Microbiol. Biotechnol. Lett. (2022), 50(1), 102–109
http://dx.doi.org/10.48022/mbl.2111.11010
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Challenging the Hypothesis of *de novo* Biosynthesis of Bile Acids by Marine Bacteria

Felipe Gonzalo Tueros[1], Mostafa M. Hashim Ellabaan[1], Marcus Henricsson[2], Ruben Vazquez-Uribe[1], Fredrik Bäckhed[2], and Morten Otto Alexander Sommer[1]*

[1]*Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kgs. Lyngby 2800, Denmark*
[2]*Department of Molecular and Clinical Medicine/Wallenberg Laboratory, Institute of Medicine, University of Gothenburg and Sahlgrenska University Hospital, Gothenburg 41345, Sweden*

Received: November 16, 2021 / Revised: January 11, 2022 / Accepted: January 19, 2022

Bile acids are essential molecules produced by vertebrates that are involved in several physiological roles, including the uptake of nutrients. Bacterial isolates capable of producing bile acids *de novo* have been identified and characterized. Such isolates may provide access to novel biochemical pathways suitable for the design of microbial cell factories. Here, we further characterized the ability of *Maribacter dokdonensis*, *Dokdonia donghaensis*, and *Myroides pelagicus* to produce bile acids. Contrary to previous reports, we did not observe *de novo* production of bile acids by these isolates. Instead, we found that these isolates deconjugated the amino acid moiety of bile acids present in the growth medium used in previous reports. Through genomic analysis, we identified putative bile salt hydrolases, which could be responsible for the different bile acid modifications observed. Our results challenge the hypothesis of *de novo* microbial bile acid production, while further demonstrating the diverse capacity of bacteria to modify bile acids.

Keywords: **Bile acids, bile salt hydrolase, marine bacteria, bile acid modification, bile acid production**

Introduction

Bile acids are amphipathic molecules that aid the digestion and uptake of fatty acids and lipo-soluble vitamins in vertebrates [1]. The amphipathic nature is a result of the opposing hydroxyl radicals in the structure, therefore, acting as a detergent and helping solubilize hydrophobic compounds by the formation of micelles [2]. These molecules are synthetized *de novo* from cholesterol in the liver of various vertebrates and are further modified by bacterial species in the gut. The first and most important modification of bile acids, also known as the "gatekeeper of bile acid metabolism", involves deconjugating the amino acid moiety from the steroid body, which is performed by bile salt hydrolase (BSH) positive bacteria [3], allowing further metabolism of bile acids by other species. BSHs have been shown to be enriched in gut microbiome species; however, their presence in marine metagenomes have been functionally identified as well [4].

Reports of bacteria capable of producing bile acids were published by several groups (Table 1), some claiming that bile acids were synthetized *de novo* by their isolates. Bile acids are a product of a complex metabolic pathway in vertebrates, where at least 14 different enzymes are involved [5]. So far, there is no clear explanation of the beneficial role these molecules could have for free-living marine bacteria producing them nor their role in the environment.

Implications of microbial production of bile acids are of high biotechnological interest, as several diseases are influenced by endogenous levels of these molecules [6].

***Corresponding author**
Tel.: +45 21 51 83 40
E-mail: msom@bio.dtu.dk

Table 1. Reported species capable of producing bile acids or bile acid derivatives.

