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A COMPARATIVE MOLECULAR STUDY OF THE GENE ENCODING THE ENZYME CYTOSINE DEAMINASE EXTRACTED FROM SACCHAROMYCES CEREVISIAE

Muna A. Mutter[1], Milad A. Mezher[2], Zainab Y. M. Hasan[3]

[1,2]Coll. Edu.Pure Sciences\ Univ. Tikrit, Salah al-din , Iraq, munaamutter@gmail.com
[3]Biotech. Res. Center \ Univ. Al-Nahrain, Baghdad – Iraq.

Abstract

This study's experiment searched for to extract the Cytosine Deaminase enzyme from Saccharomyces cerevisiae bread yeast from a variety of commercial varieties that were readily available in local markets, including Turkish, Iranian, and Russian varieties in addition to the manually produced Iraqi yeast, Another objective of the study was to perform a comparative molecular analysis of the nucleotide sequences of the genes encoding this enzyme in the yeast species under consideration. According to the findings, the enzyme's specific activity in the raw extract for the Turkish type was 10.3 units/mg of protein, for the Iranian type it was 11.32 units/mg of protein, and for the Russian type it was 9.51 units/mg of protein. The specific activity for the local type was 13.205 milliunits/mg of protein. In order to complete the next steps of enzyme purification, which involved precipitating using ammonium sulphate at saturation rates (60%), the local Iraqi yeast was selected because it exhibited the highest specific activity when compared to other types. This allowed the enzyme's specific effectiveness to reach 17.68 units/mg of protein. There have been 1,338 purifying cycles. 7,553 is the enzyme yield. the results of the molecular study of the nucleotides present in the gene encoding this enzyme showed that the size of the gene segment extracted through electrophoresis amounted to 120 base pairs. The results of the comparison in GenBank also demonstrated that the gene studied and in all types of yeast studied had mutations. (Variations) in the studied nitrogenous bases. This indicates that it is a new registration for the first time due to mutations found in the nitrogenous bases. These mutations were recorded in the American GenBank under the accession numbers: PP278029, PP278030, PP278031, and PP278032.

Key word: Saccharomyces cerevisiae, Bread Yeast, Cytosine Deaminase, Molecular study.

Introduction

Saccharomyces cerevisiae, the yeast that makes bread, is a significant microbe that is high in antioxidants because it includes compounds that boost immunity (1, 2,). Being one of the most common kinds of cyst fungus in the natural world, it is also a useful tool for the majority of initial studies on these creatures, which frequently oversimplify things. It offers evidence that all eukaryotic biological processes are present and conserved in bread yeast, making it a useful model for eukaryotic cells as they contain Numerous fundamental structures are present, including 12,068 kilobases of nuclear genomic DNA arranged in 16 chromosomes and about 6,000 genes, of which 5,570 are predicted to be protein-coding genes (3,4). Yeast plays a role in the breakdown of more complex carbohydrates into glucose and fructose. Enzymes convert carbon dioxide and ethanol, and they also produce proteins, vitamins, and lipids (5,6). The majority of fungi are capable of converting the nucleic acid cytosine into the isotopes uracil or thymine by removing the amino group from it. Consequently, the substance 5-fluorocytosine, which is used as an antifungal medication for specific types of fungi, is sensitive to them and is converted to the compound 5-fluorouracil. Bacillus cerevisiae Yeast is unique in that it can generate a large number of hydrolytic enzymes. To competition, parasitism, and the host cell wall (7,8). Among the significant enzymes in the hydrolytic deamination category is Cytosine Deaminase. The enzyme is distinguished by its capacity to eliminate the amine group from 5-methylcytosine by hydrolyzing it and converting it to either Thymine or Uracil, a process that occurs in both coliform bacteria and bread yeast (9,10). Additionally, it functions to change the chemical 5-fluorocytosine (FC-5) into 5-fluorouracil (FU-5). One of the most well-known medicines used in chemotherapy to treat various forms of cancer is this molecule, a fluorinated pyrimidine that resembles the naturally occurring nitrogenous base uracil (11,12). The bases of the nucleotides adenine, guanine, cytosine, and uracil in a DNA molecule can be arranged in a variety of ways using DNA sequencing, as is well known. Mutants of baking yeast that do not have Cytosine Deaminase activity exhibit tolerance to high quantities of 5-fluorocytosine FC-5 and are unable to convert it to 5-fluorouracil (FU-5), which is its counterpart. The Cytosine Deaminase enzyme's entire activity is lost due to this resistance to the altered FCY1 gene, which also inhibits the cells' capacity to metabolize 5-fluorocytosine into any other chemical (13,14). The chemical approach (Maxam–Gilbert method) and the chain termination method (Sanger dideoxy method), which was used in this study (15,16,17), are the two primary widely recognized methods used in DNA sequencing. Because of its potential therapeutic use, the Cytosine Deaminase gene was cloned and identified from primitive species. The encoding gene was then transferred to higher organisms, including plants and some mammals (18, 19).In this investigation, the local manually produced kind of Cytosine Deaminase was contrasted with the commercially available, purified form suitable for use in

