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Phylogenetic trees of mutant *Staphylococcus aureus* produce recombinant thrombolytic enzyme

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Abstract

The aim of study make Phylogenetic trees of mutant *Staphylococcus aureus* produce recombinant thrombolytic enzyme. Isolation *S.aureus* from different clinical sources from human that identified by vitek2-GP : 280 (56%) tonsils , 100 (20%) nose, 40 (8%) tumors, 17 (3.4%) urine, 27 (5.4%) skin(Acne) and 36 (7.2%) Blood. Genotypic analysis of *sak* gene showed presence of *sak* gene in all mutant VSSA and MSSA *S.aureus* after done chemical mutagenesis compared with control before chemical mutagenesis, size of *sak* gene (492bp) compared with DNA Ladder (1500bp).DNA Sequencing of *S.aureus* elucidate the coding region for *Sak* gene amplified from mutant VSSA and MSSA compared with analogous sequence of the same gene from standard strain of *S.aureus* possessed in BLAST / NCBI on the web site. Register strain in NCBI(National Center of Biotechnology Institute) Strain carry ID in NCBI/Gene Bank : strain1 ID=MN907801.1,strain2 ID=MN907802.1, strain3 ID=MN907803.1, strain4 ID=MN907804.1, strain5 ID=MN907805.1 and strain6 ID=MN907806.1; convert nucleotide sequence to protein by using Mega 6 program software. Phylogenetic trees drawn by neighbor-joining trees of strain based on spa-X Gene sequences for all strain that recording in NCBI/GeneBank hold accession number MN907801.1, MN907802.1, MN907803.1, MN907804.1, MN907805.1, MN9078026.1 *Staphylococcus aureus* IRAQ, the relativity between strain 0.9893-1.5814(99%); the relativity between 6 strain with strain in Australia 1.916(100%) 12/ CP043389.1:209416-2095276 *Staphylococcus aureus* Australia Meliboume; the relativity with strain 8 USA:KY America (100%), also the relativity with strain19 (100%) CP020960.1:153230:953990 *Staphylococcus aureus* Canada: Calgary (100%) relativity with strain(12,13,14,15,16,17,21,18), this results relativity with other strain in the world.The relativity of IRAQ strain between strain MN907801.1 with strain MN907804.1 0.8960(100%); the relativity between MN907804.1 with strain MN907805.1 is 0.9000(99%); the relativity between strain MN907801.1 and MN907805.1 is 0.3175(99%); the relativity of strain MN907801.1 with (relativity between MN907806.1, MN907805.1, MN907804.1, MN907801.1 is (99%)) 0.3038 (99%); the relativity between MN907803. (relativity between all strain) 0.2933(99%) and shown link in NCBI/GeneBank for strain recording with number 060753351. Compatibility IRAQ strain with other strain in the world is 99% in France, Japan, China(Zhejiang) province, USA (New York city),Ghana: Accra, Japan:Kyoto, Australia:Melbourne, USA:Detroit, USA:VA, China, USA:California, Canada:Calgary, USA, USA:SanDiego, Canada: Calgary.

Keywords: Phylogenetic trees, DNA Sequencing, Mutant *sak* gene, Fibrinolytic enzyme.

Introduction

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations causing skin and tissue infection, deep abscess, wound infections,sepsis, endocarditis, septic arthritis and osteomyelitis [1,2].

Virulence factors of *S.aureus* are chromosomally encoded (intrinsic resistance) including capsules and endotoxin, others resistance by mobile genetic elements (Extrinsic resistance) such as plasmids and bacteriophages causes horizontal gene transfer that convert bacteria from harmless into dangerous pathogens [3].

Thrombolytic therapy is needing for clot specific hydrolytic to get maximum patency in a short time with fewer side effects like minimal bleeding risk. SAK (492 bp) is one of the bacterial proteins having relatively good clot specificity than t-PA produce from native *S. aureus* and from lysogenic *S.aureus*. Cloning of *sak* gene in order to production therapeutic protein from the non-pathogenic host or less virulent *S.aureus* that useful for lowering cost

with effective therapeutic protein production in the Mutagenesis is an important technique to DNA in laboratory in order to produce mutant genes, proteins, strains of bacteria or genetically modified organisms (GMOs). The mutation may produce mutant proteins with new enhanced properties or novel functions that may be important in commercial use [5].

Mutagen is a physical or chemical agent that causes mutations due to changes in the genetic material DNA and cause increase frequency of mutation, some mutagen cause cancer, there are types of mutagens effect on DNA called genotoxic, also effect on transcription, replication the DNA which causes severe infection leading to cell death result in aberrant impaired or loss of function for a particular gene and causes accumulation mutations leading to cancers [6,7].