Strain	Isolated from	Condition	Media	Peptone	Reference
Myroides odoratus	Marine sample	Aerobic	Marine Broth 2216	5 g/l	10
Myroides odoramitimus	Marine sample	Aerobic	Marine Broth 2216	5 g/l	10
Myroides pelagicus SM1	Marine sample	Aerobic	Marine Broth 2216	5 g/l	10
Dokdonia donghaensis	Sea water	Aerobic	Marine Broth 2216	5 g/l	11
Polaribacter dokdonensis	Sea water	Aerobic	Marine Broth 2216	5 g/l	11
Donghaena dokdonensis	Sea water	Aerobic	Marine Broth 2216	5 g/l	11
Maribacter dokdonensis	Sea water	Aerobic	Marine Broth 2216	5 g/l	11
Hahella chejuensis	Sea water	Aerobic	Marine Broth 2216	5 g/l	11
Rhodococcus marinonascens	Sea water	Aerobic	Marine Broth 2216	5 g/l	11
Penicillum sp.	young stems of *Scurrula atroprupurea*	Aerobic	Glucose Peptone medium	10 g/l	17
Streptococcus faecium	Soil	Anaerobic	C-SPY	5 g/l	18
Aeromicrobium halocynthiae	*Halocynthia roretzi* (Marine ascidian)	Aerobic	A1+C Broth	4 g/l	22
Psychrobacter sp	*Stelletta sp* (Marine ascidian)	Aerobic	Zobell 2216 Broth	5 g/l	23
Hasllibacter halocynthiae	*Halocynthia roretzi* (Marine ascidian)	Aerobic	A1+C broth	4 g/l	24

Bile acid variants have also proven to be therapeutic and are the base of different treatments to address pathologies affecting the liver and gallbladder [7, 8]. Thus, elucidating a microbial biosynthetic pathway could enable the development and implementation of sustainable bile acid production, without being limited by the supply of animal derived products.

In the present study, we investigate the capabilities of three previously characterized bile acid producing strains: *Maribacter dokdonensis, Dokdonia donghaensis* and *Myroides pelagicus* (*Myroides* SM-1). However, our observations indicate that bile acids are not produced by these bacteria but rather modified by the possible use of endogenous bile salt hydrolases.

Materials and Methods

Strains and culture conditions

Marine isolates *Maribacter dokdonensis* (DSM No. 17201) and *D. donghaensis* (DSM No. 17200) were ordered from DSMZ catalogue, *Myroides pelagicus* (KCTC No. 12661) was ordered from the Korean Collection for Type Cultures (KCTC). All marine strains were ordered as freeze-dried cultures and were reconstituted in the recommended media. Cultivations were carried out in Marine Broth (MB) Difco 2216 and modified citrate media [9, 10]. Strains were incubated at 30℃ and 250 RPM in a shaking incubator. The identity of all strains was confirmed by 16S Sanger sequencing, to rule out any contamination. *E. coli* TOP10 was acquired from Invitrogen and followed same culturing conditions.

Fermentations

Fermentations were performed as previously described [10, 11]. A single colony was inoculated into 5 ml of MB or modified citrate media and was grown until saturation for 48 h. Saturated culture was then used to inoculate 250 ml of MB or modified citrate media in 1 L baffled shake flask. Culture samples were taken every 8 h for OD and total bile acid quantification. Fermentations were carried out in biological triplicates for all strains, including a negative control strain (*E. coli*). For modified citrate media fermentations, the presence of cholesterol was tested, in order to decipher whether bile acids were synthetized *de novo* using cholesterol. Stock concentration of cholesterol was dissolved in absolute ethanol and supplemented in the media at 1 g/l, according to previous study [10]. Same sampling strategy as marine broth was followed.

Fluorometric quantification of total bile acids

Fluorometric quantification of total bile acids from media supernatants was performed using "Bile Acid Assay Kit" (Sigma-Aldrich) and following the manufacturer's instructions. Biological triplicates were used, all samples were diluted (1:4) and processed simultaneously.

LC-MS/MS quantification of bile acids

50 µl of bacterial broth were extracted with 3 volumes of methanol containing deuterated internal standards (d_4-TCA, d_4-GCA, d_4-GCDCA, d_4-GUDCA, d_4-GLCA, d_4-UDCA, d_4-CDCA, d_4-LCA; 50 nM of each). After 10 min of vortex and 10 min of centrifugation at 20 000 g, the supernatant was diluted 1:50 in methanol:water [1:1]. Bile acids were analyzed using ultra-performance liquid chromatography-tandem mass spectrometry (UPLCMS/MS) according to previous work [12]. Briefly, after injection (5 µl) the bile acids were separated on a C18 column (1.7 µ, 2.1 × 100 mm; Kinetex, USA) using water with 7.5 mM ammonium acetate and 0.019% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). The chromatographic separation started with 1 minute isocratic separation at 20%B. The B-phase was then increased to 35% for 4 min. During the next 10 min the B-phase was increased to 100%. The B-phase was held at 100% for 3.5 min before returning to 20%. The total runtime was 20 min. Bile acids were detected using multiple reaction monitoring (MRM) in negative mode on a QTRAP 5500 mass spectrometer (Sciex, Canada).

