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Antimicrobial Agents Produced by Yeasts

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***Abstract*—**Natural antimicrobials are used to preserve foods that can be found in plants, animals, and microorganisms. Antimicrobial substances are natural or artificial agents that produced by microorganisms or obtained semi/total chemical synthesis are used at low concentrations to inhibit the growth of other microorganisms. Food borne pathogens and spoilage microorganisms are inactivated by the use of antagonistic microorganisms and their metabolites. Yeasts can produce toxic proteins or glycoproteins (toxins) that cause inhibition of sensitive bacteria and yeast species. Antimicrobial substance producing phenotypes belonging different yeast genus were isolated from different sources. Toxins secreted by many yeast strains inhibiting the growth of other yeast strains. These strains show antimicrobial activity, inhibiting the growth of mold and bacteria. The effect of antimicrobial agents produced by yeasts can be extremely fast, and therefore may be used in various treatment procedures. Rapid inhibition of microorganisms is possibly caused by microbial cell membrane lipopolysaccharide binding and in activation (neutralization) effect. Antimicrobial agents inhibit the target cells via different mechanisms of action.

***Keywords*—**Antimicrobial agents, Glycoprotein, Toxic protein, Yeast.

I. Introduction

THE main objective of food technology is protecting consumers from fraud, ensuring the safety of food with controlling the microorganisms. Significant risks of microbial spoilage in foods are health problems and product losses. Therefore, many techniques have been developed to minimize the microbial burden. At the same time, food fermentations are carried out by the different microorganisms like bacteria, yeasts and molds. Organic acids, hydrolytic enzymes and aroma compounds produced by microorganisms are key elements for the development of physicochemical structure, nutritional value and quality of fermented foods [1]. Contamination caused by wild type yeasts or molds, in fermentations done by *Saccharomyces cerevisiae* cause serious problems. Killer yeasts can be used to reduce this contamination during fermentation or as starter cultures [2].

Killer yeasts, are naturally found in fruits and rotten vegetables and sharing the same environment can have serious effects on the development of other flora. The yeasts showing killer features, during the production of beer and wine and food preservation are applied to combat undesired microorganisms [1]. Toxic proteins or glycoproteins (killer toxins) produced by killer yeasts, can cause the death of sensitive (killer-sensitive) strains of other yeasts. The killer phenotypes belonging variety of yeast genera were isolated from different sources [3]. According to the studies about different types of yeast, double-stranded DNA viruses that located in the cytoplasm of yeast cells, plasmids and chromosomal genes responsible for the production of some toxins. Those yeasts, bind to specific receptors on the cell surface and kill microorganisms [4], [5].

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II. Killer Yeast

Killer yeasts can produce toxic proteins or glycoproteins (killer toxins) causing inhibition of sensitive (killer-sensitive) yeast isolates or some bacteria. Killer phenotypes isolated from different sources can be found in several yeast strains [3]-[6]. Many yeast strains secrete toxins which inhibit the growth of other yeast strains. Killer (K), sensitive (S) and neutral (N) phenotypes of *Saccharomyces cerevisiae* are described by Makower and Bevan firstly [7]. Studies, lately conducted on killer yeasts have shown that many other yeast species may show killer effects. These findings have led to the search for inhibitor yeast strain in other species [6]. The majority of sensitive strains died when inhibitor and sensitive yeast strains grown together in the same medium. Whereas inhibitory effect does not occur in neutral strains when grown together with the inhibitor or sensitive strains [8]. Many yeast strains secrete toxins for inhibiting the growth of other yeast strains. These yeasts have antimicrobial activity inhibiting growth of molds and bacteria [9], [10]. Killer toxin varies among species or strains in the sense of gene structure, molecular size, development and immunity. Each toxin has different recognizing and killing mechanisms on sensitive cells [6].

The killer activity of yeasts isolated from 48 fermented food samples, in Malaysia. A total of 29 (11.5%) of the yeast isolates showed killer activity to at least one *Candida* species tested; including 22 isolates of *Trichosporon asahii,* 4 isolates of *Pichia anomala,* and one isolate each of *Pichia norvegensis, Pichia fermentans* and *Issatchenkia orientalis,* respectively. 19 yeast species were identified based on sequence analysis of the ITS1-5.8S-ITS2 partial fragments of the yeasts. The anti-*Candida* activity demonstrated by killer yeasts should be further explored for development of alternative therapy against candidiasis [1].

