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ISOLATION AND MOLECULAR IDENTIFICATION OF LACTOBACILLUS DELBRUECKII SPP. FROM CURD AND CHEESE FROM DEHRADUN REGION OF UTTARAKHAND

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ABSTRACT

This study aimed to isolate and characterize a new potential probiotic Lactobacillus with the aid of a culture method and PCR amplification of the 16S rRNA gene. The study employed a selective medium to identify fifty distinct Lactobacillus bacteria from five dairy samples sourced from the Dehradun region in Uttarakhand. Since the biochemical tests on all samples were nearly equivalent, one of the top five samples was chosen to undergo 16s RNA sequencing. Genes encoding 16S rRNA were amplified from DNA extracted from microbial cultures using universal bacterium primers using PCR. To identify the distant bacteria, the 16S rRNA genes of one identical bacteria were sequenced and aligned, and data was deposited in Gen Bank for the distant bacteria.

1. INTRODUCTION

Researchers studied the health effects and advantages of lactic acid bacteria in the contemporary setting of industrial food production, where lactic acid bacteria (LAB) are essential for fermented foods to be created and preserved.[1-2] It has been suggested that probiotic supplements, like lactic acid bacteria, can enhance food's nutritional and health-promoting qualities.[3] Lactobacillus is widely utilized in the food sector to produce starter cultures. LAB has recently sparked a lot of attention due to its ability to protect the human host system against foodborne pathogens. This has led to their potential application as biopreservatives in the food industry and antibiotic substitutes in medicine.[4-6] It's wonderful to know that probiotics can assist with a wide range of health conditions, including IBS, constipation, inflammatory bowel disease, acute & antibiotic-associated diarrhea, hypertension, diabetes, and allergy-related disorders. Including probiotics in our diets can benefit our overall health and well-being.[7-8] Because of its ability to minimize the risk of infectious diseases and the need for antibiotics, probiotic supplements have received a lot of attention in recent years. This is an important advancement, as antibiotic therapy is frequently ineffective owing to drug resistance.[9] Traditional clinical microbiology lab techniques may be challenging to accurately identify lactobacilli species, necessitating molecular diagnostic techniques like 16S rRNA gene sequencing for species-level identification. Identifying lactobacilli is challenging because of their varying shapes and sizes, which resemble other organisms like Streptococcus and Leuconostoc. Since lactobacilli rarely cause illness, labs often report them as "Lactobacillus spp." (Martinez et al., 2014).[10] Lactobacilli, a non-pathogenic bacterial genus, are a common part of the human microbiota, consisting of Gram-positive, non-spore-forming, microaerophilic, and catalase-negative rods, they are known as GRAS organisms (Generally Recognized as Safe) as they do not produce pigment (De Angelis and Gobbetti, 2016).[11] Bacteria present in the gastrointestinal tract, particularly the intestines, help minimize infections and maintain a healthy gut flora balance (Viramontes-Hörner et al., 2015).[12] It is believed that the gastric microbiota contains several Lactobacillus species, which include L. fermentum, L. ultunensis, L. gastricus, L. reuteri, L. plantarum, L. kalixensis, L. salivarius, and L. gasseri, L. delbrueckii (Liévin-Le Moal, V., & Servin, A. L. 2014).[13] Lactobacillus delbrueckii is the type species of the Lactobacillus genus, categorized into 25 new species based on genetic and phylogenetic analysis (Zheng et al., 2020).[14] The aforementioned species, which consists mostly of two subspecies, bulgaricus and lactis, is critical in the manufacture of commercial fermented dairy products like as yogurt and cheese, as well as biotherapeutic techniques (De Jesus et al., 2022).[15] Several studies have classified the delbrueckii strains as probiotics because they resist stressors, inhibit pathogens, and reduce inflammation. These studies have mostly focused on treating gastrointestinal disorders including colorectal cancer, ulcerative colitis, and intestinal mucositis. (De Jesus et al., 2019).[16-17] NCBI's scientific classification database reports that Lactobacillus spp. numbers have risen to 172 species recently. Such big outcomes are required to collect and analyze large amounts of sequencing data to uncover the evolutionary links between lactic acid bacteria species. As lactic acid bacteria continue to accumulate genetic data, insights into their evolutionary past may also become available.[18]

