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Probiotic Bile Salt Hydrolase: Current Developments and Perspectives

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Abstract Probiotic has modernized the current dietetic sense with novel therapeutic and nutritional benefits to the consumers. The presence of bile salt hydrolase (BSH) in probiotics renders them more tolerant to bile salts, which also helps to reduce the blood cholesterol level of the host. This review focuses on the occurrence of bile salt hydrolase among probiotics and its characterization, importance, applications, and genetics involved with recent updates. Research on bile salt hydrolase is still in its infancy. The current perspective reveals a huge market potential of probiotics with bile salt hydrolase. Intensive research in this field is desired to resolve some of the lacunae.

Keywords Probiotics · Bile acids · Bile salt hydrolase · Cholesterol

Introduction

Bile salt hydrolase (BSH) is an enzyme produced by several bacterial species in the human or animal gastrointestinal tract that catalyzes the glycine- or taurine-linked bile salt deconjugation reaction. Since bile acts as a biological detergent by which it shows antimicrobial property, microbes produce BSH against it to cope up with its toxicity. Microbial BSH activity contributes for its probiotic properties in the gastrointestinal tract; however, the precise mechanism is not evident.

Bile consists of cholesterol, bile acids (glycocholic and taurocholic acid), phospholipids, water, and pigment biliverdin [1–3]. It is produced as a yellow or green aqueous solution in pericentral hepatocytes from the liver of most vertebrates. Bile salts are composed of a hydrophilic side and a hydrophobic side. Hence, they tend to aggregate to form micelles; the hydrophobic sides combine towards the center and hydrophilic sides towards the

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outside. In the center of these micelles are triglycerides, which are separated from a larger globule of lipid. The solubility of the hydrophobic steroid nucleus is improved by conjugation with either glycine (glycoconjugated) or taurine (tauroconjugated) prior to synthesis. Consequently, the resulting molecules are amphipathic, which can solubilize the lipids to form mixed micelles.

The human liver can produce close to 1 l of bile every day, but comparatively small amounts are lost from the body. Hence, approximately 95% of the bile acids distributed to the duodenum are reabsorbed into venous blood within the ileum, and subsequently, through the sinusoids of the liver, they arrive at the portal vein. Hepatocytes remove the bile acids capably from sinusoidal blood, but small amounts run off into systemic circulation. Bile acids are afterward transported across the hepatocytes to be resecreted into canaliculi. As a whole, the enterohepatic recirculation makes each bile salt molecule available several times during a solitary digestive stage. During these processes, an important biotransformation that must take place before subsequent modifications is termed deconjugation [4], catalyzed by the BSH. The resulting molecules are termed as unconjugated or deconjugated bile acids. Thus, bile salt tolerance is a desired property for probiotic, which comes due to BSH activity. Ordinarily, the concentration of bile salts in bile is 0.8%. Bile also emulsifies and solubilizes the lipids prior to fat digestion.

Probiotic offers several nutritional and therapeutic benefits to consumers without any side effects, which has made them popular among consumers. It has become a popular ingredient, especially in milk products, viz., ice-cream, yogurt, cheese, etc., more specifically in developed countries where educated folk prefer probiotic-based diets. Today, health practitioners prescribe probiotic to patients very commonly to restore gut-flora after antibiotic episodes. Probiotics are dietary supplements and live microorganisms containing potentially beneficial bacteria or yeasts, which also refer to a kind of nutritional therapy based on eating probiotic foods and dietary supplements. The microorganisms referred are non-pathogenic bacteria and are considered “friendly germs” due to the benefits they offer to the gastrointestinal tract and immune system. Most studied probiotics are from the genera of *Lactobacillus* sp. [5–8] and *Bifidobacterium* sp. [9, 10]. Less commonly, strains of *Enterococcus* sp. [11] *Escherichia coli* [12], *Leuconostoc* sp. [13], *Pediococcus* sp. [14], *Saccharomyces* sp. [15], *Bacillus* sp. [16–19], *Sporolactobacillus*, *Brevibacillus*, and *Streptococcus* sp. [20], and other non-dairy probiotics [21] have also been used.

