Food Research International 112 (2018) 250–262

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Review

New insights into bacterial bile resistance mechanisms: the role of bile salt hydrolase and its impact on human health

Ana Y. Bustos[a,b,*], Graciela Font de Valdez[c], Silvina Fadda[c], María P. Taranto[c]

[a] Centro de Investigación en Biofísica Aplicada y Alimentos (CIBAAL), Universidad Nacional de Santiago del Estero – CONICET, RN 9- Km 1125, 4206 Santiago del Estero, Argentina

[b] Facultad de Agronomía y Agroindustrias (FAyA), Universidad Nacional de Santiago del Estero. Av. Belgrano Sur 1912, 4200, Santiago del Estero, Argentina

[c] Centro de Referencia para Lactobacilos (CERELA-CONICET), Chacabuco 145, T4000ILC San Miguel de Tucumán, Argentina

ARTICLE INFO

Keywords:
Bile resistance
Gut microbiota
Bile salt hydrolase
Cholesterol lowering activity
Bile acid signalling

ABSTRACT

Bile acids (BA), the major components of bile, are biological detergents that facilitate the emulsification and solubilization of dietary lipids and also display potent antimicrobial activity, the bacterial membranes being their main targets. Considering the complicated nature of the stress produced by bile and BA, the microorganism tolerance requires different defence mechanisms including the presence of efflux pumps, bile salt hydrolase (BSH) enzyme, the intrinsic capacity of cells to maintain intracellular homeostasis and modifications in the architecture and composition of the cell membrane. Besides, the expression of proteins involved in carbohydrate and fatty acid metabolism, amino acid and nitrogenous base biosynthesis, and general stress response are commonly affected by the presence of bile. Among the microbial transformations, deconjugation of BA by BSH is the most important. Several studies indicate that BSH activity affects both the host physiology and the microbiota. In fact, it was strongly suggested that BSH could play an important role in the colonization and survival of bacteria in the gut. Also, recent work has shown that BSH and free BA participate in a variety of metabolic processes that include regulation of dietary lipid absorption, cholesterol metabolism, and energy and inflammation homeostasis.

In this review we summarize recent advances in the understanding of the mechanisms involved in the tolerance of bacteria to bile, with special emphasis on the contributions of studies applying an *omic* approach. Besides, the physiological and ecological role of BSH enzyme and its relevance to human health as well as the function of bile acid as metabolic regulator are also discussed.

1. Introduction

Bile acids (BA), major components of bile, are synthesized from cholesterol and conjugated to either glycine or taurine in the liver, stored in gallbladder before secretion into the duodenum *via* the common bile duct (Li & Chiang, 2015). BA are biological detergents that facilitate the emulsification and solubilization of dietary lipids and fat-soluble vitamins, favouring its absorption by enterocytes. Although the major fraction of BA are actively reabsorbed by enterocytes and sent back to the liver (enterohepatic circulation), a small fraction is modified by the indigenous microbiota (Chand et al., 2017). Among these transformations, the deconjugation of BA by bile salt hydrolase (BSH) of bacterial origin is the most important. Several studies indicate that BSH activity affects the physiology of both the host and the microbiota. In fact, it was strongly suggested that BSH could play an important role in the colonization and survival of bacteria in the gut (Kim & Lee, 2005; Ruiz, Margolles, & Sanchez, 2013). Besides, recent work has shown that BSH and free BA participate in a variety of metabolic processes that include the regulation of dietary lipid absorption, cholesterol metabolism, energy and inflammation homeostasis, and could act as signalling molecules (Li & Chiang, 2015; Martoni, Labbe, Ganopolsky, Prakash, & Jones, 2015; Ridlon & Bajaj, 2015).

Due to the lipophilic nature of the steroid ring, BA display potent antimicrobial activity, the bacterial membranes being their main targets. High concentrations of bile could dissolve phospholipids and disrupt the lipid bilayer structure of cellular membranes causing lysis.

Abbreviations: BA, bile acids; BSH, bile salt hydrolase; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, litocholic acid; MCA, muricholic acid; CYP7A1, cholesterol 7-αhydroxylase; *L.*, *Lactobacillus*; *B.*, *Bifidobacterium*; EPS, exopolysaccharide; FXR, farnesoid X receptor; TGR5, G protein-coupled receptor; SHP, small heterodimer partner; FGF, fibroblast growth factor; AGP, antibiotic growth promoters; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; SCFAs, short chain fatty acids

* Corresponding author.
E-mail address: abustos@uspt.edu.ar (A.Y. Bustos).

https://doi.org/10.1016/j.foodres.2018.06.035
Received 27 February 2018; Received in revised form 14 May 2018; Accepted 18 June 2018
Available online 20 June 2018

Moreover, BA induce protein misfolding, oxidative damage to DNA and RNA, and intracellular acidification (Bustos, Saavedra, de Valdez, Raya, & Taranto, 2012; Merritt & Donaldson, 2009; Taranto, Fernandez Murga, Lorca, & Valdez, 2003).

Given the complicated nature of bile stress, gut microorganisms, as well as those proposed as probiotics, must carry out different defence mechanisms to tolerate its presence, which have not been fully elucidated until now.

In this work the most recent advances in the understanding of the bacterial mechanisms involved in the tolerance to bile, with special emphasis on the contributions of *omic* technologies in this field are reviewed. In addition, the physiological and ecological role of BSH enzyme and its relevance to human health as well as the function of BA as metabolic regulator are also discussed.

2. Synthesis and metabolism of bile acids

BA are hydroxylated sterols synthesized from cholesterol by a multienzyme pathway taking place in hepatocytes and involves enzymes localized in the endoplasmic reticulum, mitochondria, cytosol, and peroxisomes (Russell, 2003). Hemoproteins of cytochrome P450 from the smooth endoplasmic reticulum participate in many of the BA synthesis reactions (Li & Chiang, 2015). Obtaining BA from cholesterol involves hydroxylation, saturation of the double bond at C5-C6, epimerization of the 3-hydroxyl group, and oxidative cleavage of a 3-carbon unit. After that, the two primary C24-BA chenodeoxycholic acid (CDCA) and cholic acid (CA) are obtained (Fig. 1). These compounds are the basis for the construction of all other C24-BA (Li & Chiang, 2014). In the liver, primary BA are synthesized through two major synthesis pathways: a classic and an alternative pathway.

The classic pathway, also called the neutral pathway, is considered the major biosynthetic pathway in humans since more than 90% of total BA production employed this way. The cholesterol 7α-hydroxylase (CYP7A1) is a cytochrome P450 enzyme that catalyses the conversion of cholesterol in 7α-hydroxycholesterol, the first and rate-limiting step in the classic BA synthesis pathway. Then, by consecutive dehydrogenation and dehydroxylation, the 7α-hydroxy-4-cholestene-3-one (C4), a common precursor for CA and CDCA, is obtained. The sterol 12α-hydroxylase (CYP8B1) catalyses the hydroxylation of C4 at the C12 position, followed by several reactions including the steroid side chain cleavage by the sterol 27-hydroxylase (CYP27A1) that lead to the synthesis of CA. Without 12α-hydroxylation by CYP8B1, C4 is eventually converted to CDCA. The CYP8B1 regulates the CA/CDCA ratio in the BA pool, while CYP7A1 is responsible for the overall regulation of BA synthesis. In mice, most of the CDCA is converted to α-muricholic acid (MCA) and β-MCA. In consequence, CA and α- and β-MCAs are the main primary BA in mouse (Chiang, 2013).

The alternative pathway, also called the acidic pathway due to the production of acidic intermediates, produces less than 10% of the total BA in humans. In this pathway, cholesterol is first converted to 27-hydroxycholesterol by the mitochondrial CYP27A1, which is subsequently hydroxylated at the C-7position by oxysterol 7a-hydroxylase (CYP7B1) to form 3β,7α-dihydroxy-5-cholestenoic acid, which is eventually converted to CDCA. CYP7B1 catalyses many hydroxylation reactions in steroid synthesis in steroidogenic tissues and oxysterol synthesis in peripheral tissues. The oxysterol intermediates formed in the peripheral tissues could be transported to the liver and mainly converted to CDCA. The alternative pathway may be responsible for the synthesis of about 50% of bile acids in rodents. In the large intestine, bacterial 7α-dehydroxylase converts CA and CDCA to DCA and LCA, respectively (Chiang, 2009).To increase its solubility, the steroid nucleus of BA is conjugated by peptide linkage with taurine or glycine obtaining taurocholic and taurochenodeoxycholic acids or glycocholic and glycochenodeoxycholic acids, respectively (Fig. 1). When another amino acid is mistakenly incorporated in the molecule, it is rapidly removed by peptidases. The ratio of glyco- to tauro-conjugates in human bile is usually 3:1 with variations due to race and food, while mice differ in their BA composition with predominance of tauro-

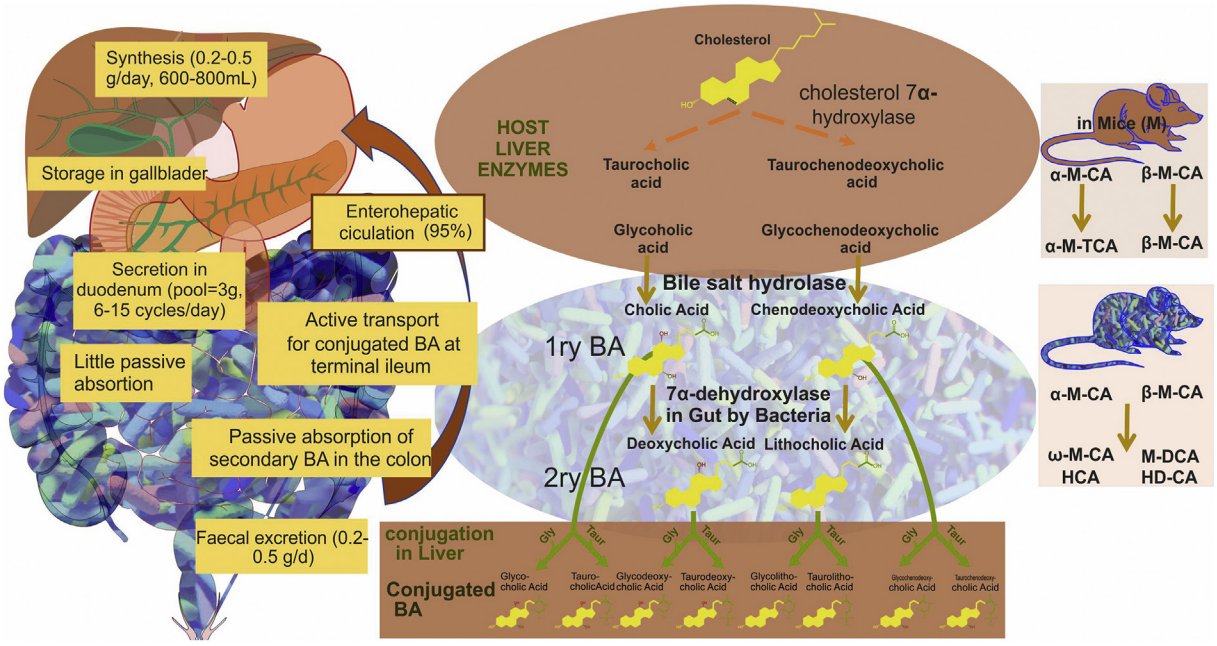

Fig. 1. Metabolism of bile acids (BA). BA are synthesized from cholesterol by a multienzyme pathway taking place in hepatocytes *via* two major biosynthetic pathways. The cholesterol 7-αhydroxylase (CYP7A1) catalyses the first and rate-limiting step in the classic BA synthesis pathway. The two primary chenodeoxycholic acid and cholic acid are conjugated by peptide linkage with taurine or glycine. Once in the gut, conjugated BA are substrates of the enzyme collectively called bile salt hydrolases. Most of the BA are returned to the liver *via* the portal circulation in the process known as enterohepatic circulation. The pool of BA, which consists of 3 g, recirculates 6 to 15 times per day, while 0.2 to 0.5 g are lost in the faeces. Conjugated BA are mainly absorbed at the terminal ileum by active transport mechanisms, while a small amount is absorbed by passive diffusion in the small and large intestines; finally, passive absorption of hydrophobic secondary BA occurs in the colon. MCA, muricholic acid; HCA, hyocholic acid; HDCA, hyodeoxycholic acid.

Table 1
Summary of BSH characteristics described in different bacterial species.

Domain	Genera	Specie	Commentaries	References
Gram positive	*Lactobacillus*	*L. acidophilus* *L. reuteri* *L. brevis* *L. salivarius* *L. casei*	Intracellular	Bustos, (Bustos et al., 2016); Chae, Valeriano, Kim, & Kang, (Chae et al., 2013); Gonzalez-Vazquez et al., (Gonzalez-Vazquez et al., 2015); Gu et al., (Gu et al., 2014); Jayashree, Pooja, Pushpanathan, Rajendhran, & Gunasekaran, (Jayashree et al., 2014)
	Bifidobacterium	*B. longum* *B. bifidum* *B. animalis*	Intracellular	Jarocki & Targonski, (Jarocki & Targonski, 2013); Kim & Lee, (Kim & Lee, 2005); Kumar et al., (Kumar et al., 2006)
	Clostridium	*C. perfringens*	Intracellular and extracellular	Gopal-Srivastava & Hylemon, (Gopal-Srivastava & Hylemon, 1988)
	Listeria	*List. monocytogenes*	Intracellular, non-intestinal environment	Dussurget et al., (Dussurget et al., 2002)
	Enterococcus	*Ent. faecalis*	Intracellular	Deepak Chand, Panigrahi, Varshney, Ramasamy, & Suresh, (Chand et al., 2018); Franz, Specht, Haberer, & Holzapfel, (Franz et al., 2001)
	Planococcus	*Planococcus antarcticus*	Intracellular, marine environment	Margolles, Gueimonde, & Sánchez, (Margolles et al., 2012)
Gram negative	*Brucella*	*Brucella abortus*	Extracellular, non-intestinal environment	Delpino et al., (Delpino et al., 2007)
	Bacteriodes	*Bact. fragilis* *Bact. vulgatus*	Intracellular, non-intestinal environment	Kawamoto, Horibe, & Uchida, (Kawamoto et al., 1989); Stellwag & Hylemon, (Stellwag & Hylemon, 1976)
	Xanthomonas	*Xanthomonas maltophilia*	Intracellular, non-intestinal environment	Dean et al., (Dean et al., 2002)
Archaea	*Methanosarcina*	*Methanosarcina acetovorans*	Intracellular, marine environment	Panigrahi, Sule, Sharma, Ramasamy, & Suresh, (Panigrahi et al., 2014)

conjugated BA (including muricholic acids) (de Aguiar Vallim, Tarling, & Edwards, 2013). In addition to increasing the solubility of BA, conjugation decreases their passive absorption in the biliary tract and small intestine, increasing their intraluminal concentration and facilitating the digestion and absorption of fats (Begley, Gahan, & Hill, 2005). The hydroxyl groups and the carboxyl group of BA on one side of the carbon skeleton form the hydrophilic face opposite the other highly hydrophobic face; they are therefore amphipathic molecules with powerful detergent properties. The concentration of BA in the small intestine varies in wide ranges from 1 mM (0.05%) to 40 mM (2%) during the first hour of digestion (Islam et al., 2011).

