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Possible Involvement of Plasmids in the Expression of Killer Toxins of *Saccharomyces cerevisiae*

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Four isolates of *Saccharomyces cerevisiae* were selected in this study for their high ability to produce killer toxin, to investigate possible correlation between their plasmids contents and killer activity. Plasmid's profiles on agarose gel revealed presence of two plasmids in each of four isolates. Yeast cells obtained after plasmid curing experiments at elevated temperature showed that cured isolates lost their killing capacity. The plasmid cured yeast cells originated from three yeast isolates (Scs7, Scvi8 and Scf14) showed the possible correlation between plasmid curing and loss of killing ability, whereas isolate Scf4 retained the killing ability in absence of plasmids. Moreover, the extracted plasmid samples were shown to be sensitive to RNase treatment. These results might suggest that the genetic determinants for toxin production in three isolates (Scs7, Scvi8 and Scf14) of selected killer's yeasts of S. cerevisiae are encoded by dsRNA plasmid(s) except one isolate (Scf4), in this isolate the killer protein may be mediated by chromosome.

Keywords: *Saccharomyces cerevisiae*, Killer toxins, Plasmids.

Killer yeasts are yeasts that secrete a number of toxic proteins which are lethal to receptive yeast cells. The term is used to describe certain strains of *Saccharomyces cerevisiae* which produce a protein toxin lethal to sensitive strains of the same genus and some other related genera[1].

Genetic studies showed that killer toxin phenomenon of *S. cerevisiae* is linked with presence of three types of toxins (K1,K2 and K28) encoded by different cytoplasmically inherited satellite dsRNA (M1,M2 and M28) and encapsidated in virus like particles (VLPs)[2]. It was also established that M-dsRNA is dependent on another group of helper virus (L-A) for their replication and capsidation, so M-dsRNA particles are responsible for killer activity and self immunity[3].

Two other killer toxins were described in *S.cerevisiae* differ in their thermo stability, designated KHR (killer of heat resistance) and KHS (killer of heat sensitive), in which the genetic determinants are located on chromosomal DNA[4].

Several yeasts including *S. cerevisiae* were identified to play a significant role in control of a range of important pathogenic fungi of agronomic, environmental and clinically aspects[5], and might have other application against yeasts that might cause contamination trouble in food industry[6].

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Therefore, the objective in this study was to determine the possible involvement of plasmids or chromosome in the killer activity of *S. cerevisiae* isolated from local habitat, as start step for further studies to use the promising potentials of killing system of *S. cerevisiae* in biological control and industrials fermentation.

MATERIAL AND METHODS

Yeast isolates

Four local isolates of *S. cerevisiae* were selected in this study (Scf4, Scs7, Scvi8 and Scf14) which exhibit high ability to produce killer toxin protein, and were active against the following:

Acinotobacter sp.(gram negative bacteria), *Lactococcus* sp.(gram positive bacteria), three isolates of *Candida albicans*, and two isolates of *Cryptococcus neofermaus* provided from Central Laboratory of Ministry of Health, Iraq.

Plasmid extraction

The extraction was performed according to Soares and Sato[7], then an aliquot of each extract samples were analyzed on 1% agarose gel[8].

Curing test

Curing test was done using Soares and Sato method[7] with some modification as following:

Killer yeast cells were grown in 30 ml of YEPD medium (20g glucose, 19g peptone, 19g yeast extract and 1L D.W.), and were incubated at 28ºC (Control), 37º C and 42º C for three days. After incubation period the plasmids were isolated and the profile was examined.

Killer activity assay

Killer activities of the yeasts were assayed before and after curing experiment to determine the possible curing of the killer activities among untreated (control) and cured isolates by using two methods, first method by growing on YEPD-MB agar[9] and second method was carried out by using cap assay method[10].

RNase digestion

Plasmids extracted from agarose gel were treated with RNase enzyme (Sigma, England) with final concentration of 300µg /ml for 1hr at 37°C and then analyzed on 1% agarose gel[9].

RESULTS AND DISCUSSION

Killer toxin genes may be mediated by either virus like particles, plasmid or chromosome[7], in this study, results revealed that all selected isolates of *S.cerevisiae* had two plasmid bands (Fig. 1,A,B,C,D). Same results were obtained by Soares and Sato[7] and Wickner[11], Who showed that the large band is L-dsRNA and the small bands represent M1,M2 or M28 dsRNA, and were identified as plasmids.

However, when the killer local yeast isolates were cured after exposure to elevated temperature (37 º C and 42ºC), three of killer toxin producing isolates of *S. cerevisiae* (Scs7, Scvi8 and Scf14) lost their killing capacity after treatment at 42ºC, only Scf4 kept its ability against tested yeast with observed reduction in killing capacity (Table 1).

Table 1. Results of plasmid curing experiment expressed as decrease in the percentage of the diameter of zone of inhibition of test yeast and bacteria, in comparison with control (28°C)

Curing temp./ Test strains	Killer's toxin producing strains of *S. cerevisiae*							
	Scf4		Scs7		Scvi8		Scf14	
	37 ºC	42 ºC	37 ºC	42 ºC	37 ºC	42 ºC	37 ºC	42 ºC
Acenitobacter sp.	45.4	-	-	-	42.8	-	-	-
Lactococcus sp	38.8	-	-	-	-	-	-	-
C. albicans (A)	60	50	77.7	-	86.3	-	89.4	-
C. albicans (B)	79.1	46.6	75	-	71	-	85	-
C. albicans (C)	82.3	61.76	46.4	-	65.9	-	52.3	-
C. neofermaus	55.8	66.66	48.6	-	52.3	-	55	-
C. neofermaus	80	-	60	-	46.4	-	70.5	-

(-): No zone of inhibition

The results of treatment of yeast cells at 37ºC showed that the test yeast strains were still sensitive to the killer activity but with significant decrease in their activity. Moreover , not all producing isolates of *S. cerevisiae* inhibited the growth of test bacteria at 37ºC. So, the obtained result showed that the test bacteria were more resistance to killer toxin than test yeast and the Scf4 producing isolate was more resistant to curing than other isolates.

