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Review

The Effect of Probiotic Supplementation in Cholestasis Liver Disease: A Systematic Review of Animal Studies

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Abstract. Gut microbiome is essential in maintaining metabolism, gut barrier homeostasis, inflammation, and hematopoiesis. Several factors affect gut microbiome composition, including genetics, lifestyle, external factors, and disease. Cholestasis liver disease promotes gut dysbiosis via abnormal bile production or flow to the intestine and disrupts the gut microbiome. This condition leads to intestinal leakage, which enables bacterial and endotoxin translocation to the liver through the portal vein. Bacterial translocation promotes inflammatory responses, which worsen liver damage in cholestasis. Moreover, probiotic supplementation in other diseases has been shown to preserve gut microbiome composition. While such studies have documented probiotics' beneficial effects, no adequate clinical trials support probiotics' potency as a cholestasis treatment. Hence, this systematic review aims to provide an in-depth analysis of probiotic supplementation as a therapy for cholestasis liver disease in animal models. The search strategies were conducted based on PRISMA methodologies based on various academic literature. The selected studies have shown improvements in bile acid metabolism, microbiota-gut-liver axis, gut epithelium integrity, liver damage and inflammation response, and liver fibrosis progression, which need to be confirmed in human clinical trials.

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1. Introduction

The gut microbiota consists of bacteria, fungi, viruses, and archaea that inhabit and establish colonies in the digestive tract. An adult's digestive system contains around 100 trillion microorganisms and takes about 2 kg of body weight. Compared to the number of host cells, and this number is about ten times higher [1]. The gut microbiota is critical to both good health and disease conditions. The gut microbiome's interactions with other organs, such as the brain and liver, are critical for metabolism, gut barrier homeostasis, inflammation, and hematopoiesis. However, the gut microbiome's overall composition is influenced by various variables, including diets, lifestyle, drugs, and other external influences [2].

Dysbiosis, or altered microbial composition, has been related to various disorders. Numerous studies provide evidence of disturbance of the gut microbiota in metabolic disorders, liver illnesses, and atherosclerosis [3–5]. The gut microbiome not only ferments ingested food but also produces metabolites that affect other organs, such as Lipopolysaccharide (LPS) and Trimethylamine N-oxide (TMAO) [5]. Through the gut-liver axis, gut microbiota and its metabolites can induce inflammation in the liver, thus aggravating liver damage after bile buildup in cholestasis liver disease.

Cholestasis diminished bile production and/or flow, and it may affect both intrahepatic and extrahepatic bile ducts or be restricted to one or the other [6]. According to Feldman et al., (2021) [7], cholestasis is clinically indicated when the levels of conjugated or direct bilirubin exceed 1 mg/dL, provided that the total bilirubin is less than 5 mg/dL. Alternatively, cholestasis is indicated when the levels of conjugated or direct bilirubin exceed 20% of the total bilirubin, if the total bilirubin is larger than 5 mg/dL [7]. According to a retrospective study, cholestasis can be attributed to various factors, including parenteral nutrition-associated cholestasis (PNAC) 48.8%, cardiovascular and circulatory disorders 18.1%, biliary anatomic obstruction 12.5%, infection 8.7% and genetic disorders 5.6% [8].

Cholestasis can result in the buildup of harmful bile acids, bilirubin, and other substances in the liver, thus elevated bile acids level in the blood circulation. However, bile acids level in the digestive system usually reduced due to bile blockade or decrease bile production [9]. An overabundance of bile acids in the liver can trigger immunogenic processes involving immune cells and releasing cytokines, chemokines, and other immunoregulators. Hepatocytes can be damaged due to an excessive buildup of immune cells in the liver [10]. Cholestasis may progress to hepatitis, cirrhosis, liver fibrosis, liver failure, hepatocytes or cholangiocyte carcinomas in without effective intervention [5].

Bile acids are formed from cholesterol metabolism in the liver regulated by three major regulatory enzymes: cholesterol 7α-hydroxylase (CYP7A1), cholesterol 27-hydroxylase (CYP27A1), and sterol 12α-hydroxylase (CYP8B1) [11]. The farnesoid X receptor (FXR) is the first identified bile acid receptor. FXR has been identified as a regulator of cholesterol and bile acids homeostasis in liver and small intestinal cells [12-13]. FXR plays a vital role in the manufacture of bile acids in the liver, the absorption of bile acids in the intestines, and the uptake of bile acids in the liver [11]. FXR suppresses the production of bile acids in the liver by reducing the expression of CYP7A1 and CYP8B1. In turn, FXR induced bile acid transporters and increase the expression of fibroblast growth factor 15 (FGF15) in the ileum and small heterodimer partner (SHP) in the liver. These changes help modulate bile acid synthesis, breakdown, secretion, and absorption [14-15].

Advances in metagenomic sequencing have discovered gut microbial changes in many types of cholestasis liver diseases. As an illustration, in primary sclerosing cholangitis (PSC), the gut microbiome has been altered, with *Clostridium* enrichment, while *Eubacterium spp.* and *Ruminococcus obeum* reduced compared to a healthy control [16]. Song et al., (2021) [17] discovered the domination of *Klebsiella, Streptococcus, Veillonella*, and *Enterococcus* in the early and late phases of biliary atresia.

Multiple studies have demonstrated the beneficial impact of probiotic supplementation on liver disease. For instance, administering *Pediococcus pentosaceus* in alcoholic liver disease (ALD) has improved liver injury by decreasing transaminase enzyme and pro-inflammatory cytokines, improving tight junctions, and restoring beneficial bacteria [18]. Cao et al. identified improvement of non-

alcoholic liver disease (NAFLD) after supplementation of *Lactobacillus plantarum* ZJUIDS14 by increasing the short-chain fatty acids (SCFA) concentration, enhancing the integrity of the gut barrier, diminishing, or preventing inflammation in the gut and liver, and influencing lipid metabolism [19].

