



The Evaluation of the Antagonistic Effect of Certain Strains of *Lactobacillus* spp. on Various Virulence Factors of Foodborne Pathogenic Bacteria

Adil Turki Al-Musawi ^{1*} , Sudad Jasim Mohammed ² , Mohammad A. Alsoufi ³ 

^{1*} Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq.
E-mail: adilalmusawi@mracpc.uobaghdad.edu.iq

² Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq.
E-mail: sudad@mracpc.uobaghdad.edu.iq

³ Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq.
E-mail: alsoufim@mracpc.uobaghdad.edu.iq

Abstract

This study included the isolation and diagnosis of *Lactobacillus* spp. Bacteria It produces an enzyme called lactase, which breaks down the bonds between the lactose in milk and produces lactic acid. This breakdown process also produces hydrogen peroxide, which limits the growth of harmful bacteria in the intestine. Beneficial bacteria generally have many benefits for the health of our bodies. They help treat or prevent stomach and vaginal candidiasis, which affects women at high rates. It is worth noting that many studies have previously focused their research on this matter, and the results were good, with a positive relationship between reducing fungal infections. Biofilm formation spending cylinder scheme disclosed variation in biofilm foundation, as weak biofilm formation was observed for *Escherichia coli* (*E. coli*), *Salmonella* spp. (*Sal. spp.*) and *Bacillus cereus* (*B. cereus*), while no biofilm formation was observed for *Staphylococcus aureus* (*Staph. aureus*). The results also confirmed that the one-and-a-half concentrated supernatant of *Lactobacillus* spp. Showed increased inhibitory activity, as determined by the disk diffusion method, against the characterized and isolated strains from some fast-food restaurants.

Keywords:

The concentrated cell-free supernatant, lactobacillus spp., anti-biofilm agent, antimicrobial agent against certain pathogenic bacteria associated with food poisoning.

Article history:

Received: 13/03/2025, Revised: 29/05/2025, Accepted: 30/06/2025, Available online: 30/08/2025

Abbreviations: CFS - concentrated cell-free supernatant; GRAS- Generally Recognized as Safe; MRS- Mon Rugose Sharp agar; BHI- Brain Heart Infusion broth.

*Corresponding Author: Adil Turki Al-Musawi, E-mail: adilalmusawi@mracpc.uobaghdad.edu.iq

Introduction

The species of *Lactobacillus* bacteria are considered the most important probiotics present in diverse environments, especially the Normal flora of humans, animals, and other living creatures. They are also found in fermented fruits and vegetables (Dempsey and Corr, 2022). These bacterial species have been used for many years in the food industry, dairy products, and the production of fermented foods due to their medical and health benefits, such as their effect on cancer tumors, immune stimulation, treatment of digestive disorders, and gastric ulcers. Due to the importance of these bacteria in enzyme production, bacteriocins, and polysaccharides, researchers have been encouraged to study their therapeutic properties and mechanisms of action (Sorescu et al., 2021). Many researchers have studied their effects on a large number of pathogenic bacterial species such as *Escherichia coli*, *Vibrio cholera*, *Pseudomonas*, *Streptococcus*, and *Staphylococcus* (Sethi, & Jain 2024); Rabetafika et al., 2023; Abedi and Hashemi, 2020). They have been employed for centuries in the production of a wide range of fermented products, such as fruit juices, yogurt, cheese, sausages, pickles, and fermented vegetables (Multari et al., 2020). When studying their physiological properties, it has been found that they belong to the genus *Lactobacillus* and family *Lactobacillaceae*, order *Lactobacillales*, class *Bacilli*, which are part of the *Firmicutes* phylum colonizing the human digestive tract, the *Lactobacillus* bacteria exhibit genetic variations, such as the size of the chromosome ranging from 1885 to 3308 kilobase pairs and the GC% content, which ranges from (32.9% to 44.4%), they are Gram-positive and non-spore-forming, their cell shape can vary from rod-shaped to cocci, either anaerobic or tolerant to low levels of oxygen, they are acid-tolerant and have complex nutritional requirements (amino acids, peptides, carbohydrates, vitamins, fatty acid esters, and nucleic acid derivatives), they are classified based on their fermentability and the pathway of hexose sugars into obligately homolactic fermentation, obligately heterolactic fermentation, and facultatively heterofermentative fermentation (Duar et al., 2017). Important species of this genus (Sorescu et al., 2019; Duar et al., 2017). Based on the aforementioned information, the idea arose to investigate the effect of the concentrated live cell supernatant of *Lactobacillus* spp (de Man et al., 1960)

Material and Methods

Sample Collection

Six breast milk samples in all were obtained and properly cleaned up in sterile test tubes. Ten to twenty days following delivery, samples were retrieved from six feeding women. Upon going to the lab, each sample was stored independently in hygienic plastic bags in a specific freezer container.