Probiotics bring out dietetic means to support the balance of intestinal flora. They may be used to counteract the local immunological dysfunctions [22], improve mineral absorption [23], reduce blood pressure [24], reduce serum cholesterol concentration [25], reduce allergy [26, 27], stimulate phagocytosis by peripheral blood leucocytes [28], modulate cytokine gene expression [29], have adjuvant effects or vaccinate [30], regress tumors [31], reduce carcinogen or co-carcinogen production, stabilize the gut mucosal barrier function, and prevent infectious succession of pathogenic microorganisms [32], and influence intestinal metabolism [33].

The probiotic business runs into millions of dollars spread over the USA, Canada, Japan, Europe, Asia-Pacific, Middle-East, and Latin America. There are as many as 121 leading companies worldwide, including BioGaia Biologics AB, Chr. Hansen A/S, Danisco A/S, Groupe Danone, Institut Rosell, Lifeway Foods, Natren, Nestlé Nutrition, Probi AB, Seven Seas Ireland, Stonyfield Farm, Valio, and Yakult Honsha. Multibionta from Yakult was the first clinically proved multivitamin probiotic. Howaru (from Danisco) launched for gut health, Calpis against tooth decay, and Kirin-Noale against blood pressure are some other commercial probiotic brands.

A variety of probiotics with BSH have been recognized and characterized. Bile salt tolerance has generally been considered more important during probiotic selection in *Bifidobacterium* than that of the other properties, such as gastric and pancreatic tolerance. It has also been observed that pancreatin supported *Bifidobacterium* possesses natural tolerance to survive in the gastrointestinal tract and resist against the antimicrobial property of bile acids [34]. Bile salt tolerance of some microorganisms is possibly due to the presence of BSH and some transporter proteins, which are functionally related to each other to respond efficiently to the stress from bile salts [35]. Microbial traits, which would be expected to be appropriate for survival in the human gut, must be proficient to pathogens for bile resistance [36]. Jones et al. [37] hypothesized that BSH facilitates colonization by mediating the resistance to the conjugated bile acids (CBA).

The aim of the present study was to review the recent advances on probiotic BSH enzymes, including the biological significance of its production and utilization, and briefly demonstrate the impact of bile hydrolysis on human physiology. Future prospects and possible applications of BSH research are also discussed.

Bile Salt Hydrolase (BSH)

BSH containing probiotic strains are preferred over BSH-negative strains as they help in cholesterol removal. BSHs belong to the choloylglycine hydrolase enzymes family, which also comprises penicillin amidases (EC-3.5.1.11). Both have been classified as an N-terminal nucleophilic (Ntn) hydrolase with an N-terminal cysteine residue. BSH (cholylglycine hydrolase; EC-3.5.1.24) catalyzes the hydrolysis of amide bond in conjugated bile salts (CBS); consequently, free amino acids are released, which form the deconjugated bile acid (mainly cholic and quenodeoxycholic). These primary bile acids may afterward undergo 7α-dehydroxylation and get converted into secondary bile acids (deoxycholic and lithocholic) [38]. The conjugated salts of cholic acid (for which no deconjugation is found) are more toxic than the conjugated salts of deoxycholic acid for which the deconjugation occurs.

BSHs can identify its substrate (bile acids) on amino acid groups (glycine/taurine) and also on cholate steroid nucleus. There have been a number of reports on cholate group identification by BSH. Several descriptions have supported its recognition by most BSHs at amino acid moieties and the hydrolysis of glycoconjugated bile salts that are more efficient than the tauroconjugated bile salts [39–41]. A *Lactobacillus buchneri* JCM1069 exhibited hydrolase activity against the taurodeoxycholic acid but not against the taurocholic acid, although both acids had taurine as their amino acid moiety but varied in their steroid moieties at 7α position [42]. Mc Auliffe et al. [43] turned off *bshA* gene of *Lactobacillus acidophilus* NCFM, which decreased the ability to hydrolyze the chenodeoxycholic containing bile salts, e.g., taurochenodeoxycholic acid (TCDCA) and glycochenodeoxycholic acid (GCDCA).