2. MATERIALS AND METHODS

2.1. Sample Collection

Milk samples were collected from five different dairies in the Dehradun region of Uttarakhand (30.318°N 78.029°E), located in India, under aseptic conditions in the laboratory for immediate isolation. The samples were collected by separating 5 samples of curd and cheese in 5 ml borosil

bottles. Curd and cheese samples were inoculated in lactobacillus selection broth (MRS) for 48 hours at 37^0C for enrichment. Enriched samples were spread by a spreader and incubated at 37^0C on MRS agar media for 24 hours for recovery of isolated lactobacillus species. Isolated samples were purified by streaking and re-streaking methods. The stocks were preserved in the refrigerator and maintained for one month at 4ºC.[19]

2.2. Morphological Characteristics

Cell Morphology and Morphological Characteristics of recovered isolated colonies were studied by gram staining procedure. Serial dilutions were made using sterile saline solution in sterile tubes up to 10^{-10}. During 24 hours of anaerobic incubation in a CO_2 incubator, 1 mL of sterile MRS plate agar was inoculated and anaerobically incubated at 37°C. For the early selection of different lactobacilli isolates, bacterial purification was typically tested using morphological tests such as gram staining and catalase. Gram-positive, catalase-negative bacilli isolates were selected and stored in MRS-Glycerol (25/75 v/v) at -20 degrees Celsius.[20] The preliminary screening was conducted using well-isolated, typical colonies representative of prospective lactobacilli, following "Bergey's manual of systematic bacteriology".[21] "These tests comprised H_2O_2 catalase activity, stab method motility (0.4% agar in MRS broth), and gelatin liquefaction using 12% gelatin in MRS broth." The bacteria were identified using polymerase chain reaction (PCR) and DNA analysis.[22]

2.3. A Biochemical approach to detecting LAB species

2.3.1. Catalase test: The test assessed isolated cultures' ability to degrade hydrogen peroxide by producing enzymes. The test used a slide approach with an inoculating loop. A culture from a typical colony of isolates was placed on a clean, grease-free glass slide and exposed to 3% hydrogen peroxide to test for bubble formation. The creation of bubbles showed a positive catalase reaction, which was tested in the presence and absence of an enzyme.[23]

2.3.2. Nitrate reduction test: There are two stages to the reduction of nitrate to nitrite. Nitrate Reagents A and B convert nitrate to nitrite initially, followed by Nitrate Reagent C (zinc dust) for additional reduction if necessary. Inoculate bacterial suspensions into the nitrate broths. Incubate the tubes for 24 hours at 30°C or 37°C, respectively. Before adding chemicals, check for N2 gas following incubation. Add 6-8 drops Nitrite Reagent A, followed by 6-8 drops Nitrite Reagent B. The reaction (colour development) should take no longer than a minute. If no colour shows, apply zinc powder. Allow at least three minutes for the crimson hue to emerge.[24-26]

2.3.3. Sugar fermentation test: First, make the carbohydrate fermentation broth. Before pouring into flasks, weigh and dissolve 1g trypticase, 0.5g sodium chloride, and 0.0189 mg phenol red in 100 mL of distilled water. Each flask should contain 0.5% to 1% of the necessary carbohydrate. Before inserting the inverted Durham tubes, make sure they are all thoroughly filled with broth. Sterilize for 15 minutes at 115 degrees Celsius. Avoid boiling the Phenol Red Carbohydrate Fermentation Broth. Overheating causes molecules to degrade, yielding substances of various hues and tastes. Transfer the sugar to screw-capped or fermentation tubes, labeling them appropriately. For 15 minutes, autoclave the broth at 115 degrees Celsius. Add bacterial culture to each carbohydrate broth tube aseptically. Incubate the tubes at 37°C for 18-24 h, then monitor the reaction.[27-42]