It contains 136 amino acid residues and has a molecular mass of 15kDa, Synthesis of staphylokinase in late exponential phase, it is comparable to streptokinase[8] that act to dissolve fibrin to assistance the bacteria in spreading (invasiveness) and give rise to damage the tissue by interact with plasminogen and turn into plasmin (proteolytic enzyme) which hydrolyses fibrin clots(deactivate the fibrin meshwork),prompt Staphylococcal get away from the fibrin clots and expedite systemic bacterial spreading from the infection position, then inhibiting phagocytosis [9,10,11], it play a role in the establishing of infections in humans [12].

The parent gene for *sak* transcribes a protein SAK consisting of 163 amino acids. After being transcribed, the 163-amino acid stretch is matured subsequently and processed into a 136-amino acid protein. Essentially Staphylokinase contains an alpha helix and a beta sheet plated on to each other. SAK is a single domain protein [13].Produce a mutant requires a change in base sequence within DNA there are a numeral of distinguished mechanisms for altering the structure of DNA include base substitutions, additions or deletions base resulting by chemicals and environmentals agents[14].

Recombinant DNA technlogy substantial to produce proteins for pharmaceutical applications via biotechnology accomplished successfully in microbial cells and yeasts. In early 80's, the FDA confirmed the clinical utilize of recombinant human insulin from recombinant *E. coli* (Humulin-US/Humuline-EU) for the treatment of diabetes, being the first recombinant pharmaceutical to get in the market. 151 recombinant pharmaceuticals approved for human utilized by Food and Drug Administration (FDA) and by European Medicines Agency (EMEA) [15].

Materials and Methods

Study population: Samples were cultivated in Nutrient broth, Nutrient agar medium and Mannitol Salt agar medium incubated at 37 ºC ,Identification of *S.aureus* achieved by Vitek2-GP with specific Card for Gram Positive bacteria and by Genotypic detection used ***16srRNA*** by PCR to confirmative identification of tumor swab isolates [16].

Standard strain : Standard strain and plasmid vector used in this study were listed in table (1).

Table (1): Standard strain and plasmid vector used in this study.

No.	Standard strain	Sources
1	MN907801.1	Iraq
2	MN907802.1	Iraq
3	MN907803.1	Iraq
4	MN907804.1	Iraq
5	MN907805.1	Iraq
6	MN907806.1	Iraq

Detection of *sak* gene encode for Staphylokinase: All isolates were subjected to molecular screening study using PCR amplification technique. In this study, uniplex PCR was done to detect for *sak 2genes* of *S.aureus*

Genomic extraction of DNA: Genomic DNA of *S.aureus* was extracted according to boiling method described by [17]

1: Aliquot of 1 ml of bacterial fresh culture was spined at 8000 rpm for 5 minutes .

2: Re-suspended pellet cells in 10 ml of TE buffer and heated to boiling at 100 ºC for 10 minutes , then left to cool at 25 ºC .

3: centrifuged at 8000 rpm for 5 minutes .

4: Supernatant the pellt containing genomic DNA with TE buffer.

Genotypic detection of *S.aureus* by Polymerase Chain Reaction (PCR): PCR done amplification of *16SRNA* and *sak* genes according to [18] showed in table 2-5. The amplification of *sak* gene from mutant *S.aureus* prepared as listed in table (2). The optimal conditions of polymerase chain reactions of *sak* gene from mutant *S.aureus* determined in table (3).

Table (2): Components of reaction mixture for amplification of *sak* gene of mutant *S.aureus*.

No.	Component	Volume 25 (µl)
1	Green master mix	12.5
2	Forward Primer (10 Pmol)	1.5
3	Reverse Primer (10 Pmol)	1.5
4	DNA template	5
5	Nuclease free water	4.5
	Total volume	**25**

Table(3): PCR Amplification Program of *sak* gene .

Initial denaturation	Denaturation	Annealing	Extension	Final extension
95 °C for 5 min. 1 cycle	94 °C for 1min.or 30 sec	52 °C for 1min. 30 sec	72 °C for min. 30 sec	72°C for 10 min. 1 cycle
		35 cycle		

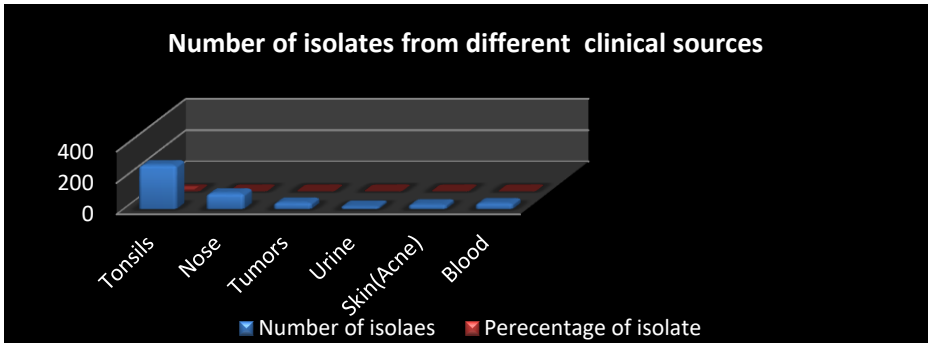
Sequencing of mutant *S.aureus*: PCR products resulted from the amplification of *sak* gene were sent to NICEM/USA ABI3730XL Applied BIOSYSTEMS to determine the complete nucleuotide sequence of *sak* gene.The results were analyzed according to National Center for Biotechnology Information (NCBI) data and Alignments of amino acid sequence were analyzed with the same software, convert nucleotide sequence to protein by using Mega 6 program software. Phylogenetic trees(evolutionary trees) of strain done by using Spa-x Gene sequences to neighbor-joining trees of strain compared with strain in UK(America), Canada, Australia and France. Also studying the protein drawing for Beta-sheet and α-Helix by using Raptoxt software program.

