



December 2024

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### Recommended Citation

Aldabbagh, Hiba Hadi; Al-Barhawee, Najwa Ibrahim; and Abed, Faten Noori Mula (2024) "Purity and Characterize the Killer Proteins Produced by *Saccharomyces cerevisiae* Strains," *BioMed Visions Journal*: Vol. 1: Iss. 1, Article 5.

DOI: <https://doi.org/10.69513/bmvj.v1.i.1.a5>

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## REVIEW

# Purity and Characterize the Killer Proteins Produced by *Saccharomyces cerevisiae* Strains

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## ABSTRACT

Identified killer toxin proteins from *Saccharomyces cerevisiae* and characterized by HPLC, Therefore, electrophoresis was performed to extract and characterize the protein on a polyacrylamide gel by method SDS – PAGE, The protein bands appeared on the gel, and determined was k28 which secreted by *S. cerevisiae* depend on molecular weight, which was estimated at 5 kilodalton. It was used in subsequent studies, and the confirmed this toxin by using HPLC which compared with the standard sample. Using bioinformatics In the analysis of the results, were identified the atomic structure and the crystalline shape of the three-dimensional protein by Pymol analysis program and also determined the K28 protein sequence which consists of 2040 nucleotides that code for the amino acids that forming the protein, and then translated protein sequence into the amino acids that forming the protein. It have ability to kill other yeast species and control yeasts that cause the spoilage of beverages and industrial foods.

**Keywords:** *Saccharomyces cerevisiae*, killer toxin K28, High-performance Liquid chromatography

## 1. Introduction

Most of the toxic substances produced by many yeasts are killer toxins of a Proteinaceous nature (Belda et al., 2017; Banjara et al., 2016). These toxic proteins were identified in *Saccharomyces cerevisiae*, and it was noted that they have the ability to kill other yeast species and control on yeasts that cause rot of drinks and industrial foods (Chessa et al., 2017). As well as their inhibition of phytopathogenic fungi thus protecting the plant from infection with these pathogens (Corbaci and Ucar, 2018). This yeast has three different types of toxins K1, K2 and K28, which have been small protein compounds with different molecular weights. Recently discovered two other types of killer toxins, but they are less effective in killing than the first three types. The killing effectiveness of K1 and K2 They are usually obtained by bonding with a compound  $\beta$ -1-6-D-glucan, this present in the wall of sensitive cells, thus disrupting

the action of the ion barrier of the plasma membrane of the target cell, While K28 is associated with  $\alpha$ -1-3 It is associated with the mannose sugar in the protein present in the sensitive cell wall, and thus this toxin enters through the Golgi apparatus and the endoplasmic reticulum, and after reaching the cytosol hydrolyze subunit  $\beta$  into small sub-units  $\alpha$  type it enters the nucleus by diffusion into the cell, the killing mechanism of this toxin depends mainly on the inhibition of DNA synthesis (Begum and Mohamudha, 2010), and these toxins have a killer efficacy against non-producing toxin strains, (Orentaite et al., 2016). *S. cerevisiae* was used in medical applications, and its positive effect was noted when given to children with acute diarrhea, as well as its ability to prevent intestinal infection with bacterial pathogens, especially *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Yersinia enterocolitica* and *Candida albicans* (Al-Dulaimi, Al-Tarjuman and Mulla Abid, 2020).

Received 21 August 2024; accepted 30 November 2024.  
Available online 8 December 2024

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<https://doi.org/10.69513/bmvj.v1.i.1.a5>  
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**Table 1.** Components of the kit used to prepare the SDS-PAGE gel.

Total volume	10 ml	5 ml
30% Acr/Bis(29:1)	4 ml	0.83 ml
1M Tris-Hcl (pH 6.8)	0	0.625 ml
1.5 M Tris HCl (pH 8.8)	2.5 ml	0
10% SDS	100 ml	50 ml
10% gel coagulator	100 ml	75 ml
Coagulation accelerator	10 ml	7.5 ml
dd H2O	3.3 ml	3.42 ml
Separation Range	12–60 KD	—

**Aim:** Investigation of killer toxins that protein nature from *Saccharomyces cerevisiae* and study characterized of it.

