Research Article

Evaluation of the Probiotic Effects of *Bacillus clausii* on the Biochemical Profile of Diarrheic Male Rats Experimentally Infected with *Escherichia coli*

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Abstract | Probiotics have shown promising therapeutic effects against colibacillosis. *Bacillus clausii* is a spore-forming Gram-positive bacterium with the unique ability to produce endospores that withstand harsh conditions such as gastric acid and bile salts, allowing it to exert probiotic and antimicrobial effects. This study aimed to evaluate the probiotic potential of *B. clausii* and investigate its therapeutic impact on *Escherichia coli*-induced diarrhea in rats, focusing on body weight, lipid profile, and protein parameters. The experiment was conducted on 15 albino Sprague-Dawley rats, randomly assigned to three groups: G1 (healthy control), G2 (infected with *E. coli*), and G3 (infected with *E. coli* and treated with *B. clausii*). The study measured body weight, lipid levels, and protein concentrations in all groups. The results revealed a significant ($P < 0.05$) increase in body weight in the treated group (G3), reaching 266.3 g with a weight gain of 90.0 g, compared to the infected and control groups. Lipid profile analysis showed reductions in cholesterol (CHOL), triglycerides (TG), LDL, and HDL levels to 64.5, 54.6, 12.13, and 36.5 mg/dL, respectively, in G3, compared to G1 (73.4, 94.0, 16.4, and 38.9 mg/dL) and G2 (74.0, 96.8, 18.4, and 17.4 mg/dL). Total protein and albumin levels were significantly ($P < 0.05$) higher in the treated group compared to G1 and G2. In conclusion, *Bacillus clausii* treatment significantly improved physiological parameters in *E. coli*-infected rats, highlighting its therapeutic potential. Further studies are recommended to explore its benefits across other health indicators.

Keywords | *Bacillus clausii, Escherichia coli*, Blood protein values, Lipid profile, Probiotics, Sprague–Dawley

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INTRODUCTION

Diarrhea is a common health problem that affects young animals and requires immediate attention to prevent complications (Asma, 2020). The standard definition of diarrhea is a condition that is distinguished by an increase in the number of intestinal motility and passing of loose and watery stools (Karima *et al*., 2023). Various mechanisms and pathogens cause diarrhea; however, the primary severity of diarrhea depends on the dose, age, and other factors. Symptoms last (1-2) weeks, and the incubation period ranges from 1-7 days (Constable *et al*., 2017). Many strains of *E. coli* have developed resistance to standard antibiotics (Hammou *et al*., 2024) that, result in hard treatment with antibiotic. Most resistance develop from misuse of antibiotic and genetic mutations. The influence of antibiotic resistance is the delay in healing accompanied with long stay in the hospital and raised the mortality rate. Therefore, probiotics are used as substitute or supportive treatment in antibiotics resistance infection to restore normal balance of gut microflora, prevent growth of pathogenic bacteria and improve the immune system (Muteeb *et al*., 2023).

According to the International Scientific Association for Probiotics, probiotics are defined as live microorganisms that grant health benefits to the host (Hill *et al*., 2014). Probiotics have shown promising therapeutic effects against colibacillosis through several mechanisms (Priyanka *et al*., 2013), such as competitive exclusion and the production of antimicrobial compounds. Competitive exclusion refers to the beneficial bacteria in probiotics competing with *Escherichia coli* for nutrients and attachment sites in the intestinal tract (Hammou *et al*., 2024). These probiotics form a protective barrier on the intestinal wall, making it harder for pathogenic *E. coli* to colonize. In addition, many probiotic strains produce natural antimicrobial agents such as organic acids, bacteriocins, and hydrogen peroxide, which can inhibit or kill pathogenic *E. coli* (Hill *et al*., 2014). Recently, there has been a growing trend in using probiotics to prevent disease, making it one of the largest sectors in the functional food industry today (Muntingh, 2017).

Bacillus spore-forming bacteria are recognized for being able to create several secondary metabolites including enzymes, antibiotics, and antimicrobial peptides. Furthermore, they play an essential role in host interaction, microbial competition and biotechnology. Metabolites characteristic has contributed to the massive potential of antibiotics in multiple fields of agriculture, industry, and medications (Bahaddad *et al*., 2023; Danilova and Sharipova, 2020). These species are considered safe and efficacious probiotics, as they do not produce toxic substances and are manageable in terms of their growth requirements and tolerance to high temperatures (Saggese *et al*., 2021), in addition to classic probiotic traits, that are characterized by high stability towards processing and storage (Bader *et al*., 2012), and at low production costs. Several modes of action mediate its beneficial effects) (de Simone, 2019; Wieërs *et al*., 2020). They are widely used in treating many conditions and have found significant use in treating various diseases (Sanders *et al*., 2019).