Once in the gut, most of the BA are returned to the liver, *via* the portal circulation in the process known as enterohepatic circulation. Conjugated BA are mainly absorbed at the terminal ileum by active transport mechanisms, while a small amount is absorbed by passive diffusion in the small and large intestines; finally, passive absorption of hydrophobic secondary BA occurs in the colon (Kim & Lee, 2005; Ridlon, Harris, Bhowmik, Kang, & Hylemon, 2016). Approximately 600 to 800 ml of bile is produced per day. The pool of BA, approximately 3 g, recirculates 6 to 15 times per day and 0.2 to 0.5 g is lost in the faeces; the BA pool must be compensated by the *de novo* synthesis to maintain the BA pool constant (Fig. 1). BA faecal excretion is the main route of cholesterol elimination from the body (Dietschy & Turley, 2002).

Early evidence showed that BA regulate their own synthesis since their administration to rats suppressed the hepatic synthesis and, on the contrary, the blockade of the intestinal reabsorption of BA increased CYP7A1 enzyme activity and subsequently BA synthesis. In fact, Chiang (2009) showed the negative feedback involved in the regulation of BA *via* the transcriptional repression of CYP7A1 gene. This mechanism allows the liver to maintain BA homeostasis, stimulating or inhibiting their synthesis in response to changes in BA levels.

3. Microbial bile acid modifications. Bile salt hydrolase activity

As was mentioned, bile and BA are synthetized in the liver to be secreted through the common hepatic duct that joins the cystic duct of the gallbladder to form the common bile duct that deposits them in the duodenum. Once in the large intestine, primary BA are susceptible to microbial modifications that include deconjugation, oxidation of hydroxyl groups at C-3, C-7, and C-12, 7α/β-dehydroxylation and epimerization (Joyce, Shanahan, Hill, & Gahan, 2014). As a result, secondary BA such as deoxycholic acid (DCA) and lithocholic acid (LCA) are produced (Fig. 1). In addition to these major BA, intestinal microbiota can generate about 20 different BA metabolites derived from CA and CDCA (Ridlon et al., 2016; Ridlon & Bajaj, 2015). In humans the BA pool, defined as the total amount present in the enterohepatic circulation, consists of about 40% each of CDCA and CA, and 20% DCA (Li & Chiang, 2014). In mice the predominant metabolite of βMCA is omega-MCA (ωMCA), which is generated by 6β-epimerization. Other less prevalent metabolites of βMCA are generated by 6β-epimerization and additional 7β-dehydroxylation producing hyodeoxycholic acid (HDCA), or additional 7β-epimerization, which produces hyocholic acid (HCA; also known as γMCA) (Degirolamo, Rainaldi, Bovenga, Murzilli, & Moschetta, 2014).

As shown in Fig. 1, the first of the microbial transformations is the deconjugation (hydrolysis) of BA. The responsible enzymes are collectively called BSH and belong to the choloylglycine hydrolase family (Long, Gahan, & Joyce, 2017). As a result of the hydrolysis, the solubility and emulsifying capacity of BA decrease.

BSH activity has been described in a wide variety of bacterial genera, mainly of gastrointestinal origin, including *Lactobacillus (L.)* sp. (Bustos, de Valdez, Raya, & Taranto, 2016; Chae, Valeriano, Kim, & Kang, 2013; Gonzalez-Vazquez et al., 2015; Gu et al., 2014; Jayashree, Pooja, Pushpanathan, Rajendhran, & Gunasekaran, 2014), *Bifidobacterium (B.)* (Jarocki & Targonski, 2013; Kim & Lee, 2005; Kumar et al., 2006), *Clostridium* (Gopal-Srivastava & Hylemon, 1988), and *Enterococcus* (Deepak Chand, Panigrahi, Varshney, Ramasamy, & Suresh, 2018; Franz, Specht, Haberer, & Holzapfel, 2001) as is shown in Table 1. Besides intestinal microbes, BSH enzyme was also reported in *Listeria monocytogenes* (Dussurget et al., 2002) and *Brucella abortus* (Delpino et al., 2007), while *Bacteriodes* (Kawamoto, Horibe, & Uchida, 1989; Stellwag & Hylemon, 1976) and *Xanthomonas* (Dean et al., 2002) represent the only Gram-negative bacteria known to exhibit BSH. Remarkably, bacteria isolated from a marine environment, *Methanosarcina acetovorans* and *Planococcus antarcticus*, also possess BSH homologues (Margolles, Gueimonde, & Sánchez, 2012; Panigrahi, Sule, Sharma, Ramasamy, & Suresh, 2014). Additionally, many *bsh* genes have been cloned, sequenced and characterized. Interestingly, the genetic organization of *bsh* genes is highly variable, and several strains possess more

than one BSH homolog, which in most cases are not identical (Begley, Hill, & Gahan, 2006). Nevertheless, in all cases only one BSH shows significant hydrolase activity, while the function of the other enzyme homologues need to be clarified (Chand et al., 2017).

Regarding the distribution of BSH activity among microorganisms, it was reported that lactobacilli are mainly responsible for the BSH activity in the intestinal tracts of mice and human (Chand et al., 2017; Li et al., 2018). Also, most of bifidobacterial species tested displayed the activity; however, *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus* were not able to deconjugate BA (Ridlon et al., 2016). These results strongly suggest a correlation between the habitat of the bacteria and the presence of BSH enzymes since it seems to be restricted to gut, an environment rich in conjugated BA, while microorganisms isolated from other sources did not normally show the enzyme. However, Reyes-Nava and Rivera-Espinoza (2014) reported the presence of BSH in microorganisms isolated from fermented milk, raw cow's milk and vegetables, among others.

In summary, the integrated metabolism of BA could be considered as a complex and transgenomic interaction between the host and its microbiome (Turroni et al., 2014).

4. Antimicrobial actions of bile acids

Besides their physiological function, BA are highly toxic to microorganisms. Fig. 2 shows their main antimicrobial actions as well as cellular mechanisms of BA response identified in intestinal bacteria. Due to its amphipathic character that confers important detersive properties, bacterial membranes are the main target of the antimicrobial action of BA. Early studies with electron microscopy revealed that strains of *L. reuteri* exposed to bile showed severe distortion of the cell envelope and membrane alterations that presented folds and buds (De Valdez et al., 1997; Taranto, Perez-Martinez, & de Valdez, 2006).

The concentration of BA is one of the most important factors involved in their toxicity. High concentrations of bile can rapidly dissolve membrane lipids, cause dissociation of integral proteins, loss of intracellular material, abolishment of glucose uptake and cell death (Kurdi, Kawanishi, Mizutani, & Yokota, 2006; Leverrier et al., 2003; Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011). Low concentrations of bile can cause disruption of the membrane and its fluidity, changes in hydrophobicity and zeta potential, alteration in the transmembrane flow of divalent cations (Kociubinski, Gómez Zavaglia, Pérez, Disalvo, & De Antoni, 2002; Pumbwe et al., 2007; Zárate, Gonzalez, Chaia, & Oliver, 2000).

An important mechanism of growth inhibition in lactobacilli and bifidobacteria is related to the dissipation of the transmembrane electrical potential ($\Delta\Psi$) and intracellular acidification caused by BA (Bustos et al., 2012; Bustos, Raya, Bru, de Valdez, & Taranto, 2011; Kurdi et al., 2006). In fact, it was reported that in some Gram-positive strains the minimum inhibitory concentration (MIC) for free BA corresponded to the concentrations that dissipated pH and $\Delta\Psi$ (Kurdi et al., 2006). Moreover, in many bacterial genera the presence of bile and BA induces oxidative damage to DNA and RNA, and protein misfolding (Begley et al., 2005; Merritt & Donaldson, 2009).

The type and structure of BA is another important factor related to their toxicity since di-hydroxylated acids (DCA) cross more quickly the membrane than tri-hydroxylates (CA) resulting, therefore, more toxic (Kurdi et al., 2006).

Enteric bacteria are usually more resistant to physiological concentrations of BA. However, it was reported that BA induce specific stress gene expression in response to perturbation in membrane functions, DNA damage and an increase in the frequency of mutations in *Escherichia coli* and *Salmonella enterica* (Sistrunk, Nickerson, Chanin, Rasko, & Faherty, 2016).

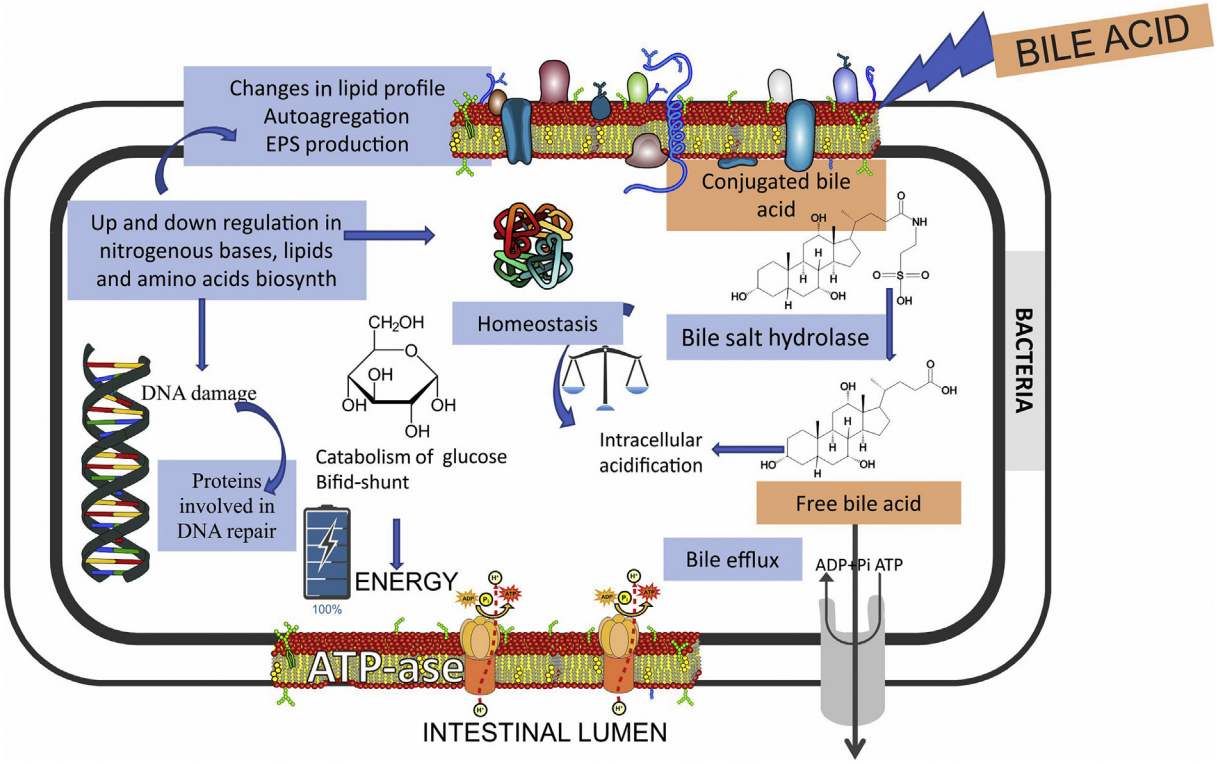

Fig. 2. Main antimicrobial actions of bile acids and bacterial adaptive mechanisms. Given the complicated nature of the stress produced by bile acids, microorganisms must deploy different defence mechanisms to overcome their presence. The main resistance mechanisms described in intestinal bacteria are mediated by the architecture and composition of the cell membrane, the presence of efflux pumps, the action of the BSH enzyme, and the intrinsic capacity of cells to maintain intracellular homeostasis. Additionally, many proteins including those involved in carbohydrate metabolism and fatty acid, amino acid and nitrogenous base biosynthesis, transporters and proteins related to general stress response are regulated by bile acids in bacteria.

5. Mechanisms of bile acid tolerance in bacteria

Given the complicated nature of the stress produced by bile and the BA component, microorganisms must deploy different defence mechanisms to overcome their presence (Fig. 2). In general, Gram-positive bacteria are more sensitive to the toxic effects of BA than Gram-negative bacteria, since the minimum inhibitory concentrations for the first microbial group are significantly lower than for the second group. These differences can be partially attributed to the presence of lipopolysaccharide that constitutes the majority of the outer leaflet of the outer membrane, Tol proteins and efflux pumps (Margolles, García, Sánchez, Gueimonde, & de los Reyes-Gavilán, 2003; Šárka, Milada, & Kateřina, 2018). However, BA tolerance is strain dependent and shows extreme variability even within the same genus or species.

The main resistance mechanisms described in intestinal bacteria are mediated by the architecture and composition of the cell membrane, the presence of efflux pumps, the action of the BSH enzyme and by the intrinsic capacity of cells to maintain intracellular homeostasis (Fig. 2) (Chen, Hsu, Chou, & Wang, 2018; Piddock, 2006; Ruiz et al., 2013).