## 2. Materials and methods

### 2.1. Preparation of *S. cerevisiae* filtrate

The filtrate was prepared by growing *Saccharomyces cerevisiae* that isolated from tangerine and orange fruit in liquid YPD medium with 6.5 PH. The liquid was incubated for 48 hours at 35 °C and then centrifuged at 5000 r/min for 20 minutes at 4 °C, and the filtrate was sterilized using Millipore filter with diameter 0.22 m $\mu$ , and the filtrate was kept at 4 °C until use (Al-Dulaimi, Al-Tarjuman and Mulla Abid, 2020).

### 2.2. Separate the proteins from the *S. cerevisiae* filtrate

Polyacrylamide gel electrophoresis (SDS-PAGE) method was used to separate proteins from two pre-prepared *S. cerevisiae* filtrate, according to the following steps:

### 2.3. SDS-PAGE gel preparation

The gel was prepared according to the information available in the kit whose components are shown in Table 1, and it was supplied by a Solarbio Life Sciences Company.

Dissolved 1 g of PAGE coagulator gel in 10 ml of Double-distilled water to make a solution 10% concentrate. If sediments appear, must expose it to a temperature of 37 °C until complete solvent, depending on the molecular weight of the protein to be separated, that chosen the concentration of the gel. The solution stored at –20 °C until use.

### 2.4. Preparation of coomassie blue stain

The stain was prepared according to the following steps:

**Fig. 1.** The kit used to separate microproteins.

1. Add 100 ml of glacial acetic acid to 450 ml of ultrapure water.
2. Dissolved 3 gm of Coomassie stain in 450 ml of methanol.
3. The solution was filtered before use.

### 2.5. SDS-PAGE electrophoresis of protein

The electrophoresis was carried out using a buffer SDS-PAGE Loading Buffer (5X) which consisted of 10% SDS, 500 mm DDT, 50% glycerol, 250 mm Tris-Hcl and 0.5% bromophenol blue (loading dye), at pH = 6.8.

### 2.6. Method of work according to the manufacturer's instructions

1. Put of a protein loading buffer SDS-PAGE (5X) at room temperature until dissolve or it is dissolve in a water bath at a temperature not exceeding 30 °C.
2. Mix one volume of Protein Loading Buffer SDS-PAGE (5X) with four sizes microliter of protein filtrate sample.
3. Put The sample in the water bath until boiling for 3–5 minutes in order to denature the protein, then put it in the water bath for an additional 3–5 minutes.
4. The sample was left to cool at room temperature.

**Table 2.** Components of the kit used to separate microproteins.

The components	The size
bufferA	10 ml
Buffer	60 ml
dry powder	9.6 g
DTT	40 mg
filter column	20
collection tube	20



**Fig. 2.** Device HPLC.

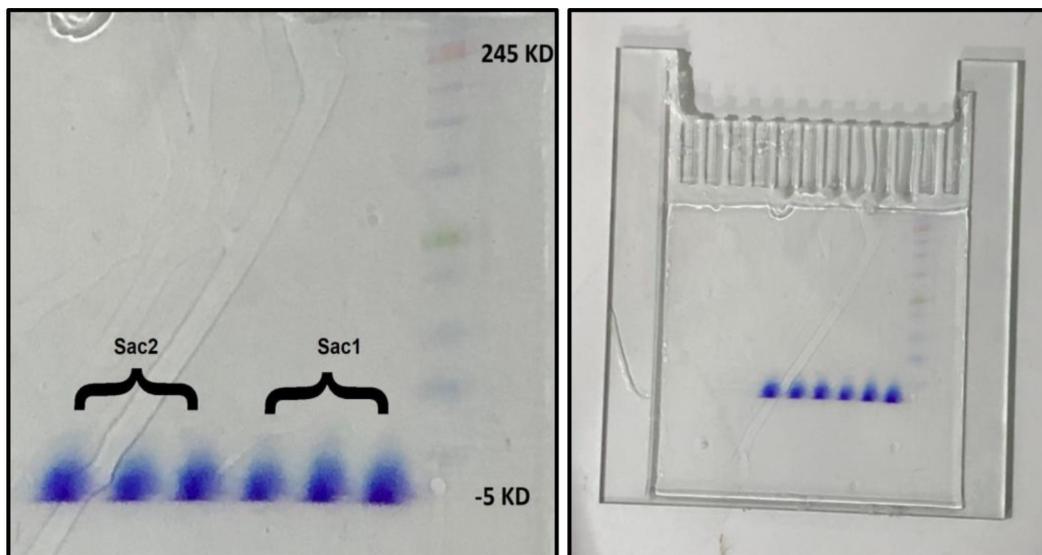
5. The sample was loaded into the pits of SDS-PAGE gel and it was electrophoresis.
6. The electrophoresis was stopped when the bromophenol blue stain started moving from the front to the end of the gel.