Most of the bacterial probiotic strains used in biomedical reviews and studies belong to the genera *Bifidobacterium* spp. and *Lactobacillus* spp. Additionally, strains such as *Bacillus clausii*, *Bacillus laterosporus*, *Bacillus coagulans*, *Bacillus subtilis*, and *Bacillus pumilus* have also been used as probiotics (Dutta *et al*., 2011; Jafar *et al*., 2019).

B. clausii is ubiquitous in nature, mobile rod cell, aerobic, gram-positive bacteria, and endospore-forming isolated from soil. It is also found naturally within the flora of the digestive system of mammals (De Jonghe *et al*., 2010). The effectiveness and safety of several *B. clausii* strains have been proven through clinical trials conducted globally (Keya *et al*., 2015). It is considered the primary causative agent to inhibit the proliferation of pathogenic microorganisms in the gastrointestinal tract. It is frequently used as a probiotic to treat conditions related to diarrhea and enteritis. In Iraq, a previous study approved that the administration of probiotics with standard rehydration therapy reduced the duration of diarrhea in children in Fallujah City, Iraq (Mukhlif and Ali, 2024).

Moreover, an anti-*Pseudomonas aeruginosa* probiotic extract was investigated for its effectiveness in postoperative wound infections in Al-Najaf province, Iraq. The results showed a synergistic antibacterial inhibition effect between *S. boundary* and *B. clausii* (Mohammed *et al*., 2021).

However, a literature review on the application of *B. clausii* in experimental animals revealed a limited number of publications. Consequently, this study aims to investigate the potential of *B. clausii* as a probiotic and explore its therapeutic effects on experimental *E. coli*-induced diarrhea in rats, focusing on body weight, lipid, and protein parameters.

MATERIALS AND METHODS

Bacterial isolate

The bacterial species used in this study was received from microbiology department, College of Veterinary Medicine, University of Kirkuk. The identification of these bacterial species were carried out at species level by Vitek 2 framework.

Characterization of probiotics

Bile salt and acid tolerance tests were used to assess the extracted probiotic according to the method previously described by Choi and Chang (2015). The survival of probiotic bacteria was determined by evaluating cell survival after 24 hours of exposure to different pH values (2, 4, 6, and 8) and various concentrations of bile salts (0.1%, 0.3%, and 0.5%). The bacterial culture was incubated under aerobic conditions at 37°C for 24 hours.

Experimental animals groups

Fifteen male Albino Sprague-Dawley rats, aged 7-8 weeks and weighing approximately 146-148g, were used in this study. The rats were allowed to acclimatize for 10 days under standard animal house conditions. *B. clausii* was tested in this study for its potential effects against *E. coli*, particularly in relation to concerns about antibiotic resistance. All rats had free access to food and water ad libitum. The rats were then randomly divided into three equal groups, each with four replicates, as follows: G1 (healthy group), G2 (infected group with *E. coli*), and G3 (infected with *E. coli* and treated with the probiotic *B. clausii*).

Every day, the rats were administered 2 ml of *B. clausii* via oral gavage, corresponding to a concentration of 1.5 x 10^8 CFU/day/rat, based on the standard turbidity of the McFarland solution. The treatment lasted for 28 days. At the end of the treatment period, the rats were subjected to a 20-hour starvation period, after which they were anesthetized with chloroform. Blood samples were collected from all rats and subjected to biochemical assays for lipid profile analysis, including low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), and triglycerides, using a standard laboratory assay with a lipid analyzer (Multichannel Abbott Spectrum Autoanalyzer, Abbott Laboratories, C4000, USA). The estimation procedures followed the manufacturer's instructions for lipid profile kits, except for LDL, which was calculated using the Friedewald equation (Friedewald and Levy, 1972). Additionally, serum total protein (TP), globulin, and albumin concentrations were estimated spectrophotometrically using available kits (Biomerex, France).