Identification of putative bile salt hydrolases

Putative BSHs were identified using the COG database [13] (COG3049), which has been associated with bile acid salt hydrolase [4]. For the genomes considered in this study, we extracted the ORFs using GenMarkS [14], ORFs were subsequently blasted against the bile acid salt hydrolase proteins using TBLASTN. Multiple alignment of the translated ORFs were performed using MUSCLE [15]. The Newick tree was generated from the alignment profiles via fasttree [16]. The tree figure generated via figtree (http://tree.bio.ed.ac.uk/software/figtree/).

Statistical analysis and software tools

Statistical analysis was carried out in GraphPad Prism 7 (USA. www.graphpad.com). Figures were created using Biorender (BioRender.com).

Results

Undetectable bile acid production by bacteria in Marine Broth Difco 2216

For determining whether marine isolates *M. dokdonensis, D. donghaensis* and *M. pelagicus* had bile acid production capabilities, fermentations in MB broth were carried out, keeping careful similarity to the setup described in the original publications [10, 11]. Surprisingly, fluorometric quantification analysis of the supernatants from different time points (24 and 48 h) did not show any significant difference compared to the 0 h sample, among all the strains tested, including the negative control (Fig. 1). Interestingly, 0 hour samples from all strains contained high amounts of bile acids (≈15 µM), including the negative control, suggesting presence of bile acids in the initial culture media (Marine Broth Difco 2216). Furthermore, we observed no difference in terms of total bile acid concentrations among the strains, including our negative control (*E. coli*) (Fig. 1).

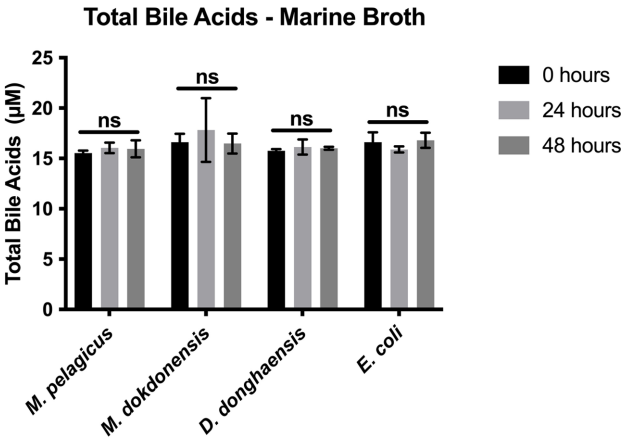

Fig. 1. Fluorometric bile acid quantification from marine broth fermentations. Each column represents the mean and error bars represent the standard deviation. Biological triplicates were analyzed for each strain. Results were compared to 0 h of each strain using Two-way ANOVA, $p < 0.05$.

Bile acids are not produced *de novo* from cholesterol

To decipher whether bile acids were produced *de novo* from cholesterol [10], marine strains were grown on modified citrate media supplemented with cholesterol [10]. From all the strains tested, only *M. pelagicus* and *E. coli* (negative control) were capable of growing in this media. The addition of cholesterol did not affect growth parameters significantly (Fig. S1). However, no bile acids were observed from any of the samples taken along the fermentation.