2.4. A molecular approach to detecting LAB species

2.4.1. DNA extraction from genomic samples:

The sample was picked up and placed in a mortar and homogenized with 1 ml of extraction buffer and the homogenate was transferred to a 2 ml-microfuge tube. An equal volume of Phenol: Chloroform: Isoamlyalcohol (25:24:1) was added to the tubes and mixed well by gently Shaking the tubes. The tubes were centrifuged at room temperature for 15 min at 14,000 rpm. The upper aqueous phase was collected in a new tube and an equal volume of Chloroform: Isoamly alcohol (24:1) was added and mixed. The upper aqueous phase obtained after centrifuging at room temperature for 10 min at 14,000 rpm was transferred to a new tube. The DNA was precipitated from the solution by adding 0.1 volume of 3 M Sodium acetate pH 7.0 and 0.7 volume of Isopropanol. After 15 min of incubation at room temperature, the tubes were centrifuged at 4ºC for 15 min at 14,000 rpm. The DNA pellet was washed twice with 70% ethanol and then very briefly with 100% ethanol and air dried. The DNA was dissolved in TE (Tris-Cl 10 mM pH 8.0, EDTA 1 mM). To remove RNA 5 µl of DNAse free RNAse. A (10 mg/ml) was added to the DNA.

2.4.2. PCR amplification of 16S rDNA gene:

PCR procedures were conducted to amplify the 16S rDNA gene in the LAB isolate. The 16S rDNA region was amplified using the universal primer set, consisting of 188 ng of Extracted DNA for amplification along with 10pM of each primer (5-GGATGAGCCCGCGGCCTA-3) and (5-CGGTGTGTACAAGGCCCGG-3)", provided by Biokart Genomic Lab INDIA. The reaction consisted of 2µl of both the "forward and reverse primers", 1µl of DNA template, and 20 µl nuclease-free water was added to bring the final volume to 50µl. The sequences were examined using the basic local alignment search tool (BLAST) to search for a comparable sequence in the National Center for Biotechnology Information database (NCBI) located at https://blast.ncbi.nlm.nih.gov/".The phylogenetic tree was constructed utilizing the neighbor-joining (NJ) technique .[43]

RESULTS AND DISCUSSION

After taking 50 Lactobacillus isolates from agar plates based on their colonial appearance, they were cultured for 24 hours at 37°C in MRS broth. Only 24 of the 50 samples, meanwhile, were accepted as legitimate. After subculturing three or four times, they spent the next 24 to 48 hours incubated at 37°C. The best five isolates' physiological and biochemical tests were used to identify and characterize the well-isolated colonies after streaking on Petri plates that had hardened anaerobically and aerobically cultivated for 48 hours.

Table I: Microbial characteristics of the isolated lactobacilli cultures from cheese and curd.

S.No.	**Name of sample**	**Shape and arrangement**	**colour**	**Margin**	**Growth temperature**		**G +ve/-ve**	**anaerobic**
					32^0C-37^0C	**37^0 C-42^0 C**		
1	4RTR	Short rod and smooth convex colonies	Greyish white	Entire	--	++	+ve	+

2	FMDY-II	Long rod and spread colonies	Milkey white	Entire	++	--	+ve	+
3	CZP 2810	Normal rod and rough irregular colonies	Light yellowish	Undulate	++	-	+ve	+
4	FMDY-I	Long rod and spread colonies	Milkey white	Entire	++	--	+ve	+
5	LCR	Normal rod and smooth convex colonies	Milkey shine	Punctiform	-+	++	+ve	+

After identifying all the isolates, we conducted all the necessary biochemical tests. All metabolic processes are amenable to biochemical analysis tests, such as catalase, fermentation, indoles, and nitrogen reduction, as shown in the following table.

Table 2**:** Microbial characteristics of the isolated lactobacilli cultures from cheese and curd.

S.No.	**Name of sample**	**Catalase test**	**Fermentation test**				**Nitrate Reduction**	**Indole**
			Glucose test		**Mannitol test**			
			A	G	**A**	**G**		
1	4RTR	-ve	++	+	-+	+	-ve	-ve
2	FMDY-II	-ve	++	-	++	-	-ve	-ve
3	CZP 2810	-ve	++	-	++	-	-ve	-ve
4	FMDY-I	-ve	++	-	++	-	-ve	-ve
5	LCR	-ve	++	-	-+	+	-ve	-ve

A – Acid production, G – Gas production.