In the last decade, the advent of *omic* technologies has allowed important advances in the characterization of bacteria responses to different stress challenges. Bile tolerance is one of the most important properties that determine the ability of bacteria to outlast in the small intestine, and consequently their capacity to develop their functional role as probiotics. Proteomics proved to be a useful tool for assessing changes in the expression levels of numerous proteins in certain conditions, allowing a holistic view of proteins in the studied organisms (Lilley, Razzaq, & Dupree, 2002). Specific proteomic findings revealed the existence of protective mechanisms allowing an adaptive response to low pH exposure that could also provide a cross-protection against other stress conditions such as the presence of bile (Lee, Lee, & Choi, 2008). Moreover, these post-genomic studies established bacterial biomarkers for the selection of the best strains with probiotic potential and were useful for a better understanding of the mechanisms used by microorganisms to tolerate this stress. The integrated transcriptomic and proteomic analyses have been the most common *omic* approaches applied to characterize the response to bile in probiotic bacteria. For instance, mechanistic insights into bile stress adaptation of *L. casei* BL23 combining these technologies were provided. This strategy allowed complementing the findings from each *omic* platform, or to unravel novel information that cannot be achieved by an individual ones (Alcantara & Zuniga, 2012). In a more recent transcriptomic and proteomic approach, Lv et al., (2017) reported 591 differentially transcribed genes and 347 differentially expressed proteins when the probiotic *L. salivarius* LI01 was subjected to bile stress. Also the protein expression pattern of *L. fermentum* NCDC 400 strain, under BA stress was analysed using a quantitative proteomic approach (Kaur, Ali, Kumar, Mohanty, & Behare, 2017). Remarkably, the shot gun proteomic approach allows identifying 10 times more proteins than classic 2D-gel-based proteomic studies (Kaur et al., 2017).

Previous and more recent studies have shown that the main proteins whose expression was regulated by bile in bacteria include proteins involved in carbohydrate metabolism and fatty acid, amino acid and nitrogenous base biosynthesis, and transporters able to extrude bile salts and proteins related to general stress response (Fig. 2) (Bustos et al., 2015; Ruiz et al., 2016; Sánchez et al., 2007; Šárka et al., 2018; Kaur et al., 2017; Lv et al., 2017). Below, some key aspects related to bile response in bacteria and the main contributions of *omic* studies in this field are revisited.

5.1. Metabolism of carbohydrates

It was reported that the challenge with bile, in different bacterial species produced changes in the expression levels of several enzymes involved in carbohydrate metabolisms. The enzymes that participate in the catabolism of glucose are often found to be overexpressed in the presence of bile, probably because the different survival strategies developed by microorganisms to tolerate its presence require energy supply. However, metabolic shifts seem to be strain-dependent. Among them, glucose-6-phosphate dehydrogenase, an enzyme that links glycolysis to the pentose phosphate pathway, was found to increase in the presence of bile in *L. reuteri* ATCC 23272 (Lee et al., 2008), *B. longum* BBMN68 and NCIMB 8809 (An et al., 2014; Sánchez et al., 2005), *B. animalis* subsp. *lactis* IPLA 4549 (Sánchez et al., 2007) but under expressed in strains sensitive to bile *L. plantarum* (Hamon et al., 2011). Lactate dehydrogenase enzyme involved in the reduction of pyruvate to lactate with the reoxidation of NADH and a related D-isomer specific 2-hydroxyacid dehydrogenase NAD-binding protein were under expressed in *L. reuteri* CRL 1098 (Bustos et al., 2015) in the presence of DCA, and in *L. rhamnosus* GG (Koskenniemi et al., 2011) and *B. longum* exposed to bile (Ruiz et al., 2009). In this case, the lower production of NADH could adversely affect the survival of the cells under this stress effector.

Remarkably, in *Bifidobacterium*, bile exposure induced metabolic shifts in many enzymes involved in the pentose phosphate pathway, the so-called bifid shunt, at the mRNA and protein synthesis level, although the response appears to be strain-dependent. Fructose-6-phosphate phosphoketolase, a key enzyme of the bifid shunt proved to play an essential role not only in the adaptation to bile but in the protection of cells from the stress generated by its presence (An et al., 2014; Sánchez et al., 2005; Sánchez et al., 2007; Sánchez, Noriega, Ruas-Madiedo, de los Reyes-Gavilán, & Margolles, 2004). In fact, *B. animalis* subsp. *lactis* showed an overexpression of many enzymes of the bifid shunt involved in ATP production by phosphorylation at the substrate level, while *B. longum* accumulates enzymes involved in glucose consumption after bile exposure, suggesting the production of ATP by a glycolytic pathway (Sanchez, Ruiz, & Margolles, 2008; Ruiz et al., 2013).

The achievement of bile tolerance was also associated with metabolic shifts. In fact, a bile-resistant *B. animalis* derivative prefers maltose over glucose compared with this parental strain, probably as a selective advantage since glucose is not available in the distal colon. Moreover, in other bile-adapted *Bifidobacterium* strains, the bifid shunt is replaced by other metabolic pathways in order to increase ATP yield (Ruiz et al., 2013). In summary, the main proteomic reports on LAB and bifidobacteria suggest that energy generation in the bile acid response is a multifactorial process with the involvement of different networks of proteins used to safeguard the cell.

5.2. Cell envelope and lipid metabolism

As was mentioned above, due to their detersive properties, bacterial cell membranes represent one of the main targets of BA since they alter both the structure of the bacterial envelopes and the morphology of the colonies. In fact, it was reported that colonies of *Lactobacillus* more sensitive to bile became rough, while the smooth colonies were more resistant. Indeed, differences in colony morphology were proposed as a marker of resistance (Šušković, Kos, Matošić, & Besendorfer, 2000). In this sense, *L. casei* BL23 cells showed a significant decrease in cell size after exposure to bile and a lower abundance of the protein LCABL_14850 detected in the membrane fraction by proteomic assays (Alcantara & Zuniga, 2012). The reduction of this structural protein affects cell wall synthesis since it participates in the complex that leads the growth of the cell wall (Jones, Carballido-López, & Errington, 2001; Young, 2010). It has been described that exposure to bile and BA induces changes in the composition of lipids and fatty acids of the membrane and surface properties in bifidobacteria and lactobacilli such as hydrophobicity and z potential reduction, which could contribute to bile tolerance (Gómez Zavaglia, Kociubinski, Pérez, Disalvo, & De Antoni, 2002; Kociubinski et al., 2002; Taranto et al., 2003). Remarkably, *L. reuteri* cells growing in the presence of bile showed a decrease in phospholipids and a low proportion of saturated fatty acids in relation to unsaturated ones and the presence of the unusual C18:0,10-

OH and C18:0,10-oxo fatty acids (Taranto et al., 2003; Taranto et al., 2006). In line with this, many lactobacilli showed lower fatty acid synthesis in response to bile stress in proteomic assays. Most genes for fatty acid biosynthesis (Wu et al., 2011) were strongly down regulated after the addition of bile in *L. delbrueckii subsp. lactis* (Burns et al., 2010), in *L. casei* BL23 (Alcantara & Zuniga, 2012), in *L. casei* (Wu et al., 2010), *L. rhamnosus* GG (Koskenniemi et al., 2011) and *L. reuteri* ATCC 55730 (Whitehead, Versalovic, Roos, & Britton, 2008). In an integrated transcriptomic and proteomic analysis of the bile stress response in *L. salivarius* LI01L, Lv et al.(2017) reported that gene expression related to peptidoglycan autolysis and biosynthesis (*mur*I, *dac*C, and *mur*E) varied strongly. This indicates the peptidoglycan layer was restructured under bile stress. Also, the biosynthesis of lipids and fatty acids was altered to cope with bile influx and enhance the hydrophobicity of the cell surface.

Some proteins related to adhesion were overexpressed in lactobacilli and bifidobacteria in the presence of bile in *in vitro* assays (Duary, Batish, & Grover, 2012; Ruiz et al., 2009). However, these findings did not correlate with the results obtained *in vivo*, and their role in the tolerance to bile and adhesiveness to intestinal cells or mucus is not clear (Burns, Reinheimer, & Vinderola, 2011; Ruiz et al., 2011).

Bile also induced exopolysaccharide (EPS) production in many bacteria, probably as a protective coat against this harmful compound (Alp & Aslim, 2010; Ruiz et al., 2013). In accordance with this, *in vitro* and *in vivo* assays revealed a correlation between EPS production and bile resistance and gut colonization in *Bifidobacterium* (Fanning et al., 2012; Ruas-Madiedo, Gueimonde, Arigoni, Clara, & Margolles, 2009). However, in *Lactobacillus* strains this link is not clear; in fact, transcriptomic and proteomic research on *L. rhamnosus* GG showed reduced production of EPS biosynthesis in the presence of bile (Koskenniemi et al., 2011). In a wild-type strain of *L. delbrueckii* no variations in EPS production were found following bile challenge, while in a bile-resistant strain significant overproduction of enzymes involved in its biosynthesis was observed (Burns et al., 2010). However, there are no available data about how bile exposure affects the composition and properties of EPS (Ruiz et al., 2013).

Finally, the cell envelope-related subunit δ of the F0F1-ATPase showed strong up regulation in the membrane fraction of *L. casei* BL23 (Alcantara & Zuniga, 2012) after exposure to bile, whereas Hamon et al. (2011) also reported a moderate increment in some *L. plantarum* strains. In *L. rhamnosus* GG, a significant increase of ATPase subunit α was observed in the cell envelope (Koskenniemi et al., 2011). These results suggest the dissipation of the proton motive force as the cell response to BA in many lactic acid bacteria (Bustos, Raya, Bru, et al., 2011; Kurdi et al., 2006; Margolles et al., 2003).

5.3. Bile efflux pumps

It has been described that multidrug resistance (MDR) transporters belonging to the ATP binding cassette or the major facilitator superfamily could play an important role in the bile resistance phenotypes in Gram-negative and Gram-positive bacteria (Šárka et al., 2018). In an earlier study, Thanassi, Cheng, and Nikaido (1997) demonstrated that two MDR transporters (AcrAB and EmrAB) were responsible for the active efflux of BA in *E. coli*. The microarray analysis of the gene expression of *Salmonella enterica* serovar Typhimurium exposed to bile reveals that the upregulation of a MDR transporter was dependent on the concentration of bile (Prouty, Van Velkinburgh, & Gunn, 2002). The CmeABC, a multidrug efflux pump system, of *Campylobacter jejuni* is overexpressed in the presence of bile, and its inactivation reduces both bile resistance and the colonization capacity of this microorganism (Lin et al., 2005).

Some MDR systems have also been identified in Gram-positive bacteria; however, in most cases, the ability of these transporters to confer resistance to bile is not clearly established. Margolles, Florez, Moreno, Van Sinderen, and Clara (2006) identified and characterized two MDR transporters (BbmAB and BbmR) in *B. breve* capable of conferring resistance to antimicrobial compounds. In *L. plantarum*, a membrane protein whose expression is induced by bile has been identified both *in vitro* and *in vivo* (Bron et al., 2004). Transporters capable of extruding BA have been described in *Lactococcus lactis* (Yokota, Veenstra, Kurdi, van Veen, & Konings, 2000), *L. johnsonii* (Elkins & Savage, 2003), two in *B. longum* (Gueimonde, Garrigues, van Sinderen, Clara, & Margolles, 2009; Price, Reid, Driessen, & Abratt, 2006), *B. breve* (Ruiz et al., 2012), *L. reuteri* ATCC 55730, (Whitehead et al., 2008). Four transporters were described in *L. acidophilus* NCFM, and the deletion of any one of them increases the sensitivity of the strain to bile and certain antibiotics (Pfeiler & Klaenhammer, 2009). Also, mutation of MDR in *L. reuteri* (Whitehead et al., 2008) and in *B. breve* (Ruiz et al., 2012; Ruiz, Zomer, O'Connell-Motherway, van Sinderen, & Margolles, 2011) reduced their ability to grow in the presence of BA. Moreover, in *L. reuteri* CRL 1098 an ATP dependent active efflux of conjugated (TCA) and free (CA) BA was reported (Bustos, Raya, de Valdez, & Taranto, 2011). Interestingly, most transporters involved in extrusion and/or tolerance to bile identified in lactobacilli and bifidobacteria exhibit some degree of inducibility. The bifidobacterial genes *Bl0920* and *Bbr_0838* showed high levels of transcriptional induction following bile exposure (Gueimonde et al., 2009; Ruiz et al., 2012). Additionally, the expression of many multidrug transporters was found to be induced in response to bile in *L. casei* BL23 (Alcantara & Zuniga, 2012), *L. rhamnosus* GG, (Koskenniemi et al., 2011) and *L. reuteri* ATCC 55730 (Whitehead et al., 2008).

5.4. General stress response proteins

Proteins involved in stress response are usually differentially expressed in strains exposed to bile as well as to other stress effectors, while their coding genes are highly conserved in bacteria. The heat shock proteins DnaK, GroEL and GroES have been frequently related to the response against several stimuli, including bile, in many bacteria genera (Ruiz et al., 2016; Siciliano & Mazzeo, 2012). GroEL and DnaK, among others, were overexpressed in *B. longum* and *Enterococcus faecalis* and *L. fermentum* after bile exposure (Bøhle et al., 2010; Kaur et al., 2017; Sánchez et al., 2005). GroEL was also overproduced in *L. reuteri* CRL1098 in the presence of DCA (Bustos et al., 2015) and was identified in two bile-sensitive strains of *L. plantarum* while not in the resistant ones (Hamon et al., 2011). GroEL and GroES were significantly upregulated upon exposure to bile in *B. longum* BBMN68 at transcriptomic and proteomic level (An et al., 2014). The overproduction of these proteins allows the accurate integrity and stability of proteins and DNA against bile stress. On the other hand, some proteins involved in the protection of DNA against UV damage and oxidative stress were overexpressed in *B. longum* NCIMB 8809 (Sánchez et al., 2005) and *Escherichia coli* (Sistrunk et al., 2016) exposed to bile.

