Study some properties of a killer toxin AY, a new killer toxin produced by Saccharomyces cerevisiae AY

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Abstract

In this study ,a killer toxin AY produced by *Saccharomyces cerevisiae* AY which have anticandida effect was partially purified by ammonium sulfate precipitation and the results showed that the killer toxin AY exhibited activity of 80 AU/ml against *Candida albicans* OR. Moreover, some properties of it was determined and the results showed that its active principle was proteinaceous in nature since it was inactivated by proteolytic enzymes. Killer toxin AY was heat –labile up to 37°C and its activity was stable at a narrow range of pH values (3-5).The effect of NaCl on Killer toxin AY was also investigated and the results showed that its activity gradually increased from 80 AU/ml in absence of NaCl to 320 AU/ml in the presence of 2-3.5% of it /.

Introduction

In contrast to antibacterial antibiotics, bacteriophages and bacteriocins, that were described at the beginning of the last century, a similar antibiotic phenomenon in yeast was only demonstrated much later .

In 1963, Bevan and Makower discovered the killer phenomenon in a *Saccharomyces cerevisiae* strain which was isolated as a brewery contaminant [1] . Killer yeasts were thereafter shown to be widespread amongst laboratory strains. These early observations led to the search for killer yeasts in other genera. Killer activity has been reported in almost 100 yeast species belonging to more than 20 genera and their number is increasing [2] .The best studied killer species is *S. cerevisiae*, which secretes different types of killer toxins [3] . The killer yeasts belonging to this species have been classified into three main groups (K1, K2, and K28) on the basis of the molecular characteristics of the secreted toxins, their killing profiles, the lack of cross-immunity, and the encoding genetic determinants [4,5] .

Studies on the nature of the killer factor produced by *Saccharomyces* have shown that this protein has a highly specific action spectrum and is dependent on specific pH, temperature and aeration conditions . The activity of the toxin is only highly stable in a narrow pH range (4.2-4.7) . The stability of the killer toxin decreases with elevation of temperature and is unstable at pH values above 5.0 [6].However, studies of a variety of killer toxins have shown that the characteristics of the K1 killer toxin and other toxins tend to be similar ,most of them are protease-sensitive heat labile proteins that are stable and active only at acidic pH values [7] , the molecular weights of them ranging from 5000-10,000 to 100,000 Da or even greater in a few cases [8] , while the genetic bases of killer characters are quite variable. There is also diversity in the mode of action of killer toxins [7] . Through the following years , killer yeasts and their toxins found applications in several fields. For example, killer yeasts have provided an interesting model for studying the mechanisms involved in the processing and secretion of extracellular proteins, and the identification of killer toxin receptors on the envelope of sensitive targets has helped in the elucidation of the structure and function of the yeast cell wall. Killer yeasts have been used to combat contaminating wild yeasts in the food and fermentation industries, and to control wood decay basidiomycetes and plant-pathogenic fungi .In the medical field, these yeasts have been used in the biotyping of pathogenic yeasts and yeast-like fungi , and in the development of novel antimycotics for the treatment of human and animal fungal infections [9] .

This study was aimed to partially purification and determination some properties of killer toxin AY, a new killer toxin from *S. cerevisiae* AY .

Materials and Methods

Yeast isolates and culture media

The killer toxin AY producer was *Saccharomyces cerevisiae* AY(commercial brand of ready powdered yeast from local market), the indicator yeast was *Candida albicans* OR, that isolated previously from oral cavity (Department of biology/College of Science/Al-Mustansiriya University) . Yeasts were inoculated and subcultured monthly on Sabouraud dextrose agar (SDA) (Hi-Media / India) slopes at 25 °C for 48 h and then maintained at 4°C until required [10] . For activation of yeasts, *C.albicans* OR was grown in Sabourouds dextrose broth (SDB) [11] and *S.cerevisiae* AY was grown in Glucose yeast extract peptone broth(GYEPB) [5] at 25 °C for 48 h .

