First Report of Draft Genome Sequencing for *Ligilactobacillus salivarius* SuAm7 from Oral Disease Patients in Iraq

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Sections Info	ABSTRACT
Article history: Submitted: May 24, 2025 Final Revised: June 15, 2025 Accepted: June 30, 2025 Published: July 19, 2025 *Keywords:* *Ligilactobacillus salivarius* Oral microbiota Genome sequencing Probiotics Phylogenetics	*Objective: The purpose of the present study is to examine the genomic composition of Ligilactobacillus salivarius SuAm7, emphasizing both its possible clinical significance and its function in oral health. **Method:** Gram-staining, 16S rRNA PCR and Whole-genome sequencing were used to identify and cultivate the saliva sample. Whole-genome sequencing with Illumina technology was done, followed by analysing the genome's features and figuring out where it fits in the picture of evolution. **Results:** SuAm7's genome was 1.84 Mb in total, smaller than most related species and it shared a lot of similarities with L. salivarius subsp. salicinius DSM 20554. Functional annotations indicate that genes for secondary metabolism, nitrogen usage, movement and chemotaxis were missing which suggests the mouth cavity is a place where the bacteria need to be specially adapted. The way the bacterium is classified was confirmed by genetic tests including ANI analysis and in silico DNA-DNA hybridization. **Novelty:** This article reports on the draught genome of Ligilactobacillus salivarius SuAm7, an oral bacterium from someone who was healthy in Iraq. The findings emphasise the value of L. salivarius SuAm7 in the oral microbiota and describe its genetic and how it functions. The study shows what types of bacteria are present in Iraqi people's mouths and indicates that L. salivarius might be useful in fighting bad breath.*

INTRODUCTION

The combination of healthy mouth and good general health is supported by the active bacteria living inside our mouths [1]. Saliva protects the mouth by containing many different kinds of bacteria that mirror all the other bacteria found in the mouth. Studying the bacteria and DNA of the microbes found in saliva is needed to learn about their influence on dental health as well as on disease development such as periodontitis and caries. Recent findings indicate that *Ligilactobacillus salivarius* was formerly called Lactobacillus and is discovered to be a facultative anaerobe in human mouths and in the intestines [2]. Though many people recognise this species for its support of the digestive system, exploring its benefits to oral health and adaptation to several types of fluid environments should get more attention. Examination of L. salivary isolates from saliva by genomic analysis helps us know what role they play, if they are resistant to antibiotics and their role in the oral microbiome [3].

Although microbial genomics attracts much interest, only a few studies have looked at the genome of oral microbes and greatly so of *Ligilactobacillus salivarius*, in Iraq. Because there isn't enough data from the region, it is clear that local studies are required to analyse the effects of geography on local microorganisms and their health impacts. Iraqi researchers were able to map and study the genome of a *L. salivarius* bacteria strain found in a patient's saliva here in Mosul. Bioinformatics was utilised to understand how this

bacterium processes food, develops resistance and developed through evolution. The main purpose of this research is to find out more about *L. salivarius* in the area, tell people about its role in health and join recent studies looking into its influence in clinics.

RESEARCH METHOD

This descriptive study involved the isolation, identification, and whole-genome sequencing of a *Ligilactobacillus salivarius* strain from saliva sample, followed by bioinformatics-based genomic analysis.

Ethical consideration

The institute's ethical review board approved the project. The University of Mosul and Iraqi Ministries of Higher Education and Scientific Study ethics committees approved the study methodology and methods. Ethical approval was given under procedure number (260), on 2/10/2024.

Isolation and identification of *Ligilactobacillus salivarius* SuAm7

Saliva sample was collected from patient with oral disease and cultured on modified Brucella agar and incubated at 37°C for 3 days under microaerophilic conditions.

Gram staining: The bacterial strain identified according to its morphology of colonies on modified Brucella agar

Molecular Techniques: Bacterial isolate were confirmed using conventional PCR based on *16S rRNA* [4]. Subsequently, genomic DNA was sent to Macrogen Biotech in South Korea where the entire genome was sequenced.

Whole Genome Based Phylogenetic Tree

Phylogenetic tree formation to determine the relationship among *Ligilactobacillus salivarius* SuAm7 and similar strains was inferred using data from the Type Strain Genome Server (TYGS) [5]. FASTA format of the genome was put on the server, using the standard settings. FastME 2.0 programme which comes with TYGS, was used to infer the tree for the analysis [6].

