https://doi.org/10.35401/2541-9897-2024-9-4-44-53

Check for updates

Illustration of the Distribution of DNA Sequences and Their Frequencies Within the *MEP2* Gene Across Ten *Candida* Species

©Ali A. Dawood

University of Mosul, Mosul, Iraq

Ali A. Dawood, Scopus *h*-index 8, University of Mosul, Al Majmoaa St, Mosul, 41002, Iraq, aad@uomosul.edu.iq

Received: July 29, 2024. Received in revised form: August 15, 2024. Accepted: October 2, 2024.

Abstract

Candida species have an impact on human skin health and occasionally can lead to catastrophic conditions, such as skin cancer. The nitrogen supply in growth media regulates *MEP2* gene expression. MEP2 protein may regulate the amount of ammonium accessible to cells by directly affecting ammonium assimilation. Reducing MEP2 expression removed its potential to drive filamentous growth.

This study aimed to examine serial imaging of the *MEP2* gene with chaos game representation (CGR) and frequency chaos game representation (FCGR). In addition, the effect of mutations in *Candida albicans* strains on MEP2 docking with lauramine oxide (LDAO) was investigated. The *MEP2* gene was selected for 10 *Candida* species from the National Center for Biotechnology Information to compare DNA sequences using conventional and portray methods (CGR and FCGR). The molecular docking between MEP2 and LDAO was determined using the HDOCK server.

CGR findings revealed that *Candida margitis, Candida orthopsilosis, Candida dubliniensis, Candida theae,* and *C albicans* had approximately 65% of the same characteristics. According to FCGR, there was a 75% similarity between *C albicans, C theae, C dubliniensis, C orthopsilosis*, and *C margitis*. In certain strains, but not all a mutation in the conserved region of the protein caused a change in the docking residue of LDAO with MEP2.

The CGR and FCGR protocols are considered practical and reliable tools for identifying protein and DNA sequence similarities. Approximately 80% of the existing algorithms for determining multiple sequence alignments are similar to traditional methods. Targeted treatment will be possible as determining MEP2 mutations is crucial for using *Candida* as a nitrogen source.

Keywords: Candida, docking, FCGR, lauramine oxide, MEP2

Cite this article as: Dawood AA. Illustration of the distribution of DNA sequences and their frequencies within the MEP2 gene across ten Candida species. *Innovative Medicine of Kuban.* 2024;9(4):44–53. https://doi.org/10.35401/2541-9897-2024-9-4-44-53

Иллюстрация распределения последовательностей ДНК и их частот в гене *MEP2* у десяти видов *Candida*

©А.А. Давуд

Университет Мосула, Мосул, Ирак

А.А. Давуд, *h*-индекс в Scopus 8, Университет Мосула, 41002, Мосул, ул. Аль-Маджмуа, Ирак, aad@uomosul.edu.iq

Поступила в редакцию 29 июля 2024 г. Исправлена 15 августа 2024 г. Принята к печати 2 октября 2024 г.

Резюме

Виды грибов рода Candida влияют на здоровье кожи человека и периодически могут приводить к таким смертельным заболеваниям, как рак кожи. Источник азота в питательной среде регулирует экспрессию гена *MEP2*. Белок MEP2 может регулировать количество аммония, доступного клеткам, напрямую влияя на ассимиляцию аммония. Снижение экспрессии MEP2 устраняет его потенциал индуцировать филаментацию.

Целью данной работы было изучение серийной визуализации гена *MEP2* с помощью подходов CGR и FCGR. Кроме того, было исследовано влияние мутаций в штаммах *C. albicans* на докинг MEP2 и оксида лаурамина (LDAO). Ген *MEP2* был выбран для десяти видов Candida из банка данных NCBI, чтобы сравнить последовательности ДНК с использованием традиционных методов и методов представления (CGR и FCGR). Молекулярный докинг между MEP2 и LDAO был осуществлен с помощью сервера HDOCK.

По результатам CGR, *C. margitis, C. orthopsilosis, C. dubliniensis, C. theae* и *C. albicans* имеют примерно 65% одинаковых характеристик. По результатам FCGR, между *C. albicans, C. theae, C. dubliniensis, C. orthopsilosis* и *C. Margitis* наблюдается 75% сходства. В некоторых штаммах, но не во всех, мутация в консервативной области белка вызывала изменение в стыковочном остатке LDAO с MEP2.

Протоколы CGR и FCGR считаются практичными и надежными инструментами для определения сходства белков и последовательностей ДНК. Примерно 80% существующих алгоритмов определения множественных выравниваний последовательностей аналогичны традиционным методам. Стоит отметить, что таргетированное лечение возможно, поскольку определение мутаций MEP2 имеет решающее значение при использовании Candida в качестве источника азота.
Ключевые слова: Candida, докинг, FCGR, оксид лаурамина, MEP2
Цитировать: Давуд А.А. Иллюстрация распределения последовательностей ДНК и их частот в гене MEP2 у десяти видов Candida. *Инновационная медицина Кубани.* 2024;9(4):44–53. https://doi.org/10.35401/2541-9897-2024-9-4-44-53

Introduction

Candida species are present all around the world; however, they primarily affect immunocompromised people who have been diagnosed with severe illnesses, such as HIV infection and cancer. *Candida* species are among the most prevalent organisms that cause hospital-acquired infections.[1]

Ammonium is a preferred nitrogen source for the majority of microorganisms inhabiting the environment, and it is assimilated into organic compounds via the glutamine synthetase-glutamate synthase pathway. In *Candida* species, ammonium permeases encoded by *MEP1* and *MEP2* genes are responsible for ammonium uptake into the cell in which it can then be utilized in a variety of biosynthetic processes.[2] The expression of the *MEP1* and *MEP2* genes is regulated by the nitrogen source in a growth medium, and the 2 permeases are thought to have a redundant function. Disruption of either gene results in an inability to grow on media with ammonium as the sole nitrogen source. The *MEP2* gene of *Candida albicans* has been cloned and functionally characterized. This isofunctional enzyme represents a potential target for development of novel antifungal therapies because its activity is required for organism growth in the host.[3,4]

Transcriptional control of MEP2 homologs is conserved, and *C albicans* MEP2 has been shown to be under control of the GATA family transcription factor Gat1 and the nitrogen catabolite repression regulator Nrg1.[5] Effects of MEP2 regulation on nitrogen catabolism and virulence are currently being investigated through construction of *C albicans* MEP2 underexpression and overexpression strains and analysis of their in vitro and in vivo behaviors. Given MEP2 involvement in ammonium and lack of current understanding of in vivo nitrogen sources for *C albicans*, it is of interest to identify specific nitrogen sources that trigger MEP2 transcription, as this may be a means to elucidate the MEP2 natural function.[6,7]

However, primary sequence similarities suggest that MEP2 may regulate the level of ammonium available to cells through direct effects on ammonium assimilation. This theory is supported by the MEP2 conservation in nitrogen catabolic repressed organisms, and data showing that *Saccharomyces cerevisiae* MEP2 is under control of both carbon and nitrogen sources at the level of transcription.