Isolation and Identification

The procedure outlined by Holt (1984) and Chowdhury et al. (2012) was used to prepare and isolate the specimens in question. The samples were activated by incubating their contents for 24 hours at 37°C. Throughout incubation, 1ml of the samples was taken out, mixed well, and added to the Mon Rugose Sharp broth. The mixture was then incubated for a whole day at 37°C. Using the exact same culture medium, this activation procedure was carried out three times. Samples were later spread-plated onto Mon Rugose Sharp agar and generated for 72 hours at 37°C.

Methods

This method is mentioned (Ramchandran and Shah, 2010). The activation was carried out by culturing the bacteria on reconstituted skim milk with a total solid content of 12% (Wt/Vol), sterilized by using an autoclave at a temperature of 121°C for 5 minutes. The milk was then cooled to a temperature of 37 to 40°C,

the recovered milk was inoculated with live cell starter at a 1% inoculum volume and incubated at 37°C until coagulation. The mixture was subsequently centrifuged using a high-speed refrigerated centrifuge (Huttich, Germany) at 14,000 rpm for 20 minutes. The upper portion was carefully withdrawn, and liquid was filtered through a 0.22µm membrane filter (Milipore). EJ-Media medium was previously prepared and used to ensure the purity of the cell-free supernatant by observing the presence or absence of growth after incubation at 37°C for 24 hours (Radford, 1976; Aween et al., 2012).

Pathogenic bacterial isolates

Four pathogenic bacterial isolates were included in the study, two of which were Gram-positive, including *Staphylococcus aureus* and *Bacillus cereus*, and two were Gram-negative, including *Salmonella* spp. and *Escherichia coli* (Table 1). These isolates were specifically isolated from some fast food restaurants related to the study conducted by (Whitmore & Fontaine 2024); (Mohammed et al., 2022; Al-Musawi, 2022) was used for specific detection of inhibitory activity of concentrated live cell supernatant of *Lactobacillus* spp.

Table 1. Type and number of pathogenic bacterial isolates used in this study

Type of Bacteria	Number of isolate	Gram stain	Isolate from
Staphylococcus aureus	1	Positive	fast food restaurants
Bacillus cereus	1		
Salmonella spp.	1	Negative	
Escherichia coli	1		

The evaluation of the effect of concentrated live cell supernatant of *Lactobacillus* spp. on Anti-biofilm formation of pathogenic Isolates

The Tube method with some modifications was employed to assess the effect of concentrated live cell supernatant of *Lactobacillus* spp (Mohammed et al., 2022). on anti-biofilm formation of the pathogenic isolate. The bacterial suspensions of each isolate were prepared by culturing them individually in Brain Heart Infusion broth (BHI) supplemented with 1% glucose and incubating them at 37°C for 24 hours. After the incubation period the growth density was compared with the McFarland standard 0.5 using a 1×10^8 cell/ml inoculum, then 1.5 ml of the bacterial suspension was transform with an equal volume of the prepared concentrated live cell supernatant of *Lactobacillus* spp. previously prepared in (1-3), which was concentrated 1.5 times using a Lyophilizer, and incubated at 37°C for 24 hours, the contents of the tubes were poured out, washed with pH 7.2 phosphate buffer saline, then dried. The tubes were stained with 0.1% Crystal violet for three minutes, the excess stain was removed, and tubes were washed with deionized water. The tubes were left to dry upside down to observe the formation or loss of biofilms on the inner walls and bottom of the tubes in the form of a violet layer (Ibraheim et al., 2023; Haghshenas et al., 2015).

