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Review

Isolation sources of bile salt hydrolase-microorganisms

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Bile salt hydrolase enzyme (BSH) is considered to be especially relevant for microbes that reside in the mammalian gastro intestinal tract. Great attention has been focused on these enzymes because of their potential to greatly influence host physiology. On the other hand, the majority of the current reviews about bile salt hydrolase (BSH) are mainly focused on either microorganisms isolated from human sources or probiotics that show this activity. However, bile salt hydrolase activity appears not to be limited to bacteria from the gastrointestinal system, since other microorganisms isolated from vegetable sources exhibit this activity as well. The present study aims to compile information on the isolation sources of microorganisms with BSH activity.

Keywords: bile salt hydrolase, bile salts, *Lactobacillus*.

INTRODUCTION

Bile acids constitute approximately 50% of the organic components of bile (Begley *et al.* 2005). In normal animals nearly all bile acids present in feces are in their free forms. This disagrees with the findings in germfree animals, where all bile acids present in feces are found as conjugates. The intestinal hydrolysis of bile salts is exclusively brought about by the enzymatic equipment action of many intestinal microbes (Tannock *et al*. 1989; Ridlon *et al*. 2006). Bile salt hydrolase (BSH) is the enzyme responsible for bile acids deconjugation in the human intestine. It acts upon a wide range of bile acid conjugates, including 6 major human conjugated bile acids (taurocholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid and glycochenodeoxycholic acid) (McAuliffe *et al*. 2005; Guo *et al*. 2011). BSH activity has been detected only in bacteria (Guo *et al*. 2011), especially in several genera of bacteria indigenous to the intestinal tract such as *Bacteroides* spp*., Enterococcus* spp*., Bifidobacterium* spp. and *Lactobacillus* spp. (Gopal-Srivastava and Hylemon 1988; Dashkevicz and Feighner 1989; Walker and Gilliland 1993; Tanaka *et al*. 1999; Franz *et al.* 2001; Dean et al. 2002; Begley *et al*. 2005; Kumar *et al*. 2007; Lambert *et al*. (a, b)2008; Sridevi *et al*. (a, b)2009).

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Often BSH activity is found in strains isolated from the intestines or from feces from mammals, which are a natural environment rich in conjugated and unconjugated bile acids (Tanaka *et al*. 1999; Kim and Lee 2005; Yildiz *et al*. 2011). However, recently BSH activity has been found in microorganisms isolated from kefir grains, pickles, hot water springs and fermented milk (Vizoso *et al.* 2006; Sridevi *et al.* 2009; Dong *et al.* 2012; Huang *et al.* 2013). Indeed, a strong correlation has been found between the habitat of a specific bacterial species or strain and BSH activity. Bile salt hydrolase activity is important in some microorganisms because certain strains of the *Lactobacillus* genus that possess BSH activity also have the capacity to decrease serum cholesterol levels in in *vivo* studies (Nguyen *et al*. 2007). The aim of this study was to compile information on microorganisms with bile salt hydrolase activity.

Bile salt hydrolase

Bile salt hydrolase (BSH) is the enzyme responsible for bile acids deconjugation during enterohepatic circulation in healthy humans. It acts upon a wide range of bile acid conjugates, including 6 major human conjugated bile acids [taurocholic acid (TCA), taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid (TCDCA), glycocholic acid (GCA), glycodeoxycholic acid (GDCA), and glycochenodeoxycholic acid (GCDCA) releasing

taurine and glycine and free bile salts (McAuliffe *et al.* 2005; Guo *et al.* 2011). Deconjugated primary bile salts are less water-soluble and are more easily excreted via the feces (Kim and Lee 2005). The amino acids can be used by several bacteria as an energy source (Cook and Denger 2002; Ridlon *et al.* 2006).

BSH activity has been widely detected in several bacterial genera of the autochthonous gastrointestinal microbiota of animals including mice, rats, humans, chickens, and swine (Kim and Lee 2005). This activity has been detected especially in several genera of bacteria indigenous to the intestinal tract such as *Bacteroides* spp., *Enterococcus* spp., *Bifidobacterium* spp. and *Lactobacillus* spp. (Gopal-Srivastava and Hylemon 1988; Dashkevicz and Feighner 1989; Walker and Gilliland 1993; Tanaka *et al.* 1999; Franz *et al.* 2001; Dean *et al.* 2002*;* Begley *et al.* 2005; Kumar *et al.* 2007; Lambert *et al.* (a, b)2008; Sridevi *et al.* (a, b)2009).