Throughout evolution, 2 primary means of nitrogen uptake have arisen. Nitrate assimilation, a nitrate to glutamine to glutamate to amino acid process, is used by plants, fungi, and microbial eukaryotes.[8] Ammonium compounds are assimilated by the biosynthetic glutamine to glutamate to amino acid pathway but, prior to reactions associated with this pathway, occur through direct incorporation into amino acids via the glutamine: 2-oxoglutarate aminotransferase and glutamate N-acetyl transferase reactions. In most organisms, the enzymes involved in nitrogen assimilation, be it nitrate or ammonium, are subject to coordinate gene expression by a nitrogen source and regulation of enzyme activity.[9]

Reducing MEP2 expression eliminated its potential to induce filamentous development. Enhanced promoter activity and messenger RNA stability both contribute to enhanced MEP2 expression levels. MEP2 fusions resulted in higher messenger RNA levels and greater cell fluorescence. MEP2 may gain structural characteristics that allow it to function as a signaling protein rather than an ammonium transporter.[10,11]

Multiple sequence alignment (MSA) is based on the assumption that genetically related sequences perform comparable tasks and conserved areas are critical to a molecule's activity. In contrast, chaos game representation (CGR) is utilized to convert a biological sequence into a visual geometric representation.[12] This approach involves repeating a basic operation to create complicated geometric patterns. Each nucleotide in the sequence indicates a certain orientation in the painting. Frequency chaos game representation (FCGR) is an extension of CGR that examines the distribution of nucleotides along a sequence. This approach calculates and depicts frequencies of nucleotides in shifting windows of the sequence.[13,14]

MSA compares multiple sequences and identifies similar and divergent regions between them, which makes it useful for studying evolution and predicting a structure, whereas CGR and FCGR analyze individual sequences and uncover underlying patterns, which is helpful in studying a structure, evolution, and nucleotide composition. In many investigations, these strategies are utilized in conjunction. For example, MSA may be used to identify conserved sections, which can be further analyzed using CGR or FCGR.[15,16]

Because the MEP2 protein is so important for *Candida* development, this study focused on diagnosing and identifying its gene using novel approaches and comparing them to old ones. The current work sought to identify changes in the DNA sequences of the *MEP2* gene between 9 *Candida* species utilizing established approaches, such as MSA vs CGR and FCGR. Furthermore, this study investigated how mutations in the *MEP2* gene affect its interaction with lauramine oxide (LDAO) through molecular docking analysis.

Methods

DNA sequences of the *MEP2* gene from 10 *Candida* species were obtained from the National Center for Biotechnology Information (NCBI) for comparative analysis. These species included *C albicans* (NC_032092), *Candida theae* (NW_026261424), *Candida dubliniensis* (NC_012863), *Candida tropicalis* (NW_003020038), *Candida parapsilosis* (NW_023503283), *Candida pseudojiufengensis* (NW_026261543), *Candida orthopsilosis* (NC_018298), *Candida margitis* (NW_026261739), *Candida oxycetoniae* (NW_026055484), and *Candida jiufengensis* (NW_026261561). *C albicans* was chosen as a wild type to compare with other species.

For DNA sequence alignment, 3 programs were employed: T-Coffee, CLUSTALW, and MAFFT version 7. A phylogenetic tree was constructed using T-Coffee.

The CGR tool provides a fractal-like visualization of DNA sequences.[17] This visualization method facilitates understanding the distribution of nucleotides within a sequence. To numerically represent nucleotides within a DNA sequence, a counting system is established. Each nucleotide is assigned a numerical value based on its position within the sequence. This system simplifies the identification of specific nucleotides within the context of the entire genome. A 2-dimensional grid or plot is utilized for visualization. Typically, the origin is placed at the center, dividing the grid into 4 quadrants representing the 4 DNA nucleotides (A, T, G, and C). Each nucleotide is plotted on the grid according to its position within the DNA sequence, using the x- and y-axes (x, y).[14,15]

To prepare it for the next iteration, the counter is increased by one after each nucleotide assignment. In the end, nucleotides are mapped to numbers based on the cumulative effect of this iterative process. This cumulative process is shown by the following accumulation equations:

$$new\_x = current\_x + (\sum(target\_x - current\_x)/2\text{^}n)$$
$$new\_y = current\_y + (\sum(target\_y - current\_y)/2\text{^}n).$$

A chaotic game plot is used to show the frequency of recurrence of a sequence, and the foundation of FCGR is counting the occurrences of a certain pattern inside the sequence.[18] This is accomplished by associating different color intensities or shades of grayscale with each computed point on the (x, y) axis. Points are defined by repeating the shape element at the given place in the sequence. The resultant FCGR approach gives visual insight into the distribution of spots within the series, with darker regions indicating higher theme frequency. The FCGR technique was implemented according to the given approach:

$$\text{Frequency}(M) = (\text{Number of times the motif M appears in the sequence S})/(n - k + 1),$$

where *S* is a sequence under study; *n*, length of the sequence (if *S* is a DNA sequence, then *n* is the number of nucleotides in the sequence); *M*, the theme to be identified inside the sequence, and *k*, length of the motif.[11,12]

Prediction of the Three-Dimensional Structure of Ten MEP2 Proteins

The SCRATCH Protein Predictor (3Dpro) program was used to predict a 3-dimensional structure of the MEP2 protein in all 9 *Candida* species. Protein sequences were graphically displayed; mutations were identified, and the PyMOL software was used to analyze the results.

Identification of Mutations in the MEP2 of C albicans Strains

The following section of the study compared 5 strains of *C albicans* for the wild type of the *MEP2* gene with mutations that impact the LDAO binding in the protein. MEP2 strains selected from the Protein Data Bank included 5AEZ wild type, 5AH3, 5FUF, 6EJH, and 6EJ6. PyMOL was used to map proteins in surface and mesh formats and discover significant strain-specific alterations. The Pipeline Builder for Identification of Targets (PBIT-V3) tool was used to identify a chemical or medication that targets MEP2. PBIT-v3 can be used to detect a target chemical by integrating and implementing distinct modules for each protein with the ability to bind to a drug-like compound. This is accomplished through the use of BLAST algorithms from sites in the DrugBank (version 5.0), Therapeutic Targets Database, and ChEMBL databases.