Partial purification of killer toxin AY

The method of De la Peña *et al.* [12] was used with some modifications, partially purified killer toxin AY was obtained from culture of *S.cerevisiae* AY grown at 25 °C in GYEPB (pH 5) for 48 h. Culture was centrifuged at 6000 rpm for 15 min. at 4°C, the supernatant was precipitated with 80% (w/v) ammonium sulfate , the mixture was stirred for 2h at 4°C and later centrifuged at 8000 rpm for 20 min at 4°C ,the precipitate was resuspended in 0.1M citrate phosphate buffer pH 4.2 and dialyzed against the same buffer for 24h at 4°C. killer toxin activity against *Candida albicans* OR was carried out by serial two-fold dilutions of partially purified killer toxin AY and assayed by the well diffusion method, Wells were cut in seeded SDA using a sterile borer (6mm diameter), then filled with 100 µl of dilutions of partially purified killer toxin AY. The plates

incubated at 25 °C for 2-3 days [10] and expressed in arbitrary units (AU) . One AU is defined as the reciprocal of the highest dilution that still resulted in a minimal level of detection [13] and calculated as: (1000 / 100) × D, where 1000: constant, 100 : volume of toxin in a well (µl) and D : the dilution factor [14] .

Effect of proteolytic enzymes

The method of Kagiyama *et al.* [15] was used, with some modification, aqueous solutions of 2mg/ml of pepsin, (Philip Harris Biological Ltd/UK), papain (BDH/England) and trypsin (Merck /Germany) were prepared .A volume of 125 µl of each enzyme solution was mixed with 500 µl of partially purified killer toxin AY then incubated for 6h. at 25 °C. Controls were performed with denatured enzymes ,heated at 100°C for 15 min. After incubation killer toxin activity was assayed [10] .

Temperature and pH stability of the killer toxin

Samples of partially purified killer toxin AY were incubated at a range of different temperatures: 25, 30 , 37 ,40 and 45°C. Aliquots (100 µl) were removed at specific intervals (30 , 60 and 120 min) .For stability to pH, Samples of partially purified killer toxin AY were adjusted to a pH range of 2 to 8 (at increments of one pH unit), all samples were incubated at 25°C for 18 h. After incubation killer toxin activity was assayed [10] .

Effect of NaCl

Solutions of NaCl concentrations (0, 1, 1.5 , 2, 2.5 , 3 and 3.5) % were prepared. A volume of 500 µl of each concentration was mixed with 500 µl of partially purified killer toxin AY then incubated for 6h. at 25 °C. After incubation killer toxin activity was assayed [10] .

Results and Discussion

Many types of salts have been employed to effect protein separation and purification through salting-out . Of these salts, ammonium sulfate has been the most widely used chemical because it has high solubility and is relatively inexpensive [16]. Since ammonium sulfate precipitation is a procedure used for the concentration of proteins [17] , partial purification of killer toxin AY was performed by precipitation with 80% ammonium sulfate . The killer activity of the toxin against *Candida albicans* OR was increased from 40 AU/ml for supernatant to 80 AU/ml for partially purified killer toxin AY (Figure 1) . Hodgson *et al.* [10] reported that addition of ammonium sulfate to culture medium stimulate protein synthesis and stabilize any killer factor produced . Since the killer toxins known so far are proteins or glycoproteins [18] , the effect of proteolytic enzymes on the killer toxin AY was assayed . killer toxin AY was totally inactivated by treatment with pepsin, papain and trypsin, indicating that killer toxin AY is protein in nature like other killer toxins [7,15,18,19] .