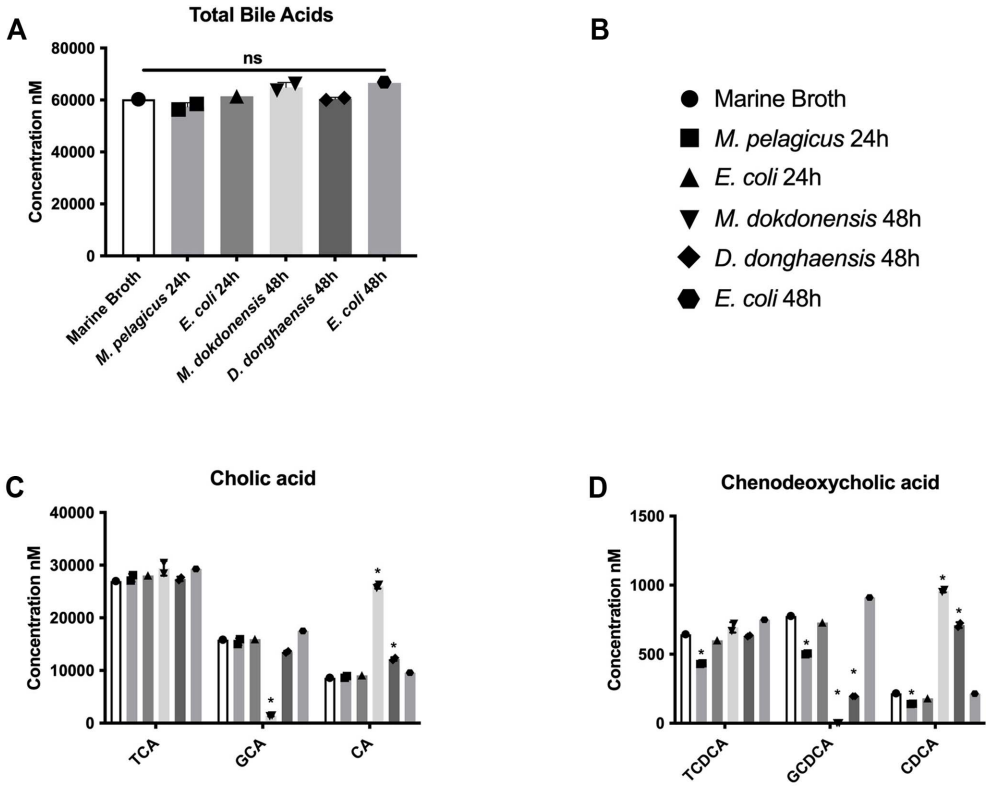

Fig. 2. Quantification of total and primary bile acids using LC-MS/MS. (A) Total bile acids. (B) Legends for the different data shown. (C and D) Conjugated and unconjugated profiles of primary bile acids, cholic and chenodeoxycholic acid. Taurocholic acid (TCA), glycocholic acid (GCA), cholic acid (CA), taurochenodeoxycholic acid (TCDCA), glycochenodeoxycholic acid (GCDCA), chenodeoxycholic acid (CDCA). Each column represents the mean and error bars represent the standard deviation. Biological duplicates were analyzed for each marine strain, only one replicate was analyzed for *E. coli* and marine broth samples. All comparisons were made against MB broth, Two-way ANOVA, $p < 0.05$.

Marine Isolates deconjugate bile acids present in Marine Broth

In order to analyze the bile acid profiles of the different samples and compare to previous reports [10, 11], samples were analyzed using liquid chromatography coupled with mass spectrometry (LC-MS/MS). No significant difference was observed in the concentration of total bile acids, validating previous bile acid quantification by fluorometric analysis (Fig. 2A). Even though no production of bile acids took place, modification of these molecules was variable among the marine strains investigated. *M. dokdonensis* showed the most activity hydrolyzing amide bonds from glycine conjugates of primary (Figs. 2C–D) and secondary bile acids (Figs. 3C–D). *D. donghaensis* and *M. pelagicus* were also capable of performing this modification. On the other hand, taurine conjugates did not seem to be greatly affected by *M. dokdonensis* and *D. donghaensis*, with the exception of taurolithocholic acid, in which *D. donghaensis* was able to deconjugate roughly 25%. *M. pelagicus*, on the contrary, had activity against most of these conjugates. None of the strains tested seemed to be able to deconjugate taurocholic acid, a comprehensive overview of all modification can be seen in Table 2. In the case of *M. dokdonensis* and *D. donghaensis*, the decrease in conjugated bile acids correlates to the increase of the deconjugated fractions, validating the bioconversion of these molecules.