Lactic acid bacteria were isolated from cheese, Curd and cultured at 37°C in anaerobic conditions. Gram-positive, catalase-negative bacteria were only evaluated for molecular identification. 16S rRNA sequence analysis at the species level is more accurate and reliable than previous methods.[45] Chen et al. and Zhang et al. found significant genetic diversity among lactic acid bacteria in milk, indicating natural selection and domestication due to geographical variations in different countries have significantly influenced their diversity.[44-47] For the 16S rRNA region, the size of the amplified PCR product was approximately 1500bp as shown in the figure of DNA gel. Comparison of 16S region sequences obtained from isolates in GenBank allows identification of isolates to the species level. Evolutionary history was estimated using the NeighborJoining method. After completion of the sequencing process, it was found that the microorganism was found to have Lactobacillus delbrueckii strain BK-4 16S ribosomal RNA gene and its sequence ID is MZ356512.1. While the next closest homolog was found to be Lactobacillus delbrueckii subsp. bulgaricus strain IMAU40168 16S ribosomal RNA gene and its sequence ID is FJ915705.1.

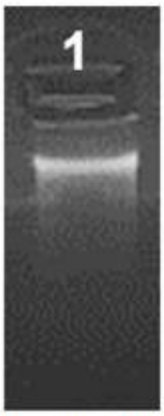
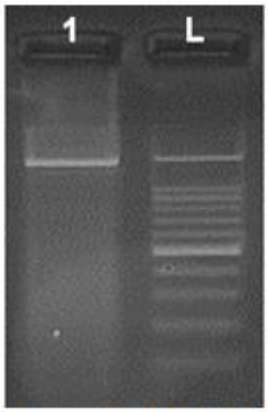
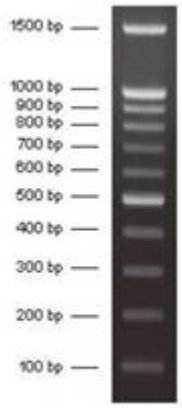


Fig 1. Genomic DNA



Gel image of isolated bacterial DNA



100 bp LADDER

The Phylogenetic Tree Builder system operates by utilizing software-aligned sequences. The Jukes-Cantor perfect distance model is employed to generate a matrix of distances, where only the alignment model positions are considered, alignment inserts are excluded, and a minimum of 200 comparable positions are set. The tree construction process involves using Weibore, which is characterized by a length of 1000 and an alphabet size of 4. Weighbor Tree is a weighted variant of Neighbour Joining that minimizes weight over longer distances. Jukes-Cantor Correction considers second replacements as sequences diverge, resulting in smaller distance-based trees. The formula accounts for nucleotide differences, removes second substitutions, and reduces underestimated distances. Bootstrapping uses resampling to estimate the sampling distribution, resulting in a distance matrix and tree. After 100 repetitions, a majority consensus tree appears.