Statistical analysis

All collected data was alnalysed using SPSS software version 22. The variables were displayed as mean± Standard deviation (SD). Moreover, the General Linear Model and Duncan test were used to estimate the significance of the various factors. Statistically, the significant result was considered at $p<0.05$.

RESULTS AND DISCUSSION

The results of the current study demonstrated the growth of *B. clausii* probiotic bacteria at various concentrations of pH and bile salts. *B. clausii* showed growth at pH 4 and 6, while no growth was observed at pH 2. These results are consistent with previous research by Al-Jandal *et al.* (2024), who reported significant differences in the total number of microorganisms in soft cheese due to the application of bacteriocins. The findings regarding acid tolerance are also in line with those of Nithya and Halami (2013), who observed that cultures of probiotic microorganisms exhibit better acid and bile tolerance. Furthermore, the results showed the growth of *B. clausii* at bile salt concentrations of 0.1% and 0.3%, while no growth occurred at 0.5%. These findings are consistent with the research of Werdi and Al-Hadidy (2023), who reported a significant increase ($P<0.05$) in the values of various liver enzymes compared to the control group. Additionally, the results align with the work of Kumar *et al.* (2022) and Nithya and Halami (2013), further supporting the beneficial properties of *Bacillus clausii* in terms of its growth under various conditions.

Table 1: The growth of *B. clausii* probiotic bacteria in various pH and different bile salt concentration.

Bile salt %			PH			
0.1	0.3	0.5	2	4	6	8
+	+	_	_	+	+	+

Table 2: The effect of *B. clausii* on body weight of the laboratory rats suffered from *E.coli* induced diarrhea.

Treatments	Initial	Final weight	Weight gain
		G	
G1	131.0 b	225.0 b	94.0 b
G2	139.0 b	267.6 a	128.6 a
G3	176.3 a	266.3 a	90.0 b

a-b different lettering in a column shows significant difference at probability level ($P<0.05$). G1: Control; G2: Infected with *E. coli*; G3: Infected with *E. coli* and treated with *B. clausii*.

The results of the current study revealed a significant variation in the body weight of rats across the different experimental groups (Table 2). The initial body weights were 131.0 g, 139.0 g, and 176.3 g for G1, G2, and G3, respectively. The results also showed an increase in the body weight of rats in G2, which were exposed to *E. coli* infection, with a final weight of 267.6 g and a weight gain of 128.6 g ($P<0.05$). However, the body weight measurement for G3, which was treated with *B. clausii*, showed a reduction compared to G1 and G2. These results may be attributed to an increase in lipid parameters such as cholesterol, triglycerides, and low-density lipoproteins (LDL). These factors likely contributed to the weight gain observed in the infected animal group, as suggested by Al-Jubouri (2009).

The findings of the current study are in agreement with (Kobyliak *et al.*, 2016), who mentioned that probiotics not only significantly affect weight loss and fat loss processes, but they also have a tendency to decrease the average size of fat cells in all fatty tissues of experimental animals.

The effect of *B. clausii* on the lipid profile of rats induced by *E. coli* infection is displayed in Table 3. A significant increase ($P<0.05$) was observed in the concentrations of cholesterol (73.4 mg/dl), triglycerides (94.0 mg/dl), and LDL-cholesterol (16.4 mg/dl) in G1 (control group). In contrast, there was a significant decrease in the concentrations of HDL-cholesterol (17.4 mg/dl) in

G2 (infected group). The concentrations for cholesterol, triglycerides, and LDL-cholesterol in G2 were 74 mg/dl, 96.8 mg/dl, and 18.4 mg/dl, respectively. These values were significantly higher compared to G3 (treated group with *B. clausii*), which had concentrations of 64.5 mg/dl, 54.6 mg/dl, and 15.13 mg/dl for cholesterol, triglycerides, and LDL-cholesterol, respectively. Additionally, HDL-cholesterol was significantly higher in G3 (36.5 mg/dl) compared to the other groups.

Table 3: The effect of *B. clausii* on Lipid profile of rats.