The largest study was conducted on the distribution and extent of BSH activity in lactic acid bacteria, involving more than 300 lactic acid bacteria strains from genera *Bifidobacterium* and *Lactobacillus*, and species *Lactococcus lactis*, *Leuconostocmesenteroides* and *Streptococcus thermophilus* (Tanaka *et al.* 1999). Lactobacilli are considered to play a major role in bile salts hydrolysis *in vivo* (Tannock *et al.* 1989 and 1994; De Smet *et al.* 1995; Elkins *et al.* 2001; Pereira *et al.* 2003; Lambert *et al.*(a)2008), e.g. lactobacilli contribute 74 and 86% of total bile acid hydrolase activity in the cecum and ileum, respectively, of mice (Tannock *et al.* 1989).

Since the free bile acids (cholic acid and deoxycholic acid) appear to be more inhibitory than the conjugated bile salts themselves, it is most likely not a detoxification mechanism for these organisms, but rather anantagonist action to enhance autochthonous microorganisms of the intestinal microbiota such as lactobacilli against pathogens in the intestines by the production of cholic and deoxycholic acid (Van der Meer *et al.* 1991; Kim and Lee 2005). It has been proposed that BSH activity of lactobacilli and enteric bacteria might be an important colonization factor in the gastrointestinal tract (Elkins *et al.* 2001; Kumar *et al.* 2012). Recently it has been shown that these enzymes play a major role in cholesterol metabolism, thereby influencing the serum cholesterol levels (Lee *et al.* 2009; Sirilun *et al.* 2010; Kumar *et al.* 2012). It has also been suggested that BSH activity should be a requirement in the selection of probiotic organisms with cholesterol-lowering properties, since microorganisms that do not deconjugate bile salts do not appear to be able to remove cholesterol from the culture medium to any significant extent (Liong and Shah 2005; Kumar *et al.* 2012).

Bile salt hydrolase function

The capacity of bacteria to express BSH contributes to their functions in the human gastrointestinal tract (Tannock *et al.* 1989; Savage 1992; De Smet *et al.* 1995), since bile salt deconjugation is a gate keeping reaction in further oxidation and dehydroxylation steps of primary bile salts (as synthesized by the host) into secondary bile salts by intestinal bacteria (Lambert *et al.* (b)2008).

Other authors have suggested some hypotheses on this matter, for example, bacteria that are able to deconjugate bile salts may be able to use the amino acid released form hydrolysis as carbon, nitrogen, and energy sources, since glycine may be metabolized to ammonia, and carbon dioxide and taurine may be metabolized to ammonia, carbon dioxide, and sulfate (Gopal-Srivastava and Hylemon 1988). Besides, BSH decrease the toxicity of conjugated bile acids for bacteria. Compared with their conjugated counterparts, deconjugated bile acids have decreased solubility and diminished detergent activity and thus may be less toxic to bacteria in the intestine. It has also been suggested that BSH are detergent shock proteins that protect their producing bacteria from the toxicity of bile acids in the gastrointestinal tract (Savage 1992; De Smet *et al.* 1995; Mosser and Savage 2001). Regarding cholesterol, it has been proposed that BSH facilitate the incorporation of cholesterol or bile into bacterial membranes. This may increase the tensile strength of the membranes or may change their fluidity or charge. Cell surface modifications that may result from BSH activity could potentially offer protection against perturbation of the structure and integrity of bacterial membranes by the immune system, and such resistance mechanisms may be important in establishing persistent infections (Kumar *et al.* 2012).

Bile salt deconjugation includes the production of secondary bile salts, which have been linked to various intestinal diseases, such as the formation of gallstones and colon cancer (Ridlon *et al.* 2006). Moreover, results of *in vitro* studies have suggested that bile salt deconjugation plays a role in mucin production and excretion in the intestinal lumen (Klinkspoor *et al.* 1999), and this could affect the nutritional environment encountered by the intestinal microbiota, as well as the intestinal transit time (Shimotoyodome *et al.* 2000).