Although the aforementioned studies have shown the wide advantage of probiotics in managing disease, no previous clinical trials have investigated the relationship between probiotics and cholestasis progression in human models. However, abundant animal model studies have been conducted, and no studies have systematically reviewed the effect of probiotics on preventing and treating cholestasis liver disease. This study will systematically assess and incorporate all known studies on the effects of probiotic treatment intended specifically for cholestasis liver disease on bile acid metabolism, liver damage, and gut barrier function.

2. Methods

This systematic review was generated using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement of the 2020 method [20]. From May to August 2024, the writers conducted a comprehensive review by examining various databases, including PubMed, ProQuest, ScienceDirect, Scopus, and Web of Science, for relevant research findings. The study topic's eligibility will be assessed utilizing the Population Intervention Comparison Outcomes (PICO) technique. The PICO method is a technique used to analyze clinical problems by identifying key terms that can be easily searched [21]. For the research to be considered valid, the studies had to satisfy the following criteria in Table 1.

The article must satisfy the inclusion criteria, which involve reporting the outcomes of probiotic supplementation in animal models of cholestasis, providing evidence of alterations in indicators of liver damage associated with cholestasis, and/or assessing the activity of enzymes related to bile acids. The exclusion criteria for this review were articles that did not utilize animal models of cholestasis supplemented with probiotics, articles that were review papers, and articles that did not disclose changes in liver damage parameters or assess bile acids-related enzymes.

Table 1. PICO table to conduct research question

PICO	Criteria
Population	Animal model studies of cholestasis liver disease of any etiology
Intervention	Any form of administration of probiotics
Comparison	Non-intervention or administration of other drugs
Outcomes	Studies should assess changes in liver damage indicators and/or bile acids-related enzymes.

Systematic reviews included only studies that fit the predefined search criteria. In this case, probiotic supplements, cholestasis, bile acids, and their synonyms were used. A total of 190 articles were retrieved after conducting a comprehensive search across all databases using the specified keywords. In addition, we eliminated fourteen duplicate articles. We examined the research paper lists of the acknowledged reports and any relevant reviews. Abstracts of titles that may be eligible were filtered.

The obtained papers were incorporated in the study based on specific criteria for inclusion and exclusion. Although 175 items could have been considered, 151 were deemed ineligible as they have yet to fulfill the criteria for both inclusion and exclusion. Nine papers satisfied the criteria for inclusion and were deemed eligible for assessment. Figure 1 depicts the methodology used to determine article selection. The investigators evaluated the chosen papers using the risk of bias (RoB) methods from the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE), which develop from Cochrane RoB framework [22].

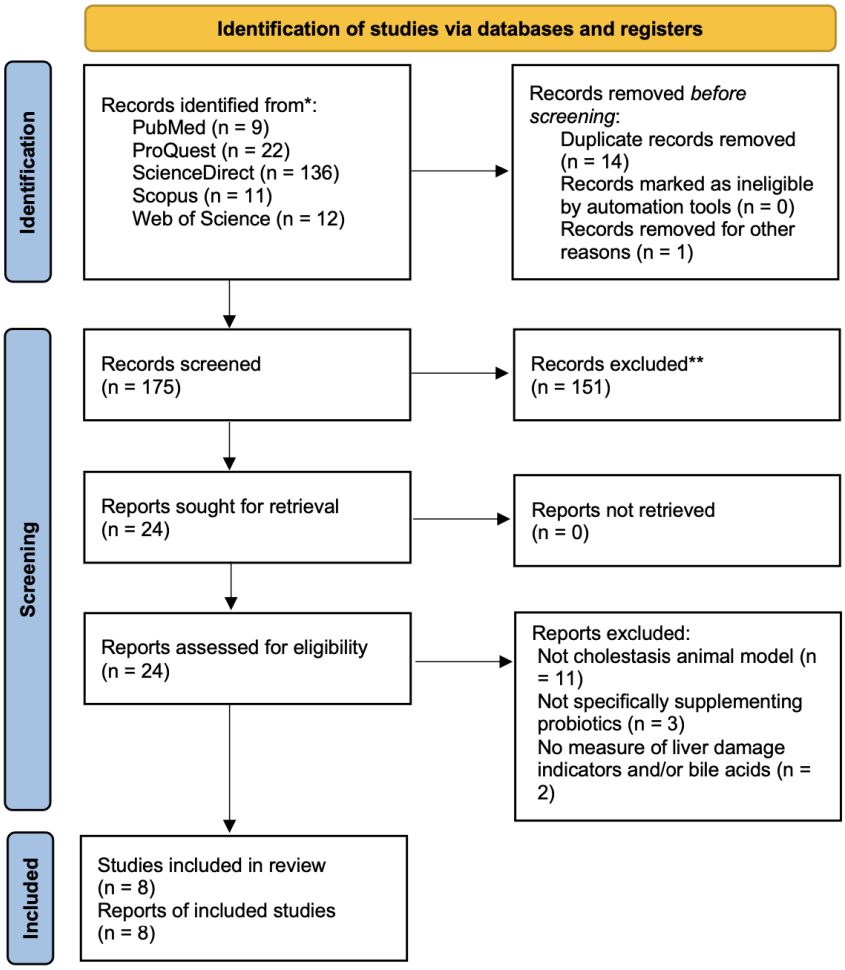

Figure 1. Study selection diagram flow

3. Results and Discussion

3.1. Risk of Bias of Included Studies

Table 2 displays the outcomes of the risk of bias evaluation for the nine studies incorporated in this systematic review. The studies included in the analysis indicate no evidence of attrition, reporting, or any other form of bias. Nevertheless, the comprehensive assessment of bias in all the research included in the analysis remained uncertain. The research listed shares common limitations. We identified inadequate randomization process, allocation concealment, random housing, and documentation of blinding of the animal assessor. The primary cause for the lack of clarity is the absence of sufficient explanations within the articles.

3.2. Cholestasis Progression

Cholestatic liver diseases might result from a genetic mutation, mechanical abnormalities, toxins, or immune system dysregulation that harms the bile ducts, leading to the accumulation of bile and the destruction of liver tissue [23]. The clinical symptoms of cholestatic liver disease may encompass pruritus (itching), weariness, jaundice (yellowing of the skin and eyes), dark urine, and acholic stools (pale or clay-colored feces). Eventually, the patient develops elevation of alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and serum bilirubin level [24].