Yeasts isolated from livestock products and their antimicrobial activity was tested towards putrefaction and pathogenic bacteria. Antimicrobial activity tested by diffusion methods against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* and then the chosen isolate identified with using 18S RNA method. The results have

shown that the total yeast population on pasteurized cow's milk were 1.2×10^6cfu/g, fruit yoghurt $5.4x10^6$cfu/g, lamb meat 1×10^5cfu/g, beef 1×10^5cfu/g and beef sausages 1×10^6cfu/g total yeast population. Fruit yoghurt isolates shown the best antimicrobial activity with 35mm clear zone diameter against *Pseudomonas aeruginosa,* 8mm clear zone diameter against *Staphylococcus aureus* and 10mm clear zone diameter against *Escherichia coli.* The 18s RNA test shown that fruit yoghurt isolate was 100% (FR3-F primer) and 99% (FR3-R primer) identical with *Candida parapsilosis* [9].

In a study apple, cranberry, chokeberry and Lithuanian red grape wine yeast populations were used for the determination of killer yeast activity. According to the tests of the killer characteristics and immunity the isolated strains were divided into seven groups. Killer toxins purified from some typical strains and they produced different amounts of active killer toxin. Total dsRNA extractions in 11 killer strains of yeast isolated from spontaneous fermentations revealed that the molecular basis of the killer phenomenon was not only dsRNAs, but also unidentified genetic determinants [11].

Wild killer yeast strains isolated from six South African wineries and they were identified using classical taxonomic methods. They were characterized according to their cross-reactions with reference killer yeasts (K1-Kll) and by electrophoresis of their double-stranded RNA molecules. All isolates belonged to the K2 phenotype as strains of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. The killer strains differed substantially in their ability to kill a sensitive wine yeast. This killer yeasts as was shown by electrophoresis of total soluble cell proteins and gas chromatographic analysis of cellular fatty acids [12].

The killer activity of 36 yeast isolates belonging to three species of *Metschnikowia pulcherrima, Wickerhamomyces anomalus* and *Torulaspora delbrueckii.* It was tested as biocontrol agents against the wine spoilage species *Pichia guilliermondii* and *Pichia membranifaciens*. The effectiveness of the classical streak-based (qualitative method) and the new semiquantitative techniques was compared. The semiquantitative technique (60%) reveals killer activity better than qualitative method (45%). The addition of 1% NaCl into the medium allowed a better observation of the killer phenomenon. Important differences were observed in the killer activity of different isolates belonging to a same killer species. The broadest spectrum of action was detected in isolates of *W. anomala* NPCC 1023 and 1025, and *M. pulcherrima* NPCC 1009 and 1013 [3].

The killer activity of 46 strains belonging to 12 yeasts isolated from water or sediment samples. *Aureobasidium pullulans*, *Hyphopichia burtonii*, *Geotrichum candidum*, *Candida krusei* and *Candida lambica* was shown killer activity but two strains of the genus *Cryptococcus* was not showed. *Sporobolomyces salmonicolor*, *Cryptococcus laurentii* and *Cryptococcus albidus* had better activity against basidiomycetous than ascomycetous species. *Hansenula anomala* showed good activity against *Geotrichum candidum* strains, *Cryptococcus albidus*, and *Sporobolomyces salmonicolor*. *Rhodotorula* species showed activity against the majority of both ascomycetous and basidiomycetous species [13].

III. Toxins Produced by Yeast

Similar to those described for the bacteriocins, K+ (killer) phenotype of inhibitor yeast, production of these toxins in natural environments, is an advantage in competition for food against susceptible organisms [14], [15]. Antimicrobial compounds which can inhibit the growth of bacteria and molds are organic acids (hexanoate, octanoate and decanoate) and proteins [9]. Inhibitory effects in yeast were discovered specific killer strains of *Saccharomyces cerevisiae* in 1963 and it is associated with the secretion of the toxin, which is in a protein structure kills target sensitive cells with a receptor without direct cell to cell contact. Cytoplasmic RNA of killer phenotype encodes the protein toxins depends on the presence of the killer virus inbaker's yeast. Until now, K1 and K28 are most commonly two killer toxins were examined [16]. Killer yeasts produce polypeptide toxins having killing activity against sensitive yeasts belonging to the same genus or different genera. *Candida, Cryptococcus, Hanseniaspora, Kluyveromyces, Pichia, Torulopsis, Ustilago, Williopsis* and *Zygosaccharomyces* species were determined to produce killer toxins [2].