5.5. Bile salt hydrolase activity

Numerous previous works suggest that BSH activity could play a role in bile tolerance in some Gram-positive bacteria and would be part of a cell detoxification strategy (Begley et al., 2006; Grill, Cayuela, Antoine, & Schneider, 2000). In fact, free BA produced by BSH activity have decreased solubility, precipitate at intestinal pH (decreased mainly by the activity of lactic acid bacteria) and leave the GIT in the faeces. In addition, the diminished detergent activity may beless toxic to bacteria in the intestine (Chand et al., 2017).

In some lactobacilli strains, a link between BSH activity and bile resistance using BSH mutant was reported (Begley et al., 2006). In *Listeria innocua* the expression of cloned BSH significantly enhance bile tolerance *in vitro* and gut colonization in mice compared to the BSH negative control strain (Jones, Begley, Hill, Gahan, & Marchesi, 2008). Numerous previous studies reported changes in the expression of BSH at genomic, transcriptomic and proteomic levels after exposure to bile;

moreover, the response observed varied according to the microorganism studied. In a bile-resistant strain of *L. plantarum* a down regulation in the expression level of BSH enzyme was observed, while no changes were found in the sensitive strains (Hamon et al., 2011). Regarding *Bifidobacterium* strains, some evidence also suggests a role of BSH in bile resistance. Positive induction of BSH was observed in a bile-adapted strain of *B. animalis* compared to its wild-type counterpart (Noriega, Cuevas, Margolles, & Clara, 2006; Sánchez et al., 2007). In addition, exposure of *B. longum* NCC2705 to the intestinal environment of a rabbit induced the over production of BSH (Yuan et al., 2007). On the contrary, in *Enterococcus faecalis* V583 and *L. reuteri* ATCC 23272, the presence of bile did not induce proteomic expression of BSH (Bøhle et al., 2010; Lee et al., 2008). In line with this, bile resistance of *L. salivarius* LI01 was found to be based mainly on a highly modified cell envelope and a potentiated bile efflux system rather than on the activity of BSH (Lv et al., 2017). It can be deduced that the regulation of the BSH enzyme is a complex process that varies according to the strain under study and could play a role in the microbial resistance to bile.

However, the mechanism by which BSH confers protection against BA is not completely clarified since the non-conjugated species are more toxic to the cell. It was proposed that conjugated BA enter the cell and are converted by the BSH enzyme into their weaker deconjugated counterparts (pKa 5) that could capture a proton preventing the pH drop caused by the presence of BA (Begley et al., 2005; Bustos et al., 2012). According to this, Bustos et al. (2012) reported that in BSH (+) strains the addition of glycodeoxycholic and taurodeoxycholic acid causes a gradual increase in the internal pH; on the contrary, in BSH(−) strains a drop in internal pH was observed, probably because of BA accumulation. Consequently, BSH (−) strains showed less survival in the presence of BA. Nevertheless, some authors did not confirm a correlation between BSH activity and bile tolerance (Ahn, Kim, Lim, Baek, & Kim, 2003; Moser & Savage, 2001) and, in addition, microorganisms of the GIT that do not exhibit BSH activity are still able to tolerate the presence of bile (Vinderola & Reinheimer, 2003).

Another theory suggests that the amino acid released by BSH from conjugated BA could be used as an electron acceptor resulting in a higher growth rate, as was observed by Van Eldere, Celis, De Pauw, Lesaffre, and Eyssen (1996) in a BSH active strain of *Clostridium*.

Remarkably, as afore mentioned, BSH enzyme has been described in pathogenic bacteria such as *Listeria monocytogenes* (Dussurget et al., 2002) and *Brucella abortus* (Delpino et al., 2007) and seems to play a role in the colonization and establishment of persistent infections of these strains. In *Listeria*, BSH levels increased under certain conditions such as low oxygen tensions prevalent in the host during infection. In addition, BSH mutants led to a substantial growth reduction of the bacteria exposed to BA (Dussurget et al., 2002). In *Brucella abortus* the deletion of *bsh* gene became the strains more sensitive to the presence of BA and affected its adhesiveness and internalization activity. Besides, Jarocki and Targonski (2013) reported the BA gel-forming ability of *Bifidobacterium* strains displaying BSH activity that could play a role in the colonization and persistence of these strains in the intestine.

A possible participation of BSH and related enzymes in disrupting the bacterial signalling mechanism in the quorum sensing has also been recently suggested (Mukherji & Prabhune, 2015).

In general, the evidence suggests that BSH enzymes play an important role for the intestinal microbiota, but also for pathogenic strains, contributing to a greater tolerance to the presence of bile, and could even represent an important factor in the initial survival and early colonization of the intestine of newborns (Joyce, Shanahan, et al., 2014).

6. Impact of bile salt hydrolase on the host metabolism

The importance of BSH in human health has been discussed extensively (Chand et al., 2017; Ettinger, MacDonald, Reid, & Burton, 2014; Long et al., 2017). One of major beneficial effects of BA deconjugation includes the lowering of serum cholesterol levels. However, it is now becoming clear that BSH is also linked to many host physiological processes. Additionally, as was mentioned, BA deconjugation is a prerequisite for further modifications that occur in the intestine (Li & Chiang, 2015). Secondary BA resulting from this process also act as potent agonists for the receptors involved in the signalling network, so, BSH activity could modify the energy metabolism in the host, as will be discussed below.

It is known that high serum cholesterol levels are strongly associated with atherosclerosis and cardiovascular diseases, the biggest cause of deaths in the world (Yusuf, Wood, Ralston, & Reddy, 2015). Among other mechanisms, such as assimilation of cholesterol during growth, numerous previous research papers related the ability of certain LAB a strains to reduce cholesterol levels with the BSH enzyme (Chand et al., 2017; Malpeli et al., 2015; Taranto, Sesma, de Ruiz Holgado, & de Valdez, 1997). For this reason, BSH activity is a desirable condition for probiotic selection. The proposed mechanism is as follows: the BSH enzyme acts on conjugated BA in the gut, releasing free BA that due to their lower solubility precipitate and are eliminated by the faeces. In Therefore, the recycling of BA is reduced and their concentration, which should remain constant, decreases. In this condition, the liver is forced to increase the *de novo* synthesis of more BA from endogenous cholesterol, decreasing its serum level. Moreover, changes in BA metabolism, by increased BSH activity, affect their efficiency to form micelles and, in consequence decrease the solubility and subsequent absorption of dietary lipids (mainly triglycerides and cholesterol) (Choi, Lew, Yeo, Nair Parvathy, & Liong, 2015; Jones, Tomaro-Duchesneau, Martoni, & Prakash, 2013).

Research with probiotic intake carried out in animal models correlated the increase in BSH activity with lowering of cholesterol. Among others, BSH (+) strains of *Pediococcus pentosaceus*, *L. reuteri*, *L. plantarum*, *L. salivarius*, *L. rhamnosus*, *B. longum* showed a significant reduction of total cholesterol levels, and in some cases a decrease in serum LDL lipoprotein and triglycerides (Choi et al., 2015; Damodharan, Lee, Palaniyandi, Yang, & Suh, 2015; Gu et al., 2014; Joyce et al., 2014; M. Taranto, Medici, Perdigon, Holgado, & Valdez, 2000). In an earlier experiment, the daily administration of a BSH-active strain of*L. reuteri* to pigs significantly lowered total and LDL-cholesterol concentrations (Klaver & Van Der Meer, 1993).Similar results were reported in another *in vivo* study done on pigs (Park et al., 2008). The administration of *L. reuteri* has a hypocholesterolemic effect both therapeutically and preventively in mice (Taranto 1998, 2000). Other authors (Sridevi, Srivastava, Khan, & Prabhune, 2009) also reported that the oral administration of immobilized BSH reduced 50% to 58% of serum cholesterol and 15% to 45% of triglycerides, depending on the dose in hypercholesterolemic Wistar rats. In a more recent study, Tsai et al. (2014) evaluated the potential of numerous lactic strains BSH (+) to reduce cholesterol in *in vitro* and *in vivo* assays performed in male hamsters, and three strains capable of reducing apo B secretion, the concentration of cholesterol in micelles and LDL and triglycerides serum levels were found. It is important to note that human BA composition has subtle differences with other experimental models. This may influence the extrapolation of existing work in experimental models to humans and should guide the selection of probiotics with appropriate deconjugation activities.

Several assays in humans seem to confirm the effects of BSH-active strains on cholesterol metabolism. In a 6 week-randomized clinical trial with a symbiotic capsule containing a BSH-active strain of *L. acidophilus* plus inulin, total cholesterol and LDL-cholesterol serum levels were significantly reduced compared to the control group (Ooi, Ahmad, Yuen, & Liong, 2010). Similar results were reported for *L. reuteri* NCIMB 30242 administered in capsules and in yogurt formulations to hypercholesterolemic adults for 9 weeks (Jones, Martoni, Parent, & Prakash, 2012). Additionally, the effect of daily consumption of a yogurt containing *L. reuteri* CRL 1098 for 4 weeks, separated by a washout period, on the lipid profile of hypercholesterolemic adults was also

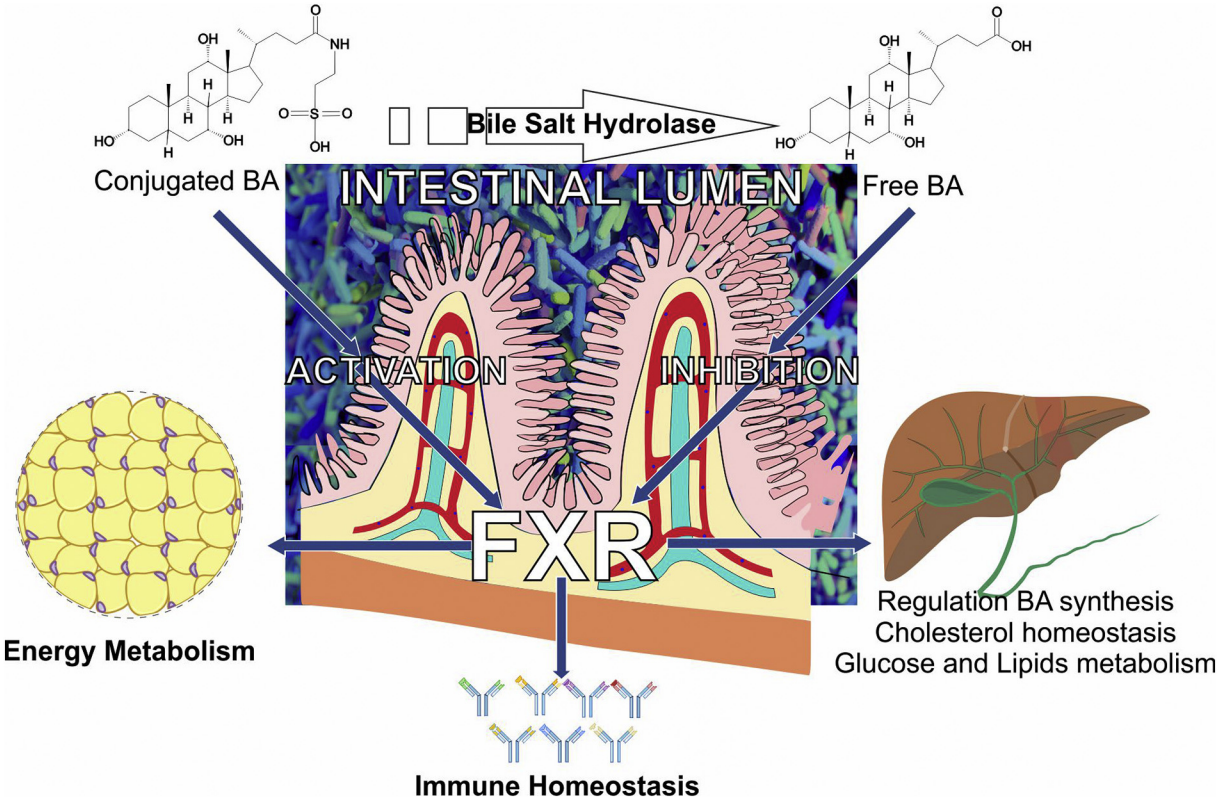

Fig. 3. Impact of bile salt hydrolase (BSH) and bile acids (BA) on the host metabolism. The major beneficial effect attributed to BSH enzyme is the lowering of serum cholesterol levels. The proposed mechanism is as follows: BSH enzyme acts on conjugated BA in the gut, releasing free BA that are eliminated by the faeces. Consequently, their concentration decreases forcing to liver to increase the *de novo* synthesis of more BA from endogenous cholesterol, decreasing its serum level. Besides their role as fat solubilizers, BA are now recognized as signalling molecules that act mainly through the nuclear farnesoid X receptor (FXR) to regulate lipid and glucose and energy metabolism. Besides, the hepatic BA synthesis is tightly regulated *via* a negative feedback mechanism that involves the FXR.

evaluated in a controlled clinical study (Malpeli et al., 2015). A significant reduction of total and LDL cholesterol was found in the group receiving *L. reuteri* compared to the placebo group, without changes in HDL-cholesterol and triglycerides levels.

Regarding the effect on weight gain, it was demonstrated that alterations in the gastrointestinal microbiota have caused obesity in mice and humans, and BSH enzyme plays a key role. In this sense, Joyce et al. (2014) reported that the controlled expression of lactic bacteria carrying a cloned BSH in the murine gut significantly altered BA serum levels and regulated the transcription of key genes involved in lipid and cholesterol metabolism, as well as overall gastrointestinal homeostasis and circadian rhythm in the liver and intestine. In summary, the expression of BSH enzyme significantly impacts on the systemic metabolic processes and adiposity in the host and results in a decrease in weight gain, as well as in serum cholesterol and liver triglycerides. These findings suggest that BSH enzyme is a potential target for weight regulation and could be applied in the design of an intervention strategy in the agricultural sector (Guban, Korver, Allison, & Tannock, 2006). In fact, the use of specific BSH inhibitors would be a promising alternative as a replacement of antibiotics for fattening animals (Chand et al., 2017).

