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Molecular identification and characterization of isolated bacteria from beef meat by 16s rRNA gene analysis

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Abstract

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The present study is focused on the molecular identification of 16s rRNA genes, identifying bacterial strains from local beef shops, and analyzing the patterns of PCR products collected from various stores in Baghdad, Iraq. For the purpose of this study, twenty beef steak samples were randomly chosen from nearby marketplaces. The total number of bacteria was counted to identify various bacterial strains presented in the samples. PCR methods were used to detect 16S rRNA extraction patterns and DNA gene primer preparation patterns, and the results revealed bacterial contamination of meat, specifically beef, sold in local butcher shops. Moreover, these 16S ribosomal RNA genes were identified via the corresponding Genbank accession codes and through molecular identification of many bacterial species, including *Staphylococcus warneri*, *Serratia marcescens*, *Klebsiella oxytoca*, *and Raoultella ornithinolytica*; however, the most dominant bacterial species were *Serratia marcescens*, which was identified utilizing 16S rRNA and DNA sequences examined utilizing nucleotide BLAST alignment techniques. In brief, the findings of this study indicated that beef exposure to various environmental factors in Boucher shops significantly influenced beef growth, especially that of several bacterial species; further research is thus required to identify additional specific genes for additional bacterial types.

Keywords: Beef meat; DNA genes; molecular detection; total bacterial count; 16S rRNA

Introduction

In most countries, meat and meat products are essential dietary items because they contain a wide range of proteins. The protein content typically compensates for nutritional deficiencies (Mahmood & Dakheel, 2023). It is a consumable food because, on the one hand, enzymes play a role in the metabolic processes that occur as food tissue develops and matures. In contrast, beef is susceptible to pathogens that might destroy it. Consequently, the beef industry is crucial, because it transforms consumable meat into permanent compounds (Mahamed et al., 2017). Various techniques are also used in food products, such as low heat, high heat, UV, and microwave radiation, to avoid or at least postpone foodstuff corruption (ALyousif, 2022)

Foodborne illnesses caused by eating unhealthy, infected foodstuffs result in significant morbidity and mortality for customers. Every year, diarrhea kills adults and children, leading to the consumption of unpleasant foods (Hadab & Dakheel, 2022). Food hygiene is becoming increasingly important, and guaranteeing quality control is a worldwide and Iraqi public health problem (Barbin, 2022). Several zoonotic illnesses, such as those caused by bacteria, viruses, and fungi, can easily spread to essential meat and animal products and may affect animal health (Dakheel et al., 2021). Bacterial species, particularly *Staphylococcus* spp., *Salmonella* spp., *Clostridi-*

um spp., *Campylobacter* spp., *Listeria* spp., *Escherichia coli*, and *Shigella* spp., cause more than 90% of foodborne illnesses (Hamzah, 2010). Some local meat markets have been affected by poor sanitation and open exhibitions. When flowing water is not accessible, hand hygiene is often accomplished in containers and occasionally without detergents. Therefore, offering meat at local stores is highly debatable from a health perspective (Barbin, 2022). Cars that are essential for the transmission of potentially fatal foodborne pathogens, can spread diseases in meat. The most common cause of foodborne diseases in underdeveloped nations is meat, which is frequently sold on the streets. It is frequently unclear if the foods in the stores are microbilogically healthy, even though their unique flavor (Qader & AlKahafaji, 2019). Therefore, contamination of meat by *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., and other bacteria during preparation, per-cooking, and practice of different phases is related to possible health hazards. As a result, foodborne tract outbreaks linked to eating cleanliness, have been reported in numerous Middle Eastern countries (Habib et al., 2020). Moreover, health risks associated with local markets are common, especially for foodborne pathogens identified in different studies (Sheng & Wang, 2021; Hamzah, 2010). In contrast, the prevalence of antimicrobial resistance among foodborne pathogens has increased in recent decades, as determined by the use of unique patterns of RNA and DNA genes via PCR (Kornienko et al., 2022; White et al., 2022; Todd, 2020).