Putative bile salt hydrolases identified in marine isolates

To determine whether the deconjugation of bile acids observed could be a result of endogenous bile salt hydrolases, bioinformatic analysis was performed. Six different putative bile salt hydrolases (BSH)s homologs

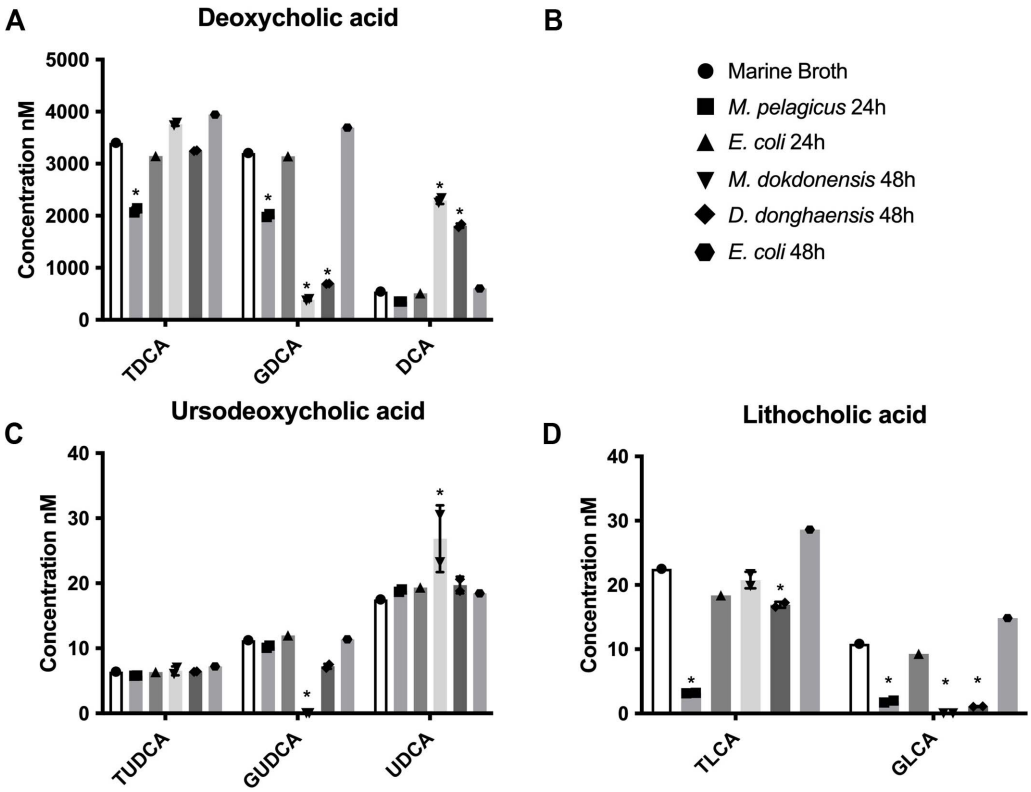

Fig. 3. Quantification of secondary bile acids using LC-MS/MS. (A, C and D) Conjugated and unconjugated profiles of secondary bile acids, deoxycholic, ursodeoxycholic and lithocholic acid. (B) Legends for the different data shown. taurodeoxycholic acid (TDCA), glycodeoxycholic acid (GDCA), deoxycholic acid (DCA), tauroursodeoxycholic acid (TUDCA), glycoursodeoxycholic acid (GUDCA), ursodeoxycholic acid (UDCA), taurolithocholic acid (TLCA), glycolithocholic acid (GLCA). Each column represents the mean and error bars represent the standard deviation. Biological duplicates were analyzed for each marine strain, only one replicate was analyzed for *E. coli* and marine broth samples. All comparisons were made against MB broth, Two-way ANOVA, $p < 0.05$.

Table 2. Deconjugation capacity of marine bacteria.