The production of yeast killer toxin is a well-established phenomenon among many yeast genera and species. The killer activity is readily detectable only when a suitable sensitive strain is tested. There is evidence that killer yeasts may secrete different killer toxins with activities specific for different yeast target cells [14]. Used in the fermentation of *Saccharomyces cerevisiae* wild yeast or fungal infection causes serious problems. Killer yeasts can be used to reduce this contamination or as a starter culture [2]. Spontaneous fermentations are carried out by a mixed microbial culture, including yeast, mold or bacteria. Organic acids, hydrolytic enzymes and aroma precursors produced by microorganisms are necessary to improve the physicochemical, nutritional and characteristic features of fermented foods. Killer yeasts are widespread in the natural structure of the fruit and putrescence vegetables and they can have a significant impact on the composition and development of other flora who share the same environment. Inhibitor properties of yeast have been used for food preservation and combating undesired microorganisms during the production of beer and wine [1].

Many yeast genera including some *Candida* species produced exotoxins. These toxins in the form of protein or glycoprotein, by binding to specific receptors on the cell surface can be killed mold, bacteria and protozoa. The toxin K28 of *S.cerevisiae,* lethal toxin secreted out of the cell, going through the cytoplasmic membrane by binding to the target cell walls, through the golgi and endoplasmic reticulum reaches the cytosol. An alpha unit of toxin transmitted to yeast cell nucleus and produces toxic signal and ultimately inhibit DNA synthesis stops at the border of the cellulose. Killer activity has emerged in temperatures as low as 25-28°C and

4.6-5.6 acidic pH [4]. Also some of the extracellular proteases obtained from yeasts such as *Candida lipolytica* [9].

The secretion of protein toxins is a widespread characteristic in environmental and laboratory yeast isolates called "killer system". The killer phenotype (K^+) can be encoded by extrachromosomal elements (EGEs) as double stranded DNA or RNA molecules (dsDNA, dsRNA) or in nuclear genes. Yeast strains *S.cerevisiae* and *P.anomala* isolated from environmental, industrial and clinical sources have not been shown same effect, but certain one from among them have killer activity and these strains are determined as K+ phenotypes. Analyses were performed in strains belonging to three yeast genera used as sensitive cells and under a wide range of pH and temperatures. Approximately 51% of isolates tested showed toxicity against at least one sensitive yeast strain under the conditions tested. The K^+ *P. anomala* isolates demonstrated a wide spectrum of action and two of them had toxic activity against strains of the three yeast genera tested, including *C. albicans* strains. In all *S. cerevisiae* K^+ isolates an extrachromosomal dsRNA molecule (4.2 Kb) was observed, contrary to *P. anomala* K^+ isolates, which do not possess any EGEs. The K^+ phenotype is produced by an exported protein factor and the kinetics of killer activity production was same in all isolates with high activity in the log phase of growth, decaying in the stationary phase [15].

Brettanomyces bruxellensis is a major problem for winemakers. Sulphur dioxide (SO2) was used for preventing this yeast, but it can elicit allergic reactions in some consumers. Biological alternatives are therefore actively sought. CpKT1 and CpKT2, which are killer toxins, isolated from wine, called *Candida pyralidae*. The two proteins had a molecular mass above 50 kDa and exhibited killer activity against several *B. bruxellensis* strains particularly in grape juice. They were active and stable at pH 3.5-4.5, and temperatures between 15-25°C which are suitable for winemaking conditions. Moreover, the activity of these killer toxins was not affected by the ethanol and sugar concentrations typically found in grape juice and wine [17].