BSH activity over different bile salts depends on several factors, such as pH and pKa values. The taurine conjugates exhibit an apparent pKa of 1.9 in aqueous solution, whereas the pKa of the unconjugated species is approximately 5.0. Glycine-conjugated bile acids exhibit pKa values of approximately 3.9 and could be partially precipitated without hydrolysis at fermentative pH values. Thus, at pH values achievable by acidic fermentative metabolism, unconjugated bile acids would be protonated and precipitate, while taurine conjugates would remain completely ionized and remain in solution (Dashkevicz

and Feighner 1989; Christiaens *et al.* 1992; De smet *et al.* (1995). This information is important when determining BSH activity.

Sources of isolation of microorganisms with bile salt hydrolase activity

BSH activity is often found in strains isolated from the intestines or feces from mammals, which are a natural environment rich in conjugated and unconjugated bile acids (Tanaka *et al.* 1999; Kim and Lee 2005; Yildiz *et al.* 2011). Strains and species of lactobacilli from other habitats like milk or vegetables—environments from which bile salts are absent—do normally not have BSH activity (Begley et al. 2006). However, as mentioned above SBH activity has been found in microorganisms isolated from kefir grains, pickles, hot water springs and fermented milk (Table 1).

Many of bacteria with bile salt hydrolase activity used in probiotic preparations have been isolated primarily from human and animal sources, to maximize the likelihood of compatibility with their gut microbiota and to ensure their survival in the gastrointestinal tract (Tannock *et al.* 1989 and 1994; De Smet *et al.* 1995; Elkins *et al.* 2001; Pereira *et al.* 2003; Lambert *et al.* (a) 2008). *Bifidobacterium and Lactobacillus* are normal inhabitants of the human and animal gastrointestinal tract and is not surprising to find them in mouth and feces. For example, in recent studies, twelve *Lactobacillus* strains (*L. reuteri, L. brevis, L. salivarius, L. gallinarum* and *L. panis* species) isolated from the gastrointestinal tract of chickens and three strains (*L. fermentum, Streptococcus bovis* ATCC 43143 and *Enterococcus faecalis* UK873) isolated from human feces were able to deconjugate sodium glycocholate and sodium taurocholate with affinity on sodium glycocholate (Pereira *et al.* 2003; Ramasamy *et al.* 2009). The ability of these strains to deconjugate bile salts is not surprising, as the strains were isolated from the gastrointestinal tract and feces, which are natural environments rich in conjugated and unconjugated bile acids (Pereira *et al.* 2003; Ramasamy *et al.* 2009). Bile from human and animal generally contains glycine conjugates in a higher proportion than taurine conjugates, and this may contribute to the higher affinity for glycine conjugates shown by these strains. In addition, their higher deconjugating activity on glyco-conjugates may be of considerable significance for their survival in the gut (Kim *et al.* 2004).

Studies report that some bacteria isolated from dairy sourcesn have the capacity to deconjugate bile salts. Dairy products play a predominant role as matrix of these microorganisms due to their nutritional composition (87.4% water, 4.7% lactose, 3.8% fat, 3.3% protein, 0.2% citrate and 0.6% minerals), which is important for microorganism metabolism. Proteolytic and lipolytic properties of microorganisms may be important for further degradation of other substrates such as bile acids (Heller 2001).

Many years ago it was believed that the intestinal tract was sterile before the individual was born, aquiring the initial microbiota of the intestinal tract from the vagina and feces of their mothers (Mandar and Mikelsaar, 1996). However, recent investigations indicate that microbial colonization may begin earlier as bacteria have been detected in the meconium, umbilical cord, and amniotic fluid (Collado *et al.* 2012; Satokari *et al.* 2008). For example, in a study, bacteria of genuses *Enterococcus* and *Staphylococcus* were isolated from umbilical cord blood of healthy neonates and from murine amniotic fluid obtained by caesarean sections (Jiménez *et al.* 2008). In addition, bifibido bacterial DNA was detected in 33 and *Lactobacillus rhamnosus* DNA in 31 human placenta samples. Therefore, elevated translocation of bacteria or their components in the mother may certainly have bearing on her immune status and may explain the physiologic activation of innate immunity that occurs during pregnancy (Satokari *et al.* 2009).