Bile acid	Specie	Deconjugation of glycine conjugate (%)	Deconjugation of taurine conjugate (%)
Cholic acid	*M. dokdonensis*	91.28	0
	D. donghaensis	14.69	0
	M. pelagicus	2.29	0
Chenodeoxycholic acid	*M. dokdonensis*	99.99	0
	D. donghaensis	74.86	1.79
	M. pelagicus	35.32	32.93
Deoxycholic acid	*M. dokdonensis*	88.02	0
	D. donghaensis	78.39	4.45
	M. pelagicus	37.58	38.11
Lithocholic acid	*M. dokdonensis*	100	7.81
	D. donghaensis	90.06	25.89
	M. pelagicus	17.17	85.90
Ursodeoxycholic acid	*M. dokdonensis*	100	0
	D. donghaensis	35.69	0
	M. pelagicus	8.8	9.8

http://dx.doi.org/10.48022/mbl.2111.11010

Table 3. Putative BSH genes identified in marine isolates.

Species	Putative BSH accession	Reference BSH accession	Species	AA identity (%)	E-value
D. donghaensis	NZ_CP015125.1	YP_006258575.1	*Solitalea canadensis* DSM 3403 (*Sphingobacteriaceae*)	46.215	1.03e-87
M. pelagicus	NZ_WMJY01000026.1	YP_004317200.1	*Sphingobacterium* sp. 21 (*Sphingobacteriaceae*)	55.620	1.48e-149
M. pelagicus	NZ_WMJY01000026.1	YP_004580371.1	*Lacinutrix* sp. 5H-3-7-4 (*Flavobacteriaceae*)	56.262	0
M. dokdonensis	NZ_FNTB01000001.1	YP_004163530.1	*Cellulophaga algicola* DSM 14237 (*Flavobacteriaceae*)	36.250	1.79e-06
M. dokdonensis	NZ_FNTB01000001.1	YP_004163530.1	*Cellulophaga algicola* DSM 14237 (*Flavobacteriaceae*)	34.940	5.10e-07
M. dokdonensis	NZ_FNTB01000001.1	YP_004163530.1	*Cellulophaga algicola* DSM 14237 (*Flavobacteriaceae*)	40.789	1.97e-09

were identified in total (Table 3). Only one putative BSH gene was identified in *D. donghaensis,* while two and three were identified in *M. pelagicus* and *M. dokdonensis,* respectively. Reference genes with highest similarity to the identified putative BSHs included BSHs from soil, fresh and marine water environments, found in isolates of *Sphingobacteriaceae* and *Flavobacteriaceae*. Lowest and highest identity (34.9% and 56.2) were observed for a putative BSHs from *M. dokdonensis* and *M. pelagicus,* respectively.

Discussion

Numerous studies have reported production of bile acids and derivatives by marine bacteria (Table 1), and a fungal specie [17]. Surprisingly, there has not been follow up studies trying to identify and uncover the bacterial or fungal biosynthetic routes for producing these metabolites, even though this would have important relevance in the biotechnological and biomedical fields.

The production of bile acids compromises a lengthy and complex pathway, which is both energy demanding and toxic to some extent, due to the production of hydrophobic molecules with surfactant properties. The fact that there might be organisms capable of this significant sacrifice would be possible, only if the advantages of producing such molecules are substantially higher for their survival or for competing in their given environment. Many theories have been formulated in order to explain the production of these molecules by these species, mostly relating to availability of insoluble carbon sources [11], competitive inhibition and symbiotic relations with insects and fishes [10]. However, there has not been any study up to date confirming these mechanisms.