Molecular Docking Between MEP2 and LDAO

Finally, after detecting the target drug LDAO, the HDOCK tool was used to dock it with MEP2 strains. The top 10 models were chosen for binding MEPT2 strains with LDAO.

Results

C albicans was used as a reference point for the rest of the *Candida* species in various tests since they are the most common species that cause disease in humans. MSA showed roughly 200 major mutations within *MEP2* gene classes. Mutations in the initial 18 nucleotides and the end section of the gene, which contained roughly 1240 nucleotides, have been identified as the cause of the variances between the species. As these mutations did not involve the conserved area, they had no direct effect on the protein's final composition. *C tropicalis* and *C oxycetoniae* had the greatest difference within the last 185 nucleotides compared with other species (Figure 1). Phylogenetic tree analysis was based on assessing the alignment of several sequences. It became evident that *C dubliniensis* was most closely related to *C albicans*. Figure 2 depicts the remaining species arranged according to their genetic closeness. When DNA sequence segments of the *MEP2* gene were compared between the 10 *Candida* species, no substantial differences were found. Figure 3 depicts the DNA sequence with an essentially identical curve and a similarity level of more than 84%.

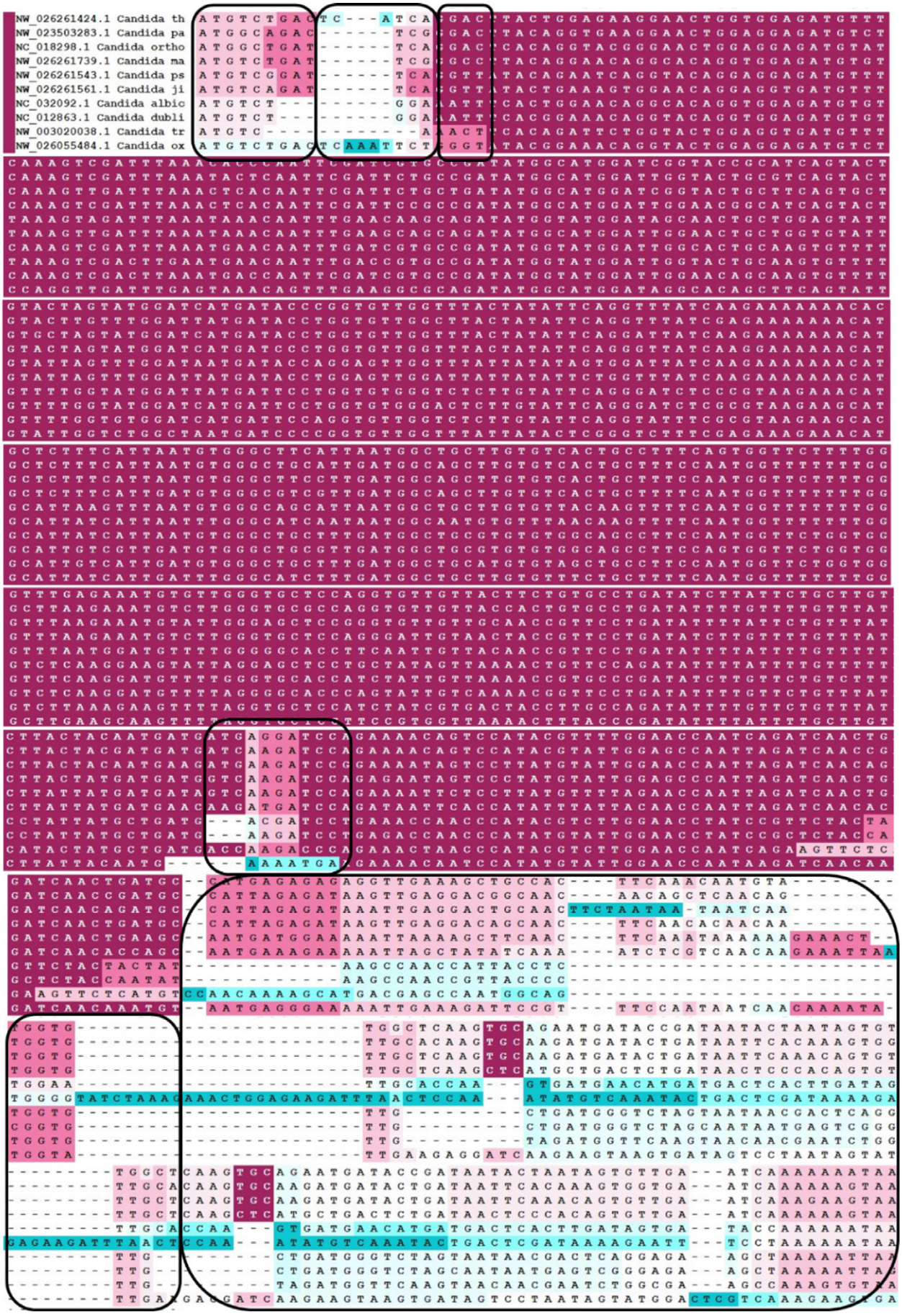

Figure 1. Multiple sequence alignment of the MEP2 gene of 10 Candida species
Рисунок 1. Множественное выравнивание последовательностей гена MEP2 десяти видов Candida