However, some recent studies suggest that the alteration of microbiota with a decrease in BSH activity reduces obesity in mice through an FXR-mediated mechanism (Li et al., 2013) as will be discussed below, suggesting that there are multiple potential mechanisms by which microbiota modifies weight gain and that the effects may be strain dependent (Joyce 2014).

6.1. BSH inhibitors as growth promoters

One of the main concerns of agricultural animal producers is to improve growth performance. For several decades, antibiotic growth promoters (AGP) have been successfully used to achieve this goal. AGP are a group of antibiotics used at subtherapeutic levels to improve the average daily weight gain and feed efficiency in agricultural animals (Dibner & Richards, 2005; Wang et al., 2012). However, recent epidemiological studies related the use of AGP to the appearance of resistant bacterial strains that can be transmitted to humans, generating an important public health problem (Marshall & Levy, 2011). For this reason, there is a worldwide trend to prohibit the use of AGP in animals intended for food and, in consequence, effective alternatives to its use are now proposed (Smith, Zeng, & Lin, 2014).

Recent studies strongly suggest that the improvement in animal growth performance by the use of AGP is related to the inhibition of the BSH enzyme. In fact, several studies (Danzeisen, Kim, Isaacson, Tu, & Johnson, 2011; Dumonceaux, Hill, Hemmingsen, & Van Kessel, 2006; Guban et al., 2006; Lin, Hunkapiller, Layton, Chang, & Robbins, 2013) showed that AGP usage significantly reduced the population of *Lactobacillus* species, the major BSH producers in the small intestine (Begley et al., 2006). As was mentioned above, deconjugation of BA compromises lipid metabolism resulting in attenuated energy harvest. Based on these findings, BSH inhibitors are proposed as alternatives to AGP for enhanced production of food animals. In this sense, Wang et al. (2012) recognized copper and zinc compounds as potent BSH inhibitor from a panel of dietary compounds. In a more recent study (Smith et al., 2014), a high-throughput screening (HTS) system was applied to identify BSH inhibitors. Among others, riboflavin and phenethyl caffeate as well as a set of antibiotics, such as tetracycline and roxarsone, were selected and validated (Smith et al., 2014). These findings allowed the authors to propose that the direct inhibition of the BSH enzyme is a new potential AGP action mechanism.

Even though these results are promising, in the future it is necessary

to validate these findings with animal trials to determine the effects of BSH inhibitors on the growth performance, as well as the ecological and physiological impact of the inhibition of BSH activity on the host.

7. Role of bile acids in metabolic regulation

7.1. Bile acids as signalling molecules

Besides their role as fat solubilizers, BA are now recognized as signalling molecules that act through the nuclear farnesoid X receptor (FXR) and the G-protein-coupled receptor (TGR5) to regulate lipid and glucose metabolism (Fig. 3) (Chiang, 2013; Degirolamo et al., 2014; Devkota & Chang, 2015). As was described above, the hepatic BA synthesis is tightly regulated *via* a negative feedback mechanism that involves CYP7A1 gene transcription inhibition (Fig. 3) (Chiang, 2013). In fact, it is now clear that the nuclear receptor FXR plays a key role in this regulatory mechanism. FXR is a ligand-activated transcription factor whose most powerful activator is CDCA followed by CA, while ursodeoxycholic acid (UDCA) inhibits FXR activation (Gonzalez-Vazquez et al., 2015). Recently, the murine taurine-conjugated primary BA (TαMCA and TβMCA) were identified as FXR antagonists (Sayin et al., 2013). FXR is highly expressed in the liver and intestine because of its continuous exposure to high levels of BA (Lu et al., 2000).

The activation of hepatic FXR stimulates the secretion of BA into the intestine and promotes the transcription of the small heterodimer partner (SHP), which reduces the activity of the CYP7A1, key enzyme of BA synthesis (Chiang, 2013). Also, SHP inhibits reabsorption of BA in intestine, increasing its excretion. Besides, the stimulation of intestinal FXR by BA induces the increase of expression and secretion of fibroblast growth factor (FGF) 15 in mice (human homolog: FGF19) and consequently, the activation of a signal cascade that ends with the transcriptional inhibition of CYP7A1 (Inagaki et al., 2006). These findings demonstrate an enterohepatic signalling axis that links the gut BA sensing to the regulation of hepatic BA synthesis. This crosstalk has important repercussions on the metabolism of lipids and carbohydrates, as will be discussed below (Fig. 3) (Gonzalez-Vazquez et al., 2015; Lan, Haywood, & Dawson, 2013; Lan, Rao, Haywood, Kock, & Dawson, 2012).

The activation of SHP by FXR has been suggested to have a role in the hepatic regulation of triglycerides and very low-density lipoprotein (VLDL) biosynthesis. SHP interferes with the expression of regulator compounds of many genes and enzymes involved in triglyceride biosynthesis and cholesterol homeostasis, leading to the reduction in hepatic fat accumulation and serum triglyceride levels (Li et al., 2013). Also, it has been suggested that FXR induction increases the clearance of LDL-lipoprotein by enlarging its receptor-mediated uptake. In addition, some research suggests that FXR diminishes HDL serum levels. Some proposed mechanisms are: decrease in apo A1 synthesis, increase in the reverse cholesterol transport both by induction of the expression of scavenger receptors that participate in HDL clearance from peripheral tissues and the increase in its hepatic uptake (Chand et al., 2017; Chiang, 2013; de Aguiar Vallim et al., 2013).

The transmembrane G protein-coupled receptor TGR5 was reported as a ubiquitous receptor present in the gastrointestinal tract and attached glands including liver, human beta cells of pancreas where its activation increases insulin secretion. TGR5 was also found in brown adipose tissue, in the central nervous system of mice, and human skeletal muscle (Hansen, Sonne, & Knop, 2014). The most potent natural agonist of TGR5 is the secondary bile acid BA, LCA, and to a lesser extent DCA, CDCA and CA. TGR5 activation promotes incretin hormone secretion and possibly energy expenditure (Kumar et al., 2012). In addition to their effect on the receptors mentioned, BA are also potent agonists of the vitamin D receptor and pregnane X receptors, which are involved in the regulation of bile and drug metabolism in the liver (Chand et al., 2017).

7.2. Relationship between, bile acids, gut microbiome and metabolism

There is increasing evidence that relates gut microbiota to energy metabolism, with special impact on lipid and glucose metabolisms (Devkota & Chang, 2015). Recent studies showed that germ-free mice have an increment in conjugated BA, especially tauro-β-muricholic acid (T-βMCA) compared to conventionally raised ones (Swann et al., 2011) probably due to the absence of BSH. Without BA hydrolysis, the subsequent microbial transformations do not occur, *i.e.* TβMCA cannot be metabolized in the absence of gut bacteria. In this sense, Li et al. (2013) reported that the administration of the antioxidant tempol reduces obesity in mice, by alteration in the gut microbiome and inhibition of BSH (+) strains. These events lead to the accumulation of TβMCA, recognized as antagonist of FXR receptor. The inhibition of FXR signalling in the intestine results in a lower diet-induced obesity in mice, showing a biochemical link between the microbiome and metabolism (Li et al., 2013). It is important to note that the modification of the composition of BA by the microbiota critically determines their hydrophobicity and therefore affects the intestinal absorption of nutrients and the activity of BA as signal molecules. However, it is not currently known whether TβMCA can antagonize FXR signalling in humans and if these findings can be extrapolated.

In addition, it was reported that the genetic *diet1* locus could confer some protection against diet-induced hypercholesterolemia enhancing BA metabolism (Reue, Lee, & Vergnes, 2014; Vergnes, Lee, Chin, Auwerx, & Reue, 2013) but the precise mechanism involved is still not completely elucidated. *Diet1* encodes a 236 KDa protein that is expressed in enterocytes of the small intestine but is not detected in the mouse liver, muscle or adipose tissues. Mutation in the *diet1* gene in a mouse strain (B6By) made it resistant to atherosclerosis and diet-induced hyperlipidaemia. When B6By mice were fed with an atherogenic diet, mRNA expression profiles revealed elevated expression of key BA synthesis proteins, including cholesterol 7α-hydroxylase and FXR receptor levels in B6By liver. In agreement with this, increased levels of BA were found in blood, faeces and urine, while plasma cholesterol levels were strongly reduced suggesting that this locus is responsible for the resistance to hypercholesterolemia in B6By mice (Li & Chiang, 2015).

On the other hand, some dietary carbohydrates can only be metabolized by the gut microbiota and as a result, short chain fatty acids (SCFAs) such as acetate, butyrate, and propionate are produced (Topping & Clifton, 2001). SCFAs are essential host energy sources and are now recognized as signal transduction molecules *via* G-protein coupled receptors (Blad, Tang, & Offermanns, 2012). In addition, SCFAs act as epigenetic regulators of gene expression by the inhibition of histone deacetylase (Kasubuchi, Hasegawa, Hiramatsu, Ichimura, & Kimura, 2015). Recent evidence suggests that dietary fibre and the gut microbial-derived SCFAs exert multiple beneficial effects on the host energy metabolism not only by improving the intestinal environment, but also by directly affecting various host peripheral tissues (Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016).

Also, the gut microbiota could modify the release of gut hormones such as glucagon-like peptide 1 (GLP1) (Long et al., 2017;; Vrieze et al., 2010) as well as glucocorticoid metabolism (Ridlon et al., 2016). In fact, recent studies reported that a bacterial metabolite of CDCA can modulate the host endogenous active steroid hormone levels (Odermatt & Klusonova, 2015).

As was described above, BA are antimicrobial agents mainly through their detergent properties but also by activation of FXR. In fact, induction of the nuclear receptor by BA promotes genes involved in enteroprotection and inhibition of bacterial over proliferation (Inagaki et al., 2006). In turn, gut microbes also modify BA metabolism and alter their composition.

Taken together, these investigations suggest that modifications in the microbiota lead to changes in the composition of BA with important consequences for the host metabolism.

7.3. Bile acid sequestrants and the treatment of type-2 diabetes mellitus

The BA sequestrants constitute a class of positively charged polymers (at intestinal pH) that bind electrostatically to BA to form a non-absorbable complex that leads to an interruption of BA enterohepatic circulation (Hansen et al., 2014). Consequently, the synthesis of BA from cholesterol increases and serum low-density lipoprotein cholesterol decreases, which is the reason why they were widely used for the treatment of hypercholesterolemia before the appearance of statin drugs (Handelsman, 2011). In recent years, interest in BA sequestrants has re-emerged due to their potential to improve glycaemic levels in patients with type-2 diabetes; however, the mechanisms involved in their glucose-lowering effect are not fully clarified. Many previous research papers showed that the concomitant consumption of statin, or other anti-diabetic drugs, with BA sequestrant improved glycaemic control in type-2 diabetes mellitus with reduction in plasma glucose and HbA1c, besides reductions in LDL cholesterol (Beysen et al., 2012; Sonne, Hansen, & Knop, 2014). Based on these findings, recently colesevelam, a BA sequestrant, was included in the diabetes management algorithm in addition to metformin or other hypoglycaemic therapies (Garber et al., 2013).

8. Other host effects of microbial BSH activity

Several reports established that gut microbiota and some probiotic bacteria provide protection against *Giardia duodenalis* infection through mechanisms that are not completely understood. Remarkably, recently Allain et al. (2017) reported that a BSH from L. *johnsonii* La1 is involved in the anti-giardia effect of the strain both *in vitro* and *in vivo*. The effect would be related to the generation of deconjugated BA, which were toxic to the parasite. In another work, the anti-giardial effects of many lactobacilli strains from different sources were tested (Allain et al., 2018). L. *johnsonii* and *L. gasseri* CNCM I-4884 showed anti-parasitic ability in *in vitro* assays, which was related to their BSH activity. Surprisingly, in a mice model only L. *gasseri* CNCM I-4884 strain was able to significantly inhibit parasite growth and faecal excretion of cysts, while with La1 strain no effect was recorded (Allain et al., 2018).

Finally, some research reported possible detrimental effects due to the action of BSH. The increase of deconjugated BA is often associated with malabsorption of lipids and steatorrhea due to their lower emulsifying capacity (Honda, Ikegami, & Matsuzaki, 2017). Besides, it has been proposed that BSH activity may also play a major role in gallstone formation and also in colon carcinogenesis. Finally, the increase of more toxic free BA could inhibit the growth of many intestinal bacteria leading to an imbalance in gut microbiota (Choi et al., 2015; Islam et al., 2011).

9. Conclusions

It is now clear that the gut is a complex ecosystem where intestinal microorganisms and BA interact with important consequences for both the host physiology and the microbiota.

These majority bile compounds influence microbial growth, physiology and ecology and in turn, gut microbes modify bile acid metabolism and composition. This relationship has consequences for the health of the host.

The understanding of the mechanisms involved in the tolerance to bile in intestinal bacteria still presents key points without elucidation. However, *omics* technologies have contributed to the identification of novel markers involved in bacterial mechanisms to survive the harsh conditions of the gastrointestinal tract. It is worth noting that it is necessary to integrate more than one single *omic* analysis to unravel the complexity of this stress. A multi-omic approach would yield a more complete picture of the biological processes occurring in bacteria at a given condition. Remarkably, the existence of common mechanisms to tolerate bile stress in bacteria belonging to phylogenetically different groups suggests the existence of convergent evolutionary tools to compete within the intestinal environment. Among them, the BSH enzyme occupies a prominent place. This enzyme seems to be promiscuous in relation to its multiple functions, participation in both complex regulatory mechanisms of microbial and host metabolic pathways. Its cholesterol lowering ability is well documented and, in addition, nowadays bile acids are recognized as molecular signals involved in the regulation of lipid, glucose and energy metabolism.

In conclusion, greater knowledge of the mechanisms involved in the bacterial tolerance to bile and about the links between microbial bile modifications and host physiology will allow us to intervene successfully in the modification of the microbiota in order to improve the health of the host.

Acknowledgments

This research has been supported by grants from Consejo Nacional de Investigaciones Científicas y Tecnológicas (PIP2015-530) and Agencia Nacional de Promoción Científica y Tecnológica (PICT2015-1705).