Data from our study shows no significant increase of bile acids compared to marine broth alone. Intriguingly, most of the studies mentioned do not measure total bile acids from marine broth Difco 2216 alone, assuming bile acids come only from the different bacterial species. Nevertheless, having bile acids in culture media is not uncommon, considering the different components used in the formulation and their origin. Most probably, bile acids identified here derive from the peptone fraction (Fig. S1). Further communication with the supplier confirmed that the composition of the Marine Broth Difco 2216 has not changed over the last 20 years and that the source of peptone is bovine and porcine. With regards to modified citrate media fermentations and *de novo* bile acid biosynthesis from cholesterol by *M. pelagicus* [10], we believe that the bile acids seen by the authors, most probably, come from contamination by the inoculum fraction used, possibly done in Marine Broth. Further investigation of additional strains was thought to be irrelevant, as most studies have used media composed of peptone or used Marine Broth Difco 2216 for their respective experiments.

While we did not observe *de novo* bile acid production,

bile acid modification capabilities were present in all strains, accounting for the deconjugation of taurine and glycine from the bile acid body. It is likely that different bile acid profiles observed in previous studies could be a result of differential BSH substrate preference, depending on the strain used. In addition, the lack of total bile acid quantifications, accounting for other types of bile acids, can easily give rise to an inadequate assessment, concluding that bacterial species have different bile acid production profiles.

Bacterial species investigated in this study showed capabilities to de-conjugate mostly the glycine fraction of primary and secondary bile acids. Our results (Tables 2 and 3) indicate that this activity might be attributed to endogenous bile salt hydrolases (BSH)s. A previous study has shown that BSHs are highly abundant and diverse in metagenomic samples from different environments, including marine sources [4]. Furthermore, BSHs have been studied in detail in probiotic strains, as it appears to be an important factor for determining colonization capability in the gut environment [19]. Up to date, there is no concrete piece of evidence outlining the role of BSHs in free living bacteria, however, studies have pointed out their close similarity, in terms of sequence identity and enzymatic activity, to penicillin V acylases (PVA) [11, 20]. PVAs have been identified in free living bacteria and are thought to be related to pathways involved in catabolism and assimilation of aromatic compounds [21]. Further investigation is required to confirm whether the identified genes are in fact BSHs, being catalytically active against conjugated bile acids, and to decipher their primary substrate preference.

Having dissected the different articles showing bile acid production in bacteria, and taking together the data generated in this study, it is unlikely that bile acids are synthetized *de novo* by previously reported marine bacteria isolates. The source of the different bile acids identified seem to be from the peptone fraction present in the culture media used (Marine Broth Difco 2216). Furthermore, differences in bile acid profiles observed among the different isolates reported in the literature could be a result of the preference of their native BSHs, steroid degrading pathways, novel bile acid modifying enzymes and the narrow selection of bile acids used as standards in their respective analytical methods. Further investigation is still required in order to properly decipher the role of novel BSHs in marine bacteria and their ecological importance.

Acknowledgments

This research was funded by The Novo Nordisk Foundation under NNF grant number: NNF20CC0035580; and The Novo Nordisk Foundation, Challenge programme, CaMiT under grant agreement: NNF17-CO0028232. F.G.T. also acknowledges NNF PhD fellowship grant no. NNF16CC0020908. M.O.A.S. acknowledges additional funding from the NNF.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Hofmann AF, Hagey LR, Krasowski MD. 2010. Bile salts of vertebrates: structural variation and possible evolutionary significance. *J. Lipid Res.* **51**: 226-246.
2. Hofmann AF, Hagey LR. 2008. Review bile acids: Chemistry, pathochemistry, biology, pathobiology, and therapeutics. *Cell. Mol. Life Sci.* **65**: 2461-2483.
3. Foley MH, Flaherty SO, Barrangou R, Theriot CM. 2019. Bile salt hydrolases: Gatekeepers of bile acid metabolism and host-microbiome crosstalk in the gastrointestinal tract. *PLoS Pathog.* **15**: e1007581.
4. Jones BV, Begley M, Hill C, Gahan CGM, Marchesi JR. 2008. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc. Natl. Acad. Sci. USA* **105**: 13580-13585.
5. Russell DW, Setchell KDR. 1992. Bile acid biosynthesis. *Biochemistry* **31**: 4737-4749.
6. Hofmann AF. 1999. The continuing importance of bile acids in liver and intestinal disease. *Arch. Intern. Med.* **159**: 2647-2658.
7. Bowlus CL. 2016. Obeticholic acid for the treatment of primary biliary cholangitis in adult patients: clinical utility and patient selection. *Hepat. Med.* **8**: 89-95.
8. Kim DJ, Yoon S, Ji SC, Yang J, Kim YK, Lee S, *et al.* 2018. Ursodeoxycholic acid improves liver function via phenylalanine/tyrosine pathway and microbiome remodelling in patients with liver dysfunction. *Sci. Rep.* **8**: 11874.
9. Passed A, Lang S, Wagner F, Wray V. 1991. Anionic trehalose tetraester from the marine bacterium *Arthrobacter* sp. EK 1. *Naturforsch* **46c**: 204-209.
10. Maneerat S, Nitoda T, Kanzaki H, Kawai F. 2005. Bile acids are new products of a marine bacterium, *Myroides* sp. strain SM1. *Appl. Microbiol. Cell Physiol.* **67**: 679-683.
11. Kim D, Kim J, Kang SJ, Yoon JH, Kim WG, Lee JS, *et al.* 2007. Biosynthesis of bile acids in a variety of marine bacteria taxa.

