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Short Communication

Isolation of Yeasts from Various Food Products and Detection of Killer Toxin Activity *In vitro*

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Abstract

Yeasts produce killer toxin, which gives them a selective advantage against susceptible yeasts. In this study we isolated 8 various non-pathogenic yeasts from 12 different food products and detected killer toxin activity in them against a clinical isolate of *Cryptococcus neoformans* by chloroform exposure method. The experiment was performed in two sets that had 3 replicates each. The first set plates and the second set plates were exposed to chloroform once and twice respectively. Among the 8 yeasts, 5 yeasts namely *Sachharomyces cerevisiae, Candida pintolopesii, Candida tropicalis, Pichia anomala* and *Dekkera spp.* showed significant amount of killer toxin activity against *Cryptococcus neoformans* in all the replicates, followed by *candida parapsilosis* and *Trichosporon asahii*, which showed killer toxin activity only in two of the three replicates. *Geotrichum candidum* showed no killer toxin activity. Killer toxin activity was observed in vitro in non-pathogenic yeast strains against medically important *Cryptococcus neoformans*.

Keywords: Yeasts; Killer toxin; Chloroform exposure.

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1. Introduction

Yeasts are ubiquitous in environment and at least 200 species of yeasts have been found in food [1]. The possibility exists that yeasts interference, competition and mutualism are both important determinants of the yeast communities and are related to variations among the habitats in which the yeast co-exist [2].

Killer Toxin production is a frequently realized intra and interspecies strategy among yeasts to restrict the growth of competitors [3]. Killer activity has been reported in almost 100 yeast species belonging to more than 20 genera and their number is increasing [4].

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The killer character confers a considerable advantage on yeast cells in competitive situations against other sensitive microbial cells in their ecological niches. This advantage has various scientific and commercial applications [5]. Killer yeasts have been used in biotyping of pathogenic yeasts as well have been proposed as antimycotic agents [6].
Our study is aimed at finding killer phenotypes in yeast species isolated from various food samples and interaction of killer toxin with medically important yeast, *Cryptococcus neoformans*.

2. Materials and Methods

2.1. *Isolation of yeast from various food products*

The food samples used for isolation of yeasts were olive brine, honey, chavanprash, lime juice, orange juice, soya sauce, saffron, grapes, bakers yeast, sour dough, curd and milk. They were kept in Sabourauds Dextrose Broth (SDB) and were incubated for 24 hours at 37°C. The suspension from the broth was streaked on Sabourauds Dextrose Agar (SDA) by quadrant streak method and their replicates were incubated at 37°C and room temperature for 1-3 days till isolated colonies appeared.

The isolated yeasts were identified by using HiCandida Identification Kit (HiMEDIA Laboratories Pvt. Ltd.), Germ tube production, morphology on corn meal agar and ascospore production.

2.2. *Screening for killer toxin*

Methylene Blue Agar (MBA) plates were taken and the diameter of the plates were streaked with the yeast strains isolated from various food samples. Plates were incubated for 48 hours at 37°C. The plates were exposed to chloroform in two sets having three replicate each. First set of plates were exposed to chloroform only once for a duration of 15 minutes and second set of plates were exposed to chloroform twice for a duration of 15 minutes and 30 minutes, respectively, to see whether longer duration of exposure to chloroform has any effect on the killer toxin activity. The killer strain was scrapped off gently with the help of a sterile microslide without breaking the agar. Next, with the help of a bacteriological loop, the test strain of *Cryptococcus neoformans* clinical isolate was streaked over the scrapped area. Plates were incubated for 48 hours at 37°C and checked for any growth inhibition of the test strain near the scrapped area, which denotes the killer toxin activity.

3. Results

From the 12 various food samples, 8 different types of yeasts namely *Saccharomyces cerevisiae*, *Candida pintolopesii*, *Candida parapsilosis*, *Candida tropicalis*, *Pichia*

anomala, Dekkera spp., Geotrichum candidum and *Trichosporon asahii* were isolated as shown in Table 1.

Table 1. Showing various types of yeast isolated from their respective food samples.

Organism	Food product
Saccharomyces cerevisiae	Grapes
Candida pintolopesii	Grapes
Candida parapsilosis	Lime juice
Candida tropicalis	Curd
Pichia anomala	Sour dough
Dekkera spp.	Orange juice
Geotrichum candidum	Milk
Trichosporon asahii	Olive brine

Among the 8 yeasts isolated from the food samples, 5 species of yeasts namely *Sachharomyces cerevisiae, Candida pintolopesii, Candida tropicalis, Pichia anomala* and *Dekkera spp*. showed killer toxin activity against *Cryptococcus neoformans* in all the three replicates of both the sets, while *candida parapsilosis* and *Trichosporon asahii* showed killer toxin activity only in two replicates in both the sets. Though *Geotrichum candidum* showed no killer toxin activity in any of the replicates of the first set, it showed growth inhibition in *Cryptococcus* neoformans in one of the replica of the second set.

Table 2. Showing growth inhibition in Cryptococcus neoformans due to killer toxin activity of different yeasts isolated from the food samples in both the sets of experiment.

Yeast Isolate	Set 1			Set 2		
	Replica 1	Replica 2	Replica 3	Replica 1	Replica 2	Replica 3
Saccharomyces cerevisiae	+	+	+	+	+	+
Candida pintolopesii	+	+	+	+	+	+
Candida parapsilosis	+	+	-	+	+	-
Candida tropicalis	+	+	+	+	+	+
Pichia anomala	+	+	+	+	+	+
Dekkera spp.	+	+	+	+	+	+
Geotrichum candidum	-	-	-	+	-	-
Trichosporon asahii	+	+	-	+	+	-

'+' denotes growth inhibition and **'–'** denotes growth

There were no significant difference in results of single exposure to chloroform (set 1) and two exposure to chloroform (set 2). The result of the screening for killer toxin activity in both the sets is shown in Table 2.

4. Discussion

Though the killer phenotype was discovered in the 1963 [2,3,7-9], there is yet a lot to be explored on the killer toxin production of non-pathogenic strains. The probability that a killer toxin produced by yeast may kill certain susceptible yeasts would also depend on the ecological characters such as the region, the host plant and the habitat from which the killer yeasts were collected [10]. In particular, fruits appear to be a very important habitat for the killer phenomenon in yeast communities, since one quarter of the yeast strains isolated from them are killers. This habitat is characterized by low pH and high sugar concentration [2]. So was seen in this study where killer phenotypes were isolated from fruits and fruit juices.

Yeast being ubiquitously found in the environment was successfully isolated from the various food products and significant killer toxin activity was observed in *Saccharomyces cerevisiae*, *Candida pintolopesii*, *Candida tropicalis*, *Pichia anomala* and *Dekkera spp.*, most of which are medically non important. There was no significant difference observed in the killer toxin activity in longer duration of exposure to chloroform.

Although in vivo yeast killer toxin activity was demonstrated, but its relevance to the mechanism of infection associated with killer yeasts, its involvement in pathogenicity, the virulence of toxigenicity itself and the elimination of the susceptible resident microbiota remains questionable [2] and there is a need to carry out more research on killer toxin activity of non pathogenic yeasts on various pathogenic strains of yeasts.

Thus to find a killer toxin from a non pathogenic strain is of utmost medical importance in combating fungal diseases, avoiding many toxic effects associated with anti-fungal medicines. Even killer toxin activity can be a base for development of simple and low cost typing systems.

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