Treatments	CHOL	TG	LDL	HDL
	mg/dl			
G1	73.4 a	94.0 a	16.4 a	38.9a
G2	74 a	96.8 a	18.4 a	17.4 b
G3	64.5 b	54.6 b	12.13 b	36.5 a

a-b different lettering in a column shows significant difference at probability level (P<0.05). CHOL: Cholesterol; TG: Triglyceriods; LDL: Low-density lipoprotein; HDL: High-density lipoprotein (HDL); G1: Control; G2: Infected with *E. coli*; G3: Infected with *E. coli* and treated with *B. clausii.*

Previous study approved that the high ratio in cholesterol levels is attributed to an increment within the action of the chemical cholesterol acyltransferase, which is sensible for the assimilation of cholesterol (Jumaa and Naji, 2021). It is worth to mentioned that previous studies explained the several mechanisms used by probiotic to lower the cholesterol levels such as the bile salt decongygation, cholesterol assimilation, short chain fatty acid production, and modulation of gut microbiota (Kumar *et al*., 2012).

Moreover, Hassan and Frank (2001) explained that the decrease in lipid markers may be attributed to disturbances in the lipid digestion system as a result of stress and the formation of lipid peroxides and unsaturated fatty acids. This mechanism can lead to the inhibition of steroid and bile salt secretion and excretion, as well as the occurrence of various absorption and digestion disturbances (Hassan and Frank, 2001).

The effect of probiotics on triglyceride levels varies depending on the type and strain of bacterial agent, and causes of decrease in TG value may be contributed in stimulation of enzyme lipase resulting fatty acids formation that absorbed by fat cells lead to inhibit the synthesis of fatty acids which enter the liver's procedure for generating fat through the activity of key enzymes preventing (Li *et al*., 2020).

While, Trieb *et al*. (2016) found that the levels of HDL decreased because the liver being exposed for some diseases or due to the high concentrations of other lipid profile.

According to previous publication, the causes of decrease in cholesterol concentration, is the cholesterol dehydrogenase enzyme synthesized by probiotic bacteria. This enzyme is activated the converting cholesterol process into Cholest-4-en -3-one, and in turn is used as a mediating factor in converting cholesterol into smaller units Coprostanol, which makes it easier to break it down for use. Accompanied with a decrease in cholesterol retention and a rise within the accompanied with a low value in absorption of cholesterol and a rise within the sum of cholesterol the body excretes (Ahn *et al*., 2003; Werdi and Al-Hadidy, 2023).

The results of the current study also showed the effects of *B. clausii* on the protein parameters in *E. coli*-infected rats. A significant reduction (P<0.05) in total protein and albumin levels was observed in G2, the infection-induced group, with concentrations of 5.00 and 4.20 dL/g for total protein and albumin, respectively. However, the total protein and albumin values in G1 (healthy group) and G3 (treated with *B. clausii*) were 5.30 and 5.50 dL/g, and 5.9 and 5.10 dL/g, respectively. These results demonstrated a significant increase in total protein and albumin levels in the treatment group with *B. clausii*, while no significant differences were observed between the groups in globulin values (Table 4).

Table 4: The effect of *B. clausii* on the serum Proteins of infected rats.

Treatments	Total protein	Albumin	Globulin
	g/dL		
G1	5.30 b	5.50 a	1.20
G2	5.00 b	4.20 b	1.00
G3	5.9 a	5.10 a	1.20

a-b different lettering in a column shows significant difference at probability level (P<0.05). G1: Control; G2: Infected with *E. coli*; G3: Infected with *E. coli* and treated with *B. clausii.*

Previous studies (Żołnierkiewicz et al., 2024), explained the reasons of low blood proteins in the infected group. The experimental animal exposed to stress that decrease using of glucose as an energy source and consequently, resort to the use of alternative sources, including proteins, like diabetes. These mechanisms lead to loss of total proteins (Proteinuria) through Urination (Żołnierkiewicz et al., 2024), and several pathological conditions, such as plasma hemolysis, decreased liver protein synthesis, disruption of kidney function, increased concentrations of free radicals, and exposure to oxidative stress were also occurred. However, other researcher showed that the Inhibition of enzymes associated with protein synthesis may be also the reason for the decrease in total protein concentration (Groener *et al*., 1989).

On the other hand, the results of the current study showed a decrease in albumin levels with no significant variation between the different experimental groups. According to a previous study by Demir *et al.* (2009), this observation may be attributed to the loss and infiltration of protein into damaged tissues. Additionally, the consumption of certain antioxidants in the body leads to a decrease in calcium concentration, as 50% of calcium is bound to albumin in the blood plasma (Abd El-Hack *et al.*, 2021; Anraku *et al.*, 2011). Low albumin protein values are also considered a clinical sign of severe liver disease, which occurs due to obstruction of its synthesis or glomerulopathy, leading to an increase in albumin loss (Belinskaia *et al.*, 2021). Furthermore, the results of this study agree with previous research that indicated the use of probiotics can lead to an increase in albumin and total protein values (Abd El-Hack *et al.*, 2021).