baking. The goal of the work was to clone and describe the FCY1 gene, which encodes the Cytosine Deaminase enzyme, in bread yeast at the genetic level for each type and compare its genetic sequence.

Material and Methods

Yeast samples used in the study

The S. cerevisiae bread yeast isolates used in this study were as follows: the first type was Turkish, the second was Iranian, the third was Russian, and the fourth was handmade local (Iraqi) yeast; the first three types were purchased premade from nearby markets.

Extraction and purification of Cytosine Deaminase

The procedure defined by Ipata and Cerignani in 1978 was implemented. For the purpose of extracting the enzyme, 100 grams of premade bread yeast for each type was combined with 50 milliliters of toluene solvent. The mixture was then placed in a water bath at 45°C for an hour, allowed to sit at room temperature at 25°C for three hours, and finally 100 milliliters of cold distilled water was added to the mixture. The mixture should be put in a glass separating funnel, shaken vigorously for 30 minutes, and then incubated for 18 hours at 4°C. After filtering the mixture, gather the separated aqueous phase and centrifuge it for 20 minutes at 4°C at 10,000 rpm in a chilled centrifugeTests were performed to determine the protein concentration and the activity of the enzyme cytosine deaminase after the filtrate was discarded. The four varieties of yeast crude extract were subjected to the following purification procedures (20,21). The extract was precipitated by adding ammonium sulfate to the crude extract, and then the dialysis process was carried out (22).

Molecular Study

DNA Extraction

In order to study the detection of the gene encoding the enzyme Cytosine Deaminase, DNA was extracted from the four types of bread yeast S. cerevisiae using the steps of the Wizard® Genomic DNA Purification Kit, produced by Promega (23).

In brief, the DNA extraction process was carried out by preparing a medium of yeast extract - peptone - dextrose. This medium was prepared according to the company's instructions, and then the DNA was extracted according to the steps of the kit, then the required gene was duplicated using the polymerase chain reaction technique and according to the primers used and mentioned in Table (1). The results were then electrophoresed on an agarose gel and the results were sent to Macrogen Company in Korea in order to determine the sequence of the nitrogenous bases of the desired gene (24).

Table(1) primers FCY1 (25)

Name of Primer	Sequence of Primers	Tm (C^0)	GC (%)	Product Size
FCY1-Forward	5-ATGGCAAGCAAGTGGGATCAG-3’	58	50%	120bp
FCY1-Reverse	5-GACCACGACCGAGAACACTTC-3’	61	57%	

Polymerase chain reaction (PCR)

The FCY1 Gene was amplified using the PCR technique with the primers FCY1-F and FCY1-R. PCR amplification was carried out using a total of 25 microliters, which included 7 microliters of DNA, 5 microliters of Taq enzyme, and 1 microliter of each primer (10 µM). After that, distilled water was poured into the tube. After the volume reached 25 microliters, electrophoresis was performed (24).

Next, use the Sanger sequencing method to examine the nucleic acid sequence (nucleotides) of the gene segment encoding the enzyme Cytosine Deaminase that was produced by the polymerase reaction in the preceding step. A technique for ascertaining the nucleotide sequence of DNA is the Sanger for DNA sequencing method via chain termination method, or simply the "chain termination method." The sequencing output of this technique, which was created by the Macrogen Company, was compared to sequences of species of yeasts that are known to exist worldwide in GenBank (26).