Phylogenetic trees drawing compared with other strain in NCBI in the world(UK(America), Canada, France and China): Phylogenetic trees drawn by neighbor-joining trees of strain based on spa-X Gene sequences for all strain that recording in NCBI/GeneBank

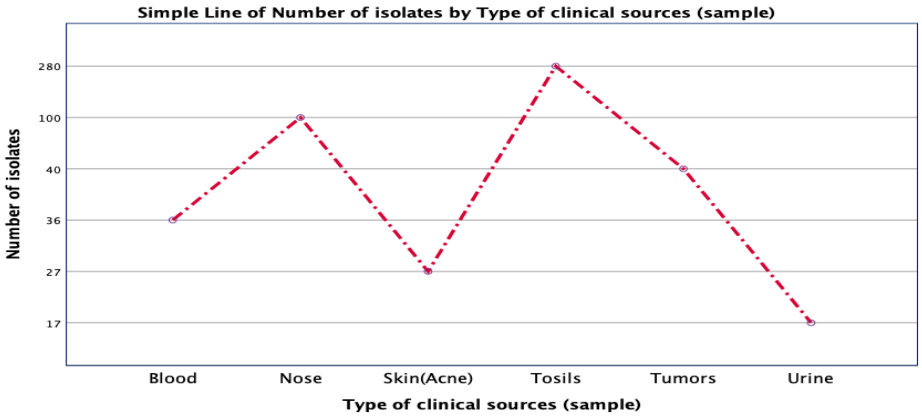
Statistical Analysis: The Statistical Analysis System-SAS [19] program was used to detect the effect of difference factors in study parameters in this study done according to SPSS by ANOVA test and T-test.

Results and Discussion

Isolation and Identification of *Staphylococcus aureus*: The results of isolation from different clinical sources of human were identified by vitek2-GP : 280 (56%) tonsils , 100 (20%) nose, 40 (8%) tumors, 17 (3.4%) urine, 27 (5.4%) skin(Acne) and 36 (7.2%) Blood as shown in figure (1)and (2).



Figure(1):Prevalence of *S.aureus* from different clinical sources with percentage of isolates.



Figure(2):Simple line number of isolates by type of clinical sources (sample) of *S.aureus* from different clinical sources(SPSS,2020).

Genotypic detection for mutant *sak* gene from mutant VSSA and MSSA *S.aureus* by PCR: Genomic DNA form 100 mutant VSSA and MSSA *S.aureus* extracted, purity of genomic DNA 1.8.

Results in figure (3) shown the genotypic analysis of *sak* gene showed presence of *sak* gene in all mutant VSSA and MSSA *S.aureus* after by Hydroxylamine(HA), Acridine Orange(AO) and Ethylemethansulfonate(EMS) chemical mutagenesis compared with control before chemical mutagenesis accomplished in agarose gel electrophoresis, size of *sak* gene (492bp) compared with DNA Ladder (1500bp).

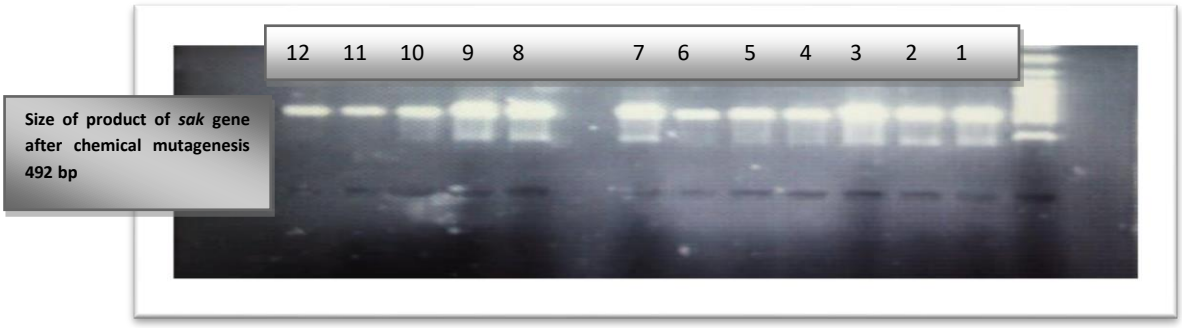


Figure (3) : Gel electrophoresis for detection amplified mutant*sak* gene (492bp)on agarose gel (1%) , 50V for 1 hr., Lane (1 – 12) represents mutantVSSA and MSSA *S.aureus* , (M) DNA Ladder (1500 bp).