### 2.7. Extraction of micro protein PAGE method

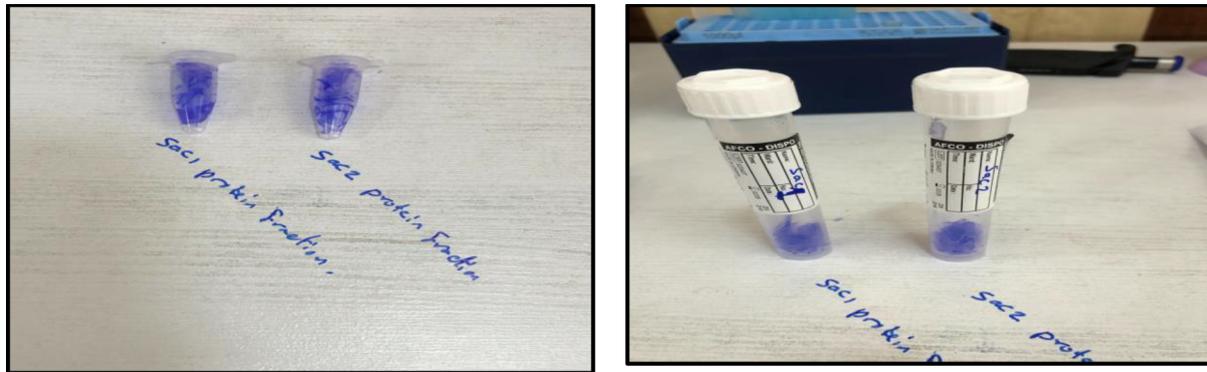
After completing the migration on a polyacrylamide gel, the gel was removed and dyed with Coomassie blue stain for about two hours, after which the separation process by using a special kit as shown in Fig. 1 prepared by Solarbio Life Sciences consisting of the materials as shown in Table 2.