In order to survive well, the bacteria perform their metabolic activities to acquire the beneficial growth, and their genomes tend to possess diverse BSHs, which may confer additional advantages. BSHs are very specific for certain bile types, and their duration of contact to bile ensures the bacterial survival into varying bile environments. Studies carried out in *Lactobacillus plantarum* WCFS1, which had four *bsh* genes, and *L. acidophilus* NCFM, which had two *bsh* genes, supported this assumption [43, 44]. Bile addition may sometimes have inducing or inhibiting effects on BSHs as they are highly substrate-specific. Studies have shown that the expression of *bsh* 1 by the bile was induced ~sixfold,

while of *bsh* 3 was reduced ~fivefold. Two *bsh* genes of *L. acidophilus* NCFM were inactivated, which indicated that the encoded enzymes possessed different substrate specificities [44]. It is also known that different parts of bile stimulate different BSHs. BSH A activity was stimulated by the steroid nucleus of bile salts, while the activity of BSH B was induced by the amino acid side chain [43].

Studies have also been conducted on the structure of BSHs which exhibited slight differences. The crystal structure of BSH from *Bifidobacterium longum* revealed that it was a member of the N-terminal nucleophil hydrolase superfamily, possessing the characteristic $\alpha\beta\beta\alpha$ tetra-lamellar tertiary structure arrangement in it, and also demonstrated the evolutionary relationship with penicillin V acylase [45]. Other amino acids, viz. Asp-20, Tyr-82, Asn-175, and Arg-228 are believed to take part actively along with Cys in catalysis of bile salts. Rosocha et al. [46] made the structural comparison of BSH in *Clostridium perfringens* with other members of Ntn hydrolase family and found them very well conserved in the geometry of active sites [46]. BSH from *Bifidobacterium* showed the tetrameric structure with native molecular weight ranging between 125,000 to 150,000 and 35,024 of its subunit molecular weight. The SH group in the N-terminal cysteine was responsible for BSH activity of *Bifidobacterium bifidum* [40, 41]. This is the reason that BSH are vigorously inhibited by thiol enzyme inhibitors. The substitution of Cys with Ser or Thr results in an inactive enzyme, which emphasizes the importance of the presence of cysteine. BSH can hydrolyze all the six major human bile salts and at least two animal bile salts [41]. *Bifidobacterium* also embraces the diverse BSH types A, B, and C. The isoelectric point of BSH type B from *B. bifidum* ATCC 11863 was 4.45; other BSH (types A and C) had pI values approx. 4.65. N-terminal amino acid sequencing of types A and C BSH revealed that six out of 20 amino acid residues were unalike and highly conserved. All the BSH from five strains showed a better deconjugation rate on glycine-conjugated bile salts than that of taurine-conjugated forms [40]. Two different antigenic conjugated BSHs, viz. α and β had been expressed from *Lactobacillus johnsonii* strain 100–100 that combined to form native homo- and heterotrimers [47]. The alignment of BSH protein sequence of probiotic strains revealed that many probiotics acquired more than one BSH homolog [48].

BSHs described in several probiotic organisms have been produced at intracellular space, exhibited pH maxima in acidic range (5.0–6.0), and have been purified [40, 41, 49–51]. A thermophilic *Brevibacillus* sp. has been reported, producing BSH (56 kDa, homodimer), hydrolyzing all of the six major human bile salts. The enzyme preferred glycine-conjugated substrates with apparent K_m and k_{cat} values of 3.08 μM and 6.32×10^2 s^{-1}, respectively, for glycodeoxycholic acid [52]. A comparatively lower molecular weight BSH (37 kDa) was purified from *L. plantarum* CK102, which maximally hydrolyzed the glycocholate with apparent K_m and V_{max} values of 0.5 mM and 94 nmol/min/mg, respectively, at pH values between 5.8 and 6.3. This activity was greatly inhibited by iodoacetate and periodic acid [53].

The immobilization of BSH has also been tried out for the economic utilization in both ways, that is, the protein and the organism itself. BSH isolated from *L. buchneri* ATCC 4005 was immobilized in 0.5% gellan gum gel [54]. BSH, microencapsulated by food-grade whey protein-gum arabic, was compared for its efficacy with living *L. plantarum* WCFS1 in vitro that would produce BSH endogenously. BSH efficacy was better against pancreatin and low gastric pH initially, but its activity decreased further due to proteolytic degradation, whereas *L. plantarum* WCFS1 withstood such conditions [55]. For the first time, the BSH active strains, *Lactobacillus reuteri* and *B. longum*, were immobilized by micro-encapsulation [56].