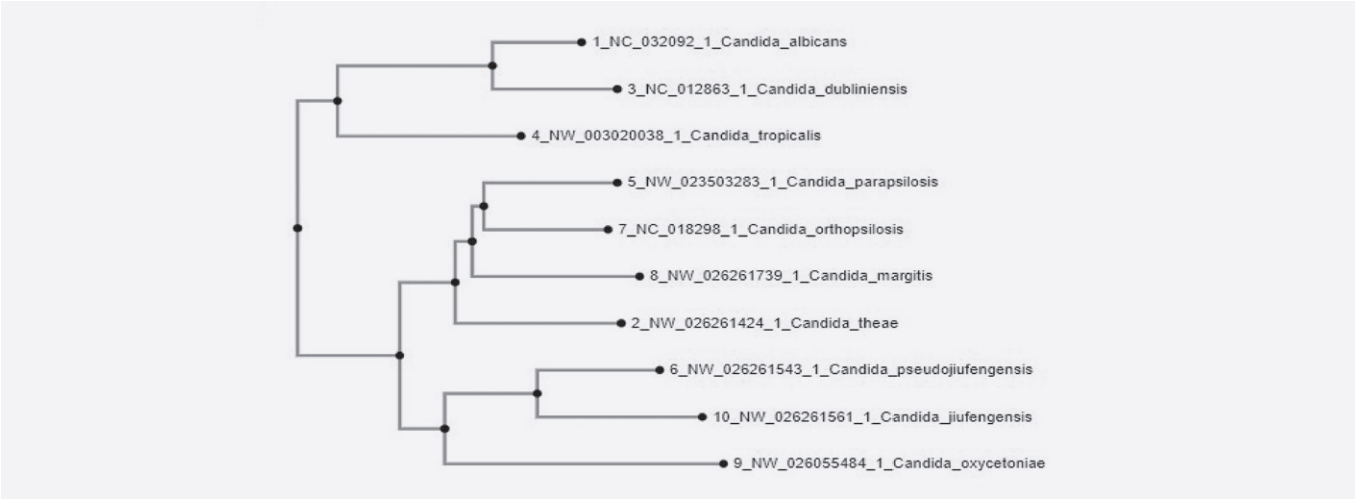

Figure 2. Construction of a phylogenetic tree depicting the relationships among the MEP2 gene of 10 Candida species
Рисунок 2. Построение филогенетического дерева, изображающего отношения среди генов MEP2 десяти видов Candida

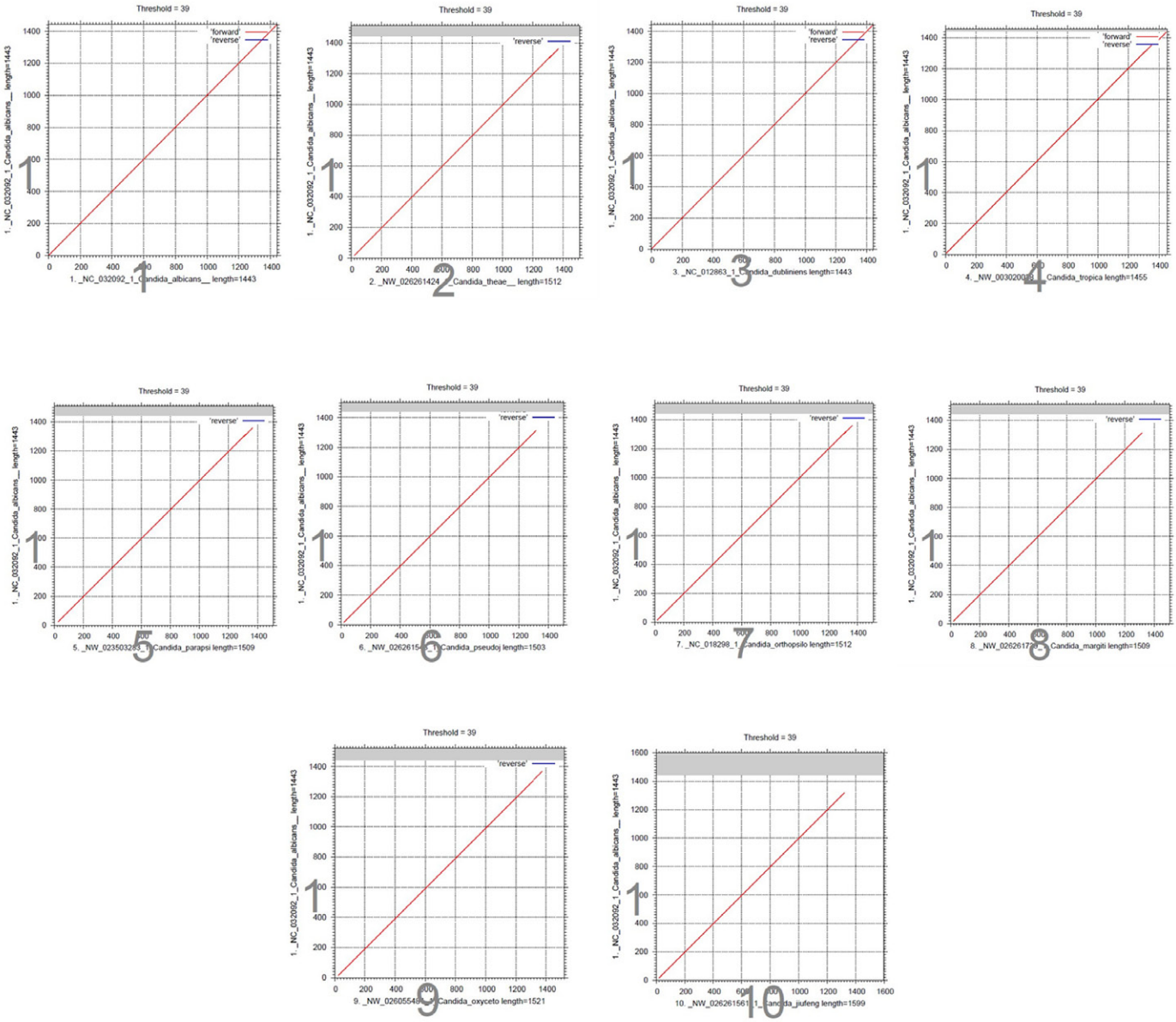

Figure 3. Graphical representations of the MEP2 gene in 9 Candida species compared with C albicans
Рисунок 3. Графические представления гена MEP2 у девяти видов Candida в сравнении с C. albicans

Following a complete portrayal of the DNA sequences of the *MEP2* gene across several *Candida* species, with *C albicans* as a template for the principal model (Figure 4), *C albicans, C theae, C dubliniensis, C orthopsilosis*, and *C margitis* were revealed to share around 65% commonality. The similarities reached 55% with *C parapsilosis* and *C oxycetoniae*. In contrast, *C tropicalis, C pseudojiufengensis*, and *C jiufengensis* differed from the rest of the species by 30%.