### 2.8. Detection of protein K28 using a high-performance Liquid Chromatography (HPLC)

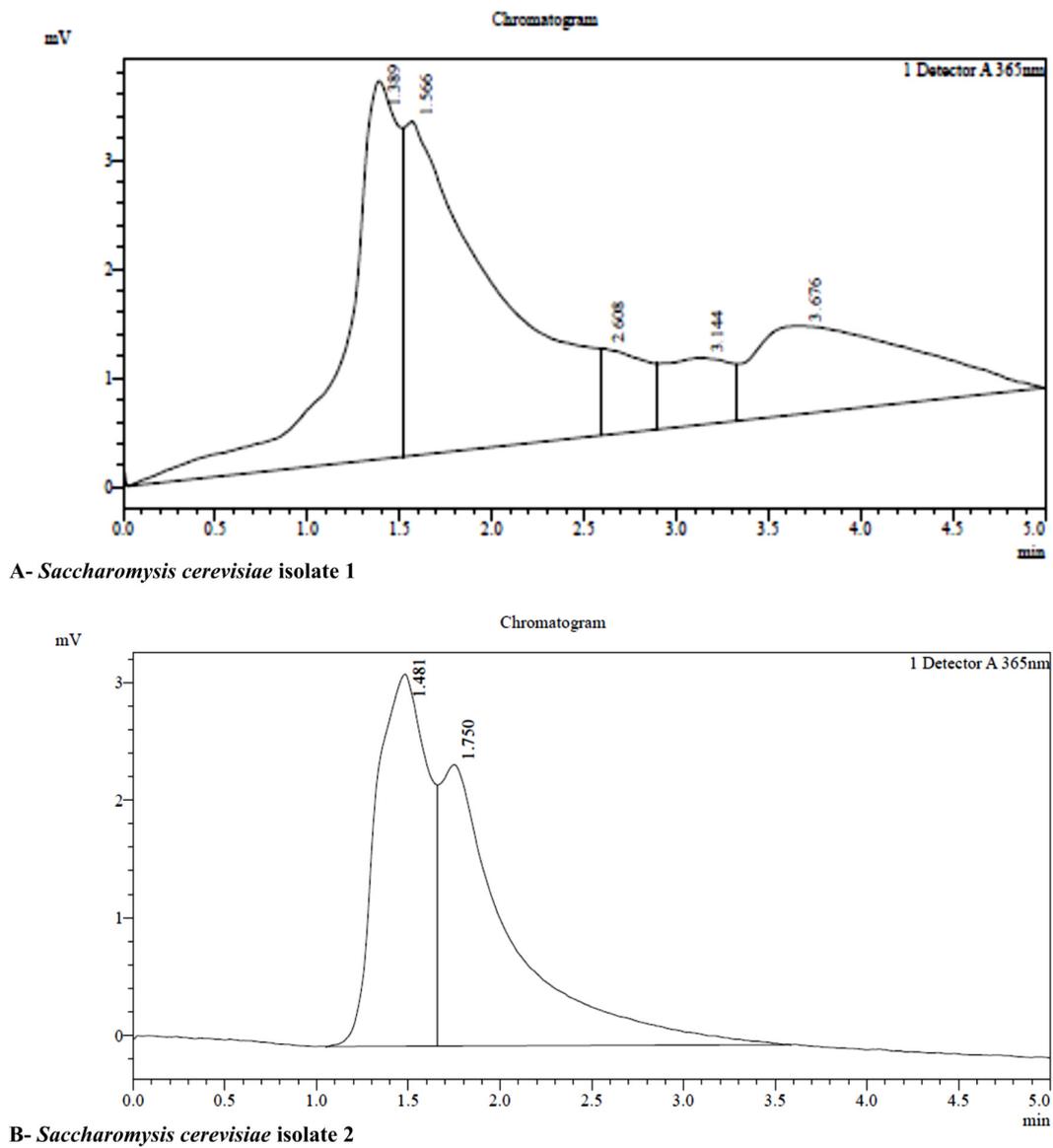
K28 protein is detected in the filtrate of baker's yeast using the HPLC as shown in Fig. 2 and extracted by electrophoresis method on a polyacrylamide gel with a molecular weight of 5 kilodaltons by (Extraction protein) technique, and using column C18 at the mobile phase (Methanol: water) with a volume of 80:20 respectively, at a rate flow 0.8 ml/min and at a wavelength of 365 nm and were injected about 20 microliters of the proteins extracted in the previous paragraph.



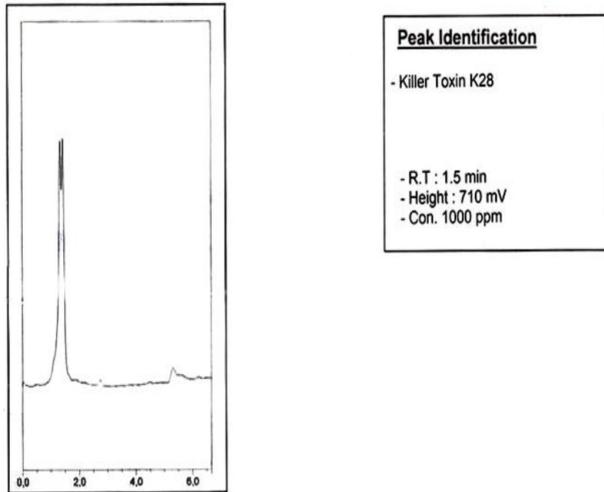
**Fig. 3.** Protein bundles are a type K28 after electrophoresis on a polyacrylamide gel.