CONCLUSION

In conclusion, this study revealed the effects of *B. clausii* bacteria as a probiotic in experimental *E. coli* infected rats. The results showed significant reduction in the negative effects of *E. coli* and beneficial effect of *B. clausii* on lipid and protein parameters in experimentally infected rats, showing a direct link to the modulation of *E. coli* infection. The authors recommend further studies on *B. clausii* probiotics to explore their impact on a wider range of physiological and biochemical parameters.

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NOVELTY STATEMENT

The novelty of this study is the investigation of the therapeutic potential of *Bacillus clause* as probiotic to treat the *E.coli* induce diarrhoea in rat , which have not been extensively studied in vivo. The research contributes to the field by approving the role of *Bacillus clause* role in modulating gut microbiota and enhancing the immune response.

AUTHOR'S CONTRIBUTION

Almas M. Al-Bayati: conceptualization and experimental study and writing manuscript draft.
Ali H. Awad: experimental study and laboratory analysis and statistical analysis.
Ahmed abdulhameed jabbar : Laboratory analysis and writing draft.
Al Salihi karima Akool : Experimental study and editing the final version of the manuscript.
Luay Jumaah Jihad: experimental study and laboratory analysis and statistical analysis.

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Ethical statement

This study was carried out in compliance with institutional guidelines and ethical standards stated in the Instructions of the Scientific Research and Higher Education Ministry.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abd El-Hack ME, Alaidaroos BA, Farsi RM, Abou-Kassem DE, El-Saadony MT, Saad AM, Shafi ME, Albaqami NM, Taha AE, Ashour EA (2021). Impacts of supplementing broiler diets with biological curcumin, zinc nanoparticles and *Bacillus licheniformis* on growth, carcass traits, blood indices, meat quality and cecal microbial load. Animals, 11(7): 1878. https://doi.org/10.3390/ani11071878

Ahn YT, Kim GB, Lim KS, Baek YJ, Kim HU (2003). Deconjugation of bile salts by *Lactobacillus acidophilus* isolates. Int. Dairy J., 13(4): 303–311. https://doi.org/10.1016/S0958-6946(02)00174-7

Al-Jandal AHM, Jassim MA, Al-Dulaimi FK (2024). Effect of adding of bacteriocin produced from *Streptococcus thermophilus* bacteria on the microbial content and some pathogenic bacteria in soft cheese. NTU J. Agric. Vet. Sci., 4(3). https://doi.org/10.56286/vz6r7j68

Al-Jubouri MAJ (2009). Effect of feeding field pistachios on the nature of blood lipids and some biochemical values in growing rats. Master thesis, College of Agriculture and Forestry, University of Mosul.

Anraku M, Takeuchi K, Watanabe H, Kadowaki D, Kitamura K, Tomita K, Kuniyasu A, Suenaga A, Maruyama T, Otagiri M (2011). Quantitative analysis of cysteine-34 on the anitioxidative properties of human serum albumin in hemodialysis patients. J. Pharma. Sci., 100(9): 3968–3976. https://doi.org/10.1002/jps.22571

Asma SAK (2020). Screening study for some strains of *E. coli* collected from five regions in Kurdistan-Iraq for its sensitivity, resistance and MDR against thirteen antibiotics. Medico Legal Update, 20(1): 526–531. https://doi.org/10.37506/mlu.v20i1.413

Bader J, Albin A, Stahl U (2012). Spore-forming bacteria and their utilisation as probiotics. Beneficial Microbes, 3(1): 67–75. https://doi.org/10.3920/BM2011.0039

Bahaddad SA, Almalki MHK, Alghamdi OA, Sohrab SS, Yasir M, Azhar EI, Chouayekh H (2023). Bacillus species as direct-fed microbial antibiotic alternatives for monogastric production. Probiot. Antimicrob. Proteins, 15(1): 1–16. https://doi.org/10.1007/s12602-022-09909-5

Belinskaia DA, Voronina PA, Shmurak VI, Jenkins RO, Goncharov NV (2021). Serum albumin in health and disease: Esterase, antioxidant, transporting and signaling properties. Int. J. Mol. Sci., 22(19): 10318. https://doi.org/10.3390/ijms221910318

Choi EA, Chang HC (2015). Cholesterol-lowering effects of a putative probiotic strain *Lactobacillus plantarum* EM isolated from kimchi. LWT Food Sci. Technol., 62: 210–217. https://doi.org/10.1016/j.lwt.2015.01.019

Constable PD, Kenneth WH, Stanley HD, Walter G (2017). Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs, and goats. Vet. Med. Elsevier. pp. i–ii.