In vitro inhibitory activity test of concentrated live cell supernatant of *Lactobacillus* spp. on growth and efficacy of pathogenic bacterial isolates

The sensitivity of the pathogenic bacterial isolates to the concentrated live cell supernatant of *Lactobacillus* spp. was studied using the method described by (Mao et al., 2023) with some modifications. A volume of 100 ml of the pathogenic isolate inoculum was transferred, based on the bacterial turbidity and compared to the McFarland standard 0.5, with an inoculum size of 1×10^8 cells/ml, then spreaion onto Muller Hinton Agar medium. The disk diffusion method was used to test the concentrated live cell supernatant of *Lactobacillus* spp. by immersing placing 6 mm diameter disks made from Whatman No.1 filter paper, sterilized by autoclave and saturated with the mentioned bacterial supernatant (T Al-Musawi, 2022). The disks were dried using gentle airflow and then placed in the center of the agar medium. This process was repeated three times. The plates

were incubated at room temperature for 30 minutes and then further incubated at 37°C for 24 hours. The inhibition zones' diameters were measured and compared to the control isolate plate.

Result and Discussion

Isolation and identification of Lactobacillus bacteria

The identification process at this stage relied on the colony, shape, edge, color, and elevation, as described by (Chowdhury et al., 2012) on MRS agar medium. These isolates were characterized as small circular colonies, some convex and others flat, smooth shiny, and creamy in color. Microscopic examination revealed diverse cell shapes, with some being rod-shaped while others were spherical. Some cells were single, while others were in pairs, and some formed long or short chains. The isolates stained positive with Gram staining (Figure 1) and were non-motile and catalase-negative, the optimal pH in the MRS broth medium ranged from 5.5 to 6.5 at 37°C.



Figure 1. Cultural and microscopic characteristics of lactobacillus spp. Colonies and cells

Testing the effect of concentrated supernatant of live Lactobacillus spp. cells on anti-biofilm formation of pathogenic isolates

The results revealed the formation of biofilms using the tube method, which involved mixing equal volumes (1.5 ml concentrated supernatant: 1.5 ml BHI nutrient medium). There was variation in the formation of biofilms, with weak biofilm formation observed for *Escherichia coli*, *Salmonella* spp. and *Bacillus cereus*. However, no biofilm formation was observed for *Staphylococcus aureus* (Al-Rawi et al., 2023).

Inhibition assay of concentrated supernatant of Lactobacillus spp. on growth and activity of pathogenic bacterial isolates

The antimicrobial activity is an important characteristic of *Lactobacillus* spp. Strains, the results showed that the 1.5 times of concentrated supernatant of *Lactobacillus* spp. exhibited increased inhibitory activity using the disk diffusion method against the tested and isolated strains from some fast-food restaurants (Table 2).

Table 2. Inhibitory effect of the concentrated filter against *Lactobacillus* spp. on the growth and effectiveness of pathogenic bacterial isolates (mm)

Tested pathogenic strains with zone of diameter in mm			
<i>E. coli</i>	<i>Sal. spp.</i>	<i>B. cereus</i>	<i>Staph. aureus</i>
+++17	+++15	++13	+++19

+++ diameter of inhibition zone between (15-23) mm

++ diameter of inhibition zone between 10-13 mm

The isolates belong to *Lactobacillus* spp. specifically, the process of isolation and selection of the microbial creature is the backbone from which other studies emerge, the idea of isolating *Lactobacillus* bacteria as a good and safe source for isolating these bacteria, which can be used in various fields, is significant. The use of selective culture media such as MRS broth and MRS agar is highly suitable for isolating these bacteria as they provide all the nutritional requirements. The presence of sodium acetate and the low pH of 5.4, along with anaerobic growth conditions, contributed to obtaining selectively good bacterial isolates with characteristics consistent with scientific references (Carvalho & Perscheid 2025; Karimov et al., 2015; De-man et al., 1960)

The biochemical tests showed negative results for gelatin liquefaction, indicating the absence of Gelatinase enzyme production this is consistent with the findings reported by (Khagwal and Sharma, 2019) (Al-Musawi et al., 2022) for all *Lactobacillus* species, where the absence of this enzyme activity is an important criterion for determining the non-pathogenic nature of the isolates (Gupta and Sharma, 2017), the isolates also tested negative for nitrate reduction, indicating the absence of Nitrate reductase enzyme production, they demonstrated the ability to hydrolyze arginine amino acid and ferment various sugars such as glucose, xylose, sucrose, fructose, lactose, maltose, trehalose, raffinose, rhamnose, mannitol, and dextrose according to the classification in Bergey's Manual of Determinative Bacteriology (Chowdhury et al., 2012).