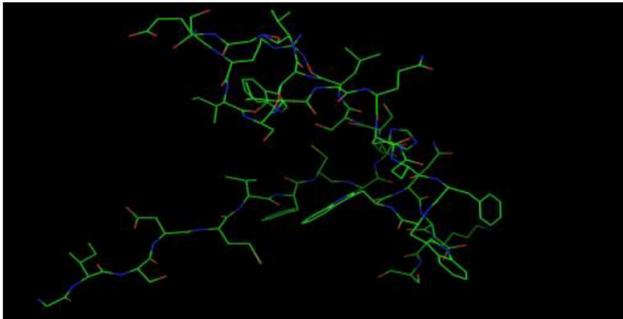
**Fig. 4.** Extracted protein K28 from the gel Determination of protein type using high-performance liquid chromatography (HPLC).



**Fig. 5.** Separation of protein K28 using HPLC from two isolates of *Saccharomyces cerevisiae*, isolate (1,2) at a retention time of 1.389 and 1.481, respectively, at a wavelength of 365 nm, at a flow rate of 0.8 ml/min, in a mobile phase of methanol: water 80:20.



**Fig. 6.** Standard protein sample K28.



**Fig. 7.** The atomic structure of a protein K28 using Pymol analysis.

### 2.9. Use bioinformatics for results analysis

A program Pymol Analysis was used to determine the atomic structure and crystalline structure of the protein extracted from baker's yeast, as well as de-

termination of the sequence of the nitrogenous bases constituting this protein.

## 3. Results and discussion

### 3.1. Protein separation by electrophoresis method

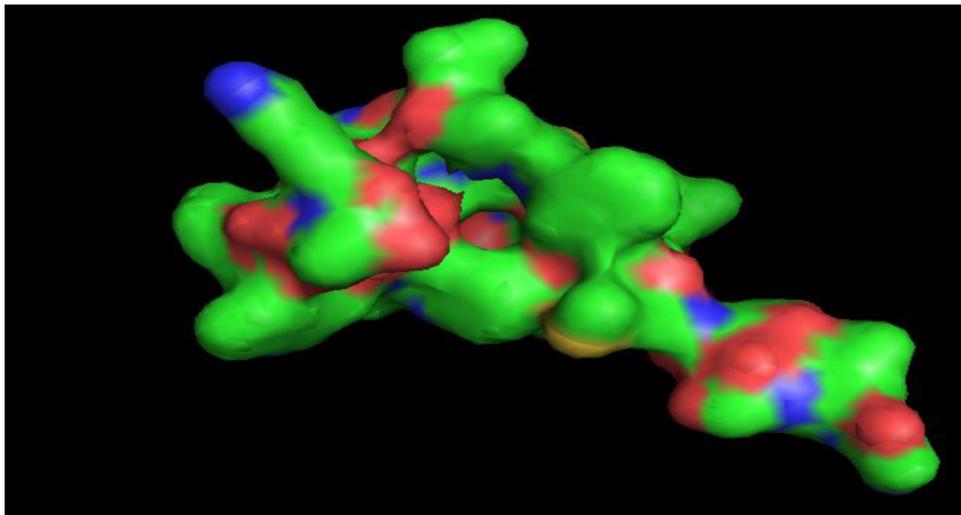
#### 3.1.1. Protein electrophoresis on a polyacrylamide gel

The electrophoresis of the proteins from the filtrate of two Baker's yeast (1 and 2) isolates on a polyacrylamide gel SDS-PAGE using Coomassie Brilliant Blue stain, and after the electrophoresis process, the protein bands appeared on the gel, and the protein was identified based on the molecular weight, which was estimated at 5 kilodaltons, and it was found to be a type of killer toxins secreted by baking yeast type K28 this result agree with (Begum and Mohamudha, 2010). As shown in the Fig. 3.

### 3.2. Extraction of protein

The proteins were extracted according to the instructions provided in the special kit, as shown in Fig. 4, and the pure protein was kept after obtaining in the refrigerator at a temperature of 4 °C until was used. subsequent experiments.