Occurrence of BSH Among Probiotics

BSH activity has been found mostly in Gram-positive commensals (except a few *Bacteroides*), and they also acquire genome homolog, whereas it is lacking in Gram-negative commensals of the gastrointestinal tract. *Escherichia coli* and *Salmonella enterica* serovar *typhimurium* are reported as BSH-negative strains [57]. The prevalence of BSH is well recognized among the established probiotic genera. It is reported foremost in the majority of species from genera *Lactobacillus* [43, 47, 50, 58–63], *Bifidobacterium* [34, 35, 40, 61, 64], *Bacteroides* [51], and *Enterococcus* [65–67]. Tanaka et al. [60] screened more than 300 strains from *Bifidobacterium*, *Lactobacillus*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus* and found BSH activity in 273 strains in *Bifidobacterium* and *Lactobacillus* but missing in *L. lactis*, *L. mesenteroides*, and *S. thermophilus* [60].

Both *Lactobacillus* and *Bifidobacterium* strains are used regularly as safe probiotics. Other probiotic species are also commensal residents of the gastrointestinal tracts of vertebrates and exhibit positive effects in limited aspects, seeking their suitable niche in the world probiotic market.

Among the several isolates of acid- (pH 2.5 and 3.0) and bile- (0.3% oxgall) tolerant strains from *Streptococcus* (61.1%), *Lactobacillus* (71.8%), and *Bifidobacterium* (27.9%) genera, three strains, *Streptococcus* HJS-1, *Lactobacillus* HJL-37, and *Bifidobacterium* HJB-4, were BSH-positive probiotic strains [61]. Schillinger et al. [62] detected BSH activity in various strains of *L. acidophilus* and *L. johnsonii* but not in *Lactobacillus casei* strains group. These lactobacilli showed various degrees of adherence on HT29 MTX cells and on human collagen type IV, fibrinogen, and fibronectin as well [62]. However, *L. lactis* and *S. thermophilus* isolated from bile-salt-originated environments and from outside the gut did not exhibit such activity [42, 59, 68].

Lactobacilli were principal genera among the gut microbiota leading the maximum BSH activity in mice gut, which was proved by eliminating the lactobacilli from the total gut microbiota. It was an ideal bile salt whose hydrolase activity was reduced by 86% and was larger than 98% when both lactobacilli and enterococci were eliminated [69]. High levels of BSH activity have been reported in enterococci genus [66, 70]. Intracellular BSH production at higher temperature was reported in thermophilic *Brevibacillus* spp. [52, 71]. Table 1 shows several important BSH-positive strains from diverse bacterial genera.

Molecular and Expression Studies of BSH Genome

Microbial genetics have not been explored enormously in order to get the adequate information about BSH evolution in various genera. Various studies have reported diverse BSH homologs in *Lactobacillus*, *Bifidobacterium*, and other genera using molecular technique-based approaches. There is little information about its constitutive or inductive conservation or mode of gene transfer. Metagenomic approaches have been exploited to identify the functional BSH in all the major gut-associated bacterial divisions and archaeal species, which have shown that BSHs are enriched in the human gut microbiome. Phylogenetic studies have explained their selection pressure in the form of conjugated bile acid, which has driven the evolution of members of the Ntn_CGH-like protein family towards BSH activity [37].

There has been little information regarding BSH genetic evolution between various genera. Lambert et al. [72] predicted *L. plantarum* WCFS1 genetics, carrying four *bsh*

Table 1 List of BSH positive bacterial strains.

Bacterial strain	Reference
Bacteroides sp.	[51]
B. vulgatus	[51]
B. fragilis	[108]
Brevibacillus sp	[52, 71]
Bifidobacterium sp.	[34, 40]
Bifidobacterium longum	[41, 43, 45]
B. bifidum	[64]
B. animalis	[109]
Lactobacillus sp.	[63]
Lactobacillus acidophilus	[43, 110]
L. johnsonii	[47, 59]
L. plantarum	[72]
L. casei Shirota ACA-DC 6002	[63]
L. plantarum ACA-DC 146	[63]
L. paracasei subsp. *tolerans* ACA-DC 4037	[63]
Enterococcus faecium	[66, 67, 70]
Clostridium perfringens	[66, 103, 108]
Streptococcus macedonicus ACA-DC 198	[111]
Brucella abortus	[57, 112]
Listeria monocytogenes	[57, 112]