J. Microbiol. Biotechnol. **17**: 403-407.
12. Tremaroli V, Karlsson F, Werling M, Ståhlman M, Kovatcheva-Datchary P, Olbers T, *et al.* 2015. Roux-en-Y gastric bypass and vertical banded gastroplasty induce long-term changes on the human gut microbiome contributing to fat mass regulation. *Cell Metab.* **22**: 228-238.
13. Tatusov RL, Galperin MY, Natale DA, Koonin EV. 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res*. **28**: 33-36.
14. Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. *Nucleic Acids Res.* **29**: 2607-2618.
15. Madeira F, Park YM, Lee J, Buso N, Gur T, Madhusoodanan N, *et al.* 2019. The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* **47**: W636-W641.
16. Price MN, Dehal PS, Arkin AP. 2009. FastTree: Computing large minimum-evolution trees with profiles instead of a distance matrix. *Mol. Biol. Evol.* **26**: 1641-1650.
17. Ohashi K, Miyagawa Y, Nakamura Y, Shibuya H. 2008. Bioproduction of bile acids and the glycine conjugates by *Penicillium* fungus. *J. Nat. Med.* **62**: 83-86.
18. Park SC, Kim CJ, Uramoto M, Yun HI, Yoon KH, Oh TK. 1995. Antibacterial substance produced by *Streptococcus faecium* under anaerobic culture. *Biosci. Biotechnol. Biochem*. **59**: 1966-1967.
19. Hill C, Gahan CGM. 2006. Bile salt hydrolase activity in probiotics. *Appl. Environ. Microbiol.* **72**: 1729-1738.
20. Panigrahi P, Sule M, Sharma R, Ramasamy S, Suresh, CG. 2014. An improved method for specificity annotation shows a distinct evolutionary divergence among the microbial enzymes of the cholylglycine hydrolase family. *Microbiology* **160**: 1162-1174.
21. Valle F, Balbás P, Merino E, Bollvar F. 1991. The role of penicillin amidases in nature and in industry. *Trends Biochem. Sci*. **16**: 36-40.
22. Kim SH, Yang HO, Sohn YC, Kwon HC. 2010. *Aeromicrobium halocynthiae* sp. nov., a taurocholic acid-producing bacterium isolated from the marine ascidian *Halocynthia roretzi*. *Int. J. Syst. Evol. Microbiol*. **60**: 2793-2798.
23. Li H, Shinde PB, Lee HJ, Yoo ES, Hong J, Choi SH, *et al.* 2009. Bile acid derivatives from a sponge-associated bacterium. *Arch. Pharm. Res.* **32**: 857-862.
24. Kim SH, Yang HO, Shin YK, Kwon HC. 2012. *Hasllibacter halocynthiae* gen. nov., sp. nov., a nutriacholic acid-producing bacterium isolated from the marine ascidian *Halocynthia roretzi*. *Int. J. Syst. Evol. Microbiol*. **62**: 624-631.