To gain a better understanding of the similarity degrees among *Candida* strains, frequency imaging required the incorporation of 4 nucleotides into the nitrogenous base sequence representation. By evaluating the frequency imaging data of the 10 *Candida* species, it became obvious that *C albicans, C theae, C dubliniensis, C orthopsilosis*, and *C margitis* had approximately 75% in common. In comparison to the other species, *C parapsilosis* and *C oxycetoniae* had 65% of similarities. In contrast, *C tropicalis, C pseudojiufengensis*, and *C jiufengensis* were more than 40% different from the rest of the species (Figure 5). The genetic diversity within the *MEP2* gene

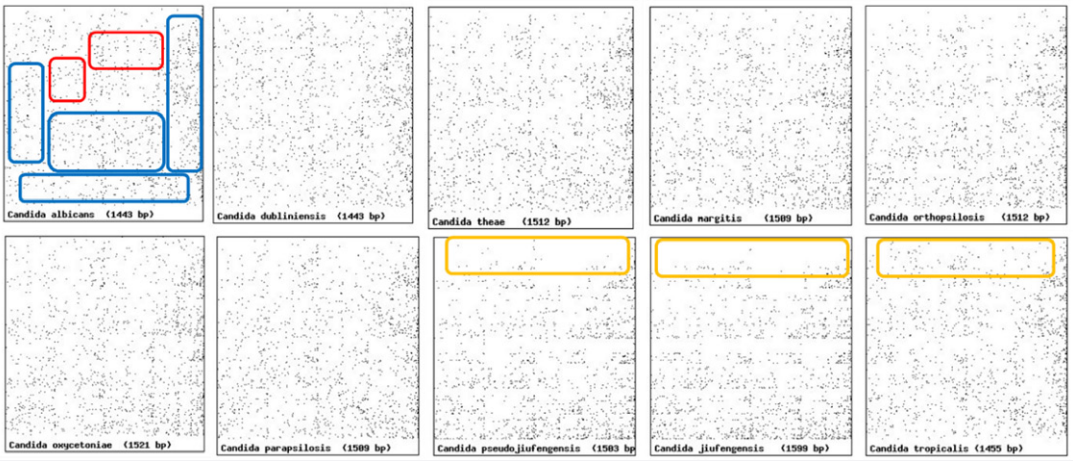

Figure 4. Chaos game representation analysis of the MEP2 gene across Candida species revealed different variants. The blue rectangles emphasize the closest similarities between the first 5 species in at least 5 bps. The red rectangles represent variances of 4 bps across the species. The yellow rectangles demonstrate changes of at least 6 bps in the final 3 species relative to the other species
Рисунок 4. CGR-анализ гена MEP2 у видов Candida выявил разные варианты. Синие прямоугольники указывают на наиболее близкие сходства между первыми пятью видами в не менее 5 п.н. Красные прямоугольники – различия (4 п.н.) между видами. Желтые прямоугольники – изменения (не менее 6 п.н.) в последних трех видах по сравнению с другими видами

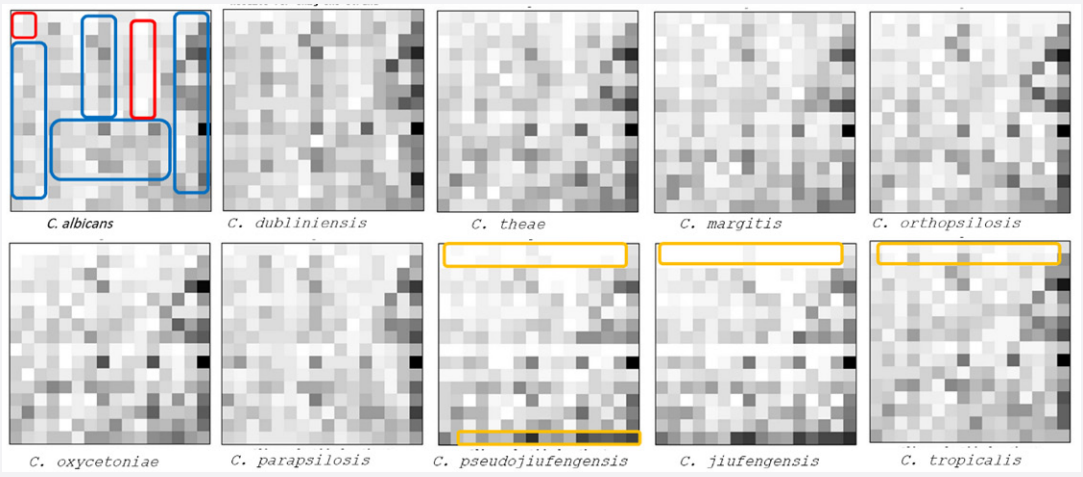

Figure 5. Frequency chaos game representation of the MEP2 gene across Candida species, characterized by the progressive accumulation of 4 bps over the entire sequence. The blue rectangles represent similarities in one set of base pairs between the species, emphasizing small differences. The red rectangles demonstrate variations in 2 sets of base pairs among the first 5 species. The yellow rectangles indicate similarities in at least 2 sets between the final 3 species and the rest
Рисунок 5. FCGR гена MEP2 у видов Candida, характеризующееся прогрессивным накоплением 4 п.н. по всей последовательности. Синие прямоугольники отражают сходство в одном наборе пар оснований между видами (небольшие различия). Красные прямоугольники – вариации в двух наборах пар оснований среди первых пяти видов. Желтые прямоугольники указывают на сходство по крайней мере в двух наборах между последними тремя и остальными видами

between the species is successfully illustrated using a 4-bps stepwise accumulation method, offering insight into the degree of genetic variation across various species.

Our investigation revealed that there was a variation in the evaluation of sequence similarity between *Candida* species when comparing the alignment of MEP2 strains using MSA compared with both CGR and FCGR. One nucleotide resolution, for instance, prevented MSA from clearly differentiating between *Candida* species. Conversely, it was simple to identify *Candida* species from CGR and FCGR images at first sight. Furthermore, FCGR was visually more accurate than CGR.

Five strains of *C albicans* MEP2 were selected based on 5AEZ being the wild strain and were compared with the mutant strains. Two clear mutations could be observed for 5AH3 strain: the conversion of arginine to aspartic acid (R452D) and serine to aspartic acid (S453D). 5FUF strain had the same mutation at residue 453 (S453D). 6EJH had a change of glysine to cysteine (G343C). As for 6EJ6 strain, asparagine was converted to alanine (N240A). Mutations caused a conformational shift in the protein chain as seen in Figure 6.

PBIT-v3 selected methylamine and LDAO, which were the best potential target medicines to bind to the MEP2 protein, from DrugBank. Molecular docking between LDAO and MEP2 was accomplished using HDOCK. Five different MEP2 strains were tested with the top 10 docking models. Table below displays the docking analysis findings for the 10 models for each strain, along with the docking and confidence scores and root mean square deviations. PyMOL was used to determine where LDAO docked in MEP2 for the models under consideration. Each MEP2 strain is located in one of the top 10 LDAO docking areas. Our findings indicated that the arginine in residue (452) was docked with the LDAO in wild-type strain 5AEZ. An aspartic acid mutation at position (452) altered the docking site in strain 5AH3; hence the docking site changed as a result of the mutation. For the other strains, our data revealed no influence of strain-specific mutations on the docking position change as shown in Figure 7.

