Advances in Microbiology 微生物前沿, 2024, 13(2), 100-118
Published Online June 2024 in Hans. https://www.hanspub.org/journal/amb
https://doi.org/10.12677/amb.2024.132012

Hans汉斯

天然溴化：酶、底物和溴化机制

胡文达，郑哲麟，何亚文*

上海交通大学生命科学技术学院，微生物代谢国家重点实验室，
教育部代谢与发育科学国际合作联合实验室，上海

收稿日期：2024年4月2日；录用日期：2024年6月20日；发布日期：2024年6月28日

摘　要

溴在自然界主要以可溶或不可溶的溴化物形式存在，溴化物在农业、化工、医药等领域具有广泛的应用潜力。动植物和微生物也可以利用溴化酶合成天然有机溴化物。随着越来越多的溴化酶被发现，溴化现象被认为是生物合成与代谢途径中的修饰策略之一。本文从以下五个方面综述了天然溴化研究领域的最新进展，包括：(1) 溴化物的分布；(2) 天然溴化物的生物学功能及其对生态环境的影响；(3) 天然溴化物的临床应用潜力；(4) 溴化酶的分类与作用机理；(5) 近期报道的溴化酶。最后，讨论了溴化酶研究领域未来急需解决的科学问题。

关键词

溴，天然有机溴化物，溴化酶，溴过氧化酶，黄素依赖型溴化酶

Naturally Occurring Bromination: Enzymes, Substrates and the Underlying Mechanisms

Wenda Hu, Zhelin Zheng, Yawen He*

Joint International Research Laboratory of Metabolic and Developmental Sciences, State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University (SJTU), Shanghai

Received: Apr. 2nd, 2024; accepted: Jun. 20th, 2024; published: Jun. 28th, 2024

Abstract

Bromine is found in nature dispersed throughout Earth's crust only in compounds as soluble and insoluble bromides. Bromides have application potentials in agriculture, chemical engineering, and medicine. Animals, plants and microorganisms can synthesize organobromine compounds via the brominases. With the discovery of more and more brominases, the naturally occurring bro-

*通讯作者。

文章引用: 胡文达, 郑哲麟, 何亚文. 天然溴化: 酶、底物和溴化机制[J]. 微生物前沿, 2024, 13(2): 100-118.
DOI: 10.12677/amb.2024.132012

mination has been recognized as one of the modification strategies in organobromine biosynthesis and metabolism of all organisms. In this review, we summarized the latest findings in the area of naturally occurring bromination, including: (1) distribution of bromides; (2) biological functions of organobromine compounds and their ecological impacts; (3) clinical potential of organobromine compounds; (4) brominases and the underlying bromination mechanisms; (5) recently reported brominases. Finally, the future prospects and challenges in the area of naturally occurring bromination were discussed.

Keywords

Bromine, Organobromine Compounds, Brominase, Bromoperoxidase, Flavin-Dependent Brominase

1. 前言

卤族元素在自然界广泛分布，天然卤代有机物广泛存在于细菌、真菌、动植物乃至人类体内。目前已经发现一万多种天然卤代有机物，主要包括氯代有机物、溴代有机物、碘代有机物和氟代有机物，其数量和种类每年还在稳定增长[1] [2] (图 1)。目前发现的天然卤化物大多为氯化物，溴化物数量仅次于氯化物，且几乎都存在于溴元素丰度较高的海洋环境；天然碘化及氟化物则较为稀少[3] [4]。砹及以上的卤族元素因无稳定同位素和高毒性，无法被生物体利用进行卤化物合成。

卤族元素在自然界广泛分布，天然卤代有机物广泛存在于细菌、真菌、动植物乃至人类体内。目前已经发现一万多种天然卤代有机物，主要包括氯代有机物、溴代有机物、碘代有机物和氟代有机物，其数量和种类每年还在稳定增长[1] [2] (图 1)。目前发现的天然卤化物大多为氯化物，溴化物数量仅次于氯化物，且几乎都存在于溴元素丰度较高的海洋环境；天然碘化及氟化物则较为稀少[3] [4]。砹及以上的卤族元素因无稳定同位素和高毒性，无法被生物体利用进行卤化物合成。

卤化酶负责天然卤代有机物的生物合成。目前研究的卤化酶中，绝大部分为专性或兼性氯化酶[5]。随着溴化酶在动物、植物及微生物中被相继发现，溴化现象也被认为是生物合成与代谢中广泛应用的修饰策略之一[6]。天然溴化有机物种类繁多、化学结构复杂，意味着很多功能迥异的新型溴化酶还有待进一步发掘。考虑到溴化物在当今农业、化工、医药等领域具有广阔的应用潜力，溴化酶的改造与应用值得受到与氯化酶同样的重视。本综述主要总结了活性天然溴代化合物研究的最新进展，并聚焦介绍自 2014 年以来发现的新型黄素依赖型溴化酶，为回答“为何选择溴化”这一科学问题提供思路。天然溴化物的分布截止 2023 年底，天然产物词典中已收录了超过 4600 种溴代化合物，其中绝大多数为生物体产生的次级代谢产物(图 1)。目前，海洋依然是溴化有机物的主要来源，海洋中的细菌、真菌、植物、无脊椎动物、鱼类等皆可合成或摄入各类具有高生物活性的溴化有机物，以至于“含有溴元素”时常被视作海洋来源天然产物的标志[2]。陆地来源的天然溴化物相比之下报道较少。部分高等植物能够利用土壤中的微量溴元素合成一溴甲烷，并释放至大气中[7]。在植物枯萎时，溴离子以有机形式保留于植物组织中，成为陆地环境中的有机溴源[8]。极少数陆生细菌及真菌也合成溴代有机物。植物病原菌黄单胞菌(*Xanthomonas* sp.)合成的菌黄素(Xanthomonadin)可能是陆生细菌中发现最早的溴化代谢物[9] [10]。新几内亚蘑菇 *Boletopsis* sp. 合成的两种溴代三联苯 Boletopsins 13 和 14 则是首批陆生真菌内发现的溴化代谢物[11]。