This study used PCR products to collect, characterize, and analyze the bacteria, found in several local beef markets and their patterns of 16s rRNA and DNA gene expression. These samples were obtained from different Al-Karkh, Baghdad, and Iraqi stores, with a focus on the molecular identification of various genes detected in the bacterial spp.

Materials and Methods

Sample preparation

Fifty beef rounds, which were large pieces of the rear leg, were provided for this investigation during January and February, 2022, from local markets. These samples were collected to determine whether 1 kg of fresh meat was sold from different local shops in Al-Karkh, Baghdad, Iraq. Twelve beef flesh samples were used to identify the type of bacteria.

Isolation of bacteria

One hundred grams of each beef sample were sliced and dipped in 250 mL of hygienic water before sterilization. To accomplish tenfold hygiene dilution, 1 mL of this mixture was placed in a different tube that contained 9 mL of distilled water (Koluman & Dikici, 2013).

Total bacterial counts

The total bacterial count was determined by scattering 0.1 mL from proper dilutions on the superficial surface of the nutrient agar dishes. The dishes were incubated overnight at 37°C. A colony counter was used to calculate the number of dissimilar colonies on each plate, and the number of colonies on each plate ranged from 25 to 250. The number of colony-forming units (CFU) per gram of sample was calculated using the formula for CFU in grams, indicated by (total colonies were multiplied by an inverted dilution factor and then divided by inoculum volume), as described previously (Cauchie et al., 2020).

Purification and amplification of DNA

To prepare the agarose solution, 1 g of agarose powder was dissolved in a vial of 100 ml containing 1x TBE. Agarose was heated in a hot bath until the solution was pure. The agarose mixture was cooled to between 50°C and 55°C. heated gel was stained with 3 µl of red stain, and the ends of the casting tray were taped with 2 layers of paper. The trays used for gel casting included combs.

The initial phase of denaturation at 94°C for 5 min was accompanied by thirty cycles of denaturation at 90°C for 1 min, annealing at 50–55°C for a min, and extension at 72°C for a minute to improve the reaction conditions. Ultimately, an extra five minutes at 70°C were reached. A 1.5% agarose gel was electrophoresed with the PCR result at 100 V. Ethidium bromide (0·5 $\mu g.mL^{-1}$) was used to color the agarose, which was then seen using a gel documentation apparatus.

Finally, the DNA quality was determined using a UV transilluminator as previously described (ALyousif, 2022)

RNA extraction and primer preparation

Lyophilized primers were diluted in ddH2O to create a standard solution of 100 pmol/l, stored at -20°C. A working primer solution of 10 pmol/l was prepared. For this, 10 mL of standard solution was added to 90 mL of ddH2O. The 16s RNA primers targeted bacterial species. PCR was conducted using the Geneaid bacterium kit containing Maxime PCR PreMix Kit(i-Taq).

Polymerase chain reaction (PCR) was used to generate RNA using universal primers obtained from Sequence-F (5'-AGAGTTTGATCCTGGCTCAG-3') and Sequence-R (5'-GGTTACCTTGTTACGACTT-3'). The responses were given in a total volume of 25 µL. Initial denaturation was performed at 95°C for 5 min, followed by 1 cycle of 95°C for 45 s, 56°C for 45 s of annealing, 1 minute of extension-1 at 72°C, and 5 min of extension-2 at 72°C; the RNA gene was subsequently recovered as indicated previously (Barbin et al., 2013).

Results

Counting the total bacteria

The number of pathogens and assessing the suitability of the foods for human consumption was determined by the total number of microorganisms, identified in beef flesh. The animal's exposure to diseased conditions both before and after slaughter is reflected in a large number of germs, whereas the animal's exposure to health status is reflected in the lower figure. The total number of bacteria in the local beef samples sold in the Baghdad/Al-Al-Karkh markets is shown in Table 1. The results showed that the total bacterial count of the beef samples was between 4.50 * 10^7 and 4.91 * 10^8 CFU/g.