Danilova I, Sharipova M (2020). The practical potential of bacilli and their enzymes for industrial production. Front. Microbiol., 11. https://doi.org/10.3389/fmicb.2020.01782

De Jonghe V, Coorevits A, De Block J, Van Coillie E, Grijspeerdt K, Herman L, De Vos P, Heyndrickx M (2010). Toxinogenic and spoilage potential of aerobic spore-formers isolated from raw milk. Int. J. Food Microbiol., 136(3): 318–325. https://doi.org/10.1016/j.ijfoodmicro.2009.11.007

de Simone C (2019). The unregulated probiotic market. Clin. Gastroenterol. Hepatol., 17(5): 809–817. https://doi.org/10.1016/j.cgh.2018.01.018

Demir H, Topkaya BÇ, Erbay AR, Doğan M, Yücel D (2009). Ischaemia-modified albumin elevation after percutaneous coronary intervention reflects albumin concentration rather than ischaemia. Ann. Clin. Biochem. Int. J. Lab. Med., 46(4): 327–331. https://doi.org/10.1258/acb.2009.008238

Dutta P, Mitra U, Dutta S, Rajendran K, Saha TK, Chatterjee MK (2011). Randomised controlled clinical trial of Lactobacillus sporogenes (*Bacillus coagulans*), used as probiotic in clinical practice, on acute watery diarrhoea in children. Trop. Med. Int. Health, 16(5): 555–561. https://doi.org/10.1111/j.1365-3156.2011.02745.x

Friedewald WI, Levy RIFD (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499–502. https://doi.org/10.1093/clinchem/18.6.499

Muntingh GL (2017). Ok, so who wants to colonise my gut? Overview of Probiotics in certain gastro-intestinal disorders. South Afr. Family Pract., 59(5): 29–34. https://doi.org/10.4102/safp.v59i5.4757

Groener JEM, van Gent T, van Tol A (1989). Effect of lipid transfer protein on plasma lipids, apolipoproteins and metabolism of high-density lipoprotein cholesteryl ester in the rat. Biochim. Biophys. Acta Lipids Lipid Metab., 1002(1): 93–100. https://doi.org/10.1016/0005-2760(89)90070-2

Hammou HA, Benabdallah R, Heleili N, Ayachi A, Bennoune O (2024). Antimicrobial resistance of *E. coli* isolated from broiler flocks and slaughterhouses in Batna District, Algeria. J. Appl. Vet. Sci., 0(0): 0–0. https://doi.org/10.21608/javs.2024.324530.1433

Hassan AN, Frank JF (2001). Applied dairy microbiology. Appl. Dairy Microbiol. Marcel Dekker, Inc., New York, pp. 151–206.

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME (2014). The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol., 11(8): 506–514. https://doi.org/10.1038/nrgastro.2014.66

Jafar NB, Ghaleb ZT, Fadhil ZH (2019). Production of fermented red beet juice using probiotic lactobacilli bacteria. Ann. Trop. Med. Publ. Health, 22(7): 91–95. https://doi.org/10.36295/ASRO.2019.220712

Jumaa, J.M.M. Naji EZ (2021). Study of the effect of ethanolic extract of pomegranate peels on some blood serum biochemical parameters in alloxan induced diabetes male rats. Tikrit J. Agric. Sci., 21(1): 138–151. https://doi.org/10.25130/tjas.21.1.14

Karima ALS, Younise MH, Ali AMA, Ali AJ, Mahdi ZH (2023). Isolation and identification of bacterial causes of diarrhea in children less than 2 years old in Al-Muthanna Governorate. Muthanna Med. J., 10(1): 1–13. https://doi.org/10.52113/1/1/2023-1-13

Keya L, Kapil J, Pallavi G, Fehmida N (2015). Bacillus clausii as an adjuvant therapy in acute childhood diarrhoea. IOSR J. Dental Med. Sci., 14(5): 74–76.