Results and Discussion

For the *S. cerevisiae* yeast under investigation, Figure (1) illustrates the rate of specific activity of the crude extract as well as the rate and concentration of protein. For those varieties of bread yeast, the following are the protein concentration of the crude extract and the rate of specific activity of the enzyme cytosine deaminozoate: The protein content is 0. 7967 mg/ml and the Turkish type is 9.91 units/mg. The protein content was 0.8567 mg/ml and the Iranian type was (11.15) units/mg. The protein concentration was 0.6167 mg/ml and the Russian type was 9.4367 units/mg. The specific activity for the raw local type was (12.683) units/mg. The protein content per milliliter is 0.8167.

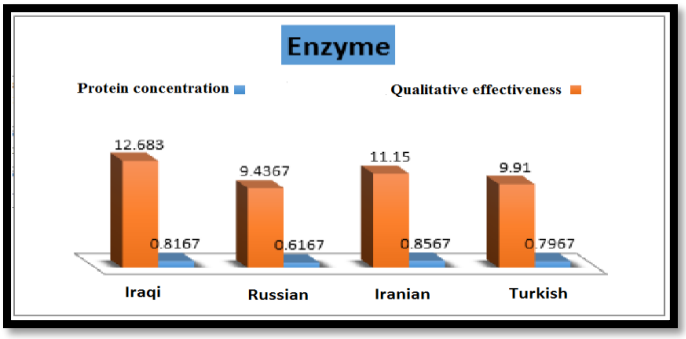

Figure (1) shows the specific activity rate of the crude extract and the rate and protein concentration of baking yeast *S. cerevisisae* for the four species under study.

These results were similar to a study by researcher Abbas *et al.* (25) in extracting the enzyme cytosine from manufactured bread yeast, as the specific activity of the crude extract reached (9.6) mIU/mg protein.

Figure (2) also shows the rate of specific activity of the enzyme after the salt precipitation process, as well as the rate of protein concentration for the bread yeast *S. cerevisisae* under study. The rate of specific activity of the enzyme cytosine deaminoiso and the protein concentration in those types of bread yeast were as follows: The Turkish type (12.8834). (unit/mg and protein concentration is 0.4067 mg/ml and the Iranian type is 13.18) units/mg and the protein concentration is 0.4834 mg/ml and the Russian type is (10.7534) units/mg and the protein concentration is 0.39 mg/ml and as for the raw local type it was The specific activity is (13.8567) units/mg, and the protein concentration is 0.55 mg/ml.

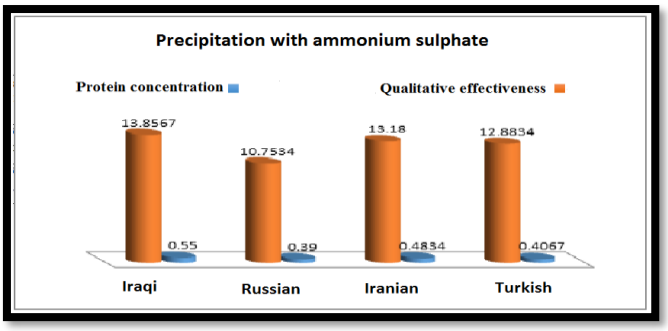

Figure (2) shows The specific activity rate of the enzyme after the precipitation process with ammonium sulfate and the protein concentration rate of baking yeast *S. cerevisisae* for the four species under study.

To compare the outcomes of the sedimentation procedure with a 60% saturation rate, the most effective isolates were chosen from duplicates of the four different varieties of yeast. Out of the four varieties, the local yeast exhibited the highest specific activity, number of purification times, and enzymatic yield. At 17.68 milliunits/mg of protein, the crude extract exhibited the highest specific activity. Its enzymatic yield was 11.335%, and its number of purification cycles was 1.341. This allowed the enzyme's specific effectiveness to reach 17.68 units/mg of protein. There have been 1,338 purifying cycles. 7,553 is the enzyme yield.

The choice of ammonium sulfate in concentrating the crude extract during the purification steps is due to several reasons, the most important of which are its availability, ease of obtaining, appropriate cost, high ability to dissolve and not harming the crude extract. Sulfate salts also work to displace and replace enzymes and precipitate them in the medium in which they are present. The basis of the precipitation process is with salts. It depends on the phenomenon of salting out due to the neutralization of the charges on the surface of the protein due to the salt and the disruption of the water layer surrounding the protein, which leads to its dissolution. The shape of the protein, its size, and the presence of other compounds with it also affect the speed of its dissolution because the salt concentration depends on the charges of the protein in terms of their number and distribution as well. About nonionic groups and the distribution of hydrophobic groups (26).