Biofilm formation is considered a virulence factor that helps microorganisms resist the environment, biofilm is a collection of microorganisms and their extracellular secretions that adhere to each other or surfaces, the adhered cells are an integral part of a dense matrix of extracellular polymeric substances (EPS) also referred to as mucus (Min et al., 2025). The supernatant produced by *Lactobacillus* strains has anti-biofilm activity, so the sensitivity of the microorganisms composing the biofilm to the supernatant of *Lactobacillus* strains is attributed to various factors, including the isolation site, which is undoubtedly influenced by the species and strain type (Khalaf et al., 2016).

Studies have shown that bacteriocins mechanism roll, which are natural molecular compounds such as proteinaceous and peptide molecules, target the cell membrane (Bradford, 1976), they remove ions from the membrane and prevent cell wall formation, one type of these peptides is classified as bacteriocin and is named after the producing organism. Additionally, the organic acids produced are believed to inhibit metabolic functions, lower pH values, inhibit active transport, and contribute to maintaining the cell membrane (Saidi et al., 2023). Also indicated that *Lactobacillus* strains that are strictly accompanying per hominid are frequently secondhand as probiotics so these strains act as antibiotics by inhibiting the growth of potential pathogens through competition for nutrients and space. (Bala Krishna 2021; Guan et al., 2023).

In other studies, a unique relationship was found between the health of the digestive system and its richness in beneficial bacteria and the psychological state of the person. Consequently, some conclusions came to indicate that beneficial bacteria, including *L. acidophilus*, may alleviate cases of depression. However, more studies and research are still needed to determine the nature of the relationship between them more precisely. (Fernandes et al., 2023, Zheng et al., 2020 ;Parente et al., 2023 ; Dempsey & Corr 2022) . These findings are consistent with previous studies by (Karimov et al., 2015), which indicated that *Lactobacillus* spp. isolated from fermented dairy products showed varying antimicrobial activity against different pathogens.

Conclusions

The results of the study demonstrated that the concentrated supernatant of *Lactobacillus* spp. isolated from maternal milk exhibited the ability to inhibit the growth of pathogenic isolates *E. coli*, *Sal. spp.*, *B. cereus*

and Staph. aureus using the tube method for biofilm inhibition. Meanwhile, the inhibition assay test results showed an increased inhibitory activity of the concentrated supernatant of Lactobacillus spp. using the disk diffusion method against the tested and isolated strains from some fast-food restaurants.

Recommendations

1. Research efforts should focus on the potential use of purified active compounds for food preservation.
2. The live cell supernatants of Lactobacillus strains used in the current study should be carefully analyzed to identify the effective inhibitory compounds. These compounds should be tested to determine if they have any toxic effects or other harmful side effects on patients.
3. It is necessary to raise awareness about health and discourage the indiscriminate use of antibiotics without doctor consultation.

Conflict of interest

No conflicts of interest are disclosed by the writers.

Ethical approval

This study received at (MRCPC-57).

Author Contributions

As the corresponding author, SJM helped with the concept and revision of the manuscript. ATM contributed with writing and gathering data. MAA assistance with the proofreading and editing.

Reference

- Abedi, E., & Hashemi, S. M. B. (2020). Lactic acid production–producing microorganisms and substrates sources-state of art. *Helicon*, 6(10). <https://doi.org/10.1016/j.helicon.2020.e04974>
- Al-Musawi, A. T., Mohammed, S. J., & Abdul-Razzaq, A. S. (2022). Evaluation of rats' biochemical markers in artemisia annua Linn. extract after lead toxicity. *Journal of Medicinal and Chemical Sciences*, 5, 1247-1252. <https://doi.org/10.26655/JMCHMSCI.2022.7.14>
- Bala Krishna, M. (2021). A Robust Strategic Theory based Load Balancing and Resource Allocation in Cloud Environment. *International Academic Journal of Science and Engineering*, 8(1), 19–30. <https://iaiest.com/iaj/index.php/IAJSE/article/view/IAJSE0803>
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, 72(1-2), 248-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Carvalho, F. M., & Perscheid, T. (2025). Fault-tolerant embedded systems: Reliable operation in harsh environments approaches. *SCCTS Journal of Embedded Systems Design and Applications*, 2(2), 1-8.
- Chowdhury, A. (2012). Screening of Lactobacillus spp. from buffalo yoghurt for probiotic and antibacterial activity. *Journal of Bacteriology & Parasitology*, 3(08). <https://doi.org/10.4172/2155-9597.1000156>