Kobyliak N, Conte C, Cammarota G, Haley AP, Styriak I, Gaspar L, Fusek J, Rodrigo L, Kruzliak P (2016). Probiotics in prevention and treatment of obesity: A critical view. Nutr. Metab., 13(1): 14. https://doi.org/10.1186/s12986-016-0067-0

Kumar M, Nagpal R, Kumar R, Hemalatha R, Verma V, Kumar A, Chakraborty C, Singh B, Marotta F, Jain S, Yadav H (2012). Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Exp. Diab. Res., 2012: 1–14. https://doi.org/10.1155/2012/902917

Kumar M, Nagpal R, Kumar R, Hemalatha R, Verma V, Kumar A, Chakraborty C, Singh B, Marotta F, Jain S, Yadav H (2022). Corrigendum to cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. J. Diab. Res., pp. 1–14. https://doi.org/10.1155/2022/3952529

Li Y, Liu M, Liu H, Wei X, Su X, Li M, Yuan J (2020). Oral supplements of combined bacillus licheniformis Zhengchangsheng® and Xylooligosaccharides improve high-fat diet-induced obesity and modulate the gut microbiota in rats. BioMed. Res. Int., 2020(1). https://doi.org/10.1155/2020/9067821

Mohammed S, Ruaa S, Atheer B (2021). Activity assay of some probiotics extract as anti-pseudomonas aeruginosa that isolated from postoperative wound infection in Alnajaf Govrnorate, Iraq. Bacterial Empire, 4(1): 16–19. https://doi.org/10.36547/be.2021.4.1.16-19

Mukhlif Z, Ali WI (2024). An interventional study to measure the role of probiotics in treating diarrhea in sample of children in Fallujah City, Iraq. Int. J. Innov. Res. Med. Sci., 9(5): 289–294. https://doi.org/10.23958/ijirms/vol09-i05/1895

Muteeb G, Rehman MT, Shahwan M, Aatif M (2023). Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. Pharmaceuticals, 16(11): 1615. https://doi.org/10.3390/ph16111615

Nithya V, Halami PM (2013). Evaluation of the probiotic characteristics of Bacillus species isolated from different food sources. Ann. Microbiol., 63(1): 129–137. https://doi.org/10.1007/s13213-012-0453-4

Priyanka P, Miral P, Krishnamurthy R (2013). Saccharomyces boulardii a probiotic of choice. CIBTech J. Biotechnol., 2(2): 1–6.

Saggese A, Baccigalupi L, Ricca E (2021). Spore formers as beneficial microbes for humans and animals. Appl. Microbiol., 1(3): 498–509. https://doi.org/10.3390/applmicrobiol1030032

Sanders ME, Merenstein DJ, Reid G, Gibson GR, Rastall RA (2019). Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. Nat. Rev. Gastroenterol. Hepatol., 16(10): 605–616. https://doi.org/10.1038/s41575-019-0173-3

Tietz Y (n.d.). Clinical biochemistry, 6th ed. McGraw-Hill, New York, p. 825.

Trieb M, Horvath A, Birner-Gruenberger R, Spindelboeck W, Stadlbauer V, Taschler U, Curcic S, Stauber RE, Holzer M, Pasterk L, Heinemann A, Marsche G (2016). Liver disease alters high-density lipoprotein composition, metabolism and function. Biochim. Biophys. Acta Mol. Cell Biol. Lipids, 1861(7): 630–638. https://doi.org/10.1016/j.bbalip.2016.04.013

Werdi CM, Al-Hadidy YI (2023). The effectiveness of the probiotic *Bacillus* spp. in induced infection with *Staphylococcus aureus* and the study of its effect on liver enzymes, kidney function and microbial balance of the gut in male rats. IOP Conf. Ser. Earth Environ. Sci., 1158(11): 112013. https://doi.org/10.1088/1755-1315/1158/11/112013

Wieërs G, Belkhir L, Enaud R, Leclercq S, Philippart FJM, Dequenne I, de Timary P, Cani PD (2020). How probiotics affect the microbiota. Front. Cell. Infect. Microbiol., pp. 9. https://doi.org/10.3389/fcimb.2019.00454

Żołnierkiewicz O, Rogacka D. Hyperglycemia (2024). A culprit of podocyte pathology in the context of glycogen metabolism. Arch Biochem Biophys. 2024 Mar;753:109927. doi: 10.1016/j.abb.2024.109927. Epub 2024 Feb 11. PMID: 38350532.