Toxin secreting strains of killer yeasts were initially identified more than 40 years ago in *Saccharomyces cerevisiae* strains infected with a double-stranded RNA "killer" virus. The mechanism of protecting immunity by which toxin-producing cells evade the inhibitory activities of these proteins has remained elusive. The mechanism leading to protecting immunity in a killer yeast secreting a viral α/β protein toxin (K28) that enters susceptible cells by receptor-mediated endocytosis and, after retrograde transport into the cytosol, blocks DNA synthesis, resulting in both cell-cycle arrest and caspase-mediated apoptosis. Toxin immunity is effected within the cytosol of a toxin-secreting yeast and occurs through the formation of complexes between reinternalized toxin and unprocessed precursor moieties that are subsequently ubiquitinated and proteasomally degraded, eliminating the active form of the toxin. Interference with cellular ubiquitin homeostasis, either through overexpression of mutated ubiquitin or by blocking deubiquitination, prevents ubiquitination of toxin and results in an impaired immunity and the expression of a suicidal phenotype. The results presented reveal the unique elegant and efficient strategy that killer cells have developed to circumvent the lethal effects of the toxin they produce [16].

Killer yeasts isolated from flowers of Indian medicinal plants and their killer toxin was determined on sensitive yeast cells as well as fungal pathogens. The toxin of *Saccharomyces cerevisiae* and *Pichia kluyveri* inhibited *Dekkera anomala* accumulating methylene blue cells on Yeast Extract Peptone Dextrose agar (*p*H 4.2) at 21°C. *S. cerevisiae* and *P. kluyveri* were found to tolerate 50% and 40% glucose, while *D. anomala* tolerated 40% glucose. Both *S. cerevisiae* and *P. kluyveri* was not inhibited the growth of *Aspergillus niger*, C*andida albicans* and *Fusarium* spp. [2].

The interactions between 20 killer yeasts of various genera and species were examined. Ten distinct groups were recognized with respect to killer activity and 10 distinct groups with respect to resistance to killer action. Using both killing and resistance phenotypes, 13 classes of killer yeast were found. With the exception of *Torulopsis glabrata* NCYC 388, non-*Saccharomyces* strains of yeast were not killed by a member of the genus *Saccharomyces*.The killer character of the 3 killing groups of *Saccharomyces* identified could be cured by treatment with cycloheximide or incubation at elevated temperature and the effectiveness of these procedures was indicative of the category of killer yeast examined. Killer yeasts not belonging to the genus *Saccharomyces* could not be cured of their activity. Double-stranded ribonucleic acids were extracted only from *Saccharomyces* spp. and the molecular weights of the species present were a function of the killer class to which a strain belonged. By an analysis of the effects of proteolytic enzymes, temperature and pH on killer activity and by gel chromatography of crude preparations of killer factors, the toxins of different killer classes were shown to be biochemically distinct. However all toxins had certain properties in common, consistent with there being a protein component essential to killer action [18].

Streptococcus pneumonia represents an important human bacterial pathogen, and the increase in antibiotic resistance demands the development of new antimicrobial compounds. Several reports have suggested that yeast killer toxins show activity against bacteria and we therefore investigated the activity of K9 killer toxin from the yeast *Williopsis saturnus* var. *mrakii* NCYC 500 against *S.pneumoniae*. However, no inhibition of bacterial growth was observed with concentrated K9 preparations in agar diffusion assays and in liquid culture. Although the cell morphology was slightly affected by K9 treatment, no effect on cellular viability was detectable, and K9 had no stimulatory effect on cell lysis induced by beta-lactams or TritonX100. This indicated that K9 did not contribute to cell wall damage. Moreover, flow cytometry was used as a sensitive assessment of the integrity of cells exposed to killer toxin. No significant damage of *S. pneumonia* cells was evident, although minor changes in fluorescence suggested that K9 killer toxin may interact with bacterial surface components [19].

IV. Conclusions

Research on killer yeasts for industry is almost new. Killer yeasts can produce toxic proteins or glycoproteins causing inhibition of sensitive yeast isolates or some bacteria. Many yeast strains secrete toxins which inhibit the growth of other yeast strains. This increasing knowledge will allow the selection or construction of industrial yeasts with killer activities targeted against a wide range of wild-type yeasts.

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