genes (*bsh1*, *bsh2*, *bsh3*, and *bsh4*), where only *bsh1* exhibited the majority of its BSH activity and the other genes appeared to be conserved. These conserved genes might be involved to encode penicillin V acylase rather than BSH activity and responsible for its physiology and mode of living [72]. Attainment of the *bsh* gene takes place by horizontal (lateral) gene transfer in lactobacilli [59]. A similar mechanism of gene transfer has been observed in *Listeria monocytogenes* [73]. Apart from the constitutive BSH production, plasmid-mediated activity of BSH in *L. acidophilus*, *L. plantarum*, *Lactobacillus brevis*, and *Lactobacillus fermentum* has also been observed [74]. However, there is no clear information of gene transfer in *Bifidobaterium*.

The nucleotide sequence of the BSH gene of *L. acidophilus* PF01 was analyzed, which revealed its location and showed that it was surrounded by 951 nucleotides in a single open reading frame (ORF) and encoding a 316 amino acid protein. BSH promoter was located upstream of the start codon. The expressed protein exhibited high homology with BSHs from other source organisms. Four amino acid motifs FGRNXD, AGLNF, VLTNXP, and GXGXGXXGXPGD, located around the active site, were highly conserved. The BSH gene was cloned into the pET21b expression vector and expressed in *E. coli* BLR (DE3). The purified recombinant BSH enzyme exhibited hydrolase activity against tauroconjugated bile salts, but not against the glycoconjugated bile salts [39]. Two genes, bshA and bshB, have been identified encoding BSH in the genome sequence of *L. acidophilus* NCFM, where different substrate specificities of two enzymes were observed [43].

Vector-free engineering on *L. plantarum* by chromosomal integration of an exogenous gene without inactivation of physiological traits has been reported [75]. *Lactobacillus plantarum* cbh gene, encoding the conjugated BSH, was cloned and successfully expressed in

the heterologous host *L. casei* LK1 with the aid of pSMA23-derived vectors [76]. BSH genes (bsh) of *B. longum* was cloned and expressed in *E. coli*. Expressed BSH protein was employed for further biochemical studies under thermal, chemical, and pH-mediated denaturation conditions prior to get the information of active site, mechanism of bile hydrolysis, and BSH properties [77].

Scope of Probiotic BSH on Host Health

Cholesterol Lowering Property

Today, hypercholesterolemia is a major challenge for human health worldwide and a central cause of coronary heart diseases. It is treated with pharmacological drugs, which are very expensive and cause a number of redundant side effects. Probiotic-based oral therapy has proved remarkable efficacy to reduce the blood cholesterol level up to 33% [58, 77–79], where BSH activity is significantly liable for the benefit. Human feces isolate *L. plantarum* CK 102 has been found to reduce the blood cholesterol level, triglyceride, LDL-cholesterol, and free-cholesterol in rats [80]. Cholesterol lowering activity of BSH by probiotic organisms has been widely discussed [54, 81]. Cell free supernatant from *L. acidophilus* ATCC 43121 exhibited cholesterol removing activity, which was different from earlier reports, suggesting this mechanism from live cells. The molecular mass of a protein with cholesterol-removing activity was estimated at 12 kDa [82]. Infant feces isolate *L. plantarum* PH04 was able to reduce the serum cholesterol and triglycerides to levels of 7% and 10%, respectively, when it was fed to hyper-cholesterolemic mice at numbers of 10^7 CFU per mouse per day for 14 days [83]. *Lactobacillus acidophilus* strains isolated from Maasai traditional fermented milk "Kule naoto" showed BSH activity and exhibited the ability to assimilate the cholesterol in vitro [84]. Sridevi et al. [54] fed Wistar rats with the immobilized *Lactobacillus* BSH, which induced hypercholesteremia by triton X-100. The serum cholesterol and triglycerides were reduced by 50% and 15%, respectively, in the group fed with immobilized enzyme at 10 IU/kg dose, whereas administration of 20 IU/kg immobilized enzyme resulted in the reduction of serum cholesterol and triglycerides by 58% and 45%, respectively [54].