A high-performance liquid chromatography device was used to confirm the quality and quantity of Killer Toxin type 28 protein. And observed that the curve highest of was at the retention time 1.389/min in yeast isolate1, and at 1.481/min in yeast isolate 2 as shown in Fig. 5A,B and it belonged to the killr toxin type 28 compared to the standard sample K28 with retention time 1.5/min as shown in Fig. 6, this indicates that it has been completely separated from the first peak and that it has a high molecular weight and high purity.



**Fig. 8.** The three-dimensional crystalline form of the protein K28 using Pymol analysis.

### 3.3. Analyze the results according to bioinformatics

Bioinformatics was used to determine the protein atomic structure of protein K28 that extracted from the filtrate of baker's yeast, and it was noted that it possesses many free terminal ends as shown in Fig. 7 that have the ability to bind to receptors on the surface of the target cell to be able to enter the cell and kill it.

The crystalline shape of the protein and its three-dimensional structure were also identified as shown in Fig. 8, which were determined based on the wrapping pattern in the protein, which is one of its general

characteristics, the presence of a large number of disulfide bonds with in the molecule, which is likely to contribute to the extreme thermal stability and the characteristic pH of some toxins.

The nucleotide sequence of protein K28 was determined by reverse bioengineering and it was shown that it consists of 2040 nucleotides and is responsible for encoding the formation of the amino acids constituting this protein, as every three nucleotides is a codon that encodes for one amino acid, in addition to translating the sequence Nucleotides to the amino acids that make up the protein, as shown in Fig. 9A,B.

#### A- *Saccharomyces cerevisiae* (Killer toxin type 28)Nucleotides seq.

```
ATGCTGAGGTTCTGACCAAGAACAGCCAGGACAAGAGCAGCGACCTGTTTCAGCATCTGCAGCGACAGGGGCACCTTCGT
GGCCCAACAGGGTGAGGACCGACTTCAAGTTCGACAACCTGGTGTCAACAGGGGTACGGCGTGAGCCAGAAGTTCA
CCCTGGTGGGCAACCCACCGTGTCTCAACGAGGGCAGCAGCTACCTGGAGGGCATCGCCAAGAAGTACTGACCCTG
GACGGCGCCTGGCCATCGACAACATCCTGAACGAGCTGAAGAGCACCTGCGGCATCCCGGCAACGCCGTGACCAGCCA
CGCTACAACATCACCAGCTGGAGGTGGTACGACAACACGCTGGCCCTGCTGATGAACATGCTGAGGGCCCTACCACCTGC
AGGTGTGACCGAGCAGGGCCAGTACAGCGCCGGCAGTACCCCATGTACCACGACGGCCACATCAAGATCAAGCTGGAC
GTGGCCGTGGCCGACGACAGCGCCCAACGGCTTCAGGTGGCCCGGCGACAGGGTGAGCGACAGCTTCCCGAGTGGGG
CCAGTTCAGCGAGAGCTTCCCGAGCATCGAGCTGCCCTACATCGACGTGAGGCCCTGACCCGTGACCGAGGTGAAGTTCG
TGCTGATGATGATGAGCAAGTGGCACAGGAGGACCAACCTGGCCATCGACTACGAGGCCCCGTGCTGGCCGACAAGTTC
GCCTACAGGCACGCCATCACCGTGCAGGACGCCGACGAGTGGATCGAGGGCGACAGGACCGACGACAGTTCAAGCCCCC
CAGCAGCAAGGTGATGCTGAGCGCCCTGAGGAAGTACGTGAACCAACAACAGGCTGTACAACAGTTTACACCGCCGCC
AGCTGCTGAGCCAGATCATGATGAAGCCGTGCCAACTGCGCCGAGGGCTACGCCTGGTGTGACGACGCCCTGGTG
AACATCCCCAAGTTCGGCAGCATCAGGGCAGGTACCCCTTCTGCTGGCCGGCGACGCCCCCTGATCCAGGCCACCCG
CCTGGAGACTGGAGGCCATCATGGCCAAGCCCGAGCTGATCTTACCTACGCCATGCAGGTGGCCGTGGCCCTGAACA
CCGGCCTGTACTGTGAGGGGTGAAGAAGACCGGCTTCGGCACCACTCGACGACAGCTACGAGGACGGCGCCTTCTCTG
CAGCCCGAGACCTTCGTGACGGCCCTGGCCTGCTGCACCGCCAGGACGCCCCCTGAACGGCATGAGCGACGTGTA
CGTGACCTACCCGACCTGCTGGAGCTGACCGCCTGACAGGGTGCCCGTACCGTGTGACCGCCGCGGCTACAACA
TCGTGGACGGCCCTGGAGGTGACCGCGTGCCTACGCTGCAGCCCTACATGATCTTCCCGTGGCCGCTTCGAC
AAGGCCAACCCCTACAGCGCAACTTCGTGATCCAGCCGCCCTGAAGTACCTGAGGAAGGGCCCTGTACGACAAGCT
GGAGGCCTGGAAGCTGGCCATGAGGATCGCCGGTACGACACAGCTTCAAGGCCTTCGGCGACGTGCACGGCC
TGACCAAGTTTACGCCGACAACAGCGACAGCTGGACCCATCCCGAGTTCGTGACCGACGGCGACATCATGGAGGTG
TACGTGACCGCCATCGAGAGGAGGGCCAGGCACTTCGTGGAGCTGCCAGGCTGAACAGCCCGCCTTCTTCAAGAGCGT
GGAGGTGAGCACCACTTACGACACCTACGTGCAGGCCGGCAGCTTACGCGTGTACCACGCCAGCAGGATCAACCTGG
ACTACGTGAAGCCCGTGAAGCCGGCATCCAGGTGATCAACGCCGGCAGCTGAGGAAGTACTGGGGCAGCGTGAGGAGG
ACCCAGCAGGGCCTGGGCGTGGTGGCCCTGACCATGCCCGCGTGTATGCCACCGCGAGAGGACCGCCGACCGCCCA
CGAGGAGCTGATCGAGCAGGTGGACGAGGTGAGCGTGGAG
```