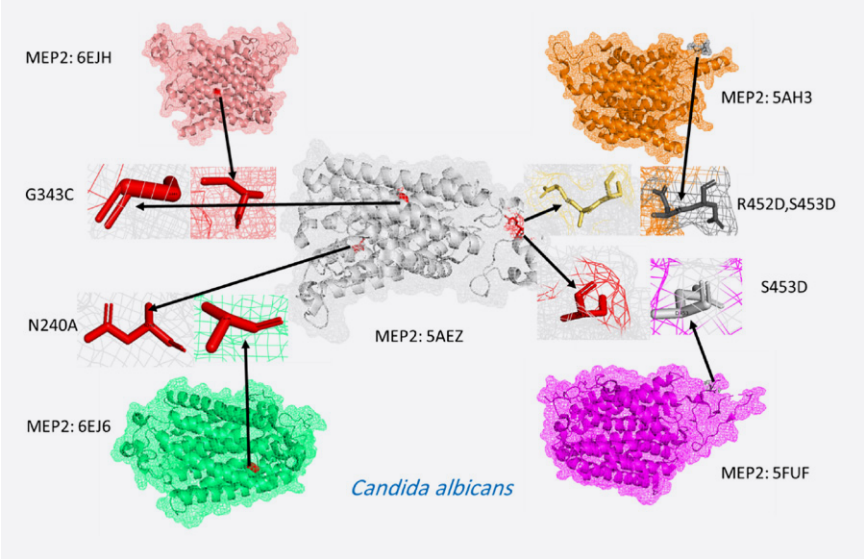

Figure 6. Key mutations that caused conformational changes in 5 C albicans MEP2 strains. 5AEZ: the wild type. 5AH3 with mutations (R452D and S453D). 5FUF with the mutation (S453D). 6EJH with the mutation (G343C). 6EJ6 with the mutation (N240A)

Рисунок 6. Ключевые мутации, вызвавшие конформационные изменения у пяти штаммов MEP2 вида C. albicans. 5AEZ: дикий тип. 5AH3 с мутациями (R452D и S453D). 5FUF с мутацией (S453D). 6EJH с мутацией (G343C). 6EJ6 с мутацией (N240A)

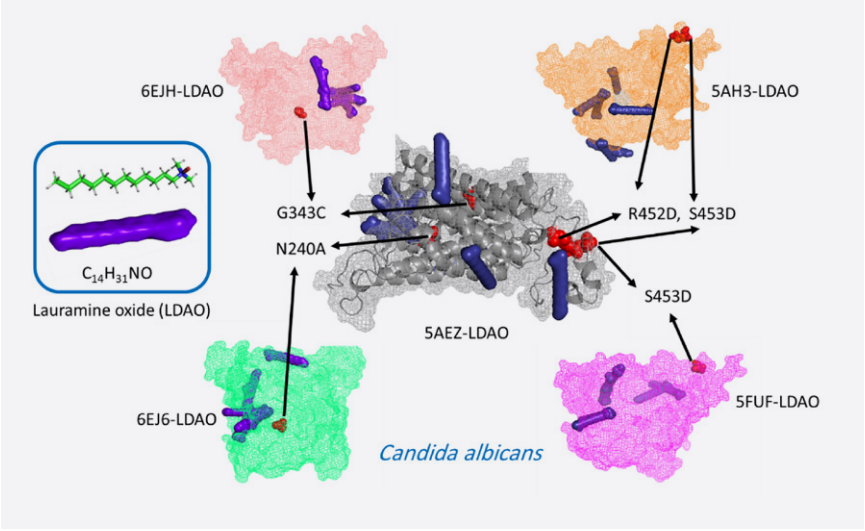

Figure 7. Molecular docking between LDAO and 5 MEP2 strains of C albicans (5AEZ, 5AH3, 5FUF, 6EJH, and 6EJ6). For each strain, there were 10 optimal docking models. The arginine at position (452) was docked with LDAO in 5AEZ. The docking location in 5AH3 strain was changed by an aspartic acid mutation at residue (452); thus LDAO did not bind to this residue

Рисунок 7. Молекулярный докинг между LDAO и пятью штаммами MEP2 вида C. albicans (5AEZ, 5AH3, 5FUF, 6EJH и 6EJ6). Для каждого штамма было десять оптимальных моделей докинга. Аргинин в позиции (452) был состыкован с LDAO в 5AEZ. Место стыковки в штамме 5AH3 было изменено мутацией аспарагиновой кислоты в остатке (452), поэтому LDAO не связывался с этим остатком

Table

Analysis of molecular docking between *C albicans* MEP2 strains and lauramine oxide

Таблица

Анализ молекулярного докинга между штаммами MEP2 *C. albicans* и оксидом лаурамина

PP-docking	Description	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
5AEZ-LDAO	Docking score	−109.42	−99.53	−98.93	−95.9	−95.85	−95.47	−93.38	−92.94	−91.82	−91.73
	Confidence score	0.3075	0.2671	0.2648	0.2531	0.2529	0.2515	0.2437	0.2421	0.238	0.2377
	RMSD (A°)	58.28	61.24	56.63	61.43	72.46	60.09	59.37	62.2	52.58	73.98
5AH3-LDAO	Docking score	−105.47	−101.26	−98.36	−97.04	−96.16	−95.23	−93.25	−92.59	−91.04	−88.96
	Confidence score	0.291	0.2739	0.2625	0.2575	0.2541	0.2506	0.2432	0.2408	0.2352	0.2278
	RMSD (A°)	52.2	54.28	69.98	54.41	71.58	52.77	71.33	38.43	73.87	72.77
5FUF-LDAO	Docking score	−109.02	−106.33	−104.59	−102.05	−100.11	−97.9	−96.82	−96.34	−95.73	−95.59
	Confidence score	0.3058	0.2945	0.2874	0.2771	0.2694	0.2608	0.2566	0.2548	0.2525	0.252
	RMSD (A°)	51.09	39.75	39.15	52.74	42.69	52.05	53.31	56.17	37.51	59.51
6EJ6-LDAO	Docking score	−107.11	−104.63	−99.12	−98.43	−97.49	−95.04	−92.6	−91.91	−91.68	−91.14
	Confidence score	0.2978	0.2875	0.2655	0.2628	0.2592	0.2499	0.2409	0.2383	0.2375	0.2356
	RMSD (A°)	38.5	44.35	43.94	42.21	37.36	39.81	39.47	38.09	40.08	32.07
6EJH-LDAO	Docking score	−109.22	−105.34	−102.13	−100.14	−100.02	−99.02	−96.86	−95.44	−94.04	−93.15
	Confidence score	0.3067	0.2904	0.2774	0.2695	0.269	0.2651	0.2568	0.2514	0.2462	0.2429
	RMSD (A°)	57.82	58.51	61.29	60.57	56.4	55.83	61.54	63	60.71	58.88

