marki* NCYC500 to kill *Streptococcus penumoniae* was recently demonstrated by **Ochigava *et al.*[56]**.

C. In Plant Protection and Post-Harvest Diseases Protection:

Some yeast is potential as biological control agents against plant pathogenic fungi. *Pichia membranifaciens* might have the potential to control *Botrytis cinerae*, which causes the gray mold disease[50]**.** In addition, yeast killer toxins have been shown to have inhibitory effects on some wood decay and plant pathogenic fungi. In post-harvest diseases: The suspension of *Candida guilliermondii* killer yeast was effective in reducing decay caused by *Aspergillus niger* and Rhizopus stolonifer. The suspension of *Kloeckera apiculata* killer yeast was effective in reducing post harvest decay of grape, peach, and apple fruits[57]**.** Killer yeasts are also attractive factors for bio-control objectives in agriculture. Several of the glucanase toxins from various strains of *Wickerhamomyces anomalu*s (formerly *Pichia anomala*) are described by a broad antimicrobial activity, which is pointed not only versus other yeasts but also prevents path organic bacteria or mycelia fungi and even protozoans. The activity versus mycelia fungi has been exploited for bio-control of

postharvest diseases reasons by plant pathogenic fungi on commercially serious fruits[58]. In specific, green mold disease happened by *Penicillium digitatum* improving on citrus fruit through postharvest storage could be controlled by *W. anomalous* toxin[58.] Presently potentially are undertaken to embed killer yeasts in eaten coverings made of sodium alginate and locust bean gum, which findings in great retention of the killer strain on the fruit surface and was observed to strongly decrease green mold improvement [59].

D. In Biotyping:

Accurate identification, recognition or comparison of pathogens is mandatory for epidemiology connected evaluate. Biotyping methods required to be an advantage that is sensitive, increasing, soft, and economical. Moreover, the methods that are advantage should have the implementation vision to a broad domain of distinct pathogens[60]. Killer system may be effective and inexpensive tool for yeast finger printing and could be used for intra-specific characterization of industrially and clinically interesting yeast cultures[56]. This system was necessitated to be a very functional epidemiological tool for earmarking particularly the fungal connected nosocomial infections situation[61]. The killer system has confirmed to be protective not only in the discrimination of significant slowly growing pathogenic, like the mycobacterium but also in the discrimination of faster-growing gram-positive and Gram-negative bacteria[54]. Proof that chosen killer yeasts may display their inhibitory influence on various types of molds other than yeasts induced estimation of the possibility of the yeast killer system to distinguish strains of *Pseudallescheria boydii, Aspergillus niger* and *Sporothrix schenckii.* The opportunity to distinguish members of the genus *Aspergillus* was of high value for research the effect of fungal pathogen *A. fumigatus* in hospitalized patients through the outbreak of aspergillosis[22]. The differentiation of various Candida species or other pathogenic microorganisms can be carried out by utilizing the toxins from earmarked killer yeast having a broad range of antimicrobial possibility. This process has successfully been used for differentiation/careful consistency of fungal pathogens from clinical/nosocomial sources. This process of bio-typing pathogens is believed typically safe, cost-effective, and unique particularly for the laboratory-establishments which have meager resources to suffer developed molecular methods for careful consistency. *Staphylococcusepidermidis* strains originated from Brazilian hospitals and clinical sources were characterization based on the antagonistic action of eleven selected killer yeasts[61]. Based upon the antimicrobial action of selected killer yeasts sensitive *S. epidermidis* strains and *coagulase-positive Staphylococcus* strains were distinguished with absolute actuality and reliability. Consequently, the killer yeast-based bio-typing appears a legitimate, straightforward and low-cost system for distinguishing action of pathogenic microorganisms. Molecular tools though have gained immense significance in recent years for bio-typing of yeast and other microorganisms. Meanwhile, the process based on the system of the killer/sensitive phenotype of yeast may play a vital function in supplementation of molecular identification information for yeast20. The strains of *Candida dubliniensis* from *Candida albicans* were appreciably differentiated utilizing a versioning process that was based upon sensitivity various killer toxins. Similarly, the anti-mycotic possibility of killer yeasts was exploited for categorization of fungal pathogens obtained from various environmental niches and clinical sources[63].

E. In Recombinant DNA Technology:

Killer plasmids of *Saccharomyces cerevisiae*, which code for killer toxins, have been used as cloning vectors in recombinant DNA technology for the expression of foreign protein. Killer toxins which are naturally produced and secreted by virus-infected strains of the fungal pathogen *U. maydis* have been observed to be attractive and unique model for the introduction of fungal resistance into tobacco plants[64-65-66]. As stated above, killer toxins kill sensitive cells by prevention of DNA replication, induction of membrane permeability alterations and the detention of the cell cycle. Furthermore, in some issue, a toxin can interfere with cell-wall synthesis by inhibiting-1,3-glucan syntheses or by hydrolyzing the great cell wall components, b-1,3 glucansandb-1,6 glucans. Moreover, it is yet unknown what the receptors of much other killer toxins on the sensitive cells are and how the killer toxins kill the sensitive cells66. Furthermore, small has been recognized about the connection among the structure of killer toxins, their killer activity and bounding to targets on the sensitive cells[67]. Killer plasmids of *Saccharomyces cerevisiae*, which code for killer toxins, have been used as cloning vectors in recombinant DNA technology for the expression of foreign protein. Killer toxins which are naturally produced and secreted by virus-infected strains of the fungal pathogen U. maydis have been observed to be appealing and unique model for the introduction of fungal resistance into tobacco plants[5].

Conclusion remarks:

A yeast killer toxin represents a selective property to yeast species producing it. A number of these toxins are of viral origin. As they kill sensitive cells by prevention of DNA replication, induction of membrane permeability alterations and the detention of the cell cycle. Variable genetic basis for killer

phenotype have been studied, they are either cyto-plasmically inherited encapsulated double-stranded RNA (dsRNA) viruses, linear dsDNA plasmids or chromosomal genes. Many clinical applications for these toxins have been useful in medicine, agriculture and food industries.

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