Bilirubin is a byproduct of heme metabolism. Bilirubin excretion is initiated by the breakdown of red blood cells (RBC), which enables heme metabolism to generate biliverdin. Biliverdin reductase enzymatically transforms biliverdin into unconjugated bilirubin, which later attaches to albumin during transport to the liver [25-26]. Hepatic microsomes utilize the uridine diphosphate (UDP)-glucuronyl transferase to combine bilirubin with glucuronic acid by conjugation [26].

Conjugated bilirubin then released into the bile and carried to the small intestine. In intestine, bilirubin transformed into urobilinogen by bacterial enzymes. Urobilinogen eliminated from the body through feces as stercobilin and urine as urobilin [27]. In cholestatic liver diseases, an increase in direct bilirubin levels suggests a disruption in the normal secretion of bilirubin into the bile due to functional issues with hepatocytes or a disturbance in the bile flow.

Nevertheless, it is crucial to accurately determine the specific cause of elevated bilirubin levels. Insufficient management can result in severe liver damage, potentially leading to the death of those affected [9]. Cholestasis can be treated with a variety of specific therapies, including antibiotics for infection-induced cholestasis and cholangitis, Kasai portoenterostomy for biliary atresia, and corticosteroids for autoimmune cholangitis [20, 30]. Ursodeoxycholic acid (UDCA) is a general drug that stimulates bile flow in the liver [28, 30].

Tabel 2. Risk of bias assessment

First author, year	Selection bias			Performance bias		Detection bias		Attrition bias	Reporting bias	Other
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Jiang et al. 2022 [11]	?	+	?	?	?	+	?	+	+	+
Kong et al. 2022 [31]	?	?	?	?	?	?	?	+	+	+
Sun et al. 2023 [15]	?	+	?	?	?	?	?	+	+	+
Han et al. 2023 [14]	+	+	?	?	?	+	?	+	+	+
Ren et al. 2019 [32]	?	+	?	?	?	+	?	+	+	+
Lin et al. 2022 [33]	?	?	?	?	?	+	?	+	+	+
Liu et al. 2020 [34]	?	+	?	?	?	?	?	+	+	+
Kabiri-Arani 2024 [35]	+	?	?	?	?	?	?	+	+	+

(1) Sequence generation; (2) Baseline characteristics; (3) Allocation concealment; (4) Random housing; (5) Blinding; (7) Random outcome assessment; (7) Blinding; (8) Incomplete outcome data; (9) Free of selective outcome reporting; (10) Other sources of bias; ? indicated unclear; + indicated the requirement is fulfilled; - indicated the requirement is not fulfilled.

Cholestasis can result in the accumulation of bile in the liver. Over time, this can cause liver and the bile ducts damaged [9]. Hepatocytes and cholangiocytes can proliferate in response to injury, resulting in the development of periductular fibrosis, biliary fibrosis, and cirrhosis [29, 36]. Bile is a complex mixture of endogenous metabolites and xenobiotics, and it acts as an essential excretory pathway in the body [37]. The obstruction of bile flow and subsequent buildup can have significant toxicity due to the presence of many chemicals, such as bile acid [9].

Bile acids, derived from cholesterol metabolism, are the main component of bile and have a crucial function in facilitating bile flow [37]. In humans, the first primary bile acids secreted are chenodeoxycholic acid (CDCA) and Cholic acid (CA). Those bile acids are later converted into secondary bile acids in the form of lithocholic acid (LCA) and deoxycholic acid (DCA), with bacterial enzymes as the key regulators [9].

To conclude, cholestasis is a complex condition with many contributing factors, mechanisms, and risks. Given that some cholestasis is benign, and others are malignant, it is critical to evaluate the cholestasis etiology thoroughly. The symptoms of bilirubin and bile acid buildup were often equal in cholestasis, although the specific symptoms were more prevalent in some etiologies. Probiotics potentially improve the general cholestasis symptom through bile acid regulation.

3.3. Probiotics

Probiotics were initially characterized as commensal microorganisms typically contained in food and live in the digestive system. Probiotics primarily comprise gram-negative bacteria from two genera: *Bifidobacterium* and *Lactobacillus* [38]. Later, probiotics were defined as any beneficial microorganisms that can be safely ingested to improve individual health. Numerous bacteria have been recognized as probiotics due to their positive health impacts. *Clostridium butyricum*, *Pediococcus*, and *Streptococcu*s genera are among examples of commercial probiotics [39–41].

Probiotics have many beneficial effects on immune response, inflammation, and metabolism [38]. Tian et al., (2019) [42] revealed the beneficial effect of *Bifidobacterium* (*B. longum subsp. infantis* E41 and *B. breve* M2CF22M7) in reducing depression and anxiety behaviors. *Lactobacillus rhamnosus* supplementation improved lung inflammation and mitigated gut dysbiosis in the asthma lung disease model [43]. In liver disease, several studies have shown beneficial effect of different stain of probiotic such as *B. breve* CKDB002, *B. longum* CKDB004, *B.adolescentis*, *P. pentosaceus* CGMCC 7049, and *Clostridium butyricum* [44–46].

Specific strain of probiotic might induce different beneficial effect. As an example, different strain of *Pediococcus pentosaceus* found beneficial as putative probiotic and antioxidant, while the others were able to improve obesity and fatty liver [41]. Han et al., (2023) [14] identified beneficial effect of 4 weeks treatment of *Pediococcus pentosaceus* Li05 in bile-duct ligation (BDL) Cholestasis model through inflammatory regulation, bile acids metabolism, tight junction integrity, and SCFA production. In addition, *Lactobacillus* and *Streptococcus* have widely used for yoghurt fermentation, which beneficial in enhancing metabolism and improving bacterial balance [39].