These results were consistent with a study (25). Different saturation percentages of ammonium sulfate were used to precipitate the enzyme extracted from Turkish bread yeast, including (20-40-60-80)%. The specific effectiveness was calculated after performing the dialysis process of the model for each saturation percentage, to determine The best percentage of saturation. The results showed that the best percentage of saturation was 60%, as it gave the highest effectiveness and specificity amounting to 12.5 milliunits/mg of protein, with a number of purification times of 1.302 times, and an enzymatic yield of 4.82%.) in that study.

Regarding the results of the comparative molecular study, it revealed the results of doubling of the FCY1 gene using forward and reverse primers for this target gene and for 4 isolates of the yeast *S. cerevisiae* (the first Turkish type, the second Iranian type, the third Russian type, and the fourth type of local (Iraqi) yeast. PCR technology and electrophoresis of the replication products. The appearance of bands for all samples at a molecular size of approximately 120 bp compared to the DNA ladder (1000 plus), which indicates the binding of the primers and the occurrence of replication in all samples, as shown in Figure (3).

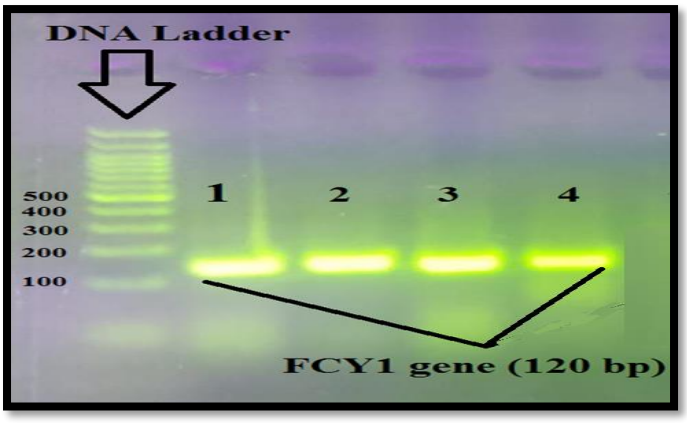

Figure (3) Electrophoresis of PCR duplication products with primers FCY1-F and FCY1-R on a 2% agarose gel at 5 V for 1.5 h and the appearance of bands at ~120 bp compared to the DNA ladder (1000 plus).

The results of the analysis of the sequences of the nitrogenous bases, after comparing them with the sequences of the isolates and species registered at the National Center for Biotechnology Information in America (NCBI), proved that all the sequences belong to the yeast Saccharomyces cerevisiae, and their classification according to GenBank appeared as follows:

Eukaryota ; Fungi; Dikarya ; Ascomycota ; Saccharomycotina Saccharomycetes; Saccharomycetales; Saccharomycetaceae; *Saccharomyces*.

The comparison's findings in GenBank further showed that the investigated gene contained mutations (variations) in the nitrogenous bases under investigation in all yeast species under investigation. Given that two mutations were found in the Turkish and local type yeast (Iraqi), with similarity rates reaching 98%, and one mutation appeared in both Iranian and Russian yeast, with matching rates reaching 99%, Table (2) indicates that this is a new registration for the first time due to mutations present in the nitrogenous bases.

Table (2) International isolates, their accession numbers and the name of the country in NCBI compared with them by the BLAST programme, showing the type of variation in the rules, as well as the percentage of identity with the isolates studied.

No	Type of substitution	Nucleotide	Sequence ID with compare	Country	Source	Identities
1	Transition	G\A	ID: CPO36481.1	USA	*Saccharomyces cerevisiae*	98 %
	Transvertion	T\G				
2	Transvertion	A\T	ID: CP046096.1	Netherlands	*Saccharomyces cerevisiae*	99%
3	Transition	C\T	ID: CP024010.1	South Korea	*Saccharomyces cerevisiae*	99%
4	Transvertion	A\C	ID: CP046459.1	India	*Saccharomyces cerevisiae*	98%
	Transvertion	A\T				

Molecular amplification techniques also allow rapid and sensitive detection and identification by directly detecting small amounts of fungal DNA present in a sample and analyzing them without the need for prior culture, which makes these tests

attractive for early diagnosis of disease or new traits formed as a result of mutations (27).