Molecular identification via PCR

16S rRNA gene detection

In the present study, five bacterial species were isolated using polymerase chain reaction (PCR) and 16s ribosomal RNA (rRNA) for genus-specific confirmation. The 574 bp segment of the studied gene was designed for amplification. The results were shown following the PCR results (Figures 1, 2, 3, 4, and Table 1). These samples and the 16s ribosomal-RNA gene sequences were uploaded into the GenBank-Graphics/Next MatchPrevious database with the corresponding GenBank accession codes. Exposure to chemical mutagens may alter gene sequences and disrupt hereditary rRNA (Doulgeraki et al., 2012). These results included 16S rRNA gene sequence data from beef in Al-Karkh/Baghdad/Iraq.

16_s RNA gene detection

The most common bacterial species, *Serratia marcescens*, was identified by molecular characterization utilizing 16S rRNA sequences examined via nucleotide BLAST alignment techniques. This analysis revealed the most generating isolate, which was detected as *Serratia marcescens*. The database included the sequences of the bacterial 16S

Table 1. The total bacterial count of beef meat (CFU/ g)

No of Sample	Total bacterial counting (CFU/ g)
Sample 1	4.50 * 10^7
Sample 2	4.49 * 10^7
Sample 3	4.51 * 10^7
Sample 4	4.53 * 10^8
Sample 5	4.91 * 10^8

Score	Expect	Identities	Gaps	Strand
1541 bits(834)	0.0	838/840(99%)	0/840(0%)	Plus/Plus

Query 301 TCGGATCGTAAAACTCTGTTATCAGGGAAGAACAAATGTGTAAGTAATTGTGCACATCTT 360
Sbjct 408 .....C..... 467
Query 421 GTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTCTTAAGTCT 480
Sbjct 528 .....T..... 587

Score	Expect	Identities	Gaps	Strand
1240 bits(671)	0.0	677/680(99%)	0/680(0%)	Plus/Plus

Query 301 CAGCTCGTTTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGT 360
Sbjct 321 .....**G**..... 380

Query 541 ATTGGAGTCTGCAACTCGACTCCATAAAGTCGGAACCGCTAGTAATCGTAGATCAGAATG 600
Sbjct 561 .....**G**.....**T**..... 620

Fig 1. *Staphylococcus warneri* strain DK131 16S ribosomal RNA gene, partial sequence; Sequence ID: MT642942.1 Length: 1495; Number of Matches: 1; Range 1: 108 to 947 GenBankGraphics; Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1541 bits(834)	0.0	838/840(99%)	0/840(0%)	Plus/Plus

Query 121 AAAGCGCACGCAGGCGGTCTGTCAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAAC 180
Sbjct 535 .....**G**..... 594
Query 601 TGCATGGCTGTCGTCAGCTCGTGTTGGGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAA 660
Sbjct 1015 .....**T**..... 1074

Fig. 2. *Serratia marcescens* (subs. Marcescens) 16S ribosomal RNA gene, partial sequence; Sequence ID: KU237235.1 Length: 726 Number of Matches: 1; Range 1: 21 to 700 GenBankGraphics; Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1483 bits (803)	0.0	809/812 (99%)	0/812 (0%)	Plus/Plus

Query 121 ATAACGTCGCAAGACCAAGGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGA 180
Sbjct 157 .....**A**..... 216
Query 421 TTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCAAGCAGCCGCGGTAATACGG 480
Sbjct 457 .....**C**..... 516
Query 661 CCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAGGCGTGGGGAG 720
Sbjct 697 .....**A**..... 756

Fig. 3. The partial sequence of the *Klebsiella oxytoca* 16S ribosomal RNA gene; Sequence ID: MT326229.1 Length: 1443; Number of Matches: 1; Range 1: 415 to 1254 GenBankGraphics; Next Match Previous Match