Nutritional Relevance and Substrate Specificity

Bile salt deconjugation process generates amino acids, which serve as a carbon, nitrogen, and energy sources. Glycine is hydrolyzed into ammonia and carbon dioxide, whereas taurine is hydrolyzed into ammonia, carbon dioxide, and sulfate. Bile salt deconjugations take place by BSH-positive strains that confer a nutritional advantage. Deconjugation of taurocholate (TCA) and glycocholate (GCA) has been reported in *L. acidophilus* SNUL020 and SNUL01 at similar rates, whereas *L. acidophilus* FM01 deconjugated the GCA more rapidly than TCA [68]. Van [85] also supported this hypothesis; he found that certain BSH-positive *clostridium* strains utilized the liberated taurine as an electron acceptor and exhibited improved growth in the presence of taurine or taurine-conjugated bile salts in the medium.

Modification of Membrane Integrity

Some of the friendly bacteria are important for host gut health and their hydrolytic enzymes, viz. lysozyme, phospholipase A2, α-defensins, etc., are key bio-molecules that

play a crucial role in intestinal defense. Survival of these microbes in the gut conditions is pretty challenging where compositional integrity, permeability, fluidity, hydrophobicity, and net charge of membranes establish the degree of strength against the host defenses [86]. Studies have revealed the involvement of BSHs for carrying out bile or cholesterol integration into bacterial cell membranes, which then augment further potency of the membranes by BSH-mediated lipid intermolecular hydrogen bonding [87–89], or could amend its fluidity or charge. These modifications take place due to BSH activity that recommends safety against the perturbation of the configuration and integrity of bacterial membranes by the immune system that ensures to set up the prolonged persistence. This mechanism confers BSH-positive bacteria to dominate over the BSH-negative pathogens or other transients.

Bile Detoxification/Deconjugation

Both bile salt hydrolysis and bile tolerance envelop separate ambiguity in bile science. Bile acids are potentially toxic end products of cholesterol metabolism. Several studies undertaken using the wild-type and *bsh* mutant combinations recognize a connection between the bile salt hydrolysis and bile tolerance in probiotics organisms. Mutant isolate *Lactobacillus amylovorus* (modified with *N*-methyl-*N* 1-nitro-*N*-nitrosoguanidine mutagenesis), in which BSH activity was partly reduced, exhibited reduced growth rates in the presence of bile salts [50]. Mutation of *bsh* in *L. monocytogenes* and [73] *L. plantarum* [58] made their cells more susceptible to bile salts. There are reports comparing the susceptibility of few bifidobacterial strains with conjugated primary (glycocholate and taurocholate) and secondary (glycodeoxycholate and taurodeoxycholate) salts. The level of the hydrolase activity with acquired bile resistance was found, and it was found that none of them displayed deconjugation against the primary salts, while most of them deconjugated the secondary salts [90]. The accurate BSH mechanism by which the bacteria exhibit the tolerance against bile is not yet known. However, it was proposed that the protonated form of bile salts might exhibit toxicity through intracellular interface, and BSH-positive cells might protect themselves by the formation of the weaker unconjugated counterparts [58]. This mechanism could facilitate to wipe out the acidification by bringing back and exporting the co-transported proton [58].

Microcapsulation of probiotic cells has also been attempted to achieve better BSH deconjugation rates. *Lactobacillus reuteri* microcapsules, which metabolize glyco- and tauroconjugated bile salts at rates of 10.16±0.46 and 1.85±0.33 μmol/g microcapsule per hour, respectively, showed better acid resistance, whereas *B. longum* showed low BSH activity and acid resistance. *Lactobacillus reuteri* microcapsules also exhibited an improved deconjugation with 49.4±6.21% of glycoconjugates per hour in a simulated human gastrointestinal (GI) model and complete deconjugation after 4 h [56]. Bile salt deconjugation rates of microencapsulated BSH overproducing cells of *L. plantarum* 80 were 4.87±0.28 μmol/g microcapsule per hour towards glycoconjugates and 0.79± 0.15 μmol/g microcapsule per hour towards tauroconjugates in the simulated gastro-intestine [91].