A previous study by [20] amplification of *Sak* gene by PCR technique was carried out for (A15-M1 , A15-M4 , A15 , A31, A34, A43) mutant *S.aureus* when exposure to UV.light.

There are several types of PCR have sensitivity, especially with small numbers of bacteria, Nested PCR is one of detection only in a few bacteria found in clinical specimens[21,22].

Nucleotides Sequencing of *sak* gene from mutant VSSA and MSSA *S.aureus* with types of mutations: The Results of DNA Sequencing of *S.aureus*elucidate the coding region for *Sak* gene amplified from mutant VSSA and MSSA compared with analogous sequence of the same gene from standard strain of *S.aureus* possessed in BLAST / NCBI on the web site shown in figure (4).

Register strain in NCBI(National Center of Biotechnology Institute) Strain carry ID in NCBI/Gene Bank : strain1 ID=MN907801.1,strain2 ID=MN907802.1, strain3 ID=MN907803.1, strain4 ID=MN907804.1, strain5 ID=MN907805.1 and strain6 ID=MN907806.1; convert nucleotide sequence to protein by using Mega 6 program software; also Protein drawing done by using Raptoxt software program.

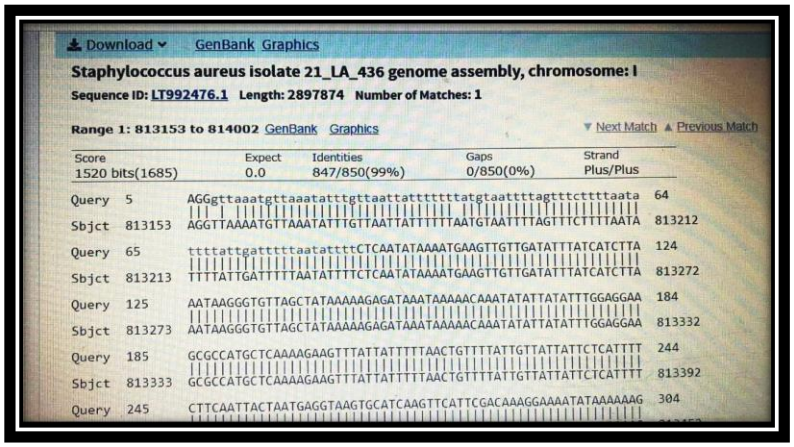
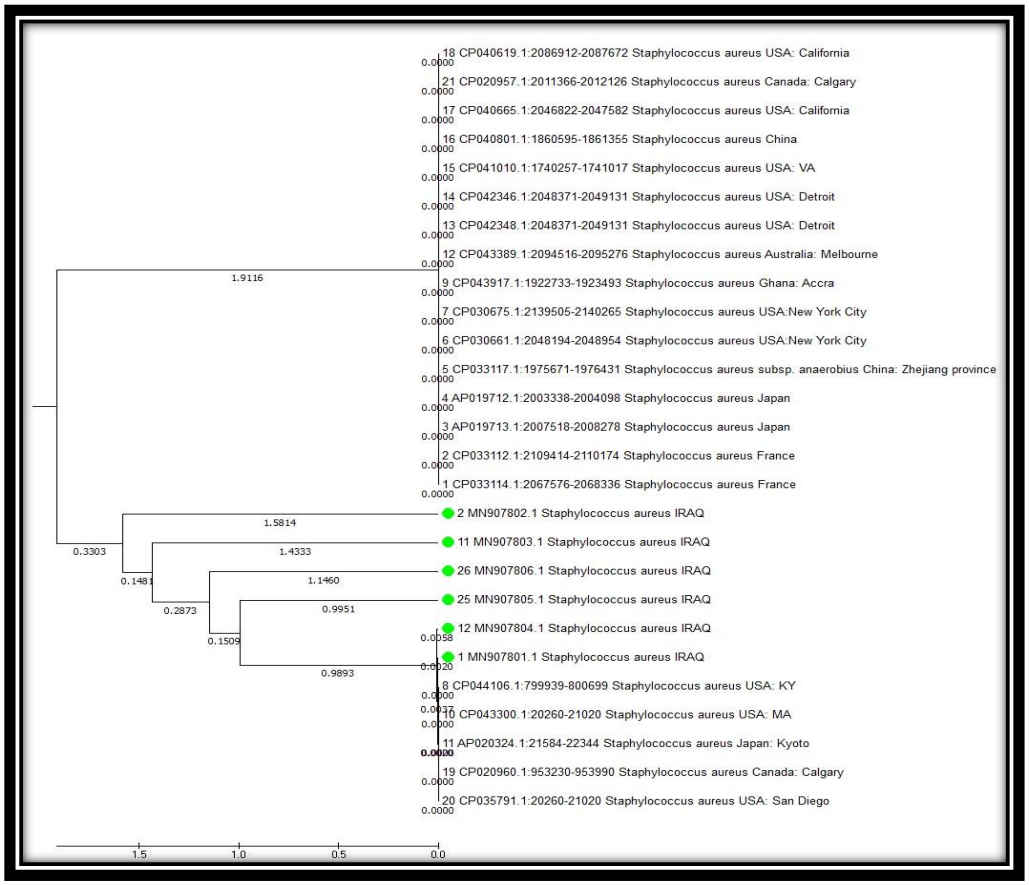


Figure (4):Matching of mutant *S.aureus* with strain ID in NCBI/Gene Bank ID=LT992476.1, Length:2897874, number of matching:1 in NCBI.