No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Sequence ID with submission	Source
1	Transvertion	230	A\T	ID: MT642942.1	ID: ON366401.1	Staphylococcus warneri
	Transition	380	A\G			
	Transvertion	570	A\T			
2	Transition	455	C\T	ID: MT642942.1	ID: ON366402.1	Staphylococcus warneri
	Transition	579	T\C			
3	Transvertion	329	G\T	ID: KU237235.1	ID: ON366403.1	Serratia marcescens
	Transition	586	G\A			
	Transition	596	T\C			
4	Transition	563	G\A	ID: MT326229.1	ID: ON366404.1	Klebsiella oxytoca
	Transvertion	1041	T\G			
5	Transition	175	A\G	ID: MT568560.1	ID: ON366405.1	Raoultella ornithinolytica
	Transvertion	497	C\A			
	Transition	746	A\G			

Fig. 4. *Raoultella ornithinolytica* strain RT1902 16S ribosomal RNA gene, partial sequence; Sequence ID: MT568560.1 Length: 1478 Number of Matches: 1; Range 1: 37 to 848 GenBankGraphics; Next Match Previous Match

rRNA sequences with the registration numbers (Table 2 and Figure 5).

Discussion

The total bacteria count

The overall bacterial count was 10^7 – 10^8, which was within permissible limits, and some of these shops' beef was affected by germs (Rani et al., 2023). These findings were consistent with those of (Sofos, 2014), who conducted prior research in Baghdad. Another study examined the possibility that frozen meat is less likely to be infected than fresh meat (Mahmood & Dakheel, 2023). As a result, the number of bacteria, period, weather, storage conditions, and beef's bio-

Table 2. The characterizations of *Serratia marcescens* using the 16S rDNA gene for different isolates in various countries that are related strains in GeneBank

	1	2	3	4	5	6	7	8	9	10
1. ON366403.1:S.marcescens:IRAQ										
2. 1 KU237235.1:S.marcescens:Egypt	0.004									
3. 2 KC212077.1:S.marcescens:China	0.004	0.000								
4. 3 MT386168.1:S.marcescens:Panama	0.006	0.001	0.001							
5. 4 TM3639S.l:S.marcescens:India	0.006	0.001	0.001	0.000						
6. 5 MT424300.1:S.marcescens:Nigeria	0.006	0.001	0.001	0.000	0.000					
7. 6 CPOS0960.1:S.marcescens:USA	0.006	0.001	0.001	0.000	0.000	0.000				
8. 7 MT12S920.1iS.marcescens:Bangladesh	0,006	0.001	0.001	0.000	0.000	0.000	0.000			
9. 8 MN36S937.1:S.marcecens:South Korea	0.006	0.001	0.001	0.000	0.000	0.000	0.000	0.000		
10. 9 MN763847. l:S.marcescens:Saudi Arabia	0,006	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	
11. 10 AP024847. l:S.maricscens:Japan	0.006	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000

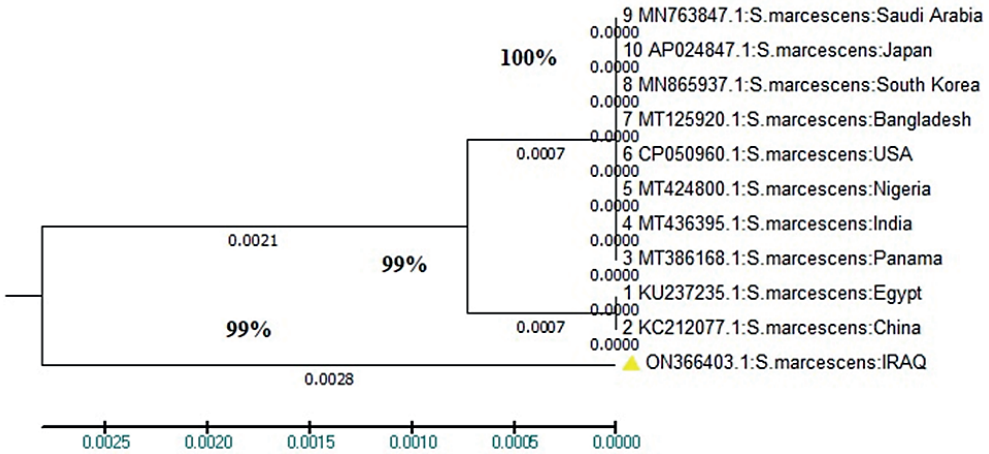

Fig. 5. Analysis of the phylogenetic tree for *Serratia marcescens* isolates based on 16S rRNA for different isolates in various countries that are related strains in GenBank

logical and physical characteristics might affect how quickly it spoils due to germs (Ali, 2013).