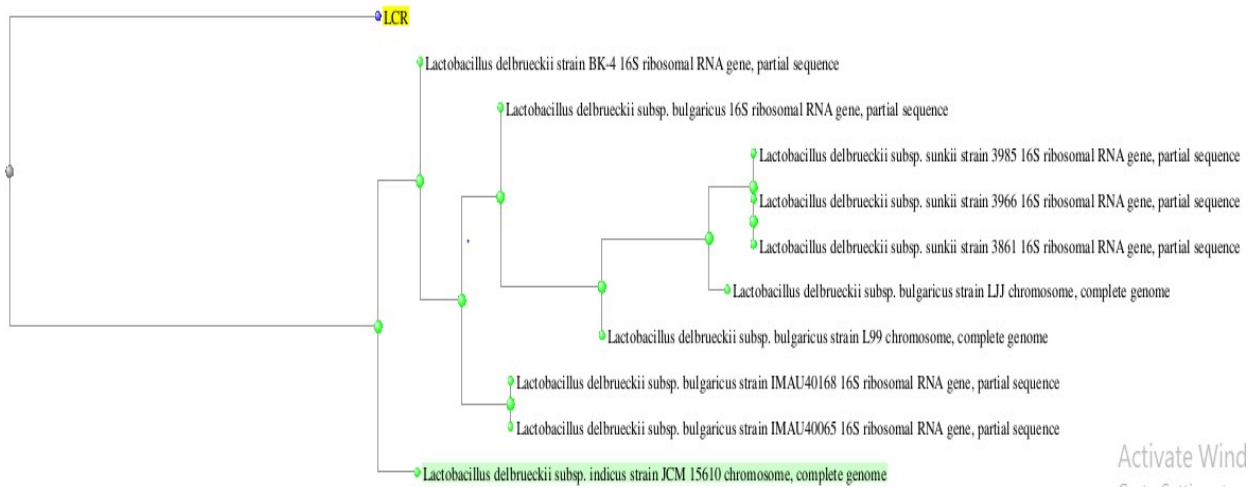


Fig 2. The Phylogenetic Tree with 10 Neighbour of the isolated sample LCR

The sequences were checked using the National Center for Biotechnology Information (NCBI) database and the bacterial 16S rRNA sequenced and aligned genes were deposited followed by gene processing and an accession number OR660397 and version number OR660397.1 were obtained, which is mentioned on the website of NCBI.

CONCLUSION

Scant data exist regarding the probiotic properties of lactobacillus species. Lactobacillus species found in fermented foods (yogurt, paneer, and sourdough) improve digestion. A recent study found that Lactobacillus species and the probiotics they produce strengthen the immune system and

reduce the risk of many diseases. Further research on Lactobacillus species as probiotics is required, however, this study identified different Lactobacillus species from fermented and non-fermented foods locally. Ultimately, the Lactobacillus bacteria were identified based on 16s RNA sequencing, which will be beneficial in future discoveries. This study also discovered several Lactobacillus delbrueckii species. Overall, the study has shown the ability to identify Lactobacillus species. This is the first step in the discovery of Lactobacillus.

Data availability statement

This investigation utilizes datasets from online repositories, which provide names and accession numbers for the datasets. https://www.ncbi.nlm.nih.gov/genebank/,OR660397.1; https://www.ncbi.nlm.nih.gov/gene/?term=OR660397.1

REFERENCES

1. Lavanya B, Sowmiya, S, Balaji S, Muthuvelan, B. Screening and characterization of lactic acid bacteria from fermented milk. Br J Dairy Sci. 2011;2:5-10.
2. Klaenhammer T, Altermann E, Arigoni F, Bolotin, A, Breidt F, Broadbent J, Cano R, Chaillou S, Deutscher J, Gasson M, van de Guchte M, Guzzo. Discovering lactic acid bacteria by genomics. Antonie van Leeuwenhoek. 2011;82:29-58.
3. Fu, N., Huang, S., Xiao, J., & Chen, X. D. (2018). Producing powders containing active dry probiotics with the aid of spray drying. Advances in Food and Nutrition Research, 85, 211-262.
4. Lashani, E., Davoodabadi, A., and Soltan Dallal, M. M. (2020). Some probiotic properties of Lactobacillus species isolated from honey and their antimicrobial activity against foodborne pathogens. Vet. Res. Forum. 11, 121–126.
5. Salim, A., Nadri, S., Hosseini, M. J., Rokni-Zadeh, H., and Mohseni, M. (2020). Protective effect of probiotic Lactobacillus acidophilus against the toxicity of beauvericin mycotoxin on the Caco-2 cell line. Toxicon 185, 184–187.
6. Rashed, S. S., Ghaffari, M., Moghadam, N. B., Ebrahimi, M. T., and Keshtmand, Z. (2022). Effects of a novel probiotic mixture on the modulation of brain and intestine Aquaporin-4 gene expression in rats exposed to Cadmium. Metab. Brain Dis. 37, 2777–2782.
7. M'Hamed, A., C., Ncib, K., Merghni, A., Migaou, M., Lazreg, H., et al. (2022). Characterization of probiotic properties of Lacticaseibacillus paracasei L2 isolated from a traditional fermented food "Lben". Life 13, 10021.
8. A'inurrofiqin, M., Rahayu, E. S., Suroto, D. A., Utami, T., and Mayangsari, Y. (2022). Safety assessment of the indigenous probiotic strain Lactiplantibacillus plantarum subsp. plantarum Kita-3 using Sprague–Dawley rats as a model. AIMS Microbiol. 8, 403–421.
9. Das, S., Vishakha, K., Banerjee, S., Bera, T., Mondal, S., and Ganguli, A. (2022). A novel probiotic strain of Lactobacillus fermentum TIU19 isolated from Haria beer showing both in vitro antibacterial and antibiofilm properties upon two multi resistant uro-pathogen strains. Curr. Res. Microb. Sci. 3, 100150.
10. Martinez, R. M., Hulten, K. G., Bui, U., & Clarridge III, J. E. (2014). Molecular analysis and clinical significance of Lactobacillus spp. recovered from clinical specimens presumptively associated with disease. Journal of clinical microbiology, 52(1), 30-36.
11. Gobbetti, M., Minervini, F., Pontonio, E., Di Cagno, R., & De Angelis, M. (2016). Drivers for the establishment and composition of the sourdough lactic acid bacteria biota. International journal of food microbiology, 239, 3-18.