The most widely used genus as a probiotic, *Lactobacillu*s, has been found to be beneficial in cholestasis improvement. A two-days treatment of *L. rhamnosus LRX01* in Intrahepatic cholestasis of pregnancy (ICP) have improved interstinal immunity and regulate FXR expression [33]. Liu et al., (2020) [34] showed eleven days intervention of *L. rhamnosus GG* BDL cholestasis, which prevents liver fibrosis through bile acids regulation. In addition, 28 days administration of *L. plantarum* in BDL cholestasis have protect the liver damage with its antioxidant and anti-inflammatory effect [35]. Even though there is only one species administered, the probiotics might interact with other microbes and develop cross-feeding mechanism to shaping community composition, stability, and resilience [47].

Probiotic intervention may have an impact on the overall structure of the gut microbiome. In addition, many factors, including the composition and dosage of probiotic bacteria, patients' gut microbiota features, and disease severity, might affect intervention results. Probiotics can mitigate cholestasis liver disease by modulating the microbiome's composition, enhancing the gut barrier, reducing or preventing liver damage and inflammation, and regulating bile acid metabolism via gut-liver communication pathways. Table 3 summarizes the papers that have been included in this review.

3.4. Bile Acids Metabolism

Cholestasis leads to elevated amounts of bile acids in the liver, thus increasing bile acid concentration in the circulation. Conversely, the levels of bile acids in the intestine dropped [9]. Humbert et al. demonstrated a notable increase in bile acids, including taurocholic acid (TCA), glycocholic acid (GCA), tauro chenodeoxycholic acid (TCDCA), glycochenodeoxycholic acid (GCDCA), and tauro

deoxycholic acid (TDCA), in the blood serum of cholestasis patients. In contrast, the levels of UDCA, DCA, LCA, and hyodeoxycholic acid (HDCA) were reduced in these patients' blood serum [48].

Wang et al., (2019) [49] showed that cholestasis patients had lower concentration of primary bile acids, including allocholic acid (ACA), CA, CDCA, alpha muricholic acid (α-MCA), and beta muricholic acid (β-MCA), as well as secondary bile acids, including alpha and beta hyodeoxycholic acid (α- and β-HDCA), in their fecal samples. The cholestasis model showed lower levels of CA, CDCA, TCA, UDCA, LCA, TCDCA, α-MCA, and β-MCA, which were then recovered by supplementation of *Pediococcus pentosaceus* Li05. Interestingly, we observed elevated concentrations of taurine-α-muricholic acid (T-αMCA) and taurine-β-muricholic acid (T-βMCA) in 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)-induced cholestasis model [14]. Tang et al., (2023) [50] showed that T-αMCA and T-βMCA operate as endogenous antagonists of FXR. These compounds prevent the activation of FXR by other bile acids by binding to their exact location.

Multiple investigations have identified reduced FXR levels in animal cholestasis models. The FXR is a bile acid receptor crucial in regulating bile acid levels in the liver and intestine [12-13]. In cholestasis, the interaction of FXR with bile acids triggers the production of liver SHP, intestinal FGF15, and Fibroblast growth factor receptor 4 (FGFR4). These substances then suppress the expression of bile acids (BA) synthases like CYP7A1, CYP27A1, and CYP8B1 [14, 15, 31]. The addition of probiotics significantly enhanced the activation of bile acid receptors, including FXR, liver X receptor beta (LXR-β), rakeda G protein-coupled receptor 5 (TGR5), retinoid-related orphan receptor alpha (ROR-α) and retinoid-related orphan receptor gamma (ROR-γ) [11,33]. Furthermore, the administration of probiotics in the cholestasis model leads to decreased bile acid synthase enzymes, such as CYP7A1, CYP27A1, and CYP8B1 [11, 15, 31, 34].

After probiotic supplementation, another enhancement was observed in the expression of BA uptake protein and BA transporter. Probiotic supplementation was found to lower the expression of Na^+ dependent taurocholic acid transporter (NTCP) as BA uptake transporters. This condition helps maintain a low bile acid level in hepatocytes and prevents cholestatic liver injury [11]. Furthermore, there was a modest variation in BA transporters level between the liver and ileum.

Probiotic supplementation enhances bile transport in the liver by upregulating the expression of multidrug resistance protein 1 (MDR-1), bile salt export pump (BSEP), multidrug resistance protein (MDR-2), peroxisome proliferator-activated receptor alpha (PPAR-α), organic solute transporter subunits alpha (OST-α), sulfotransferase family 1A member 1 (SULT1A1), matrix metalloproteinase-2 (MRP-2), and matrix metalloproteinase-3 (MRP-3), which are involved in the bile acids transport [11,14,31]. In contrast, hepatocytes upregulate the export transporters MRP3, MRP4, OSTα, and organic solute transporter subunits beta (OSTβ) as a response to cholestasis. The expression of several BA transporters, including OSTα, OSTβ, and MRP2, was decreased after probiotic supplementation in the liver and intestine [14]. Along with Sodium–bile acid transporter (ASBT) elevation in the ileum, probiotic supplementation can enhance the elimination of toxic bile acids through feces. Sodium–bile acid transporter (ASBT) can co-transport sodium ions (Na+) with bile acids at the apical membrane. After intestinal epithelial cells absorb the BAs, their removal is facilitated by OSTα/β and MRP3 [51].

In conclusion, the absence of bile acids in the intestines has affected the gut microbiota. Over time, gut dysbiosis can cause inflammation in the intestines and liver. Probiotic intervention has been shown to help mitigate the negative effects of an inadequate supply of bile acids in the intestine. Furthermore, alterations in the gut microbiome following probiotic intake may be attributed to raised gut bacterial metabolism. Further research must investigate the metabolomic changes and their relationship to bile acids following probiotic treatment in patients with cholestasis liver disease.

3.5. Changes in Microbial Composition

Gut-liver interactions are closely associated with the gut microbiome [52]. Liver disease is followed by dysbiosis, which refers to microbial disturbance, alterations in bacterial metabolic activity, or changes in the distribution of bacteria within the intestine [53]. Dysbiosis can lead to the disruption of the intestinal epithelial barrier integrity, allowing intestinal bacteria and their byproducts to enter the liver through the portal veins. Bacterial toxins, particularly LPS, enter the liver through portal vein circulation and integrate to form the LPS-Cluster of Differentiation (CD)-14 complex, triggering the secretion of pro-inflammatory cytokines such as interleukin (IL)-1 and (IL)-6, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α [17].