In silico DNA-DNA (*is*DDH) Hybridization analysis

With the help of the GGDH bioinformatics tool, the values of *is*DDH were determined between *Ligilactobacillus salivarius* SuAm7 and the top most related strains using whole genome sequences data [7].

16S rRNA gene phylogenetic tree analysis

To identify similarities between *Ligilactobacillus salivarius* SuAm7 and other DNA sequences, the Nucleotide Basic Local Alignment Search Tool (BLASTn) was employed against GenBank entries. A bootstrap analysis (1000X) with the MEGA-11 software [8] was used to build the phylogenetic tree.

Antibiotic resistance genes are found in the Genome of *Ligilactobacillus salivarius* SuAm7:

CARD (version 3.2.6) is a programme developed by [9] that was used to detect resistance genes to antibiotics, disinfecting agents and antiseptics in the genome of *Ligilactobacillus salivarius* SuAm7.

Comparisons of the Genome

Using the BLAST Ring Image Generator (BRIG) software [10], the genome sequence of *Ligilactobacillus salivarius* SuAm7 was compared to the closest species and the results shown as a set of circles.

RESULTS AND DISCUSSION
Results

When referring to the NCBI website, it was noted that there was a registration for the first genome (reference genome) was on Jan 5, 2024, and the number of total genomes of this bacterium deposited on the site was (354) around the world. and the registration of our genome was on the 25-Feb-2025, (20/4/2025).

**Genome of *Ligilactobacillus salivarius SuAm*7 Description:**

In our study, genome of *L. salivarius* SuAm7 including 50 contigs sequences as a final assembly. The contigs sequences (bp) were between 505 and 557,020, and length of total Genome sequence was 1,883,876 bp, with GC content (%) as 32.84, protein-coding sequence (CDSs) as 1,779 and tRNA genes numbers was 48. To enable a thorough assessment of genome assemblies, the N50 metric should be used in concert with other metrics, see Table 1.

Table 1. General genome features of *L.salivarius* SuAm7 generated using QUAST software and RAST server.

Feature	Value
Genome total length (bp)	1,883,876
Number of contigs	50
Largest contig (bp)	557,020
Smallest contig (bp)	505
GC content (%)	32.84
Total of protein-coding sequences (CDSs)	1,779
Number of tRNA genes	48
Number of rRNA genes	2, 3, 2 (5S, 16S, 23S)
N50	137,869

Analysis of Genome and Closely Related Strain:

The Rapid Annotation System Technology (RAST) server was used for genome annotation. Subsystem characteristics and the coverage of each subsystem feature were shown using the SEED viewer using a pie chart view. The green bar means the percentage of proteins belonging to the subsystems and the blue bar shows the percentage not included in the subsystems. Furthermore, there were no genes for nitrogen metabolism, motility and chemotaxis, photosynthesis, secondary metabolism, nodulation, phages, prophages and plasmids in our bacterium's genome. With 118 genes, the highest gene group is found in protein. Sulfur metabolism and potassium metabolism (3) genes as the smallest gene groupings, Figure 1.
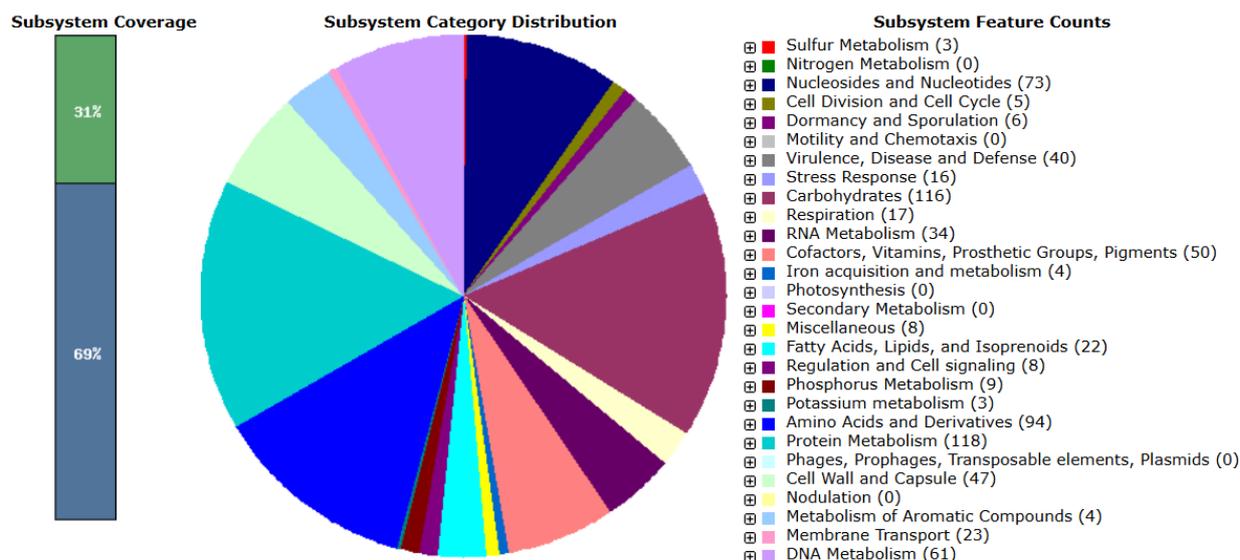
Figure 1. Subsystem category distribution statistics of *L.salivarius* SuAm7.