Su and Freudenreich (28) showed that the FCY1 gene encodes a yeast Cytosine Deaminase, which exhibits biochemical activity in vitro to convert cytosine to uracil. Leonhardt et al. (29) were able to study the sequences of the FCY1 gene encoding the cytosine enzyme in bread yeast *S. cerevisiae*.Durand et al. (30) demonstrated that Cytosine Deaminase is encoded by the FCY1 gene in *Saccharomyces cerevisiae*. Perhaps the reason for the occurrence of mutations in the gene under study is what was confirmed by late studies of *Saccharomyces cerevisiae,* that transcription-related mutations increase when error repair pathways are disrupted or bypassing error-free damage, and mutations also decrease and decrease when translation synthesis processes are disrupted or reduced. (31) It was also found that transcription increases the exposure of the basic DNA template to internal and external damage, and the process of converting cytosine to uracil during the transcription process leads to spontaneous, spontaneous and immediate mutations such as CG to TA (32).

Figure (4) shows the genetic tree of the genetic sequences of the FCY1 gene in four species of *S. cerevisiae* yeast: the first Turkish type, the second Iranian type, the third Russian type, and the fourth local type. The kinship tree shows the branching of origin into two groups, the first group included the first Turkish type, which was listed under the name *S. cerevisiae* Muna1. As for the second group, it divided into two secondary groups, one of which included the second Iranian type, *S. cerevisiae* Muna2, while the second secondary group included the third Russian type and the fourth local type, which indicates the closeness of these last species together.

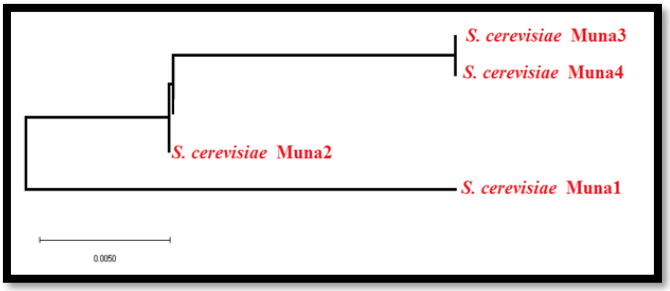

Figure (4) Phylogenetic tree showing the genetic relationship between the S. cerevisiae studied with the Mega-x programme.

Once the analysis of the nitrogenous base sequences of the samples analysed was completed, these sequences were registered in the American GenBank and the data were assigned accession numbers for identification. As shown in (Table 3), each isolate has an identification accession number.

Table (3) NCBI Accession Numbers for S. cerevisiae

No	Type of *S. cerevisiae*	Accession number
1	***S. cerevisiae* Muna1**	**PP278029**
2	***S. cerevisiae* Muna2**	**PP278030**
3	***S. cerevisiae* Muna3**	**PP278031**
4	***S. cerevisiae* Muna4**	**PP278032**

Building an evolutionary tree is also an excellent way to find out how different or related the sequences of genes or organisms are to each other. The Molecular Evolutionary Genetic Analysis (MEGA) program can be used, which is a free program that allows anyone to build evolutionary trees in an easy way. The most common options in building genetic trees are neighbor joining and maximum likelihood, both of which give good estimates of the relationship between different molecular sequences (33).

Conclusion:

The cytotoxic medicine 5-fluorouracil (5-FU), which is used to treat several forms of cancer, is produced when the non-toxic molecule 5-fluorocytosine (5-FC) is hydrolyzed by the enzyme Cytosine Deaminase, which is isolated from baker's yeast Saccharomyces cerevisiae. The local Iraqi yeast, which was made by hand, showed the highest qualitative effectiveness compared to the Turkish, Iranian, and Russian types. The results of the molecular comparison also showed the presence of mutational variations in all the types studied, and these variations were recorded in the American Gene Bank.

References

1- Marta, M.; Lorena, C.; Susana, M.; Anaelle, M.; Olivia, C.; Ananda, P. F. and Achraf, A. L. 2023 Potential benefits of yeast Saccharomyces and their derivatives in dogs and cats. Frontiers in Veterinary Science. 25 October 2023.Front. Vet. Sci. 10:1279506. Doi: 10.3389/ fvets. 2023. 1279506.