Disruption in gut microbiome diversity was observed in both DDC-induced cholestasis and LCA-induced cholestasis. According to alpha diversity analysis, the cholestasis liver disease model exhibited diminished microbial diversity. However, its condition recovered following *Prevotella copri* treatment [11]. Interestingly, Kong et al., (2022) [31] discovered that Shannon and Simpson levels decreased in a snakehead fish cholestasis model and decreased following *Lactococcus lactis L19* supplementation. However, there was an improvement in the gut microbiota structure, and the intestinal microbiome produced in LCA group was reduced after *Lactococcus lactis L19* treatment.

The supplementation of *Lactococcus lactis L19* reduced the presence of *Chlamydiae* and increased the abundance of Firmicutes in snakehead fish with cholestasis liver disease [31]. Jiang et al., (2022) [11] demonstrated a reduction in the prevalence of Family Muribaculaceae, Genus *Lactobacillus, Bifidobacterium, Turicibacter, Clostridium sensu stricto 1, Odoribacter,* and *Alistipes* in the cholestasis group. However, they fully restored in the *Prevotella copri* treatment group [11]. The bacterial taxa Filum Firmicutes, Genus *Lactobacillus, Bifidobacterium, Clostridium, Alistipes,* and *Odoribacte*r have been identified as producers of short-chain fatty acids (SCFA) [54-55].

Han et al., (2023) [14] found that SCFA propionic and butyric acid levels increased in BDL-induced cholestasis rodents following treatment with *Pediococcus pentosaceus* Li05. The SCFA are the byproduct of the fermentation of indigestible carbohydrates in the intestine. Previous studies showed the roles of SCFA in lipid, cholesterol, and glucose metabolism [44]. They also help preserve the intestinal mucosal barrier integrity and suppress the generation of pro-inflammatory cytokines and immunological responses [56]. Several studies have demonstrated the beneficial impact of SCFA supplementation on different liver diseases. The improvement was observed in the regulation of lipid metabolism, the prevention of hepatic inflammation, the enhancement of mucosal barrier function, and the composition of intestinal flora [57-58].

Gut dysbiosis seems to mediate the progression from early to severe liver damage in cholestasis. Furthermore, probiotic supplementation could mitigate this issue. Interestingly, multiple studies have demonstrated that probiotics intervention improves SCFA levels. It is critical to determine the beneficial effects of SCFA in ameliorating cholestasis liver disease.

Table 3. Probiotics supplementation effect in various cholestasis liver disease

No	Disease Type	Study Design	Probiotics, dosage	Treatment duration	Significant Effects	Ref
1	Primary sclerosing cholangitis (PSC)	C57BL/J mice, 7 weeks old	*Prevotella copri*, 1×10^8 CFU /day	one week	• ALP, ALT, AST, TBI, and hepatic TBA ↓ • Collagen 1a1 and Timp-1↓ • Hydroxyproline↓ • CA and T-α-MCA↑ • FXR, LXRβ, TGR5, RORα, FGF15 and FGFR4 ↑ • Liver organic anion transporting polypeptide 4 (OATP4), BSEP, MRP2, MRP3, PPARα ↑ • Ileal ileal bile acid binding protein (IBABP), ASBT ↑, and MRP2↓ • CYP7A1↓ • microbiota diversity↑	Jiang et al. 2022 [11]
2	Cholestatic liver injury	*Cephalopholis argus* (Snakehead fish)	*Lactococcus lactis* L19, 1.0×10^8 CFU/g	8 weeks	• AST, ALT, ALP, TBA, TBIL, and DBIL ↓ • vacuolar degeneration↓ • 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), CYP7A1, CYP8B1, and CYP27A1 ↓ • FXR and SHP ↑ • BSEP, MRP2, MRP3, MRP4, OSTB, SULT1A1 ↑, and NTCP↓ Shannon and Simpson index↓	Kong et al. 2022 [31]
3	Intrahepatic cholestasis of pregnancy	Sprague Dawley rats, 8-week-old	*Roseburia intestinalis,* 2 x 10^9 colony forming units (CFU)	11 days	• ALT, TBA, and CDCA↑ • Hepatic lobular structure disorder, necrosis, and inflammatory infiltration ↓ • LPS ↓ • ZO-1, Occludin, and Claudin-1 ↑ • IL-1β, IL-6, IL-10, TNF-α, and TH17 ↓ • Treg ↑ • CYP7A1↓ • Ileum FXR, SHP, FGF15, and FGFR4 ↑ • Liver SHP, BSEP, FGFR4 ↑, and NTCP ↓	Sun et al. 2023 [15]