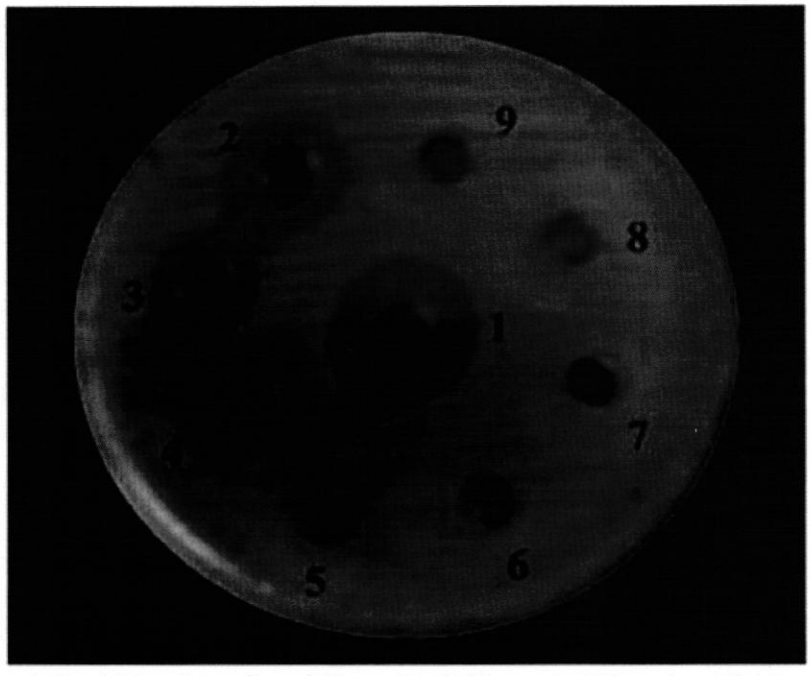

Figure 1: Antimicrobial activity of partially purified killer toxin AY produced by Saccharomyces cerevisiae AY against Candida albicans OR by the agar well diffusion assay (1: partially purified killer toxin AY, 2 - 9 : serial two-fold dilutions of partially purified killer toxin AY)

The stability of all killer toxins is strongly dependent on pH and temperature [19] . As shown in table 1, the killer activity was stable at 25 ,30 and 37°C for 30 , 60 and 120 min., while whole activity was lost at 40 and 45°C for all incubation periods used in this study. When proteins are exposed to increasing temperature,

losses of solubility or activity occurs over a fairly narrow range. Depending upon the protein studied and the severity of the heating, these changes may or may not be reversible [20] .

Table 1:Effect of temperature treatment on the killer toxin AY activity produced by Saccharomyces cerevisiae AY against Candida albicans OR

Temperature (°C)	Incubation period (minutes)	killer activity (AU/ml)
25	30	80
	60	80
	120	80
30	30	80
	60	80
	120	80
37	30	80
	60	80
	120	80
40	30	0
	60	0
	120	0
45	30	0
	60	0
	120	0

Figure 2 illustrates the pH effect on killer toxin stability, the killer toxin was stable at pH values 3, 4 and 5 but it retained 25 and 50 % of its activity at pH values 2 and 6, respectively. No activity was observed at pH values 7and 8. At lower pH values , the solubility is often increased , less aggregation of hydrophobic peptides occurs, and thus , more molecules should be available to interact with sensitive cells [21] .

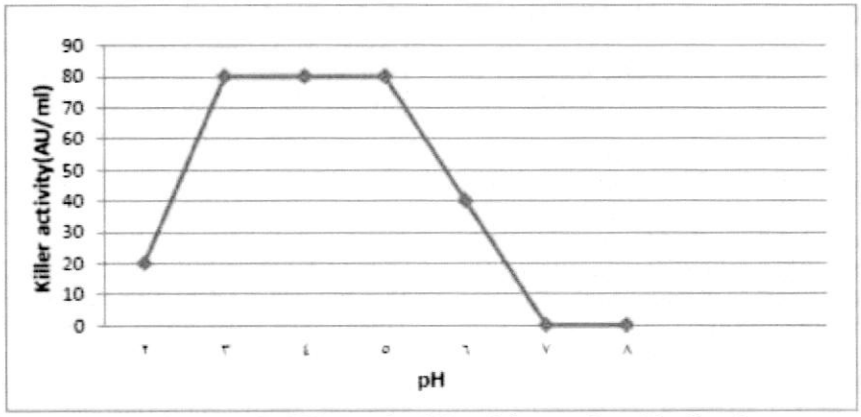

Figure 2: Effect of pH treatment on the killer toxin AY activity produced by Saccharomyces cerevisiae AY against Candida albicans OR

Such as most of the killer toxins [6,18], killer toxin AY was unstable at both high temperatures and high pH values. Marquina *et al* . [3] reported that the toxins from all killer strains studied are protease-sensitive, heat-labile macromolecules and most of them are stable and act only at acidic pH values .