Phylogenetic trees drawing compared with other strain in NCBI in the world(UK(America), Canada, France and China): Phylogenetic trees drawn by neighbor-joining trees of strain based on spa-X Gene sequences for all strain that recording in NCBI/GeneBank hold accession number MN907801.1, MN907802.1, MN907803.1, MN907804.1, MN907805.1, MN9078026.1 *Staphylococcus aureus* IRAQ, the relativity between strain 0.9893-1.5814(99%); the relativity between 6 strain with strain in Australia 1.916(100%) 12/ CP043389.1:209416-2095276 *Staphylococcus aureus* Australia Meliboume; the relativity with strain 8 USA:KY America (100%), also the relativity with strain19 (100%) CP020960.1:153230:953990 *Staphylococcus aureus* Canada: Calgary (100%) relativity with strain(12,13,14,15,16,17,21,18), this results relativity with other strain in the world shown in figure(5).

In figure(6) the relativity of IRAQ strain between strain MN907801.1 with strain MN907804.1 0.8960(100%); the relativity between MN907804.1 with strain MN907805.1 is 0.9000(99%); the relativity between strain MN907801.1 and MN907805.1 is 0.3175(99%); the relativity of strain MN907801.1 with (relativity between MN907806.1, MN907805.1, MN907804.1, MN907801.1 is (99%)) 0.3038 (99%); the relativity between MN907803. (relativity between all strain) 0.2933(99%) and shown link in NCBI/GeneBank for strain recording with number 060753351.

Results in table (4) shown compatibility IRAQ strain with other strain in the world is 99% in France, Japan, China(Zhejiang) province, USA (New York city),Ghana: Accra, Japan:Kyoto, Australia: Melbourne, USA: Detroit, USA:VA, China, USA: California, Canada: Calgary, USA, USA: San Diego, Canada: Calgary.