No	Disease Type	Study Design	Probiotics, dosage	Treatment duration	Significant Effects	Ref
4	Bile Duct Ligation (BDL) Cholestasis	C57/BL6 J mice, 8-week-old	*Pediococcus pentosaceus* Li05, 1 × 10^9 CFU (colony-forming units)/mL	28 days	● TB, DB, AST, ALT, ALP and TBA ↓ ● Bile duct proliferation, inflammatory infiltration, and hepatocellular necrosis ↓ ● Hepatic F4/80, IL-1β, IL-6, TNF-α↓ ● Serum IL-1β, IL-2, IL-6, MCP-1, INF-γ, G-CSF ↓, and IL-10 ↑ ● Hepatic Collagen1a1, Collagen3, α-SMA, TGF-β, CTGF, and TIMP1↓ ● CYP7A1↓ & CYP27A1 ↑ ● FXR, SHP, and FGF15 ↑ ● NTCP, BSEP, MDR2, MRP2 ↑ ● Ileal ASBT, OSTα, OSTβ, and MRP2 ↓ ● CA, CDCA, DCA, HCA, LCA, TCA, UDCA, α-MCA, β-MCA, and HDCA ↑ ● T-αMCA and T-βMCA↓ ● Propionic and butyric acids↑ ● ZO-1, occludin, claudin1, and MUC2 ↑ ● Serum LBP↓ ● Ileal TGR5 and LGR5 ↑	Han et al. 2023 [14]
5	Intrahepatic cholestasis of pregnancy (ICP)	C57BL/6 male mice, 8–10-week-old	*Lactobacillus rhamnosus* GG *(*LGG*)*, 10^9CFU/day	7 days	● ALT, TBIL, DBIL ↓ ● IL-1β↓ ● serum and hepatic BA ↓ ● BSEP and MRP3↑ ● Liver FXR, and SHP ↑	Ren et al. 2019 [32]
6	Intrahepatic cholestasis of pregnancy (ICP)	Sprague-Dawley rats, 9–12-week-old	*Lactobacillus rhamnosus* LRX01, 2 × 10^8CFU	2 days	● FXR ↓ in ileum ● Ileal RORγ-T, Foxp3, & NLRP3 ↓ ● IL-1β & TNF-α ↓ (ileal & serum) ● Epithelial degeneration↓	Lin et al. 2022 [33]

No	Disease Type	Study Design	Probiotics, dosage	Treatment duration	Significant Effects	Ref
7	Bile Duct Ligation (BDL) Cholestasis	Mdr2-/- mice and C57BL/6 J mice, 7- and 8-week-old	*Lactobacillus rhamnosus* GG, 10^9 CFU/day	11 days	• AST, ALP and DBIL ↓ • Hepatic necrosis, bile duct hyperplasia, portal edema, infiltrates & fibrosis portal tract ↓ • Collagen-I, α-SMA, TGF-β, and TIMP-1 ↓ • Hepatic and ileal F4/80, TNF-α, IL-6 and IL-1β ↓ • cytokeratin 19↓ • Hepatic bile acids↓ • T-αMCA and T-βMCA↓ • CYP7A1, Cyp27a1, Cyp7b1 ↓ • SHP and FGF15↑	Liu et al. 2020 [34]
8	Bile Duct Ligation (BDL) Cholestasis	Albino Wistar rats, 180–200 g	*Lactobacillus plantarum,* 1×10^{10} CFU/mL	28 days	• AST, ALT, ALP, LDH, TBIL, and DBIL ↓ • Malondialdehyde (MDA), Nitric oxide (NO), protein carbonyl, and total oxidant status (TOS) ↓ • GSH, total antioxidant capacity (TAC), superoxide dismutase (SOD), and Catalase (CAT) ↑ • ZO-1↑ • α-SMA↓ • TNF-α, IL-6 ↓, and IL-10↑ • Liver fibrosis, inflammation, necrosis, and infiltration ↓	Kabiri-Arani et al. 2024 [35]

3.6. Antioxidant Effect

Oxidative stress has recently been identified as a potent mediator of liver injury. Oxidative stress is defined as an imbalance between oxidant and antioxidant agents [59]. Reactive oxygen species (ROS) naturally synthesize from incomplete reduction of molecular oxygen by mitochondria during aerobic respiration [60]. Normally, ROS are necessary for signal transmission, defense against pathogenic microorganisms, and gene expression regulation in response to growth factors, hormones, cytokines, and extracellular ATP [59]. Nonetheless, high levels of ROS can be extremely hazardous and cause oxidative stress in all cellular macromolecules, including proteins, nucleic acids, and lipids. This circumstance may lead to protein dysfunction, DNA mutagenesis, and lipid peroxidation, resulting in cell death [59-60].

Liver is among the organs with the highest mitochondrial numbers [61]. Multiple factors, including environmental pollutants, alcohol, and drug overconsumption, can promote the production of ROS in the liver [62]. Furthermore, ROS may stimulate Kupffer cells to release cytokines, resulting in liver inflammation [63]. Kabiri-Arani et al., (2024) [35] found an elevation of Malondialdehyde (MDA), Nitric oxide (NO), protein carbonyl, and total oxidant status (TOS) in the BDL-cholestasis model. Those outcomes are consistent with Delli-Bovi et al. (2021) [63] remark that elevated ROS levels in the liver may increase lipid peroxidation and the creation of MAD, a highly reactive aldehyde product.

In response to oxidative stress, several compounds including superoxide dismutase (SOD), catalase, glutathione peroxidase, tocopherol, vitamin E, beta-carotene, ascorbate, and glutathione (GSH), have been identified as protective agent against ROS [59]. Recent studies have shown that probiotics may possess antioxidant properties. After four weeks of *L. plantarum* treatment in BDL-cholestasis rats, there was an increase in GSH, total antioxidant capacity (TAC), superoxide dismutase (SOD), and catalase (CAT) levels [35]. In addition, administering *L. plantarum* for ten days in BDL-cholestasis increased GSH levels, while oxidized glutathione (GSSG) levels decreased [64].

Recent studies also reviewed the correlation between gut microbiome and oxidative stress. The gut dysbiosis condition is commonly defined as an increase in pathogenic bacteria, potentially producing LPS, TMAO, and other toxins. Those toxins are possibly recognized by pattern recognition receptors (PRRs) and induce ROS production [63]. Taken together, probiotic treatment in cholestasis liver disease promotes a high possibility of oxidative stress mitigation through gut microbiome regulation.

3.7. Gut Barrier Permeability

Intestinal permeability has long been used as an indicator to classify healthy and diseased conditions. As connected by the gut-liver axis, the intestinal barrier has been identified as an essential area in developing liver disease, thus becoming one of the primary target therapies for liver disease. Tight junctions (TJ) in the intestinal mucosa are essential for preventing unwanted leakage, such as endogenous endotoxin, a substrate essential for the progression of liver disease [11, 65]. The intestinal barrier comprises the epithelial cells maintained by TJ proteins such as Zonula occludens-1 (ZO1), Claudin-1, Claudin-4, Occludin, and Mucin 2 (MUC2). Disruption of its structural integrity can result in the gut leaking, which enables harmful microorganisms and their endotoxins to move to the liver.