12. Viramontes-Hörner, D., F. Márquez-Sandoval, F. Martín-delCampo, B. Vizmanos-Lamotte, A. Sandoval-Rodríguez, J. Armendáriz-Borunda, H. García-Bejarano, K. RenoirteLópez, G. García-García (2015). Effect of a symbiotic gel (Lactobacillus acidophilus+Bifidobacterium lactis+inulin) on presence and severity of gastrointestinal symptoms in hemodialysis patients. J. Renal Nutr. 25: 284-291.
13. Liévin-Le Moal, V., & Servin, A. L. (2014). Anti-infective activities of lactobacillus strains in the human intestinal microbiota: from probiotics to gastrointestinal anti-infectious biotherapeutic agents. (Clinical microbiology reviews, 27(2), 167-199.
14. Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M., Harris, H. M., Mattarelli, P., ... & Lebeer, S. (2020). A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. International journal of systematic and evolutionary microbiology, 70(4), 2782-2858.
15. De Jesus, L. C. L., Aburjaile, F. F., Sousa, T. D. J., Felice, A. G., Soares, S. D. C., Alcantara, L. C. J., & Azevedo, V. A. D. C. (2022). Genomic characterization of Lactobacillus delbrueckii strains with probiotics properties. Frontiers in Bioinformatics, 2, 912795.
16. De Jesus, L. C. L., de Jesus Sousa, T., Coelho-Rocha, N. D., Profeta, R., Barroso, F. A. L., Drumond, M. M., et al. (2021a). Safety Evaluation of Lactobacillus delbrueckii subsp. lactis CIDCA 133: a Health-Promoting Bacteria. Probiotics Antimicro. Prot. doi:10.1007/s12602-021-09826-z.
17. De Jesus, L. C. L., Drumond, M. M., de Carvalho, A., Santos, S. S., Martins, F. S., Ferreira, Ê., et al. (2019). Protective Effect of Lactobacillus delbrueckii subsp. lactis CIDCA 133 in a Model of 5 Fluorouracil-Induced Intestinal Mucositis. J. Funct. Foods 53, 197–207.
18. Bergey's manual of systematic bacteriology; Volume 3: The Firmicutes: (464-513); SpringerVerlag, New York. 2009.
19. Kouadri Boudjelthia, N., Belabbas, M., Bekenniche, N., Monnoye, M., Gérard, P., & Riazi, A. (2023). Probiotic Properties of Lactic Acid Bacteria Newly Isolated from Algerian Raw Cow's Milk. Microorganisms, 11(8), 2091.
20. Kouadri Boudjelthia, N., Belabbas, M., Bekenniche, N., Monnoye, M., Gérard, P., & Riazi, A. (2023). Probiotic Properties of Lactic Acid Bacteria Newly Isolated from Algerian Raw Cow's Milk. Microorganisms, 11(8), 2091.
21. Bergey's manual of systematic bacteriology; Volume 3: The Firmicutes: (464-513); SpringerVerlag, New York. 2009.
22. Idbeis, H. I. (2023). Molecular Identification and Phylogenetic Analysis of Lactic Acid Bacteria Isolated from Cow Raw Milk in Al-Kut-Iraq. Central Asian Journal of Medical and Natural Science, 4(5), 623-631.
23. Reiner, K. (2010). Catalase test protocol. American society for microbiology, 1-6.
24. https://microbenotes.com/nitrate-reduction-test-objectives-principle-procedure-and-results/
25. https://www.austincc.edu/microbugz/nitrate_reduction.php
26. Buxton, R. (2011). Nitrate and nitrite reduction test protocols. American Society for Microbiology, 1-20.
27. Barker HA. 1956. Bacterial fermentation. John Wiley & Sons, Inc., New York, NY.
28. Bartelt M. 2000. Diagnostic bacteriology, a study guide. F. A. Davis Co., Philadelphia, PA.
29. Becton Dickinson. 1953. Difco manual of dehydrate media and reagents for microbiological and clinical laboratory procedures, 9th ed. Difco Laboratories, Detroit, MI.