- de Man, J. D., Rogosa, D., & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *Journal of applied microbiology*, 23(1), 130-135. <https://doi.org/10.1111/j.1365-2672.1960.tb00188.x>
- Dempsey, E., & Corr, S. C. (2022). Lactobacillus spp. for gastrointestinal health: current and future perspectives. *Frontiers in immunology*, 13, 840245. <https://doi.org/10.3389/fimmu.2022.840245>
- Duar, R. M., Lin, X. B., Zheng, J., Martino, M. E., Grenier, T., Pérez-Muñoz, M. E., ... & Walter, J. (2017). Lifestyles in transition: evolution and natural history of the genus Lactobacillus. *FEMS microbiology reviews*, 41(Supp_1), S27-S48. <https://doi.org/10.1093/femsre/fux030>
- Fernandes, M. S. M., Rios, J. C., Vasconcelos, B. M., Lourenço, M. L. M. C., Matos, M. N. C., Cavalcante, R. M. B., ... & Carneiro, V. A. (2023). Effect of Lactobacillus spp. cell-free supernatant against planktonic growth and biofilm formation of foodborne Escherichia coli isolates. *Letters in Applied Microbiology*, 76(1), ovac006. <https://doi.org/10.1093/lambio/ovac006>
- Guan, C., Zhang, W., Su, J., Li, F., Chen, D., Chen, X., ... & Zhang, C. (2023). Antibacterial and antibiofilm potential of Lactocaseibacillus rhamnosus YT and its cell-surface extract. *BMC microbiology*, 23(1), 12. <https://doi.org/10.1186/s12866-022-02751-3>
- Gupta, A. & Sharma, N. (2017). Characterization of Potential Probiotic Lactic Acid Bacteria- Pediococcus acidilactici Ch-2 Isolated from ChuliAtraditional Apricot Product of Himalayan Regions for the Production of Novel Bioactive Compounds with Special Therapeutic Properties. *J Food Microbiol Saf Hyg*. 2(1): 1-11. <https://doi.org/10.4172/2476-2059.1000119>
- Haghshenas, B., Nami, Y., Haghshenas, M., Abdullah, N., Rosli, R., Radiah, D., & Yari Khosroushahi, A. (2015). Bioactivity characterization of Lactobacillus strains isolated from dairy products. *Microbiologyopen*, 4(5), 803-813. <https://doi.org/10.1002/mbo3.280>
- Holt, J.G. (1984). *Bergey's Manual of Systematic Bacteriology*. Williams and Wilkins, Baltimore
- Ibraheim, H. K., Madhi, K. S., Baqer, G. K., & Gharban, H. A. (2023). Effectiveness of raw bacteriocin produced from lactic acid bacteria on biofilm of methicillin-resistant Staphylococcus aureus. *Veterinary World*, 16(3), 491. <https://doi.org/10.14202/vetworld.2023.491-499>
- Karimov, N., Kulmetov, M., Safarova, N., Jumaev, K., Fayzullaev, M., Sultanov, S., ... & Yakhshieva, Z. (2024). The Ecotourism Industry's Role in Environmental Stewardship. *Natural and Engineering Sciences*, 9(2), 293-308. <https://doi.org/10.28978/nesciences.1574450>
- Khagwal, N & Sharma P.K (2019). Antimicrobial Profiling of Lactobacillus Spp. Isolated from Indian Fermented Food. *Int. J. Pure App. Biosci.* 7 (2):182-189. <https://doi.org/10.18782/2320-7051.7389>
- Khalaf, K. J., Mahmood, N. N., Ajah, H. A., & Salman, J. A. (2016). Effect of Lactobacillus salivarius supernatant against growth and biofilm formation of some pathogenic microorganisms. *Baghdad Science Journal*, 13(2), 6. <https://doi.org/10.21123/bsj.2016.13.2.0247>
- Manfi, A. R. S. S., Salahdin, O. D., Al Alaq, F. T., Abdulazeem, L., Kzar, M. H., Khattab, E. S., & Naje, A. S. Antibacterial Activity of Bacteriocin-Isolated from Lactobacillus Spp. Against Some Pathogenic Bacteria.