On the other hand, the intestine is a selection system that whittles down the microbial diversity reaching the gut, which comes from the environment and different kinds of foods (Ley *et al.* 2006). Adult's microbiota is dominated by members of just two divisions of bacteria, the *Bacteroidetes* and *Firmicutes* (Eckburg *et al.* 2005). *Firmicutes* include lactic acid bacteria (LAB), a group that comprises species such as lactobacilli, enterococci, pediococci, lactococci among others. Generally, LAB species are found living in a wide variety of environments such as the mammalian intestines, vagina, and mouth; in milk products; fermented sausages; sewage and fermented vegetable material. Strains of species belonging to *Lactobacillus* (*L. plantarum*) and *Leuconostoc* (*L. mesenteroides*) are the most common bacteria in natural vegetable lactic acid fermentation (Cleveland et al., 2001; Yan et al., 2008), but *L. casei, L. delbrueckii* and *L. brevis* have been reported as well (Czy_zowska et al., 2006). In naturally fermented olives, five strains of *L. plantarum* and 7 of *L. pentosus* were isolated, which exhibited partial bile salt hydrolase activity (Argyri *et al.* 2013).

Therefore, microbiota inherent in the human intestinal tract capable of hydrolyzing conjugated bile salts could be acquired from different types of food, including vegetables. Due to the stress conditions in the gut, these microorganisms have had to express the genes encoding for the bile salt hydrolase enzyme (Elkins *et al.* 2001) which enable them to counteract the antimicrobial activity of bile salts. This could explain why microorganisms found in vegetable sources are capable to deconjugating bile salts.

CONCLUSIONS

Recent research shows that microorganisms capable of dissociating conjugated bile salts are found not only in

Table 1. Source of isolation of microorganisms with bile salt hydrolase.

Microorganism	Source	Special characteristic	Reference
Lactobacillus plantarum Lp09 and Lp45	Kefir grains	Tolerance to low pH levels and high bile salt concentrations. Showed potential bile salt hydrolase activity, bile salt deconjugation activity, and cholesterol coprecipitation ability.	Huang *et al.* 2013
Lactobacillus acidophilus LA15, *Lactobacillus plantarum* B23 and *Lactobacillus kefiri* D17	Tibetan kefir grains	Showed potential bile salt hydrolase (BSH) activity, cholesterol assimilation and cholesterol co-precipitation ability	Zheng et al. 2013
Lactobacillus Johnsoni PF01 (previously *L. acidophilus*)	Piglet feces	High bile resistance *in vitro* through bile salt hydrolase (BSH) activity against tauroconjugated bile salts.	Lee *et al.* 2011; Chae *et al.* 2013
5 *Lb. plantarum* and 7 *Lb. pentosus* strains	Fermented olives	Exhibited partial bile salt hydrolase activity.	Argyri et al. 2013
Lactobacillus plantarum BBE7	*Pickles*	High BSH activity, a higher cholesterol-removing activity (about 72.8 %) from cholesterol (100 µg/mL)-containing MRS.	Dong *et al.* 2012
Bacillus sp., namely, *B. licheniformis* Me1, *B. subtilis* Bn1	Milk and fermented beans	Showed bile salt hydrolase activity	Nithya and Halami 2013
Lactobacillus plantarum ST-III	Chinese pickles	Four bile salt hydrolases, high hydrolysis activity for glycodeoxycholic acid.	Ren *et al.* 2011
Brucellaabortus 2308	----------	Choloylglycinehydrolase gene	Delpino *et al.* 2007; Marchesini *et al.* 2011
Lactobacillus plantarum Lp91	Indian gut population	Exhibit tolerance to low pH and high bile salt concentrations. Show potential BSH activity, cholesterol assimilation and cholesterol co-precipitation ability.	Kumar *et al.* 2011
Lactobacillus strains	Human fecal samples	Detection of bile salt hydrolase by plate, thin-layer chromatography and colorimetric assay upon different bile acid conjugates (glycocholic acid, glycochenodeoxycholic acid, glycodeoxycholic acid, taurocholic acid, taurochenodeoxycholic acid and taurodeoxycholic acid).	Guo *et al.* 2011
Brevibacillus sp	Hot water springs, Pali Maharashtra , India	Intracellular enzyme, with a molecular mass of 28 kDa, native molecular mass 56 kDa, homodimer K_M and K_{cat} values of 3.08µM and 6.32 x 10^2 s^{-1}, respectively, for glycodeoxycholic acid.	Sridevi *et al.* 2009