Improvement in GIT Condition

Intestinal bacteria have been reported to be involved in the production of secondary bile acids (via successive unconjugated bile salts modifications), which were accumulated in the entero-hepatic circulation of some individuals and caused gallstones, gastrointestinal tract

(GIT) dysfunctions, colon cancer, and other GIT diseases. Kurdi et al. [92] studied the cholic acid transportation and accumulation in *B. bifidum* and showed that cholic acid (liberated bile acid from BSH) accumulated inside the bacterial cell in the host intestine. The entrapment of these free bile acids by bifidobacteria resulted in the decreased assembly of secondary bile acids, which were usually considered as cytotoxic and precarcinogenic [92]. The responsible enzyme (7α-dehydroxylase) was found in *Clostridium* and *Eubacterium* species [93, 94] but not in lactic acid bacteria or bifidobacteria [92]. The majority of the intestinal anaerobic bacteria were involved in bile salt hydrolysis and hydroxyl group dehydrogenation reactions.

Reduction in Lipid Digestion

Conjugation of bile salts is a significant step prior to lipid assimilation in mammals. Bile acid deconjugation in mammalian intestine was eventually found to facilitate the intestinal bacteria [95], which is an undesired process for lipid digestion. Microbial deconjugation and dehydroxylation of conjugated bile salts by BSH-positive bacteria upset the digestion process, which results in related complications and abnormal growth. A product of microbial bile acid biotransformation in host intestine was found to be the main cause of growth reduction in chicks [96]. Microbial deconjugation and dehydroxylation of bile acids also mess up lipid assimilation in the host [97, 98]; moreover, it produces toxic products to facilitate the impaired growth [99]. Further studies anticipated the action of an antibiotic that plays a crucial role in the inhibition of microbial bile acid biotransformation in GIT and also proposed an opposite correlation between the level of cholyltaurine hydrolysis and growth rate of antibiotics fed broiler chickens in their small intestine [100, 101]. This growth reduction was reversible with antibiotic supplementation [102].

Future Prospects

Bile acid biotransformation studies have insights via crystallization, site-directed mutagenesis, and protein secondary structure analysis. Molecular cloning of genes encoding bile salt-biotransforming enzymes has assisted getting a closer view of the genetic association of related pathways and aided developing the probes for the detection of bile salt-modifying bacteria. The potential exists for altering the bile acid pool by targeting the key enzymes in the 7α/β-dehydroxylation pathway through the development of pharmaceuticals or sequestering the bile acids biologically in probiotic bacteria, which may result in their effective removal from the host after excretion [103].

Upcoming research on BSH enzymes must contemplate on determining their specific role in gastrointestinal flora, and beneficial or detrimental effects on the host as well. BSH research must spotlight a cooperative account between the BSH mutants and BSH-positive strains in the perspective of its significance for the host animal. Investigations must focus on determining the morphological (membrane integrity of bile and cholesterol or in terms of fatty acids composition) and histological changes in the organism mounting in the presence or absence of bile salts. Moreover, the ultimate providence of the final product synthesized via bile salt hydrolysis should be examined. Animal trials would provide better insights to uncover its role in gastrointestinal endurance. Most importantly, secondary bile acid levels in serum and biliary must be examined during the hydrolysis in the bile acid pool as it is considered as cytotoxic and precarcinogenic for colon cancer. It also leads to DNA damage, colonic mucosal dysfunction, and, ultimately, diarrhea [104, 105]. Gallstone patients have

been observed with elevated secondary bile salt concentrations [106, 107]. Molecular tools, such as reverse transcriptase-polymerase chain reaction (PCR), should be used to quantify BSH homologs and trace heterologous or homologous BSH incidence. Blotting techniques, microarray, metagenomics, denaturing gradient gel electrophoresis, and fluorescence in situ hybridization can be used to monitor the BSH-positive strains and their synergistic or individual impact on host health and established gut micro flora after the administration.

BSH research should lead to extensive isolation of BSH-positive microorganisms and their characterization and construct an approach to improve these strains prior to their prolonged persistence in GIT for better performances. Screening of *bsh* gene can be performed using PCR with degenerative primers, and thereafter, determining their potency using well-known simplified assay methods [74]. This could have a major perspective, especially by appealing to the budding health-conscious civilization as the intake of a probiotic-based diet might be more "promising" than the existing cholesterol-removing therapies.

Conclusion

The importance of BSH seems apparent to confer the additional benefits from novel probiotic organisms to the host when it is administered in highly adverse conditions of GIT. BSH research still in its infancy and future investigations may uncover its significance more clearly. BSH research may eventually lead to inspecting more vigorous probiotics with better health performance.

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