Zhang et al., (2010) [64] used Wistar rats to demonstrate that cholestasis induced by bile duct ligation led to significant reduction in the expression of TJ proteins claudin-1, ZO-1, and occludin, while *Lactobacillus plantarum* treatment significantly improved these conditions. Other studies also showed that TJ proteins were enhanced by the supplementation of various types of probiotics, such as *Pediococcus pentosaceus* Li05 and *Roseburia intestinalis* [14-15]. This review did not find evidence of increased intestinal permeability after probiotic supplementation. Furthermore, several studies have identified a correlation between SCFAs and intestinal TJ [57]. Therefore, further discussion regarding the effects of SCFAs on intestinal TJs in cholestasis may be warranted.

3.8. Liver Injury Markers

In response to the toxic bile accumulation in the liver, hepatocytes and cholangiocytes develop massive injuries. Liver disease often progresses from steatosis to steatohepatitis and fibrosis. Subsequently, it could progress to cirrhosis and, in certain instances, hepatocellular cancer [46]. Liver damage was detected using various clinical markers. The most frequently utilized parameters included bilirubin, transaminase enzyme, alkaline phosphatase, and bile acid level. Bilirubin was conjugated and excreted in the liver. Consequently, the damaged liver cell would inappropriately metabolize the bilirubin, thus entering the bloodstream facilitated by MRP3.

Transaminases, comprised of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are commonly employed as specific markers for detecting hepatocellular necrosis. Elevated levels of AST in the bloodstream are observed following significant tissue death, leading to an escalation in long-term liver damage [66]. The liver contains a significantly higher alkaline phosphatase concentration than other tissues such as the kidney, heart, and muscle. An increase in ALT levels typically suggests liver damage resulting from viral hepatitis, ischemia, toxin-induced liver damage, pancreatobiliary pathologies, idiophatic, and other causes [67]. Along with other parameters, bile acids produced in the liver are dietary surfactants, modulators of macronutrient metabolism, and

systemic pro-/anti-inflammatory balance. The abnormal bile acid synthesis correlates with several liver diseases [12].

Animal cholestasis models successfully established elevation in liver injury markers, including ALT, AST, ALP total bilirubin, direct bilirubin, and hepatic bile acids. Several studies showed that supplementation of different types of probiotics resulted in improvement of liver injury. Supplementation of *Lactobacillus plantarum* successfully decreased bilirubin, ALT, AST, ALP, and LDH levels in the Bile Duct Ligation-Induced Cholestasis model [35]. *Lactobacillus rhamnosus* GG also decreased ALT, AST, ALP, TBIL, and DBIL in Intrahepatic cholestasis of pregnancy and BDL-cholestasis [32, 34]. Similar results were obtained from supplementation of *Prevotella copri, Lactococcus lactis* L19*, Roseburia intestinalis,* and *Pediococcus pentosaceus* Li05 [11, 14-15, 31]. These findings indicate that the addition of probiotics to the treatment of cholestasis, regardless of the underlying causes, resulted in improved liver damage.

3.9. Hepatic Inflammatory Response

Hepatic damage in cholestasis is marked by substantial liver inflammation. Excessive accumulation of bile acids and translocated bacterial toxins to the liver might significantly increase inflammatory factor activation [14, 68]. This review identified 5 out of 8 studies that examine the alteration in liver inflammatory variables. The occurrence of cholestasis in rats effectively demonstrated an increase in several pro-inflammatory factors, such as, IL-1β, IL-2, IL-6, INF-γ, TNF-α, granulocyte-colony stimulating factor (GCSF), monocyte chemoattractant protein-1 (MCP-1) and F4/80 [14-15, 32, 34].

Nevertheless, Sun et al., (2023) [15] found the elevation of anti-inflammatory cytokines IL-10 in the intrahepatic cholestasis of pregnancy model. Conversely, Han et al., (2023) [14] and Kabiri-Arani et al., (2024) [35] conducted investigations that yielded contrasting findings. In the cholestasis model, the expression of IL-10 may be increased as a reaction to liver inflammation.

Following various administrations of probiotic supplements, this review demonstrated enhancements in markers of inflammation and levels of immune system cytokines. Sun et al., (2023) [15] showed a decreased level of pro-inflammatory, including IL-1β, IL-6, IL-10, TNF-α, and T helper 17 cells (TH17). In another study, Liu et al., (2020) [34] demonstrated that the supplementation of *Lactobacillus rhamnosus* GG resulted in a reduction in hepatic and ileal F4/80, TNF-α, IL-6, and IL-1β. The fermented-milk produced by LGG contains two distinct proteins, p40 and p75, which can inhibit inflammation, prevent cell apoptosis and tight-junction disruption in the intestinal epithelium [69-70]. This effect is achieved by activating the epidermal growth factor receptor (EGFR) [71].

In addition, Han et al., (2023) [14] and Kabiri-Arani et al., (2024) [35] investigations observed an increase in IL-10 levels following probiotic administration. Interleukin 10 is an anti-inflammatory cytokine that plays important role in arresting inflammation and autoimmunity. Increased concentrations of IL-10 may inhibit the immune response to microbial pathogenesis and delay the healing process of tissue injury and disruptions in blood flow [72]. Despite the knowledge of multiple pathways that support a potential key role of probiotics in liver inflammation, studies directly exploring the role of probiotics as potential mediators of the effects of microbiota-targeted interventions on cholestasis liver disease are sparse.

3.10. Hepatic Fibrosis Marker

Cholestasis liver disease is associated with a sequence of liver deterioration, beginning with steatosis and progressing to fibrosis, cirrhosis, and cancer [46]. Fibrosis occurs due to an aberrant healing process that arises from inflammation that is not managed correctly. Fibrotic tissue marked by excessive accumulation of fibrous connective tissue in the extracellular matrix (ECM) [73]. Producing inflammatory cytokines leads to prolonged damage to hepatocytes, triggering activation of myofibroblasts and subsequent formation of fibrous scars.