18 CP040619.1:2086912-2087672 Staphylococcus aureus USA: California
21 CP020957.1:2011366-2012126 Staphylococcus aureus Canada: Calgary
17 CP040665.1:2046822-2047582 Staphylococcus aureus USA: California
16 CP040801.1:1860595-1861355 Staphylococcus aureus China
15 CP041010.1:1740257-1741017 Staphylococcus aureus USA: VA
14 CP042346.1:2048371-2049131 Staphylococcus aureus USA: Detroit
13 CP042348.1:2048371-2049131 Staphylococcus aureus USA: Detroit
12 CP043389.1:2094516-2095276 Staphylococcus aureus Australia: Melbourne
9 CP043917.1:1922733-1923493 Staphylococcus aureus Ghana: Accra
7 CP030675.1:2139505-2140265 Staphylococcus aureus USA:New York City
6 CP030661.1:2048194-2048954 Staphylococcus aureus USA:New York City
5 CP033117.1:1975671-1976431 Staphylococcus aureus subsp. anaerobius China: Zhejiang province
4 AP019712.1:2003338-2004098 Staphylococcus aureus Japan
3 AP019713.1:2007518-2008278 Staphylococcus aureus Japan
2 CP033112.1:2109414-2110174 Staphylococcus aureus France
1 CP033114.1:2067576-2068336 Staphylococcus aureus France
2 MN907802.1 Staphylococcus aureus IRAQ
11 MN907803.1 Staphylococcus aureus IRAQ
26 MN907806.1 Staphylococcus aureus IRAQ
25 MN907805.1 Staphylococcus aureus IRAQ
12 MN907804.1 Staphylococcus aureus IRAQ
1 MN907801.1 Staphylococcus aureus IRAQ
8 CP044106.1:799939-800699 Staphylococcus aureus USA: KY
10 CP043300.1:20260-21020 Staphylococcus aureus USA: MA
11 AP020324.1:21584-22344 Staphylococcus aureus Japan: Kyoto
19 CP020960.1:953230-953990 Staphylococcus aureus Canada: Calgary
20 CP035791.1:20260-21020 Staphylococcus aureus USA: San Diego
1.9116
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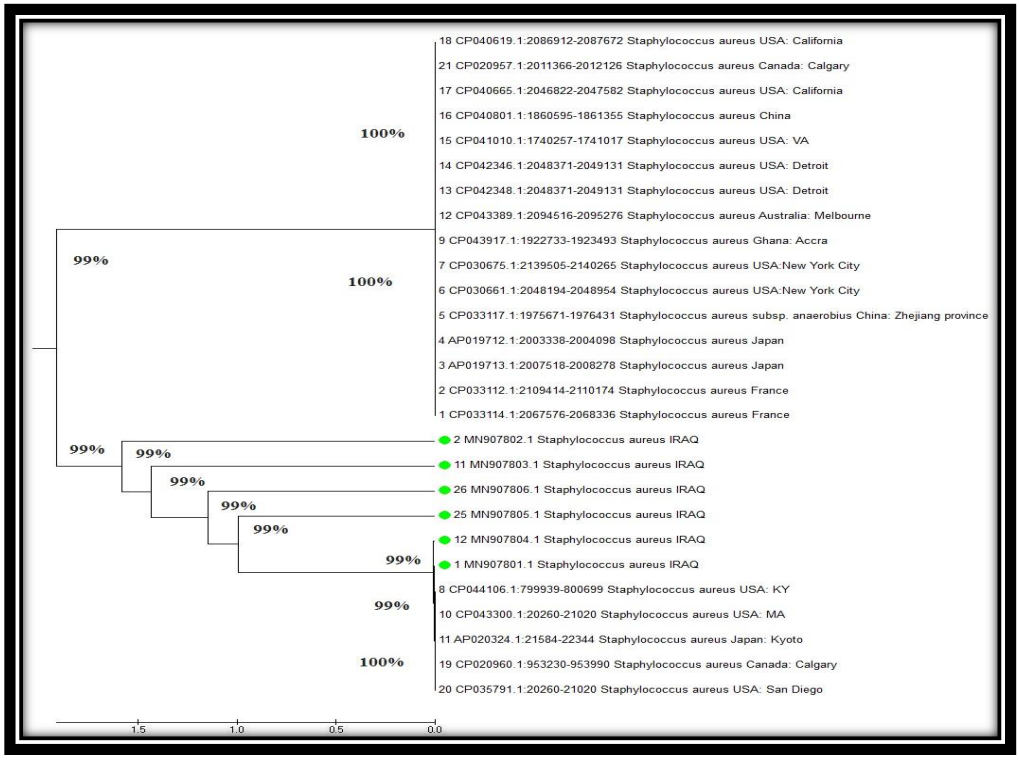
18 CP040619.1:2086912-2087672 Staphylococcus aureus USA: California
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17 CP040665.1:2046822-2047582 Staphylococcus aureus USA: California
16 CP040801.1:1860595-1861355 Staphylococcus aureus China
15 CP041010.1:1740257-1741017 Staphylococcus aureus USA: VA
14 CP042346.1:2048371-2049131 Staphylococcus aureus USA: Detroit
13 CP042348.1:2048371-2049131 Staphylococcus aureus USA: Detroit
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26 MN907806.1 Staphylococcus aureus IRAQ
25 MN907805.1 Staphylococcus aureus IRAQ
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11 AP020324.1:21584-22344 Staphylococcus aureus Japan: Kyoto
19 CP020960.1:953230-953990 Staphylococcus aureus Canada: Calgary
20 CP035791.1:20260-21020 Staphylococcus aureus USA: San Diego
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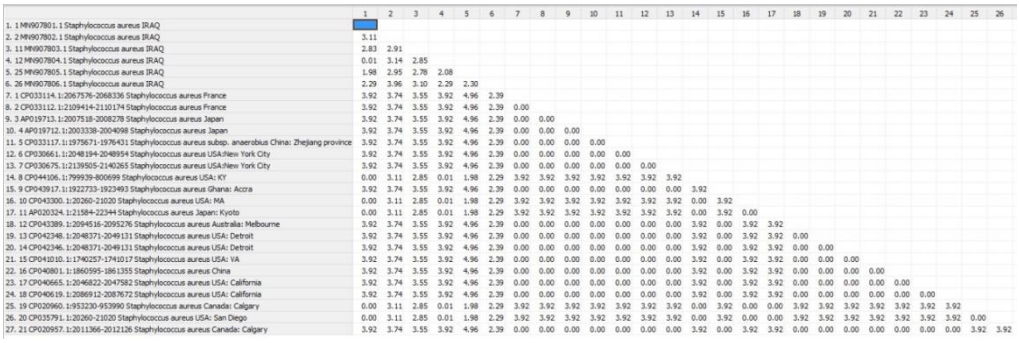