#### B- *Saccharomyces cerevisiae* (Killer toxin type 28) protein translate seq.

```
MLRFVTKNSQDKSSDLFISCDRGTGFVAHNRVRTDFKFDNLVFNRYVGVYQKFTLVGNPTVCFNEGSSYLEIAKKYLT
DGLLAIDNINLNLKSTCGIPGNAVTSWAYNITSWRWYDNHVALLMNLRLAYHLQVLTQQQYSAGEYPMYHDGHKIKLD
VAVADDSAPNGFRWPGRVSDSFPFEWAQFSEFSPIDVPYIDVRLTVTEVNFVLMMSKWHRRNLDAIDYEAPVLADKF
AYRHAIYQDADEWIEGDRDQFKPPSSKVMLSALRKYVNHNLRYNQFYTAQQLSQIMMKPVPNCAEGYAWLMDALV
NIPKFGSIRGRYPFLLAGDAALIQATALEDWSAIMAKPELIFTYAMQVAVALNTGLYLRRVKKTFGTTIDDSYEDGAF
QPETFVQAALACCTGQDAPLNGMSDVVYTPDLELDALTRVPVTVIEPAGYNIVDGALEVTGVPACSPYMIFVAAF
KANPYSGNFVIQALKYLRKALYDKLEAWKLAWMRIAAGYDTSFKAFGDVHGLTKFYADNSDSWTHIPEFVTDGDIMEV
YVTAIERRARHFVELPRLNSPAFFKSVEVSTTIYDYVQAGSFSVYHASRINLDYVKPVSAGIQVINAGELRNYWGSVRR
TQQGLGVVGLTMPAVMPTGERTAGTAHEELIEQVDEVSVE
```

Fig. 9. A: nucleotide sequence of the 28 K protein extracted from *Saccharomyces cerevisiae*, B: translation of the protein from the sequenced nucleotides.

## 4. Conclusion

This research has demonstrated that the yeast *Saccharomyces cerevisiae* contains the killer toxin protein K38 and the success of bioinformatics technology in determining the sequence of its amino acid components.

## Acknowledgment

We are grateful to the University of Mosul for allowing us to conduct this study and have it published in your prestigious journal.

This research is specific to the participants and is not related to any other research, nor does it contradict the results of previous research by other researchers.

## Conflicts of interest

No any conflict of interest.

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