By using in silico DNA-DNA hybridization (*is*DDH), 15 closely associated type strains have compared with the SuAm7. When comparing the genome of bacterium under study was with what is mentioned in the (**Table 2**), pairwise comparisons of *L. salivarius* SuAm7 genome vs. type strain genomes based on *is*DDH reveals that *is*DDH value was ranging from 83.4to 14.4. Genome of *Ligilactobacillus salivarius* SuAm7 found as bigger than *Ligilactobacillus saerimneri* DSM 16049, *Ligilactobacillus hayakitensis* JCM 14209, *Ligilactobacillus aviarius* DSM 20655, *Ligilactobacillus araffinosus* DSM 20653 and *Ligilactobacillus cholophilus* BD7642T. As for the rest of the type strain, the genome of *SuAm7* was smaller than them. GC content (%), was smaller than *Ligilactobacillus saerimneri* DSM 16049, *Ligilactobacillus agilis* DSM 20509, *Ligilactobacillus aviarius* DSM 20655 and *Ligilactobacillus pabuli* AF129. protein-coding sequences of SuAm7 was higher than *Ligilactobacillus saerimneri* DSM 16049, *Ligilactobacillus cholophilus* BD7642T, *Ligilactobacillus hayakitensis* JCM 14209, *Ligilactobacillus aviarius* DSM 20655 and *Ligilactobacillus araffinosus* DSM 20653 when was compared to the 16 type strains. *Enterococcus* durans NBRC 100479 has 0.239 as the smallest nucleotide.

The building of the final tree used Fast ME 2.0 as an approach with the balanced minimum evolution method (100X pseudo-bootstrap support). The species and subspecies belonging, the genomic GC percentage, δ-values, genome size and protein count are how leaves are labelled in the metagenome.

Because they are members of the same species and subspecies clusters and have a strong bootstrap value, the phylogenetic analysis shows that *L. salivarius* SuAm7 is closely related to *L. salivarius* subsp. salicinius DSM 20554, as they share a common ancestor with strong bootstrap support (85%). Both are distinct from other Ligilactobacillus species, such as *L. sairrimneri* , *L. hayakutensis* , and *L. agilis* , which form separate clusters.

Table 2. Genome Pairwise comparisons of *Ligilactobacillus salivarius* SuAm7 genome vs. type strain genomes based on *is*DDH, GC content, δ- value, Genome size and Number of proteins.