- Mao, Y., Wang, Y., Luo, X., Chen, X., & Wang, G. (2023). Impact of cell-free supernatant of lactic acid bacteria on *Staphylococcus aureus* biofilm and its metabolites. *Frontiers in Veterinary Science*, 10, 1184989. <https://doi.org/10.3389/fvets.2023.1184989>
- Min, A. K., Thandar, N. H., & Htun, Z. T. (2025). Smart sensors embedded systems for environmental monitoring system integration. *Journal of Integrated VLSI, Embedded and Computing Technologies*, 2 (3), 1–11.
- Mohammed, S. J. (2019). Evaluation of the microbial and chemical quality of feed available in local markets in Baghdad City. *Plant Archives* (09725210), 19(2).
- Mohammed, S. J., Al-Musawi, A. T., Al-Fraji, A. S., & Kareem, H. S. (2022). Comparison of three culture media in assessing the sensitivity of antibiotics to common foodborne microorganisms. *Journal of Medicine and Life*, 15(5), 645. <https://doi.org/10.25122/jml-2021-0404>
- Multari, S., Carafa, I., Barp, L., Caruso, M., Licciardello, C., Larcher, R., ... & Martens, S. (2020). Effects of *Lactobacillus* spp. on the phytochemical composition of juices from two varieties of *Citrus sinensis* L. Osbeck: ‘Tarocco’ and ‘Washington navel’. *Lwt*, 125, 109205. <https://doi.org/10.1016/j.lwt.2020.109205>
- Parente, E., Zotta, T., Giavalisco, M., & Ricciardi, A. (2023). Metataxonomic insights in the distribution of Lactobacillaceae in foods and food environments. *International Journal of Food Microbiology*, 391, 110124. <https://doi.org/10.1016/j.ijfoodmicro.2023.110124>
- Rabetafika, H. N., Razafindralambo, A., Ebenso, B., & Razafindralambo, H. L. (2023). Probiotics as antibiotic alternatives for human and animal applications. *Encyclopedia*, 3(2), 561-581. <https://doi.org/10.3390/encyclopedia3020040>
- Ramchandran, L., & Shah, N. P. (2010). Characterization of functional, biochemical and textural properties of synbiotic low-fat yogurts during refrigerated storage. *LWT-Food Science and Technology*, 43(5), <https://doi.org/10.1016/j.lwt.2010.01.012>
- Saidi, N., Saderi, H., Owlia, P., & Soleimani, M. (2023). Anti-biofilm potential of *Lactobacillus casei* and *Lactobacillus rhamnosus* cell-free supernatant extracts against *Staphylococcus aureus*. *Advanced Biomedical Research*, 12(1), 50. https://doi.org/10.4103/abr.abr_156_21
- Sethi, K., & Jain, R. (2024). Smart Grid Technologies and Renewable Integration: Contributions to the Periodic Series in Electrical and Environmental Innovation. In Smart Grid Integration (pp. 26-32). *Periodic Series in Multidisciplinary Studies*.
- Sorescu, I., Dumitru, M., & Ciurescu, G. (2019). *Lactobacillus* spp. and *Enterococcus faecium* strains isolation, identification, preservation and quantitative determinations from turkey gut content. *Biotechnol. Lett*, 24(1), 41-49.. 24(1):41-49. <https://doi.org/10.26327/RBL2019.252>
- Sorescu, I., Dumitru, M., & Ciurescu, G. (2021). *Lactobacillus* spp. strains isolation, identification, preservation and quantitative determinations from gut content of 45-day-old chickens broilers. *Brazilian Journal of Poultry Science*, 23(01), eRBCA-2020. <https://doi.org/10.1590/1806-9061-2020-1378>

- T Al-Musawi, A. (2022). Inhibitory activity of curcumin extract against some bacteria causing food poisoning isolated from some ready-to-eat meals. *Caspian Journal of Environmental Sciences*, 20(5), 1047-1052. <https://doi.org/10.22124/CJES.2022.6078>
- Whitmore, J., & Fontaine, I. (2024). Techniques for Creating, Extracting, Separating, and Purifying Food and Feed Using Microalgae. *Engineering Perspectives in Filtration and Separation*, 28-33. <https://filtrationjournal.com/index.php/epfs/article/view/EPFS24505>
- 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. <https://doi.org/10.1099/ijsem.0.004107>