Summary

(Since many beneficial bacteria fall within the lactic acid bacteria (LAB group, the focus has been on the isolation of LAB from various sources and ،assessing their probiotic qualities in vitro. By using 16S rRNA gene sequencing the strains were identified. Following this, whole genome sequencing was used to identify the strain level, investigate possible therapeutic applications, and .evaluate safety

.samples were collected of traditional yogurt, cheese, and infant feses ١٥٠ The De Man Rogosa and Sharpe (MRS) selective medium was used to separate the bacteria, and then they were purified. 161 isolates were obtained, divided ،into 47 from infant feces, 51 from yogurt, and 63 from cheeses. Initially identification was accomplished by morphological and biochemical ،investigations using colony and cells characteristics Gram staining, oxidase .and catalase

Multiple phases were used to assess the probiotic properties of the bacterial isolates. First, as a precaution, a hemolysis test was done. The majority of the isolates exhibited no blood hemolysis. The efficiency of the agar diffusion, agar spot, and broth microdilution methods was then evaluated against Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus faecalis, and Escherichia coli. Only 10 of the total isolates tested exhibited inhibitory activity against the indicator bacteria. n the diffusion test against E. faecalis, the largest mean diameter of the inhibition zone was 21 mm, whereas the spot test against K. pneumoniae had the largest mean inhibition zone of 25 mm. The microdilution assay yielded a minimum inhibitory concentration of 1/16. Furthermore, the strains were evaluated for resistance to simulated stomach and intestinal environments. The logarithm of

.live cells decreased somewhat when exposed to the enzyme pepsin and pH3
However, there were no significant differences in the logarithm of viable cells
.when Oxgall was present
In both the auto-aggregation and co-aggregation ability tests, isolate HR3 had higher percentages of 65.95% and 59.32%, respectively. Anti-biofilm activities were evaluated. Cell-free supernatants (CFSs) have been shown to inhibit P. aeruginosa and S. lugdunensis biofilm formation. During the antibiotic susceptibility test, the probiotic isolates showed consistent resistance ،patterns against ciprofloxacin, cloxacillin, gentamicin, kanamycin streptomycin, and vancomycin, as well as resistance to tetracycline. These ،isolates, however, were susceptible to penicillin, imipenem, erythromycin .clindamycin, amikacin, and azithromycin
By using the 16S rRNA gene sequence the isolates HR1 and HR2 were identified as Lacticaseibacillus sp. and Lacticaseibacillus paracasei while the .other isolates were identified as L. plantarum
Whole genome resequencing was performed, and sequencing data was converted into raw data by Illumina SBS technology. After sequencing, overall reads' quality, total bases, total reads, GC (%) and basic statistics were calculated. The filtered reads were mapped to the reference genome. After the genome was assembled, it was annotated. The variants were classified by each .chromosome
Pathosystems Resource Integration Center (PATRIC) and Rapid Annotation using Subsystem Technology (RAST) were used for assembly and annotation. The National Center for Biotechnology Information (NCBI) was ،(used to register sequences of strains HA3(Isolate HR3) and HA9(Isolate HQM .under accession numbers JAUTDK000000000.1 and JAUTDL000000000.1
The genomes measured a single circular 3,314,449 and 3,312,723 base

.pairs in size. Their chromosomes contained 44.38% and 44.40% G+C content
Isolate HA3 comprised 3,253 protein-coding sequences, 3 ribosomal RNA
rRNA) genes, and 62 transfer RNA (tRNA) genes, while strain HA9 contained)
protein-coding sequences, 2 rRNA genes, and 59 tRNA genes. Both HA3 ٣٠٢٩٨
and HA9 strains were equipped with multiple antibiotic resistance genes across
different groups and stress response factors. The HA3 strain also can produce a
total of 14 bacteriocins, and eight of them exhibit immunogenicity. The HA3
.and HA9 strains could synthesize water-soluble B vitamins
(The protein sequences inside PATRIC cross-genus families (PGFams
(were aligned by multiple sequence comparison by log-expectation (MUSCLE
.tool, and the nucleotides that matched each sequence were assigned to a protein
These sequences revealed that HA3 was 100% comparable to the reference
.strain L. plantarum WCFS1, whereas HA9 was 80%