A special thanks to Dr. Guillermo Vega Lopez for his generous collaboration in the edition of the figures.

References

de Aguiar Vallim, T. Q., Tarling, E. J., & Edwards, P. A. (2013). Pleiotropic roles of bile acids in metabolism. *Cell Metabolism, 17*(5), 657–669.

Ahn, Y. T., Kim, G. B., Lim, K. S., Baek, Y. J., & Kim, H. U. (2003). Deconjugation of bile salts by Lactobacillus acidophilus isolates. *International Dairy Journal, 13*(4), 303–311.

Alcantara, C., & Zuniga, M. (2012). Proteomic and transcriptomic analysis of the response to bile stress of Lactobacillus casei BL23. [Research Support, Non-U.S. Gov't]. *Microbiology, 158*, 1206–1218. http://dx.doi.org/10.1099/mic.0.055657-0.

Allain, T., Chaouch, S., Thomas, M., Travers, M.-A., Vallee, I., Langella, P., ... Bermudez-Humaran, L. G. (2018). Bile salt hydrolase activities: A novel target to screen anti-Giardia lactobacilli? *Frontiers in Microbiology, 9*, 89.

Allain, T., Chaouch, S., Thomas, M., Vallée, I., Buret, A. G., Langella, P., ... Florent, I. (2017). Bile-salt-hydrolases from the probiotic strain *Lactobacillus johnsonii* La1 mediate anti-giardial activity in vitro and in vivo. *Frontiers in Microbiology, 8*, 2707.

Alp, G., & Aslim, B. (2010). Relationship between the resistance to bile salts and low pH with exopolysaccharide (EPS) production of Bifidobacterium spp. isolated from infants feces and breast milk. *Anaerobe, 16*(2), 101–105.

An, H., Douillard, F. P., Wang, G., Zhai, Z., Yang, J., Song, S., ... Zhang, B. (2014). Integrated transcriptomic and proteomic analysis of the bile stress response in a centenarian-originated probiotic *Bifidobacterium longum* BBMN68. *Molecular & Cellular Proteomics, 13*(10), 2558–2572.

Begley, M., Gahan, C. G., & Hill, C. (2005). The interaction between bacteria and bile. [Research Support, Non-U.S. Gov't Review]. *FEMS Microbiology Reviews, 29*(4), 625–651. http://dx.doi.org/10.1016/j.femsre.2004.09.003.

Begley, M., Hill, C., & Gahan, C. G. (2006). Bile salt hydrolase activity in probiotics. *Applied and Environmental Microbiology, 72*(3), 1729–1738.

Beysen, C., Murphy, E., Deines, K., Chan, M., Tsang, E., Glass, A., ... Hellerstein, M. (2012). Effect of bile acid sequestrants on glucose metabolism, hepatic de novo lipogenesis, and cholesterol and bile acid kinetics in type 2 diabetes: A randomised controlled study. *Diabetologia, 55*(2), 432–442.

Blad, C. C., Tang, C., & Offermanns, S. (2012). G protein-coupled receptors for energy metabolites as new therapeutic targets. *Nature Reviews Drug Discovery, 11*(8), 603.

Bøhle, L. A., Færgestad, E. M., Veiseth-Kent, E., Steinmoen, H., Nes, I. F., Eijsink, V. G., & Mathiesen, G. (2010). Identification of proteins related to the stress response in *Enterococcus faecalis* V583 caused by bovine bile. *Proteome Science, 8*(1), 37.

Bron, P. A., Marco, M., Hoffer, S. M., Van Mullekom, E., De Vos, W. M., & Kleerebezem, M. (2004). Genetic characterization of the bile salt response in *Lactobacillus plantarum* and analysis of responsive promoters in vitro and in situ in the gastrointestinal tract. *Journal of Bacteriology, 186*(23), 7829–7835.

Burns, P., Reinheimer, J., & Vinderola, G. (2011). Impact of bile salt adaptation of *Lactobacillus delbrueckii* subsp. *lactis* 200 on its interaction capacity with the gut. *Research in Microbiology, 162*(8), 782–790.

Burns, P., Sánchez, B., Vinderola, G., Ruas-Madiedo, P., Ruiz, L., Margolles, A., ... Clara, G. (2010). Inside the adaptation process of *Lactobacillus delbrueckii* subsp. *lactis* to bile. *International Journal of Food Microbiology, 142*(1–2), 132–141.

Bustos, A., de Valdez, G. F., Raya, R., & Taranto, M. P. (2016). Genetic characterization and gene expression of bile salt hydrolase (bsh) from *Lactobacillus reuteri* CRL 1098, a probiotic strain. *International Journal of Genomics, Proteomics, Metabolomics & Bioinformatics (IJGPMB), 1*(1), 1–8.

Bustos, A. Y., de Valdez, G. F., Raya, R., de Almeida, A. M., Fadda, S., & Taranto, M. P. (2015). Proteomic analysis of the probiotic *Lactobacillus reuteri* CRL1098 reveals novel tolerance biomarkers to bile acid-induced stress. *Food Research International, 77*, 599–607.

Bustos, A. Y., Raya, R., Bru, E., de Valdez, G. F., & Taranto, M. P. (2011). Application of fluorescent techniques to evaluate the survival of probiotic lactobacilli to bile acid. *Biotechnology Letters, 33*(7), 1389–1394.

Bustos, A. Y., Raya, R., de Valdez, G. F., & Taranto, M. P. (2011). Efflux of bile acids in lactobacillus reuteri is mediated by ATP. *Biotechnology Letters, 33*(11), 2265.

Bustos, A. Y., Saavedra, L., de Valdez, G. F., Raya, R. R., & Taranto, M. P. (2012). Relationship between bile salt hydrolase activity, changes in the internal pH and tolerance to bile acids in lactic acid bacteria. *Biotechnology Letters, 34*(8), 1511–1518.

Chae, J., Valeriano, V., Kim, G. B., & Kang, D. K. (2013). Molecular cloning, characterization and comparison of bile salt hydrolases from *Lactobacillus johnsonii* PF01. *Journal of Applied Microbiology, 114*(1), 121–133.

Chand, D., Avinash, V. S., Yadav, Y., Pundle, A. V., Suresh, C. G., & Ramasamy, S. (2017). Molecular features of bile salt hydrolases and relevance in human health. [Research Support, Non-U.S. Gov't Review]. *Biochimica et biophysica acta, 1861*(1 Pt A), 2981–2991. http://dx.doi.org/10.1016/j.bbagen.2016.09.024.

Chand, D., Panigrahi, P., Varshney, N. K., Ramasamy, S., & Suresh, C. (2018). Structure and function of a highly active bile salt hydrolase (BSH) from *Enterococcus faecalis* and post-translational processing of BSH enzymes. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1866*(4), 507–518.

Chen, S. W., Hsu, J. T., Chou, Y. A., & Wang, H. T. (2018). The application of digestive tract lactic acid bacteria with high esterase activity for zearalenone detoxification. *Journal of the Science of Food and Agriculture*. http://dx.doi.org/10.1002/jsfa.8904.

Chiang, J. Y. (2009). Bile acids: Regulation of synthesis. *Journal of Lipid Research, 50*(10), 1955–1966.

Chiang, J. Y. (2013). Bile acid metabolism and signaling. [Research Support, N.I.H., Extramural Review]. *Comprehensive Physiology, 3*(3), 1191–1212. http://dx.doi.org/10.1002/cphy.c120023.

Choi, S. B., Lew, L. C., Yeo, S. K., Nair Parvathy, S., & Liong, M. T. (2015). Probiotics and the BSH-related cholesterol lowering mechanism: A Jekyll and Hyde scenario. [Research Support, Non-U.S. Gov't Review]. *Critical Reviews in Biotechnology, 35*(3), 392–401. http://dx.doi.org/10.3109/07388551.2014.889077.

Damodharan, K., Lee, Y. S., Palaniyandi, S. A., Yang, S. H., & Suh, J.-W. (2015). Preliminary probiotic and technological characterization of Pediococcus pentosaceus strain KID7 and in vivo assessment of its cholesterol-lowering activity. *Frontiers in Microbiology, 6*, 768.

Danzeisen, J. L., Kim, H. B., Isaacson, R. E., Tu, Z. J., & Johnson, T. J. (2011). Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment. *PLoS One, 6*(11), 27949.

De Valdez, G., Martos, G., Taranto, M., Lorca, G., Oliver, G., & de Ruiz Holgado, A. (1997). Influence of bile on β-galactosidase activity and cell viability of *Lactobacillus reuteri* when subjected to freeze-drying. *Journal of Dairy Science, 80*(9), 1955–1958.

Dean, M., Cervellati, C., Casanova, E., Squerzanti, M., Lanzara, V., Medici, A., ... Bergamini, C. M. (2002). Characterization of cholylglycine hydrolase from a bile-adapted strain of *Xanthomonas maltophilia* and its application for quantitative hydrolysis of conjugated bile salts. *Applied and Environmental Microbiology, 68*(6), 3126–3128.

Degirolamo, C., Rainaldi, S., Bovenga, F., Murzilli, S., & Moschetta, A. (2014). Microbiota modification with probiotics induces hepatic bile acid synthesis via downregulation of the Fxr-Fgf15 axis in mice. [Research Support, Non-U.S. Gov't]. *Cell Reports, 7*(1), 12–18. http://dx.doi.org/10.1016/j.celrep.2014.02.032.

Delpino, M. V., Marchesini, M. I., Estein, S. M., Comerci, D. J., Cassataro, J., Fossati, C. A., & Baldi, P. C. (2007). A bile salt hydrolase of *Brucella abortus* contributes to the establishment of a successful infection through the oral route in mice. *Infection and Immunity, 75*(1), 299–305.

Devkota, S., & Chang, E. B. (2015). Interactions between diet, bile acid metabolism, gut microbiota, and inflammatory bowel diseases. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Digestive Diseases, 33*(3), 351–356. http://dx.doi.org/10.1159/000371687.

Dibner, J., & Richards, J. (2005). Antibiotic growth promoters in agriculture: History and mode of action. *Poultry Science, 84*(4), 634–643.

Dietschy, J. M., & Turley, S. D. (2002). Control of cholesterol turnover in the mouse. *Journal of Biological Chemistry, 277*(6), 3801–3804.

Duary, R. K., Batish, V. K., & Grover, S. (2012). Relative gene expression of bile salt hydrolase and surface proteins in two putative indigenous *Lactobacillus plantarum* strains under in vitro gut conditions. *Molecular Biology Reports, 39*(3), 2541–2552.

Dumonceaux, T. J., Hill, J. E., Hemmingsen, S. M., & Van Kessel, A. G. (2006). Characterization of intestinal microbiota and response to dietary virginiamycin supplementation in the broiler chicken. *Applied and Environmental Microbiology, 72*(4), 2815–2823.

Dussurget, O., Cabanes, D., Dehoux, P., Lecuit, M., Buchrieser, C., Glaser, P., & Cossart, P. (2002). Listeria monocytogenes bile salt hydrolase is a PrfA-regulated virulence factor involved in the intestinal and hepatic phases of listeriosis. *Molecular Microbiology, 45*(4), 1095–1106.

Elkins, C. A., & Savage, D. C. (2003). CbsT2 from *Lactobacillus johnsonii* 100-100 is a transport protein of the major facilitator superfamily that facilitates bile acid antiport. *Journal of Molecular Microbiology and Biotechnology, 6*(2), 76–87.

Ettinger, G., MacDonald, K., Reid, G., & Burton, J. P. (2014). The influence of the human microbiome and probiotics on cardiovascular health. [Review]. *Gut Microbes, 5*(6), 719–728. http://dx.doi.org/10.4161/19490976.2014.983775.

Fanning, S., Hall, L. J., Cronin, M., Zomer, A., Macsharry, J., Goulding, D., ... Dougan, G. (2012). Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. *Proceedings of the National Academy of Sciences, 109*(6), 2108–2113.

Franz, C. M., Specht, I., Haberer, P., & Holzapfel, W. H. (2001). Bile salt hydrolase activity of enterococci isolated from food: Screening and quantitative determination. *Journal of Food Protection, 64*(5), 725–729.

Garber, A., Abrahamson, M., Barzilay, J., Blonde, L., Bloomgarden, Z., Bush, M., ... Garvey, W. (2013). AACE comprehensive diabetes management algorithm 2013. *Endocrine Practice, 19*(2), 327–336.

Gómez Zavaglia, A., Kociubinski, G., Pérez, P., Disalvo, E., & De Antoni, G. (2002). Effect of bile on the lipid composition and surface properties of bifidobacteria. *Journal of Applied Microbiology, 93*(5), 794–799.

Gonzalez-Vazquez, R., Azaola-Espinosa, A., Mayorga-Reyes, L., Reyes-Nava, L. A., Shah, N. P., & Rivera-Espinoza, Y. (2015). Isolation, identification and partial characterization of a *Lactobacillus casei* strain with bile salt hydrolase activity from Pulque. [Research Support, Non-U.S. Gov't]. *Probiotics and Antimicrobial Proteins, 7*(4), 242–248. http://dx.doi.org/10.1007/s12602-015-9202-x.

Gopal-Srivastava, R., & Hylemon, P. B. (1988). Purification and characterization of bile salt hydrolase from Clostridium perfringens. *Journal of Lipid Research, 29*(8), 1079–1085.

Grill, J., Cayuela, C., Antoine, J., & Schneider, F. (2000). Isolation and characterization of a *Lactobacillus amylovorus* mutant depleted in conjugated bile salt hydrolase activity: Relation between activity and bile salt resistance. *Journal of Applied Microbiology, 89*(4), 553–563.

Gu, X.-C., Luo, X.-G., Wang, C.-X., Ma, D.-Y., Wang, Y., He, Y.-Y., ... Zhang, T.-C. (2014). Cloning and analysis of bile salt hydrolase genes from lactobacillus plantarum CGMCC no. 8198. *Biotechnology Letters, 36*(5), 975–983.