2- Nadine, F.; W.; A.; Edouard, T.; Ziad, R.; Angela, B.; Giacomo, Z.; Isabelle, M-P.; and Marilena, B. (2019). Genetic and Phenotypic Characterization of a Saccharomyces cerevisiae Population of 'Merwah' White Wine. Micro-organisms, 7, 492; doi: 10.3390/ microorganisms 7110492.

3- Parapouli, M., Vasileiadis, A., Afaendra, A-S., and Hatziloukas, E. (2020). Saccharomyces cerevisiae and its industrial applications. Journal/microbiology., 6 (1): 1–31.

4- Yin, J.W. Sun, F. Li, J. Hong, X. Li, Y. Zhou, Y.and Lu, M. (2020). "VARIDT 1.0: Variability of drug transporter database," Nucleic Acids Res., vol. 48, no. D1, pp. D1042–D1050. Doi: 10.1093/nar/gkz779.

5- Carbonetto B. Ramsayer J. Nidelet T. et al. (2018) Bakery yeasts, a new model for studies in ecology and evolution. Yeast 35: 591–603.

6- Heitmann, M. Zannini, E. Arendt, E. (2018) Impact of Saccharomyces cerevisiae metabolites produced during fermentation on bread quality parameters: A review. Crit Rev Food Sci Nutr 58: 1152–1164.

7- Whitaker, J. R. (2018). Principles of Enzymology for the Food Sciences: Marcel Dekker, Inc, New York DOI.https://doi.org/10.1201/9780203742136.

8- Volesky, B. and Luong, I. (1985). Microbial enzymes production, purification and isolation. (CRC) Critical Reviews in Biotechnology, 2: 119-146. DOI:10.3109/07388558409082583.

9- Xueqin, L.; Yaokang, W.; Mengyue, G.; Jieying, D.; Yang, G.; Yanfeng, L.; et al. (2021). Synthetic biology for future food: Research progress and future directions. Future Foods,vol. 3. 100025, 1-16.https://doi.org/10.1016/j.fufo.2021.100025.

10- Maria, P.; Anastasios, V.; Amalia - Sofia, A.; and Efstathios, H. (2020). Saccharomyces cerevi-siae and its industrial applications. AIMS Microbiology, Volume 6, Issue: 1, page: 1–31., DOI: 10.3934/ microbial. 2020001. http://www.aimspress.com/journal/microbiology.

11- Shazia, E. (2010). Production of Peroxidases from indigenous fungal Strains and their application for AZO dye decolorization. PhD thesis submitted under the Microbiology of the Quaid-I-Azam University Islamabad, Pakistan. http://prr.hec.gov.pk/jspui/handle/123456789/682.

12- Raza, A. Ghosh, S.S, (2019). Connexin - 43 Enhaces the Redesigned Cytosine Deaminase Activity for Suicide Gene Therapy Human Breast Cancer Cell. Biochemistry Insights. Volume 12: 1-8. Journal - Permissions. DOI: 10. 1177/ 1178626418818182.3.

13- Zahra, N.; Seyedeh, F. F.; Amin, M.; Saeid, N.; Majid, G-M.; and Seyyed, R. S. (2023). Advancing sustainability in the food and nutrition system: a review of artificial intelligence applications. Frontiers in Nutrition Front. Nutr. 10:1295241.doi:10.3389/fnut.2023.1295241.

14- Zymancczyk-Duda, E.; Brzezinska - Rodak, M., Klimek- Ochab., Duda, M.; and Zerka, A. (2018). "Yeast as a versatile Tool in Biotechn-ology"; Industrial application, Yeast Chapter, 10 yeast biotechnological processes and applications". Microbiologyical Research; 207; 83=90. DOI:10. 5772/intechopen.70130.

15- Corrie, S. M. (2014). A practical guide to DNA extraction, PCR, and gene-based DNA sequencing in insects. HALTERES, Volume 5, 32-42.

16- Yuedi, D.; Jun, F.; Lili, D.; Biao, H.; and Bin, Z. 2022 Antitumor efficacy of Cytosine Deaminase armed vaccinia virus plus 5 fluorocytosine in colorectal cancers Cancer Cell International. Ding et al. Cancer Cell Int (2020) 20:243. https://doi.org/10.1186/s12935-020-01340-6.