30. Clarke H, Cowan ST. 1952. Biochemical methods for bacteriology. J. Gen. Microbiol. 6:187-197.
31. Cowan ST, Steel KJ. 1965. Identification of medical bacteria. Cambridge University Press, Cambridge, England.
32. Forbes BA, Sahm DF, Weissfeld AS. 2007. Bailey and Scott's diagnostic microbiology, 12th ed. Mosby Company, St. Louis, MO.
33. Hugh R, Leifson E. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. J. Bacteriol. 66:24–26.
34. Mahon CR, Lehman DC, Manuselis G. 2011. Textbook of diagnostic microbiology, 4th ed. W. B. Saunders Co., Philadelphia, PA.
35. Morello JA, Janda WM, Bohnhoff M. 1985. Neisseria and Branhamella, p 176–192. In Lennette EH, Balows A,Hausler WJ, Jr, Shadomy HJ (ed), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, DC.
36. Müller V. 2008. Bacterial fermentation. In: eLS. John Wiley & Sons Ltd., Chichester, England. http://www.els.net/WileyCDA/ElsArticle/refId-a0001415.html. doi:10.1002/9780470015902.a0001415.pub2.
37. Stanier RY, Doudoroff M, Adelberg EA. 1963. The microbial world, 2nd ed. PrenticeHall, Englewood Cliffs, NJ.
38. U. S. Food and Drug Administration. 1998. Bacteriological analytical manual.
39. http://www.fda.gov/Food/ScienceResearch/
40. LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm.
41. Vera HD. 1948. A simple medium for identification and maintenance of the gonococcus and other bacteria. J. Bacteriol. 55:531–536.
42. Reiner, K. (2012). Carbohydrate fermentation protocol. Energy, 11, 12.
43. Bruno, W. J., Socci, N. D., & Halpern, A. L. (2000). Weighted neighbor joining: a likelihood-based approach to distance-based phylogeny reconstruction. Molecular biology and evolution, 17(1), 189-197.
44. Chen, X., Song, Y. Q., Xu, H. Y., Menghe, B. L. G., Zhang, H. P., & Sun, Z. H. (2015). Genetic relationships among Enterococcus faecalis isolates from different sources as revealed by multilocus sequence typing. Journal of Dairy Science, 98(8), 5183-5193.
45. Ren, L., & Suo, H. (2017, April). Molecular Identification of lactic acid bacteria isolated from the traditional fermented yak yogurt in western Sichuan Region. In 7th International Conference on Education, Management, Information and Mechanical Engineering (EMIM 2017) (pp. 1244-1252). Atlantis Press.
46. ZHANG W, LIU W, SONG Y, et al. Multilocous sequence typing of dairy-associated Leuconostoc mesenteroides population reveals clonal structure with intragenic homologous recombination[J]. Journal of Dairy Science, 2015, 98(6): 2284–2293
47. Sun, Z., Liu, W., Song, Y., Xu, H., Yu, J., Bilige, M., ... & Chen, Y. (2015). Population structure of Lactobacillus helveticus isolates from naturally fermented dairy products based on multilocus sequence typing. Journal of Dairy Science, 98(5), 2962-2972.