Guban, J., Korver, D., Allison, G., & Tannock, G. (2006). Relationship of dietary antimicrobial drug administration with broiler performance, decreased population levels of *Lactobacillus* salivarius, and reduced bile salt deconjugation in the ileum of broiler chickens. *Poultry Science, 85*(12), 2186–2194.

Gueimonde, M., Garrigues, C., van Sinderen, D., Clara, G., & Margolles, A. (2009). Bile-inducible efflux transporter from *Bifidobacterium longum* NCC2705, conferring bile resistance. *Applied and Environmental Microbiology, 75*(10), 3153–3160.

Hamon, E., Horvatovich, P., Izquierdo, E., Bringel, F., Marchioni, E., Aoudé-Werner, D., & Ennahar, S. (2011). Comparative proteomic analysis of *Lactobacillus plantarum* for the identification of key proteins in bile tolerance. *BMC Microbiology, 11*(1), 63.

Handelsman, Y. (2011). Role of bile acid sequestrants in the treatment of type 2 diabetes. [Review]. *Diabetes Care, 34*(Suppl. 2), S244–S250. http://dx.doi.org/10.2337/dc11-s237.

Hansen, M., Sonne, D. P., & Knop, F. K. (2014). Bile acid sequestrants: Glucose-lowering mechanisms and efficacy in type 2 diabetes. [Review]. *Current Diabetes Reports, 14*(5), 482. http://dx.doi.org/10.1007/s11892-014-0482-4.

Honda, A., Ikegami, T., & Matsuzaki, Y. (2017). Intestinal digestion and absorption. *Bile acids in gastroenterology* (pp. 27–41). Springer.

Inagaki, T., Moschetta, A., Lee, Y.-K., Peng, L., Zhao, G., Downes, M., ... Repa, J. J. (2006). Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proceedings of the National Academy of Sciences of the United States of America, 103*(10), 3920–3925.

Islam, K. S., Fukiya, S., Hagio, M., Fujii, N., Ishizuka, S., Ooka, T., ... Yokota, A. (2011). Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. *Gastroenterology, 141*(5), 1773–1781.

Jarocki, P., & Targonski, Z. (2013). Genetic diversity of bile salt hydrolases among human intestinal bifidobacteria. [Research Support, Non-U.S. Gov't]. *Current Microbiology, 67*(3), 286–292. http://dx.doi.org/10.1007/s00284-013-0362-1.

Jayashree, S., Pooja, S., Pushpanathan, M., Rajendhran, J., & Gunasekaran, P. (2014). Identification and characterization of bile salt hydrolase genes from the genome of *Lactobacillus fermentum* MTCC 8711. *Applied Biochemistry and Biotechnology, 174*(2), 855–866.

Jones, B. V., Begley, M., Hill, C., Gahan, C. G., & Marchesi, J. R. (2008). Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proceedings of the National Academy of Sciences, 105*(36), 13580–13585.

Jones, L. J., Carballido-López, R., & Errington, J. (2001). Control of cell shape in bacteria: Helical, actin-like filaments in Bacillus subtilis. *Cell, 104*(6), 913–922.

Jones, M. L., Martoni, C. J., Parent, M., & Prakash, S. (2012). Cholesterol-lowering efficacy of a microencapsulated bile salt hydrolase-active *Lactobacillus reuteri* NCIMB 30242 yoghurt formulation in hypercholesterolaemic adults. *British Journal of Nutrition, 107*(10), 1505–1513.

Jones, M. L., Tomaro-Duchesneau, C., Martoni, C. J., & Prakash, S. (2013). Cholesterol lowering with bile salt hydrolase-active probiotic bacteria, mechanism of action, clinical evidence, and future direction for heart health applications. *Expert Opinion on Biological Therapy, 13*(5), 631–642.

Joyce, S. A., Macsharry, J., Casey, P. G., Kinsella, M., Murphy, E. F., Shanahan, F., ... Gahan, C. G. (2014). Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut. [Research Support, Non-U.S. Gov't]. *Proceedings of the National Academy of Sciences of the United States of America, 111*(20), 7421–7426. http://dx.doi.org/10.1073/pnas.1323599111.

Joyce, S. A., Shanahan, F., Hill, C., & Gahan, C. G. (2014). Bacterial bile salt hydrolase in host metabolism: Potential for influencing gastrointestinal microbe-host crosstalk. [Research Support, Non-U.S. Gov't Review]. *Gut Microbes, 5*(5), 669–674. http://dx.doi.org/10.4161/19490976.2014.969986.

Kasubuchi, M., Hasegawa, S., Hiramatsu, T., Ichimura, A., & Kimura, I. (2015). Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients, 7*(4), 2839–2849.

Kaur, G., Ali, S. A., Kumar, S., Mohanty, A. K., & Behare, P. (2017). Label-free quantitative proteomic analysis of *Lactobacillus fermentum* NCDC 400 during bile salt exposure. *Journal of Proteomics, 167*, 36–45.

Kawamoto, K., Horibe, I., & Uchida, K. (1989). Purification and characterization of a new hydrolase for conjugated bile acids, chenodeoxycholyltaurine hydrolase, from Bacteroides vulgatus. *The Journal of Biochemistry, 106*(6), 1049–1053.

Kim, G., & Lee, B. H. (2005). Biochemical and molecular insights into bile salt hydrolase in the gastrointestinal microflora-a review. *Asian-Australasian Journal of Animal*

Sciences, 18(10), 1505.
Klaver, F., & Van Der Meer, R. (1993). The assumed assimilation of cholesterol by Lactobacilli and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity. *Applied and Environmental Microbiology, 59*(4), 1120–1124.
Kociubinski, G., Gómez Zavaglia, A., Pérez, P. F., Disalvo, E. A., & De Antoni, G. L. (2002). Effect of bile components on the surface properties of bifidobacteria. *Journal of Dairy Research, 69*(02), http://dx.doi.org/10.1017/s0022029901005313.
Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell, 165*(6), 1332–1345.
Koskenniemi, K., Laakso, K., Koponen, J., Kankainen, M., Greco, D., Auvinen, P., ... Salusjärvi, T. (2011). Proteomics and transcriptomics characterization of bile stress response in probiotic *Lactobacillus rhamnosus* GG. *Molecular & Cellular Proteomics, 10*(2), M110 002741.
Kumar, D. P., Rajagopal, S., Mahavadi, S., Mirshahi, F., Grider, J. R., Murthy, K. S., & Sanyal, A. J. (2012). Activation of transmembrane bile acid receptor TGR5 stimulates insulin secretion in pancreatic β cells. *Biochemical and Biophysical Research Communications, 427*(3), 600–605.
Kumar, R. S., Brannigan, J. A., Prabhune, A. A., Pundle, A. V., Dodson, G. G., Dodson, E. J., & Suresh, C. (2006). Structural and functional analysis of a conjugated bile salt hydrolase from Bifidobacterium longum reveals an evolutionary relationship with penicillin V acylase. *Journal of Biological Chemistry, 281*(43), 32516–32525.
Kurdi, P., Kawanishi, K., Mizutani, K., & Yokota, A. (2006). Mechanism of growth inhibition by free bile acids in lactobacilli and bifidobacteria. *Journal of Bacteriology, 188*(5), 1979–1986.
Lan, T., Haywood, J., & Dawson, P. A. (2013). Inhibition of ileal apical but not basolateral bile acid transport reduces atherosclerosis in apoE−/− mice. *Atherosclerosis, 229*(2), 374–380.
Lan, T., Rao, A., Haywood, J., Kock, N. D., & Dawson, P. A. (2012). Mouse organic solute transporter alpha deficiency alters FGF15 expression and bile acid metabolism. *Journal of Hepatology, 57*(2), 359–365.
Lee, K., Lee, H.-G., & Choi, Y.-J. (2008). Proteomic analysis of the effect of bile salts on the intestinal and probiotic bacterium *Lactobacillus reuteri*. *Journal of Biotechnology, 137*(1), 14–19.
Leverrier, P., Dimova, D., Pichereau, V., Auffray, Y., Boyaval, P., & Jan, G. (2003). Susceptibility and adaptive response to bile salts in *Propionibacterium freudenreichii*: Physiological and proteomic analysis. *Applied and Environmental Microbiology, 69*(7), 3809–3818.
Li, F., Jiang, C., Krausz, K. W., Li, Y., Albert, I., Hao, H., ... Gonzalez, F. J. (2013). Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. *Nature Communications, 4*, 2384.
Li, J., Jin, L., Song, Z., Cai, Y., Wang, X., Lin, X., ... Shang, J. (2018). Taxonomic profiling and populational patterns of bacterial bile salt hydrolase (BSH) genes on worldwide human gut microbiome. *bioRxiv*, 260794.
Li, T., & Chiang, J. Y. (2014). Bile acid signaling in metabolic disease and drug therapy. *Pharmacological Reviews, 66*(4), 948–983.
Li, T., & Chiang, J. Y. (2015). Bile acids as metabolic regulators. *Current Opinion in Gastroenterology, 31*(2), 159–165.
Lilley, K. S., Razzaq, A., & Dupree, P. (2002). Two-dimensional gel electrophoresis: Recent advances in sample preparation, detection and quantitation. *Current Opinion in Chemical Biology, 6*(1), 46–50.
Lin, J., Cagliero, C., Guo, B., Barton, Y.-W., Maurel, M.-C., Payot, S., & Zhang, Q. (2005). Bile salts modulate expression of the CmeABC multidrug efflux pump in *Campylobacter jejuni*. *Journal of Bacteriology, 187*(21), 7417–7424.
Lin, J., Hunkapiller, A. A., Layton, A. C., Chang, Y.-J., & Robbins, K. R. (2013). Response of intestinal microbiota to antibiotic growth promoters in chickens. *Foodborne Pathogens and Disease, 10*(4), 331–337.
Long, S. L., Gahan, C. G., & Joyce, S. A. (2017). Interactions between gut bacteria and bile in health and disease. *Molecular Aspects of Medicine, 56*, 54–65.
Lu, T. T., Makishima, M., Repa, J. J., Schoonjans, K., Kerr, T. A., Auwerx, J., & Mangelsdorf, D. J. (2000). Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Molecular Cell, 6*(3), 507–515.
Lv, L.-X., Yan, R., Shi, H.-Y., Shi, D., Fang, D.-Q., Jiang, H.-Y., & Gu, S.-L. (2017). Integrated transcriptomic and proteomic analysis of the bile stress response in probiotic Lactobacillus salivarius LI01. *Journal of proteomics, 150*, 216–229.
Maldonado-Valderrama, J., Wilde, P., Macierzanka, A., & Mackie, A. (2011). The role of bile salts in digestion. *Advances in Colloid and Interface Science, 165*(1), 36–46.
Malpeli, A., Taranto, M. P., Cravero, R. C., Tavella, M., Fasano, V., Vicentin, D., ... de Valdez, G. F. (2015). Effect of daily consumption of *Lactobacillus reuteri* CRL 1098 on cholesterol reduction in hypercholesterolemic subjects. *Food and Nutrition Sciences, 6*(17), 1583.
Margolles, A., Florez, A. B., Moreno, J. A., Van Sinderen, D., & Clara, G. (2006). Two membrane proteins from *Bifidobacterium breve* UCC2003 constitute an ABC-type multidrug transporter. *Microbiology, 152*(12), 3497–3505.
Margolles, A., García, L., Sánchez, B., Gueimonde, M., & de los Reyes-Gavilán, C. G. (2003). Characterisation of a Bifidobacterium strain with acquired resistance to cholate—A preliminary study. *International Journal of Food Microbiology, 82*(2), 191–198.
Margolles, A., Gueimonde, M., & Sánchez, B. (2012). Genome sequence of the Antarctic psychrophile bacterium Planococcus antarcticus DSM 14505. *Journal of Bacteriology, 194*(16), 4465.
Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: Impacts on human health. *Clinical Microbiology Reviews, 24*(4), 718–733.
Martoni, C. J., Labbe, A., Ganopolsky, J. G., Prakash, S., & Jones, M. L. (2015). Changes in bile acids, FGF-19 and sterol absorption in response to bile salt hydrolase active L. reuteri NCIMB 30242. [Clinical TrialResearch Support, Non-U.S. Gov't]. *Gut Microbes, 6*(1), 57–65. http://dx.doi.org/10.1080/19490976.2015.1005474.
Merritt, M. E., & Donaldson, J. R. (2009). Effect of bile salts on the DNA and membrane integrity of enteric bacteria. *Journal of Medical Microbiology, 58*(12), 1533–1541.
Moser, S. A., & Savage, D. C. (2001). Bile salt hydrolase activity and resistance to toxicity of conjugated bile salts are unrelated properties in lactobacilli. *Applied and Environmental Microbiology, 67*(8), 3476–3480.
Mukherji, R., & Prabhune, A. (2015). Possible correlation between bile salt hydrolysis and AHL deamidation: Staphylococcus epidermidis RM1, a potent quorum quencher and bile salt hydrolase producer. *Applied Biochemistry and Biotechnology, 176*(1), 140–150.
Noriega, L., Cuevas, I., Margolles, A., & Clara, G. (2006). Deconjugation and bile salts hydrolase activity by Bifidobacterium strains with acquired resistance to bile. *International Dairy Journal, 16*(8), 850–855.
Odermatt, A., & Klusonova, P. (2015). 11β-Hydroxysteroid dehydrogenase 1: Regeneration of active glucocorticoids is only part of the story. *The Journal of Steroid Biochemistry and Molecular Biology, 151*, 85–92.
Ooi, L.-G., Ahmad, R., Yuen, K.-H., & Liong, M.-T. (2010). Lactobacillus acidophilus CHO-220 and inulin reduced plasma total cholesterol and low-density lipoprotein cholesterol via alteration of lipid transporters. *Journal of Dairy Science, 93*(11), 5048–5058.
Panigrahi, P., Sule, M., Sharma, R., Ramasamy, S., & Suresh, C. (2014). An improved method for specificity annotation shows a distinct evolutionary divergence among the microbial enzymes of the cholylglycine hydrolase family. *Microbiology, 160*(6), 1162–1174.
Park, Y. H., Kim, J. G., Shin, Y. W., Kim, H. S., Kim, Y.-J., Chun, T., ... Whang, K. Y. (2008). Effects of *Lactobacillus acidophilus* 43121 and a mixture of *Lactobacillus casei* and *Bifidobacterium longum* on the serum cholesterol level and fecal sterol excretion in hypercholesterolemia-induced pigs. *Bioscience, Biotechnology, and Biochemistry, 72*(2), 595–600.
Pfeiler, E. A., & Klaenhammer, T. R. (2009). Role of transporter proteins in bile tolerance of *Lactobacillus acidophilus*. [Research Support, Non-U.S. Gov't]. *Applied and Environmental Microbiology, 75*(18), 6013–6016. http://dx.doi.org/10.1128/AEM.00495-09.
Piddock, L. J. (2006). Multidrug-resistance efflux pumps? not just for resistance. *Nature Reviews Microbiology, 4*(8), 629.
Price, C. E., Reid, S. J., Driessen, A. J., & Abratt, V. R. (2006). The *Bifidobacterium longum* NCIMB 702259T ctr gene codes for a novel cholate transporter. *Applied and Environmental Microbiology, 72*(1), 923–926.
Prouty, A. M., Van Velkinburgh, J. C., & Gunn, J. S. (2002). Salmonella enterica serovar Typhimurium resistance to bile: Identification and characterization of the tolQRA cluster. *Journal of Bacteriology, 184*(5), 1270–1276.
Pumbwe, L., Skilbeck, C. A., Nakano, V., Avila-Campos, M. J., Piazza, R. M., & Wexler, H. M. (2007). Bile salts enhance bacterial co-aggregation, bacterial-intestinal epithelial cell adhesion, biofilm formation and antimicrobial resistance of *Bacteroides fragilis*. *Microbial Pathogenesis, 43*(2–3), 78–87.
Reue, K., Lee, J. M., & Vergnes, L. (2014). Regulation of bile acid homeostasis by the intestinal Diet1–FGF15/19 axis. *Current Opinion in Lipidology, 25*(2), 140.
Reyes-Nava, L., & Rivera-Espinoza, Y. (2014). Isolation sources of bile salt hydrolase-microorganisms. *Herald Journal of Agriculture and Food Science Research, 3*, 49–54.
Ridlon, J. M., & Bajaj, J. S. (2015). The human gut sterolbiome: Bile acid-microbiome endocrine aspects and therapeutics. [Review]. *Acta pharmaceutica Sinica. B, 5*(2), 99–105. http://dx.doi.org/10.1016/j.apsb.2015.01.006.
Ridlon, J. M., Harris, S. C., Bhowmik, S., Kang, D. J., & Hylemon, P. B. (2016). Consequences of bile salt biotransformations by intestinal bacteria. [Research Support, U.S. Gov't, Non-P.H.S. Review]. *Gut Microbes, 7*(1), 22–39. http://dx.doi.org/10.1080/19490976.2015.1127483.
Ruas-Madiedo, P., Gueimonde, M., Arigoni, F., Clara, G., & Margolles, A. (2009). Bile affects the synthesis of exopolysaccharides by *Bifidobacterium animalis*. *Applied and Environmental Microbiology, 75*(4), 1204–1207.
Ruiz, L., Coute, Y., Sanchez, B., Clara, G., Sanchez, J.-C., & Margolles, A. (2009). The cell-envelope proteome of *Bifidobacterium longum* in an in vitro bile environment. *Microbiology, 155*(3), 957–967.
Ruiz, L., Hidalgo, C., Blanco-Míguez, A., Lourenço, A., Sánchez, B., & Margolles, A. (2016). Tackling probiotic and gut microbiota functionality through proteomics. *Journal of Proteomics, 147*, 28–39.
Ruiz, L., Margolles, A., & Sanchez, B. (2013). Bile resistance mechanisms in Lactobacillus and Bifidobacterium. *Frontiers in Microbiology, 4*, 396. http://dx.doi.org/10.3389/fmicb.2013.00396.
Ruiz, L., O'Connell-Motherway, M., Zomer, A., de los Reyes-Gavilán, C. G., Margolles, A., & van Sinderen, D. (2012). A bile-inducible membrane protein mediates bifidobacterial bile resistance. *Microbial Biotechnology, 5*(4), 523–535.
Ruiz, L., Ruas-Madiedo, P., Gueimonde, M., los Reyes-Gavilán, C. G., Margolles, A., & Sánchez, B. (2011). How do bifidobacteria counteract environmental challenges? Mechanisms involved and physiological consequences. *Genes & Nutrition, 6*(3), 307.
Ruiz, L., Zomer, A., O'Connell-Motherway, M., van Sinderen, D., & Margolles, A. (2011). Discovering novel bile protection systems in Bifidobacterium breve UCC2003 through functional genomics. *Applied and Environmental Microbiology, 78*(4), 1123–1131.
Russell, D. W. (2003). The enzymes, regulation, and genetics of bile acid synthesis. *Annual Review of Biochemistry, 72*(1), 137–174.
Sánchez, B., Champomier-Vergès, M.-C., Anglade, P., Baraige, F., Clara, G., Margolles, A., & Zagorec, M. (2005). Proteomic analysis of global changes in protein expression during bile salt exposure of *Bifidobacterium longum* NCIMB 8809. *Journal of Bacteriology, 187*(16), 5799–5808.
Sánchez, B., Champomier-Verges, M.-C., Stuer-Lauridsen, B., Ruas-Madiedo, P., Anglade, P., Baraige, F., ... Margolles, A. (2007). Adaptation and response of Bifidobacterium animalis subsp. lactis to bile: A proteomic and physiological approach. *Applied and*