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1. 1 MN907801.1 Staphylococcus aureus IRAQ																										
2. 2 MN907802.1 Staphylococcus aureus IRAQ	3.11																									
3. 11 MN907803.1 Staphylococcus aureus IRAQ	2.83	2.91																								
4. 12 MN907804.1 Staphylococcus aureus IRAQ	0.01	3.14	2.85																							
5. 25 MN907805.1 Staphylococcus aureus IRAQ	1.98	2.95	2.78	2.08																						
6. 26 MN907806.1 Staphylococcus aureus IRAQ	2.29	3.96	3.10	2.29	2.30																					
7. 1 CP033114.1:2067576-2068336 Staphylococcus aureus France	3.92	3.74	3.55	3.92	4.96	2.39																				
8. 2 CP033112.1:2109414-2110174 Staphylococcus aureus France	3.92	3.74	3.55	3.92	4.96	2.39	0.00																			
9. 3 AP019713.1:2007518-2008278 Staphylococcus aureus Japan	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00																		
10. 4 AP019712.1:2003338-2004098 Staphylococcus aureus Japan	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00																	
11. 5 CP033117.1:1975671-1976431 Staphylococcus aureus subsp. anaerobius China: Zhejiang province	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00																
12. 6 CP030661.1:2048194-2048954 Staphylococcus aureus USA:New York City	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00	0.00															
13. 7 CP030675.1:2139505-2140265 Staphylococcus aureus USA:New York City	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00	0.00	0.00														
14. 8 CP044106.1:799939-800699 Staphylococcus aureus USA: KY	0.00	3.11	2.85	0.01	1.98	2.29	3.92	3.92	3.92	3.92	3.92	3.92	3.92													
15. 9 CP043917.1:1922733-1923493 Staphylococcus aureus Ghana: Accra	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.92												
16. 10 CP043300.1:20260-21020 Staphylococcus aureus USA: MA	0.00	3.11	2.85	0.01	1.98	2.29	3.92	3.92	3.92	3.92	3.92	3.92	3.92	0.00	3.92											
17. 11 AP020324.1:21584-22344 Staphylococcus aureus Japan: Kyoto	0.00	3.11	2.85	0.01	1.98	2.29	3.92	3.92	3.92	3.92	3.92	3.92	3.92	0.00	3.92	0.00										
18. 12 CP043389.1:2094516-2095276 Staphylococcus aureus Australia: Melbourne	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.92	0.00	3.92	3.92									
19. 13 CP042348.1:2048371-2049131 Staphylococcus aureus USA: Detroit	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.92	0.00	3.92	3.92	0.00								
20. 14 CP042346.1:2048371-2049131 Staphylococcus aureus USA: Detroit	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.92	0.00	3.92	3.92	0.00	0.00							
21. 15 CP041010.1:1740257-1741017 Staphylococcus aureus USA: VA	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.92	0.00	3.92	3.92	0.00	0.00	0.00						
22. 16 CP040801.1:1860595-1861355 Staphylococcus aureus China	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.92	0.00	3.92	3.92	0.00	0.00	0.00	0.00					
23. 17 CP040665.1:2046822-2047582 Staphylococcus aureus USA: California	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.92	0.00	3.92	3.92	0.00	0.00	0.00	0.00	0.00				
24. 18 CP040619.1:2086912-2087672 Staphylococcus aureus USA: California	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.92	0.00	3.92	3.92	0.00	0.00	0.00	0.00	0.00	0.00			
25. 19 CP020960.1:953230-953990 Staphylococcus aureus Canada: Calgary	0.00	3.11	2.85	0.01	1.98	2.29	3.92	3.92	3.92	3.92	3.92	3.92	3.92	0.00	3.92	0.00	0.00	3.92	3.92	3.92	3.92	3.92	3.92	3.92		
26. 20 CP035791.1:20260-21020 Staphylococcus aureus USA: San Diego	0.00	3.11	2.85	0.01	1.98	2.29	3.92	3.92	3.92	3.92	3.92	3.92	3.92	0.00	3.92	0.00	0.00	3.92	3.92	3.92	3.92	3.92	3.92	3.92	0.00	
27. 21 CP020957.1:2011366-2012126 Staphylococcus aureus Canada: Calgary	3.92	3.74	3.55	3.92	4.96	2.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.92	0.00	3.92	3.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.92	3.92

Figure (5): Phylogenetic trees of Novel strain of *S.aureus* relativity with other strain in the world.