Microbial contamination of meat, specifically beef, is implicated in many factors, including shipping, preservation, maintenance, and prolonged, exposure to unsanitary environments (Mahamed et al., 2017). The meat and, more specifically, beef sold in the local butcher shops of Baghdad, Iraq, could contain different kinds of bacteria, especially on their surface. Thus, the meat products should be frozen, and hygienic safety precautions must be taken.

In the present study, the shelf life of the meat samples was one day, which could be compared to the several days needed for degradation after storage, as other studies have done (Ali, 2013). Thus, the total microbes of these samples could be affected by the time of storage and some meat parameter qualities, such as pH and lipid stability. Moreover, the treatment of the samples with natural plant extracts showed their antibacterial properties (Hadab & Dakheel, 2022).

Molecular identification via PCR

The 16s ribosomal RNA genes were loaded into a database system with chemical mutagen exposure, that may alter the gene sequence, resulting in hereditary rRNA disruption (ALyousif, 2022). When bacteria is subjected to natural conditions and molecular genetic mutations, her capacity to repair rRNA damage is lost, and this trait is subsequently transmitted (Mahamed et al., 2017). In addition, this work was supported by Hadab & Dakheel (2022), who identified many bacterial isolates as novel isolates in GenBank, based on 16S rRNA genes from shops in the Misan region of Iraq. This finding enhanced the 16S rRNA gene sequence data from beef meat in Al-Karkh/Baghdad/Iraq.

The current investigation involved molecular identification of many bacterial species, including *Staphylococcus warneri, Serratia marcescens, Klebsiella oxytoca*, and *Raoultella ornithinolytica.* The bacteria was cultured on many selective media for culture, and numerous investigators have isolated it (Ali, 2013).

The UPGMA algorithm was used to infer the evolutionary history of the genes. The optimal tree was displayed (Abdlla & Al-Sanjary, 2023), with a total branch length total of 0.00635885, in the same units, as the genetic distances used to estimate the phylogenetic tree, the tree is rendered to proportion. The evolution was estimated by the number of nucleotide substitutions persite (Tanura et al., 2004). Eleven nucleotide sequences were subjected to the study. The codon positions 1st+2nd+3rd+Noncoding can be considered. Positions with holes and incomplete data were all removed. The final dataset included 680 locations. In MEGA6, evolutionary analyses were carried out (Al-Zahrani & Bukhari, 2019).

The findings demonstrated that beef exposure to diverse conditions in Boucher shops substantially impacts beef products, particularly the growth of different bacterial types, including *S. marcescens*. According to *Serratia* species, it is one of the strains that fixes nitrogen. This finding clarified the capability of creating a medium devoid of nitrogen sources. Either the inhibition of Tran or the reduced expression of this gene's transcription that prevents the gene's expression may be attributed to the pH of the meat (Alttai et al., 2023). Studies have revealed that applicable mechanisms, including cold plasma, can inactivate contaminants when they contact biological materials and meat. These investigations have examined the effects of cold plasma on determining whether cold plasma may be useful for curing meat products (Gorbunova, 2019).

Conclusion

In conclusion, the number of total bacteria was measured using the PCR technique, including the 16s rRNA gene primer manufacturing patterns, which were used to characterize various bacterial species in beef obtained from local markets. Using the corresponding GenBank accession codes, 16S ribosomal RNA genes were discovered in the present study, and numerous bacterial species, including *Staphylococcus warneri*, *Klebsiella oxytoca*, *Raoultella ornithinolytica* and *Serratia marcescens*, were easily identified via molecular methods. However, *Serratia marcescens* was the most prevalent bacterial species and was distinguished using 16S rRNA.

Declarations

The authors confirm that they do not have any conflicts of interest.

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