Table 1. Continue

Lactobacillus plantarum WCFS1	Human saliva	Four Bsh genes were expressed during the exponential phase of growth.	Kleerebezem *et al.* 2003; Lambert *et al.* (a)2008
L. acidophilus BFE 6128 *L. acidophilus BFE 6154* *L. plantrum BFE 5878* *L. plantarum BFE 5092*	Kulenaoto (African fermented milk)	Bile salt hydrolase activity on taurodeoxycholate and high resistance to duodenum juice containing 0.5% bile salts in the gastrointestinal passage model.	Vizoso Pinto *et al.* 2006
L. plantarum BFE 5759	Kwerionik (African fermented milk)	Bile salt hydrolase activity on taurodeoxycholate and high resistance to duodenum juice containing 0.5% bile salts in a gastrointestinal passage model.	Vizoso Pinto *et al.* 2006
L. plantarum BFE 1684 *L. plantarum BFE 1685*	Children's feces BFEL culture collection	Bile salt hydrolase activity on taurodeoxycholate and high resistance to duodenum juice containing 0.5% bile salts in the gastrointestinal passage model.	Vizoso Pinto *et al.* 2006
L. fermentum KC5b	Human isolates	Bile salt hydrolase activity in taurocholate and glycocholate (3.0±1.2 and 3.1±2.0 µmol of cholic acid per 10^{10} CFU/min).	Pereira *et al.* 2003
L .acidophilus 016 *L. acidophilus* L1 *L. acidophilus* ATCC 43121	Human intestine Human intestine Porcine intestine	Bile salt hydrolase activity on taurocholate. Intracellular enzymes (optimum pH 5.5-6.5 and 3.5 .4.5), molecular mass 126 kDa.	Corzo and Gilliland 1999
Streptococcus bovis ATCC 43143	Human isolates	Bile salt hydrolase activity on: taurocholate and glycocholate (30.9±1.0 and 3.5±1.3 µmol of cholic acid per 10^{10} CFU/min).	Pereira *et al.* 2003
Enterococcus faecalis UK873	Human isolates	Bile salt hydrolase activity on: Taurocholate and glycocholate (9.5 ± 6.6 and 23.9 ±5.2 µmol of cholic acid per 10^{10} CFU/min).	Pereira *et al.* 2003
Xanthomonasmaltophilia	Nature	Bile adapted strain dimer native and subunit molecular weight 100 and 52 kDa respectively 101 U/mg activity	Dean *et al.* 2002
Lactobacillus plantarum strains	Raw cow milk	Displayed BSH activity by providing the precipitation zone around colonies on plate assay.	Sieladie *et al.* 2011.
Lactobacillus sakei (eleven different strains) and *Lactobacillus plantarum* NR74	Kimchi	Showed deconjugation ability of bile salts, as indicated by the BSH test on agar plates.	Lee *et al.* 2011

Table 1. Continue

Lactobacillus amylovorus JCM2125	Human isolates	Capacity to express TCA hydrolase activity and capacity to express TDCA hydrolase activity	Moser and Savage 2001.
L. fermentum ATCC 11976	Infant intestine	Capacity to express TCA hydrolase activity and capacity to express TDCA hydrolase activity	Moser and Savage 2001.
L. crispatus JCM 8778	Feces	Capacity to express TCA hydrolase activity and capacity to express TDCA hydrolase activity	Moser and Savage 2001.
L. brevis BCCM 18022	Zabady (Yogurt)	Capacity to express TCA hydrolase activity and capacity to express TDCA hydrolase activity	Moser and Savage 2001.
12 *Lactobacillus* strains: -*L. reuteri* (C 1, C 10 and C 16) -*L. brevis* (I 12, I 23, I 25, I 211 and I 218) -*L. salivarius* (I 24) -*L. gallinarum* (I 16 and I 26) -*L. panis* (C 17).	Gastrointestinal tract of chickens	All 12 *Lactobacillus* strains were able to deconjugate GCA (16.87–100%) and TCA (1.69–57.43%).	Ramasamy *et al.* 2009.

environments rich in bile salts, as previously thought. So it is necessary to further investigate the role of these microorganisms in some fermented foods, and the environmental conditions that favor the growth of microorganisms with BSH activity.

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