Ligilactobacillus salivarius SuAm7 vs. type strain genomes	Digital *is* DDH value (%)	Percent G+C (%)	δ- value	Genome Size (bp)	Number of proteins
Lactobacillus salivarius subsp. salicinius DSM 20554	83.4	32.61	0.279	1,971,522	1932
Ligilactobacillus salivarius DSM 20555	80.5	32.49	0.36	1,975,751	1861
Ligilactobacillus saerimneri DSM 16049	55.3	39.55	0.335	1,721,367	1756
Ligilactobacillus hayakitensis JCM 14209	53.6	33.98	0.354	1,667,319	1666
Ligilactobacillus agilis DSM 20509	50.9	39.74	0.321	2,056,971	1891
Ligilactobacillus aviarius DSM 20655	43.7	39.9	0.258	1,680,107	1585
Ligilactobacillus araffinosus DSM 20653	43.6	38.12	0.265	1,475,115	1414
Ligilactobacillus cholophilus BD7642T	39.3	31.37	0.275	1,587,935	1530
Ligilactobacillus pobuzihii E100301	35.4	37.72	0.352	2,346,370	2127
Ligilactobacillus pabuli AF129	35.2	40.51	0.298	2,326,077	2159
Marinilactibacillus piezotolerans DSM 16108	27.6	35.44	0.278	2,551,636	2473
Enterococcus durans NBRC 100479	24.8	37.79	0.239	3,017,301	2874
Enterococcus dispar ATCC 51266	21.4	37.17	0.251	2,812,918	2637
Streptococcus parauberis CCUG 39954	19.5	35.49	0.271	2,164,480	2146
etragenococcus halophilus subsp. flandriensis DSM 23766	18.9	35.81	0.297	2,520,474	2463
Bavariicoccus seileri DSM 19936	14.4	38.25	0.282	2,244,485	2128

Discussion

A deep understanding of the sequences of genome can participate for obtaining an accurate genetic analysis of bacteria, involving the characteristics of genetic that associated with favorable effects and those potentially associated with undesirable

features [11]**.** For judging the quality of a genome, the N50 statistic is helpful and it reveals how well the assembly contains continuous reads, handles repetitions and performs overall [12]. With the appearance of new sequencing methods, some researchers are suggesting the use of "in silico methods" to estimate genomic distances, instead of DDH [13]. Many people link Ligilactobacillus with vertebrate hosts because it includes species that are homofermentative, non-motile and can make urease. Being able to resist stomach acid and having a QPS status granted by the EFSA make them widely chosen as probiotics [14]. The research by [15] looked at the genome of *Ligilactobacillus salivarius* (recently renamed from *Lactobacillus salivarius*) strain B4311 which was taken from samples of faeces from broiler chickens. Building on the complete genome of *L. salivarius* B4311 which is 2,071,255 base pairs, we found it to contain three parts: a single chromosome (1,801,655 bp), a megaplasmid (247,930 bp) and a small plasmid (21,670 bp) and all this is larger than that of our current research. The (GC) content of B4311 is 33.1% which is the highest reported value and higher than that of SuAm7. Also, the genome of B4311 include 1963 protein-coding sequences (CDS), more than what was reported by [15] in SuAm7. Running the B4311 genome through the BAGEL4 platform (http://bagel4. molgenrug.nl/) revealed that it has a gene cluster for a two-peptide bacteriocin in the salivaricin P family which belongs to class IIb. In 2007, this bacteriocin family was identified for the first time in the bacteria *L. salivarius* DPC6005 [16]. In addition, the study of [17] mentioned that the total size of the genome of *Ligilactobacillus salivarius* 2102-15 was 2,017,204 bp which was bigger than genome under study (SuAm7). And the average GC content of 33.07 % which is also highest than the GC content of SuAm7. Also, the above researchers were pointed that Illumina reads were adapter trimmed using Trimmomatic 0.30 with a sliding window quality cut off of Q15. All sequencing reads were assembled by using Unicycler v.0.4.9b. The whole genome assembly coverage was 80.0x. The assembly was annotated by the NCBI Prokaryotic Genome Annotation Pipeline using the best-placed reference protein set and GeneMarkS-21. The complete genome sequence of *Ligilactobacillus salivarius* 2102-15 was represented by three circular molecules: chromosome (1,834,593 bp) and two plasmids (140,826 and 41,785 bp).

CONCLUSION

Fundamental Finding : The draft genome of Ligilactobacillus salivarius SuAm7 reveals unique genetic features, including its close relation to L. salivarius subsp. salicinius DSM 20554, smaller genome size, and specialized adaptations to the oral environment. The absence of motility and metabolism-related genes highlights its niche-specific role. **Implication :** This study enhances the understanding of oral microbiota and opens pathways for potential probiotic applications in oral health management. **Limitation :** Despite its contribution, the study is limited by the use of a draft genome, which may not capture the full complement of functional genes and regulatory elements necessary to fully characterize its biological role. **Future Research :** Further research should aim to complete the genome sequence and conduct functional analyses to validate gene expression and probiotic potential, ensuring a more comprehensive understanding of its role in oral health.

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