The effect of NaCl on Killer toxin AY was also investigated and the results showed that its activity increasing gradually with increasing of salt concentration .

In absence of NaCl, the killer activity of toxin was 80 AU/ ml while at 1 and 1.5 % of NaCl, the activity was increased to 160 AU/ ml , and to 320 AU/ml in the presence of 2- 3.5% of the salt (figure 3) . The reasons for such an increased vulnerability of the sensitive cell to the toxin's action remain to be elucidated. It is well known that the composition of the yeast plasma membrane and, therefore, its permeability depend on the specific growth rate . It has also been reported that killer toxins induce the formation of ion-permeable channels in lipid bilayer membranes. The disruption of the ionic equilibrium across the plasma membrane may well lead to an increased mortality of the intoxicated cells in the presence of NaCl [22] .

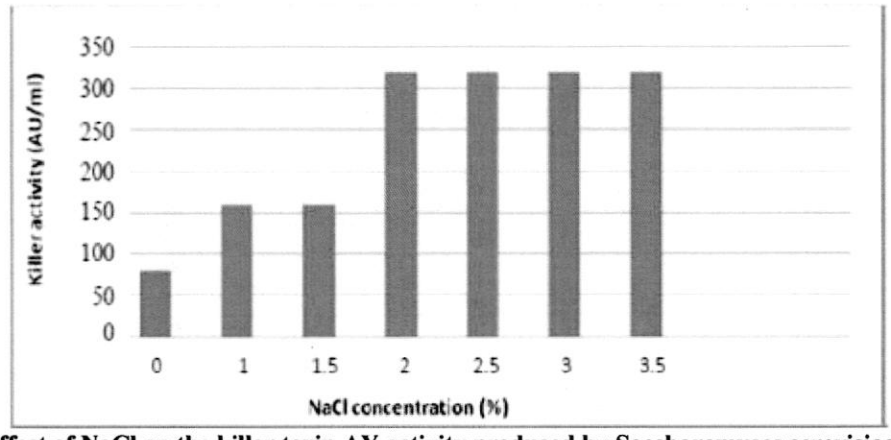

Figure 3: Effect of NaCl on the killer toxin AY activity produced by Saccharomyces cerevisiae AY against Candida albicans OR

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دراسة بعض صفات السم القاتل AY ، سم قاتل جديد منتج من خميرة الخبز *Saccharomyces cerevisiae* AY

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الملخص

تمت في هذه الدراسة تنقية السم القاتل AY المنتج من خميرة الخبز *Saccharomyces cerevisiae* AY والذي يمتلك فعالية ضد الكانديدا تنقية جزئية بواسطة الترسيب بأملاح كبريتات الامونيوم ، وأظهرت النتائج أن فعالية السم القاتل AY بلغت 80 وحدة / مل ضد *Candida albicans* OR . تم أيضا تحديد بعض صفات السم القاتل AY وأظهرت النتائج انه ذو طبيعة بروتينية إذ فقد فعاليته بعد تعرضه للإنزيمات المحللة للبروتين ، وهو حساس لدرجات الحرارة الأعلى من37 ْم ، وأظهرت فعاليته استقرارا في مدى ضيق من الرقم الهيدروجيني (pH) ما بين 5-3 .تمت أيضا دراسة تأثير ملح الطعام NaCl في السم القاتل AY ، وأظهرت النتائج أن الفعالية القاتلة للسم ازدادت تدريجيا من 80 وحدة / مل بغياب الملح إلى 320 وحدة / مل بوجود 3.5%-2 منه .