Note: LDAO, lauramine oxide; M, model; PP, protein-protein; RMSD, root mean square deviation

Прим.: LDAO – оксид лаурамина; M – модель; PP – белок-белковый; RMSD – среднеквадратическое отклонение

Discussion

Ammonium is the preferred nitrogen source for many microbes, and it enters the cell via MEP1-MEP3 proteins. Each of these transporters may enable fungal growth on media containing low concentrations of ammonium as the only nitrogen source; however, mutations in *MEP* genes prevent fungus from growing on ammonium at concentrations below 5 mM. *MEP* gene expression is enhanced in nitrogen-limited circumstances and inhibited in high ammonium concentrations. Under the latter circumstances, enough ammonium can easily move throughout the cell as ammonia or be absorbed by nonspecific transporters to promote growth.[9,19]

Furthermore, MEP2 is considered to function as an ammonium sensor, stimulating pseudohyphal development in response to extracellular ammonium. Current data show that MEP2's signaling function is linked to ammonium transport, since amino acid changes that limit MEP2's transport action also reduce pseudohyphal development.[20,21]

Although several mutations have been detected in MEP2, which are located in the protein's early sequence and final section, they differ significantly between *Candida* species and do not comprise the conserved area. When the DNA sequence segments of the *MEP2* gene were examined between the 10 *Candida* species, no significant differences were discovered; hence alternative approaches were utilized to compare the sequences between the species. By evaluating the root analysis of the strains, it became evident that they were interrelated, confirming that there was no basic difference between the strains. The DNA plot between the 9 strains and *C albicans* showed a rate of roughly 80%.

The use of coordinate imaging technology is critical in detecting the differences between the DNA sequences of the strains under investigation. The CGR study of the *MEP2* gene across the *Candida* species revealed distinct variations, as similarity was seen between the 5 species closest to *C albicans*, and there were 3 species that differ

considerably from the other 5 species in the yellow area highlighted in Figure 5.

In terms of FCGR, by inserting 4 nucleotides into the representation of the nitrogenous base sequence and analyzing the data for the 10 *Candida* species, it became obvious that *C albicans, C theae, C dubliniensis, C orthopsilosis*, and *C margitis* shared around 75% similarity. They were 65% similar to the other species, including *C parapsilosis* and *C oxycetoniae*. In contrast, *C tropicalis, C pseudojiufengensis*, and *C jiufengensis* varied from the other species by more than 40%. Clearly, the application of genetic imaging tools for strains can give results that may have a different impact than that of standard procedures.

Candida species are responsible for the vast majority of fungal pathogen-induced human illnesses. *C albicans* is the most prevalent cause of opportunistic infections. *Candida auris*, together with other developing species, such as *C tropicalis, C parapsilosis*, and *C krusei*, pose a new public health hazard worldwide. Some observations indicate that the reported antagonistic relationship between the 2 *Candida* species might be bidirectional, with *C albicans* releasing metabolites that might influence the metabolic activity of *C tropicalis* on some level.[22,23] This discovery prompted us to compare MEP2 protein levels in different *C albicans* species.

Previous research revealed that amphotericin B and ketoconazole have varied inhibitory effects on the expression of *MEP1-5* genes in *Candida epidermidis*. Amphotericin B suppressed *MEP1-4* genes more effectively; however, ketoconazole dramatically reduced *MEP*2 gene expression. This work sheds light on the possible mechanisms of action of antifungal medications on *Candida epidermidis* gene expression. It may also have ramifications for future research into more targeted antifungal treatments.[24]

Five strains of *C albicans* were chosen for comparison, with 5AEZ strain serving as the wild type. The 4 strains were found to have 4 main MEP2 mutations. The chemical substance, LDAO, was chosen because of its capacity to interact with proteins and accept the primary source of ammonium. Mutations were found in each strain. LDAO fusion sites were identified for each strain. Our findings show that the mutation in 5AH3 strain at residue 452 altered the fusion position of LDAO in MEP2, implying that this mutation eventually impacts *Candida*. Despite the advent of drug-resistant *C albicans* strains, this suggests that mutations play a basic and crucial role in modifying the position of the drug's docking with the protein.

The used software selects any chemical substance, regardless of its toxicity to the human body, that has the ability to dock to MEP2 by mathematical and chemical operations. Currently, there are no studies supporting the binding of MEP2 to LDAO in a laboratory setting or animal tests.

Conclusions

The CGR and FCGR procedures are widely regarded as useful and accurate methods for detecting DNA sequence and protein similarity. Approximately 80% of current MSA determination algorithms are comparable to conventional approaches. The docking residue of LADO with MEP2 varies as a result of a mutation within the protein's conserved region in certain strains but not all. Since identifying mutations in MEP2 is important for using *Candida* as a nitrogen source, this will allow for targeted therapy. Protein sequences from different species should ideally be compared using both CGR and FCGR, as well as by applying the MSA method.

Литература/References

1. Nobile CJ, Johnson AD. Candida albicans biofilms and human disease. *Annu Rev Microbiol*. 2015;69:71–92. PMID: 26488273. PMCID: PMC4930275. https://doi.org/10.1146/annurev-micro-091014-104330

2. Biswas K, Morschhäuser J. The Mep2p ammonium permease controls nitrogen starvation-induced filamentous growth in Candida albicans *Mol Microbiol*. 2005;56(3):649–669. Published correction appears in *Mol Microbiol*. 2006;60(6):1603–1604. PMID: 15819622. https://doi.org/10.1111/j.1365-2958.2005.04576.x

3. van den Berg B, Chembath A, Jefferies D, Basle A, Khalid S, Rutherford JC. Structural basis for Mep2 ammonium transceptor activation by phosphorylation. *Nat Commun*. 2016;7:11337. PMID: 27088325. PMCID: PMC4852598. https://doi.org/10.1038/ncomms11337

4. Dabas N, Schneider S, Morschhäuser J. Mutational analysis of the Candida albicans ammonium permease Mep2p reveals residues required for ammonium transport and signaling. *Eukaryot Cell*. 2009;8(2):147–160. PMID: 19060183. PMCID: PMC2643611. https://doi.org/10.1128/EC.00229-08

5. van den Berg B, Lister S, Rutherford JC. Ammonium transceptors: novel regulators of fungal development. *PLoS Pathog*. 2019;15(11):e1008059. PMID: 31697784. PMCID: PMC6837285. https://doi.org/10.1371/journal.ppat.1008059