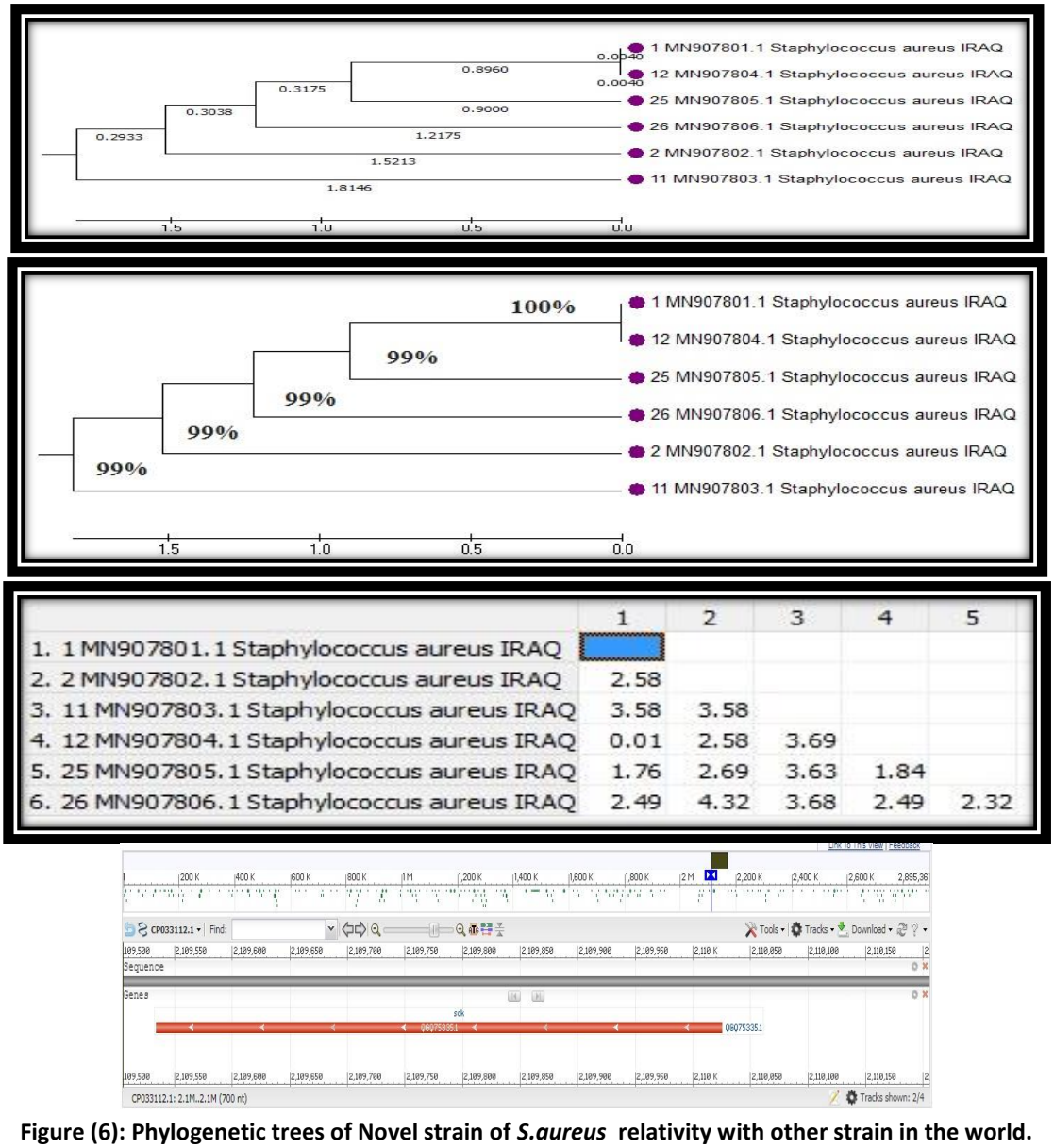

Figure (6): Phylogenetic trees of Novel strain of *S.aureus* relativity with other strain in the world.

Table(4):Percentage of Identity for Novel strain *S.aureus* with other strain NCBI in the world.

	Accession	Country	Source	Compatibility
1.	ID: CP033114.1	**France**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
2.	ID: CP033112.1	**France**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
3.	ID: AP019713.1	**Japan**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
4.	ID: AP019712.1	**Japan**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
5.	ID: CP033117.1	**China: Zhejiang province**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
6.	ID: CP030661.1	**USA:New York City**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
7.	ID: CP030675.1	**USA:New York City**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
8.	ID: CP044106.1	**USA: KY**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
9.	ID: CP043917.1	**Ghana: Accra**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
10.	ID: CP043300.1	**USA: MA**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
11.	ID: AP020324.1	**Japan: Kyoto**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
12.	ID: CP043389.1	**Australia: Melbourne**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
13.	ID: CP042348.1	**USA: Detroit**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
14.	ID: CP042346.1	**USA: Detroit**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
15.	ID: CP041010.1	**USA: VA**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
16.	ID: CP040801.1	**China**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
17.	ID: CP040665.1	**USA: California**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
18.	ID: CP040619.1	**USA: California**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
19.	ID: CP020960.1	**Canada: Calgary**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
20.	ID: CP035791.1	**USA: San Diego**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**
21.	ID: CP020957.1	**Canada: Calgary**	**Staphylococcus aureus staphylokinase SakXH gene**	**99%**

Conclusions

1-Expression of thrombolytic enzyme from *S.aureus* increased by chemical mutagenesis.

2-Presence *sak* gene of mutant S.aureus when detection by PCR.

3- Iraqi Novel strain of *S.aureus* when done DNA Sequencing of mutant *S.aureus*.

4-Phylogenetic trees of six Iraqi novel strain of mutant *S.aureus* compared with other strain in the world have different relativity between other strain in France, Japan, China(Zhejiang) province, USA (New York city),Ghana: Accra, Japan:Kyoto, Australia: Melbourne, USA:Detroit, USA:VA, China, USA:

California, Canada: Calgary, USA, USA: SanDiego, Canada: Calgary.

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