Many investigators showed that the killer potential is only observed on gram positive bacteria[12,13], however the present results showed that the spectra of killer toxin was on gram positive and negative bacteria (Table 1).

Further analysis was done to ascertain the possible involvement of plasmids with killer toxin production. The cured cells were subjected for plasmid isolation and the results indicated that all heat treated isolates lack plasmid bands (Fig. 1,A*,B*,C*,and D*).

It is clear From the above mentioned results that the killer activity in isolate Scs7, Scs8 and Scs14 is most probably coded by plasmid , so, the activity disappeared completely at 42ºC , this was accompanied with losing the plasmid bands, whereas isolate Scf4 may produce two types of toxin determined genetically by genetic determinants located both on plasmids and chromosomes. Moreover it is possible to suggest

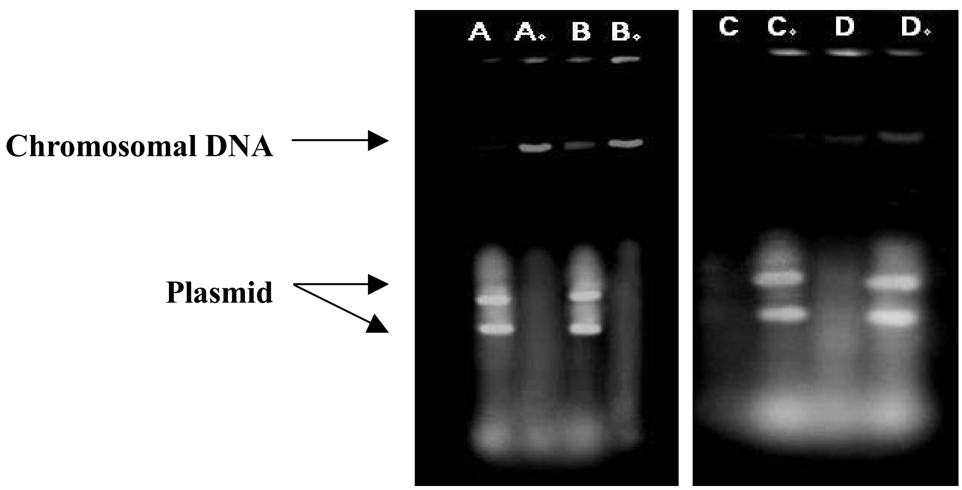

Fig. 1. Plasmid profiles on 1% agarose gel of uncured (A,B,C,D) and cured (A*,B*,C*,D*) isolates of *S.cerevisiae* Scf4, Scs7, Scvi8, and Scf14 respectively.

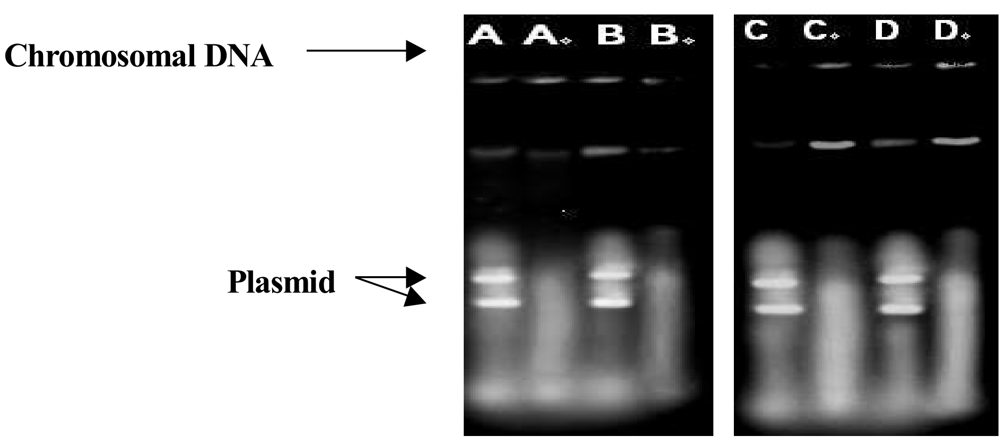

Fig. 2. Gel electrophoresis of RNase digested plasmids(A*,B*,C*,D*) of *S. cerevisiae* isolates Scf4,Scs7,Scvi8 and Scf14 Compared with undigested once(A,B,C.D)

that killer activity might come from constitutive effect of these determinants and the reduction in killer activity might arise from lack of plasmid expression.

Other suggestions in respect of the reduction on Scf4 activity may related to an alteration in some genes like KRE or KEX1, because killer activity character is governed by dsRNA plasmid and at least 12 chromosomal genes which encode protease necessary for processing the protoxins[14], as well as the proteins which are required for assembly of encapsid's cell wall[15]. Furthermore, growing the producing isolate Scf4 at 42º C may alter the plasmid encoded protein, thus, the killer yeast will secrete inactive toxin or with altered recognition site required for protein processing[15].

To evaluate whether the isolated plasmids are made of RNA or DNA, sample of plasmids were treated with RNase and gel electrophoresis analysis showed that the plasmid bands were disappeared completely and clear long smears were exhibited (Fig. 2,A*,B*,C*,and D*), these results were expected because killer character in this genus encoded mainly by dsRNA[16].

More analysis is required to determine the main character of purified toxin especially with isolate Scf4, to analyze the restriction profile and the sequences of plasmid and of most important the possibility of designing specific primer for detection the killer toxin genes.

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