Environmental Microbiology, 73(21), 6757–6767.
Sánchez, B., Noriega, L., Ruas-Madiedo, P., de los Reyes-Gavilán, C. G., & Margolles, A. (2004). Acquired resistance to bile increases fructose-6-phosphate phosphoketolase activity in Bifidobacterium. *FEMS Microbiology Letters, 235*(1), 35–41.
Sanchez, B., Ruiz, L., & Margolles, A. (2008). Proteomics of stress response in Bifidobacterium. *Frontiers in Bioscience: a Journal and Virtual Library, 13*, 6905–6919.
Šárka, H., Milada, P., & Kateřina, D. (2018). Importance of microbial defence systems to bile salts and mechanisms of serum cholesterol reduction. *Biotechnology Advances, 36*, 682–690.
Sayin, S. I., Wahlström, A., Felin, J., Jäntti, S., Marschall, H.-U., Bamberg, K., & Bäckhed, F. (2013). Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell metabolism, 17*(2), 225–235.
Siciliano, R. A., & Mazzeo, M. F. (2012). Molecular mechanisms of probiotic action: A proteomic perspective. *Current Opinion in Microbiology, 15*(3), 390–396.
Sistrunk, J. R., Nickerson, K. P., Chanin, R. B., Rasko, D. A., & Faherty, C. S. (2016). Survival of the fittest: How bacterial pathogens utilize bile to enhance infection. *Clinical Microbiology Reviews, 29*(4), 819–836.
Smith, K., Zeng, X., & Lin, J. (2014). Discovery of bile salt hydrolase inhibitors using an efficient high-throughput screening system. *PLoS One, 9*(1), e85344.
Sonne, D. P., Hansen, M., & Knop, F. K. (2014). Mechanisms in endocrinology: Bile acid sequestrants in type 2 diabetes: Potential effects on GLP1 secretion. *European Journal of Endocrinology, 171*(2), R47–R65.
Sridevi, N., Srivastava, S., Khan, B. M., & Prabhune, A. A. (2009). Characterization of the smallest dimeric bile salt hydrolase from a thermophile Brevibacillus sp. *Extremophiles, 13*(2), 363–370.
Stellwag, E., & Hylemon, P. (1976). Purification and characterization of bile salt hydrolase from *Bacteroides fragilis* subsp. *fragilis*. *Biochimica et Biophysica Acta (BBA)-Enzymology, 452*(1), 165–176.
Šušković, J., Kos, B., Matošić, S., & Besendorfer, V. (2000). The effect of bile salts on survival and morphology of a potential probiotic strain *Lactobacillus acidophilus* M92. *World Journal of Microbiology and Biotechnology, 16*(7), 673–678.
Swann, J. R., Want, E. J., Geier, F. M., Spagou, K., Wilson, I. D., Sidaway, J. E., ... Holmes, E. (2011). Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. *Proceedings of the National Academy of Sciences, 108*(Supplement 1), 4523–4530.
Taranto, M., Fernandez Murga, M., Lorca, G., & Valdez, G.d. (2003). Bile salts and cholesterol induce changes in the lipid cell membrane of *Lactobacillus reuteri*. *Journal of Applied Microbiology, 95*(1), 86–91.
Taranto, M., Medici, M., Perdigon, G., Holgado, A. R., & Valdez, G. (2000). Effect of *Lactobacillus reuteri* on the prevention of hypercholesterolemia in mice. *Journal of Dairy Science, 83*(3), 401–403.
Taranto, M., Sesma, F., de Ruiz Holgado, A. P., & de Valdez, G. F. (1997). Bile salts hydrolase plays a key role on cholesterol removal by *Lactobacillus reuteri*. *Biotechnology Letters, 19*(9), 845–847.
Taranto, M., Medici, M., Perdigon, G., Holgado, A. R., & Valdez, G. (1998). Evidence for hypocholesterolemic effect of Lactobacillus reuteri in hypercholesterolemic mice. *Journal of dairy science, 81*(9), 2336–2340.
Taranto, M. P., Perez-Martinez, G., & de Valdez, G. F. (2006). Effect of bile acid on the cell membrane functionality of lactic acid bacteria for oral administration. *Research in Microbiology, 157*(8), 720–725.
Thanassi, D. G., Cheng, L. W., & Nikaido, H. (1997). Active efflux of bile salts by Escherichia coli. *Journal of Bacteriology, 179*(8), 2512–2518.
Topping, D. L., & Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews, 81*(3), 1031–1064.
Tsai, C. C., Lin, P. P., Hsieh, Y. M., Zhang, Z. Y., Wu, H. C., & Huang, C. C. (2014). Cholesterol-lowering potentials of lactic acid bacteria based on bile-salt hydrolase activity and effect of potent strains on cholesterol metabolism in vitro and in vivo. [Research Support, Non-U.S. Gov't]. *The Scientific World Journal, 2014*(690752), http://dx.doi.org/10.1155/2014/690752.
Turroni, F., Ventura, M., Buttó, L. F., Duranti, S., O'Toole, P. W., Motherway, M. O. C., & van Sinderen, D. (2014). Molecular dialogue between the human gut microbiota and the host: A *Lactobacillus* and *Bifidobacterium* perspective. *Cellular and Molecular Life Sciences, 71*(2), 183–203.
Van Eldere, J., Celis, P., De Pauw, G., Lesaffre, E., & Eyssen, H. (1996). Tauroconjugation of cholic acid stimulates 7 alpha-dehydroxylation by fecal bacteria. *Applied and Environmental Microbiology, 62*(2), 656–661.
Vergnes, L., Lee, J. M., Chin, R. G., Auwerx, J., & Reue, K. (2013). Diet1 functions in the FGF15/19 enterohepatic signaling axis to modulate bile acid and lipid levels. [Research Support, N.I.H., ExtramuralResearch Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. *Cell Metabolism, 17*(6), 916–928. http://dx.doi.org/10.1016/j.cmet.2013.04.007.
Vinderola, C., & Reinheimer, J. (2003). Lactic acid starter and probiotic bacteria: A comparative "in vitro" study of probiotic characteristics and biological barrier resistance. *Food Research International, 36*(9–10), 895–904.
Vrieze, A., Holleman, F., Zoetendal, E., De Vos, W., Hoekstra, J., & Nieuwdorp, M. (2010). The environment within: How gut microbiota may influence metabolism and body composition. *Diabetologia, 53*(4), 606–613.
Wang, Z., Zeng, X., Mo, Y., Smith, K., Guo, Y., & Lin, J. (2012). Identification and characterization of a bile salt hydrolase from *Lactobacillus salivarius* for development of novel alternatives to antibiotic growth promoters. *Applied and Environmental Microbiology, 78*(24), 8795–8802.
Whitehead, K., Versalovic, J., Roos, S., & Britton, R. A. (2008). Genomic and genetic characterization of the bile stress response of probiotic *Lactobacillus reuteri* ATCC 55730. *Applied and Environmental Microbiology, 74*(6), 1812–1819.
Wu, R., Sun, Z., Wu, J., Meng, H., & Zhang, H. (2010). Effect of bile salts stress on protein synthesis of Lactobacillus casei Zhang revealed by 2-dimensional gel electrophoresis. *Journal of dairy science, 93*(8), 3858–3868.
Wu, R., Zhang, W., Sun, T., Wu, J., Yue, X., Meng, H., & Zhang, H. (2011). Proteomic analysis of responses of a new probiotic bacterium *Lactobacillus casei* Zhang to low acid stress. *International Journal of Food Microbiology, 147*(3), 181–187.
Yokota, A., Veenstra, M., Kurdi, P., van Veen, H. W., & Konings, W. N. (2000). Cholate resistance in *Lactococcus lactis* is mediated by an ATP-dependent multispecific organic anion transporter. *Journal of Bacteriology, 182*(18), 5196–5201.
Young, K. D. (2010). Bacterial shape: Two-dimensional questions and possibilities. *Annual Review of Microbiology, 64*, 223–240.
Yuan, J., Wang, B., Sun, Z., Bo, X., Yuan, X., He, X., ... Jiang, Z. (2007). Analysis of host-inducing proteome changes in *Bifidobacterium longum* NCC2705 grown in vivo. *Journal of Proteome Research, 7*(01), 375–385.
Yusuf, S., Wood, D., Ralston, J., & Reddy, K. S. (2015). The World Heart Federation's vision for worldwide cardiovascular disease prevention. *The Lancet, 386*(9991), 399–402.
Zárate, G., Gonzalez, S., Chaia, A. P., & Oliver, G. (2000). Effect of bile on the beta-galactosidase activity of dairy propionibacteria. *Le Lait, 80*(2), 267–276.