In the fibrotic liver, cholestasis can activate portal fibroblasts (PFs) as a substantial source of myofibroblasts. Portal fibroblasts (PFs) play a crucial function in preserving the structural integrity of the portal tract [74]. Bile duct obstruction leads to the proliferation of portal (myo)fibroblasts, which increase the expression of fibrogenic genes. Several most-known fibrogenic genes are α-smooth muscle actin (α-SMA) collagen type I, collagen type III, collagen type I alpha 1 chain (COL1A1), transforming growth factor beta 1/2 (TGF-β1/2), tissue inhibitor of metalloproteinase 1 (TIMP-1), and matrix metalloproteinase-2 (MMP-2). This process results in the formation of myofibroblasts [74-75].

Our review identified several studies demonstrating the increased expression of fibrogenic genes in the cholestasis model generated by bile duct ligation. Han et al. showed that cholestasis induced by bile duct ligation drastically raised the expression of fibroblastic genes, including Collagen1a1, Collagen3, α-SMA, TGF-β, TIMP1, and connective tissue growth factor (CTGF). However, the situation was significantly improved by the administration of *Pediococcus pentosaceus* Li05 [14]. Liu et al., (2020) [34] demonstrated that the administration of LGG significantly reduced liver fibrosis markers, including α-SMA, collagen I, TGF-β, and TIMP-1, in BDL mice. The results suggest that probiotic medication can potentially decrease liver fibrosis in cases with obstructive cholestasis.

4. Conclusion

This systematic review has discovered multiple academic sources examining the effects of probiotic treatments in Cholestasis Liver Disease. To summarize, this analysis has shown the extensive potential of probiotics as a treatment for cholestasis liver disease. Although it is premature to definitively state that probiotics supplementation has a significant impact on avoiding and improving cholestasis liver injury, it is apparent that probiotics administration is promising as a potential therapeutic strategy for supporting the treatment of cholestasis liver illness. According to the analysis, the administration of probiotics has a beneficial impact on various aspects such as the metabolism of bile acids, modulation of the microbiota-gut-liver axis, maintenance of intestinal barrier integrity, reduction of liver damage, improvement of inflammation, and delay the progression of liver fibrosis. While most studies have demonstrated a favorable outcome, there remain uncertainties regarding the most influential probiotics, precise mechanisms of action, and potential side effects associated with probiotic medication. Future research should investigate the bacterial strains that are most efficacious in improving cholestasis liver disease.

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Abbreviation

α-/β-HDCA, α-/β- hyodeoxycholic acid; α/β-MCA, α-/β-muricholic acids; α-SMA, alpha-smooth muscle actin; ACA, allocholic acid; ALD, alcoholic liver disease; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ASBT, sodium–bile acid transporter; AST, aspartate aminotransferase; BA, bile acid; BDL, bile duct ligation; BSEP, bile salt export pump; CA, cholic acid; CAT, catalase; CD14, Cluster of Differentiation 14; CDCA, chenodeoxycholic acid; CFU, colony-forming units; CK-19, cytokeratin 19; CTGF, connective tissue growth factor; COL1A1, collagen type i alpha 1 chain; CYP27A1, sterol 27-hydroxylase; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, 12-alpha-hydroxylase; DBIL, direct bilirubin; DCA, deoxycholic acid; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; EGFR, epidermal growth factor receptor; FGF15, fibroblast growth factor 15; FGFR4, fibroblast growth factor receptor 4; FOXP3, forkhead box P3; FXR, farnesoid X receptor;

GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GCSF, granulocyte-colony stimulating factor; GSH, glutathione; GSSH, oxidized glutathione; HDCA, hyodeoxycholic acid; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; IBABP, ileal bile acid binding protein; IFN-γ, interferon γ; IL-1/-2/-6/-10/1β, interleukin-1/-2/-6/-10/-1β; LBP, lipopolysaccharide-binding protein; LCA, lithocholic acid; LDH, lactate dehydrogenas; LGG, *Lactobacillus rhamnosus* GG; LGR5, leucine rich repeat containing G protein-coupled receptor 5; LXRβ, liver X receptor β; LPS, lipopolysaccharide; MCP1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MDR-1/-2, multidrug resistance protein-1/-2; MMP-2, matrix metalloproteinase-2/-3/-4; MRP-2/-3/-4, multidrug resistance–associated protein -2/-3/-4; MUC2, mucin 2; NAFLD, non-alcoholic fatty liver disease; NLRP3, NLR family pyrin domain containing 3; NO, nitric oxide; NTCP, Na + dependent taurocholic acid transporter; OATP4, organic anion transporting polypeptide 4; OST-α/-β, organic solute transporter subunits alpha/beta; PBC, primary biliary cholangitis; PF, portal fibroblasts; PICO, population intervention comparison outcomes; PNAC, parenteral nutrition-associated cholestasi; PPAR-α, peroxisome proliferator-activated receptor alpha; PRISMA, preferred reporting items for systematic reviews and meta-analyses; PSC, primary sclerosing cholangitis; RBC, red blood cells; RoB, risk of bias; ROR-α/-γ , retinoid-related orphan receptor -α/-γ; SCFA, short-chain fatty acids; SHP, small heterodimer partner; SOD, superoxide dismutase; SULT1A1, sulfotransferase family 1a member 1; T-α-/T-β-MCA, taurine-α-/β-MCAs; TAC, total antioxidant capacity; TBA, total bile acids; TBI, total bilirubin; TCA, taurocholic acid; TCDCA, tauro chenodeoxycholic acid; TDCA, tauro deoxycholic acid; TGF-β, transforming growth factor beta; TGR5, takeda G protein-coupled receptor 5; TH17, T helper 17 cells; TIMP-1, tissue inhibitor of metalloproteinase 1; TJ, tight junctions; TMAO, trimethylamine N-oxide; TNF-α, tumor necrosis factor alpha; TOS, total oxidant status; Treg, regulatory T-cells; UDCA, ursodeoxycholic acid; UDP, uridine diphosphate; ZO-1, zonula occludens-1.

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