17- Antonella, A.; and Sheryl, P. (2023). Artificial intelligence in food biotechnology: trends and perspectives. Frontiers in Industrial Microbiology, Front. Ind. Microbial. 1:1255505. DOI: 10.3389/finmi.2023.1255505.

18- Pablo, H-C.; Elena, L-R.; Juan, A. M.; and Macarena, P.2022. Unifying Different Cancer Theories in a Unique TumourModel:Chronic Inflammation and Deaminases as Meeting Points. Int. J. Mol. Sci. 2022, 23, 8720. pp;1-16. https://doi.org/10.3390/ijms23158720. e

19- Ayoub, M.-J.; Jean-Luc, L.; Pierre, A-N; Huu-Vang, N.; Rachad, S.; and Claude, G. (2021). 'Lebanon's Native Oenological Saccharomyces Cerevisiae Flora: Assessment of Different Aspects of Genetic Diversity and Evaluation of Winemaking Potential'. Journal of Fungi 7(8).,620. https://doi.org/10.3390/jof7080678.

20- Ipata, P.L. and Cercignani, G. (1978). Cytosine and Cytidine deaminase from Yeast. In: "Method in Enzymology". (Ed. Patricia, A.H. and Mary, E.J) Vol. L1. Aca. Press Inc., PP: 394-401.

21- AL- Easawi, M. A. H. (2020). Extraction and purification of beta - galactosidase from local almond and its use for lactose intolerance treatment. the Iraqi journal of agricultural science, 51(3), 767-776 .DOI:https://doi.org/10.36103/ijas.v51i3.1032.

22- Younis, R. (2020). Production, purification and inhibition of alginate Ly-ase from local isolate of pseudomonas aeruginosa NA11. Iraqi Journal of Agricultural Sciences, 51(6). 1726-1739.

23- Promega Corporation. (2019). GoTaq and Wizard are registered trademarks of Promega Corporation. Toll Free in USA 800-356-9526 · 608-274-4330 · Fax 608-277-2516 TM050. www.promega.com

24- Sambrook,J.; Fritsch, E. F. and Maniatis, A.(2012). Molecular cloning, 2nd ed. New York: Cold Spring Harbor Laboratory Press. 228 pp.

25- Abbas, L.M.R.; AL-Maeny, S.AL.; Nader, M.I. (2010). Extraction, Ourification and characterization of Cytosine Deaminase from Saccharomyces cerevisiae. Genetic Engineering and Biotechnology Institute for Post graduate Studies, Baghdad – Iraq. Iraqi Journal of Science. VOI, 51, No4, PP 606-598.

26- Bagnoli, S.; Tozzini, E.T.; and Cellerino, A. (2023). Immunofluorescence and Aggresome Staining of Nothobranchius furzeri Cryosections Cold Spring Harb Protoc; DOI: 10.1101/pdb. Prot 107791.

27- Willinger, B., Kienzl, D., & Kurzai, O. (2014). 13 Diagnostics of Fungal Infections. In Human fungal pathogens (pp. 229-259). Springer, Berlin, Heidelberg.

28- Su, X. A., & Freudenreich, C. H. (2017). Cytosine deamination and base excision repair cause R-loop–induced CAG repeat fragility and instability in Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences, 114(40), E8392-E8401.

29- Landeweert, R., Leeflang, P., Kuyper, T. W., Hoffland, E., Rosling, A., Wernars, K., and Smit, E. (2003). Molecular identification of ectomycorrhizal mycelium in soil horizons. Applied and Environmental Microbiology, 69(1), 327-333.

30- Durand, R., Jalbert-Ross, J., Fijarczyk, A., Dubé, A. K., & Landry, C. R. (2023). Cross-feeding affects the target of resistance evolution to an antifungal drug. bioRxiv, 2023-05.

31- Pani, B., & Nudler, E. (2017). Mechanistic insights into transcription coupled DNA repair. Dna Repair, 56, 42-50.

32- Williams, J. D., Zhu, D., García-Rubio, M., Shaltz, S., Aguilera, A., & Jinks-Robertson, S. (2023). Spontaneous deamination of cytosine to uracil is biased to the non-transcribed DNA strand in yeast. DNA repair, 126, 103489.

33- Newman, L., Duffus, A. L., and Lee, C. (2016). Using the free program MEGA to build phylogenetic trees from molecular data. The American Biology Teacher, 78(7), 608-612.