6. Ariz I, Boeckstaens M, Gouveia C, et al. Nitrogen isotope signature evidences ammonium deprotonation as a common transport mechanism for the AMT-Mep-Rh protein superfamily. *Sci Adv*. 2018;4(9):eaar3599. PMID: 30214933. PMCID: PMC6135547. https://doi.org/10.1126/sciadv.aar3599

7. Paul JA, Wallen RM, Zhao C, Shi T, Perlin MH. Coordinate regulation of Ustilago maydis ammonium transporters and genes involved in mating and pathogenicity. *Fungal Biol*. 2018;122(7):639–650. PMID: 29880199. https://doi.org/10.1016/j.funbio.2018.03.011

8. De Michele R, Ast C, Loqué D, et al. Fluorescent sensors reporting the activity of ammonium transceptors in live cells. *Elife*. 2013;2:e00800. Published correction appears in *Elife*. 2015;4. PMID: 23840931. PMCID: PMC3699834. https://doi.org/10.7554/eLife.00800

9. Neuhäuser B, Dunkel N, Satheesh SV, Morschhäuser J. Role of the Npr1 kinase in ammonium transport and signaling by the ammonium permease Mep2 in Candida albicans. *Eukaryot Cell*. 2011;10(3):332–342. PMID: 21278231. PMCID: PMC3067475. https://doi.org/10.1128/EC.00293-10

10. Boeckstaens M, Llinares E, Van Vooren P, Marini AM. The TORC1 effector kinase Npr1 fine tunes the inherent activity of the Mep2 ammonium transport protein. *Nat Commun*. 2014;5:3101. PMID: 24476960. https://doi.org/10.1038/ncomms4101

11. Thind AS, Sinha S. Using Chaos-game-representation for analysing the SARS-CoV-2 lineages, newly emerging strains and recombinants. *Curr Genomics*. 2023;24(3):187–195. PMID: 38178984. PMCID: PMC10761335. https://doi.org/10.2174/0113892029649902310131121 56

12. Joseph J, Sasikumar R. Chaos game representation for comparison of whole genomes. *BMC Bioinformatics*. 2006; 7:243. PMID: 16677374. PMCID: PMC1482720. https://doi.org/10.1186/1471-2105-7-243

13. Rekadwad BN, Gonzalez JM, Khobragade CN. Genomic analysis of a marine bacterium: bioinformatics for comparison, evaluation, and interpretation of DNA sequences. *Biomed Res Int*. 2016;2016:7215379. PMID: 27882328. PMCID: PMC5108866. https://doi.org/10.1155/2016/7215379

14. Löchel HF, Eger D, Sperlea T, Heider D. Deep learning on chaos game representation for proteins. *Bioinformatics*. 2020;36(1):272–279. PMID: 31225868. https://doi.org/10.1093/bioinformatics/btz493

15. Lichtblau D. Alignment-free genomic sequence comparison using FCGR and signal processing. *BMC Bioinformatics*. 2019;20(1):742. PMID: 31888438. PMCID: PMC6937637. https://doi.org/10.1186/s12859-019-3330-3

16. Dawood AA. A method utilizing an image visibility graph to portray the arrangement of genomic data sequencing, gene frequencies for the peptidoglycan-associated lipoprotein (Pal) gene in Brucella spp., and prevalence of brucellosis in Nineveh. *Medicina Moderna*. 2024;31(2):155–165. https://doi.org/10.31689/rmm.2024.31.2.155

17. Deschavanne PJ, Giron A, Vilain J, Fagot G, Fertil B. Genomic signature: characterization and classification of species assessed by chaos game representation of sequences. *Mol Biol Evol*. 1999;16(10):1391–1399. PMID: 10563018. https://doi.org/10.1093/oxfordjournals.molbev.a026048

18. Marini AM, Boeckstaens M, Benjelloun F, Chérif-Zahar B, André B. Structural involvement in substrate recognition of an essential aspartate residue conserved in Mep/Amt and Rh-type ammonium transporters. *Curr Genet*. 2006;49(6):364–374. PMID: 16477434. https://doi.org/10.1007/s00294-006-0062-5

19. Khalil MI. Molecular docking and analysis of MEP2 protein in Candida albicans membrane. *EurAsian Journal of BioSciences*. 2020;14(2):4373–4376.

20. Brito AS, Neuhäuser B, Wintjens R, Marini AM, Boeckstaens M. Yeast filamentation signaling is connected to a specific substrate translocation mechanism of the Mep2 transceptor. *PLoS Genet*. 2020;16(2):e1008634. PMID: 32069286. PMCID: PMC7048316. https://doi.org/10.1371/journal.pgen.1008634

21. Lopes JP, Lionakis MS. Pathogenesis and virulence of Candida albicans. *Virulence*. 2022;13(1):89–121. PMID: 34964702. PMCID: PMC9728475. https://doi.org/10.1080/21505594.2021.2019950

22. Roberts K, Osme A, De Salvo C, et al. Candida tropicalis affects Candida albicans Virulence by limiting its capacity to adhere to the host intestinal surface, leading to decreased susceptibility to colitis in mice. *J Fungi (Basel)*. 2024;10(4):245. PMID: 38667916. PMCID: PMC11051055. https://doi.org/10.3390/jof10040245

23. Macias-Paz IU, Pérez-Hernández S, Tavera-Tapia A, Luna-Arias JP, Guerra-Cárdenas JE, Reyna-Beltrán E. Candida albicans the main opportunistic pathogenic fungus in humans. *Rev Argent Microbiol*. 2023;55(2):189–198. PMID: 36411138. https://doi.org/10.1016/j.ram.2022.08.003

24. Hilal Hashoosh Q, Al-Aaraji AM. Metalloprotease genes expression in Trichophyton mentagraphytes and Trichophyton simii contains genetic variations isolated from Iraqi patients resistance to ketoconazole and amphotericin B. *IOP Conference Series: Earth and Environmental Science*. 2024;1325(1):012025. https://doi.org/10.1088/1755-1315/1325/1/012025

Author credentials

Ali A. Dawood, PhD, Assistant Professor, Deputy Head of the Department of Anatomy, College of Medicine, University of Mosul (Mosul, Iraq). https://orcid.org/0000-0001-8988-5957

Conflict of interest: *none declared.*

Сведения об авторах

Давуд Али Адел, PhD, ассистент-профессор, заместитель заведующего кафедрой анатомии, медицинский колледж, Университет Мосула (Мосул, Ирак). https://orcid.org/0000-0001-8988-5957

Конфликт интересов

Автор заявляет об отсутствии конфликта интересов.