2. 天然溴化物的生物学功能及其对生态环境的影响

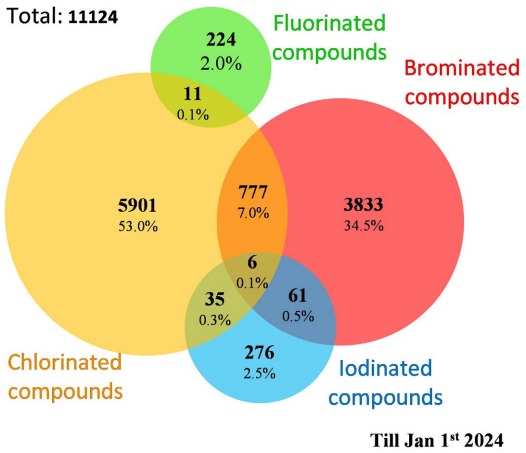


Figure 1. All halogenated natural compounds recorded in the Dictionary of Natural Products (DNP) till January, 2024
图 1. 天然产物词典(DNP)目前收录的所有天然卤化物(截止至 2024 年 1 月)

相当一部分有机溴化物具有较强的毒性，因此被广泛地用作生物种间攻防战的化学武器。海绵会合成大量溴代酪氨酸、溴酚、溴代异恶唑啉等毒性溴化物以抵御细菌侵染[12] [13]。在物理创伤、感染等条件的诱导下，一些海绵体内的有机溴代物浓度甚至可超过自身体重的 10%，而一些海绵寄生菌群也进化出相应去溴化机制，以应对海绵的化学防御[14]。海藻体内存在完整的活性氧合成酶–溴过氧化酶途径，用于生成以溴仿为主的毒性溴化物，并由专门的运输机制运至植物表面，对海藻表面的细菌生物膜起到重要的抑制作用[15] [16]。

溴化现象在高等动物体内也有重要的生理作用。由于其生理学毒性，有机溴化分子往往在感染条件下被用于抗菌、抗病毒免疫反应[17]。人体免疫系统中存在多种卤过氧化酶，倾向于利用血液中微量的溴离子合成溴代酪氨酸等多种溴化物质，以应对哮喘等过敏性炎症[18] [19]。绒毛鲨表皮的溴化犬尿素类代谢物具有抗菌活性，并且发出绿色荧光。这种表皮荧光可能被鲨鱼用作求偶过程中的视觉交流信号[20]。溴化反应在动物体内可介导胶原质的合成，对这一机制的依赖使得溴元素成为所有动物界物种的必需元素[21]。包括人类在内的一些哺乳动物脑髓液中所发现的 4-溴-3-氧丁酸-1-甲基庚酯(1-Methylheptyl 4-bromo-3-oxobutanoate)是陆地来源发现最早的溴化有机物之一。该物质可能在 REM (Rapid Eye Movement，快速眼动期)睡眠中扮演某种作用[22]。

溴化物对生态环境也有显著影响。海藻和蓝细菌等海洋光合自养生物往往会通过光合电子传递链产生过量的活性氧，被溴过氧化酶利用，氧化电负性相对较弱的溴元素，以大量合成溴代甲烷等简单溴化有机物[23] [24]。据统计，每年由海洋生物合成并释放至大气环境的二溴甲烷约 56,000 吨，海洋自然途径产生的溴仿更是达到 1~2 百万吨，对全球将近 30%的臭氧层降解现象负责[2] [25]。水体中的蓝细菌 *Aetokthonos hydrillicola* 所合成的溴化二吲哚毒素 Aetokthonotoxin (AETX)是鱼类和鸟类患脑白质空泡(Vacuolar myelinopathy, VM)的诱因[26]。上述动物往往摄取携带蓝细菌的软水草(*Hydrilla verticillata*)而中毒，严重时可导致死亡。除此以外，多溴代二苯醚(Polybrominated Diphenyl Ethers, PBDE)类物质也是一种强细胞毒性的有机残留物，可来自于工业生产，也可来源于海洋微生物的生物合成[27] [28]。藻类等植物可能通过自我合成或微生物共生的方式获得这些物质，进一步通过食物网将其传递给无脊椎动物、鱼类、乃至鲸鱼等高级消费者[29] [30]。PBDE 可通过呼吸道、食道、或皮肤进入人体，造成各种发育、神经、性功能相关慢性疾病[31] [32]。由于其广泛的健康与环境危害，PBDE 于 2009 年被《斯德哥尔摩公

约》列为持久性污染有机物[33] [34]。

3. 天然溴化物的临床应用潜力

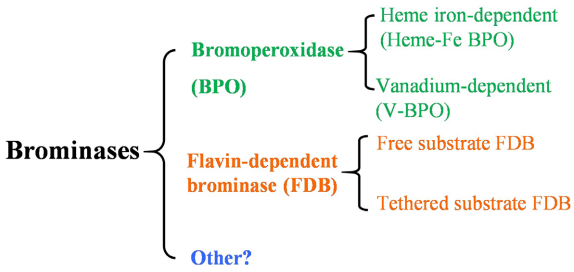


Figure 2. The proposed brominase classification based on catalytic mechanisms
图 2. 依据催化机理的溴化酶分类

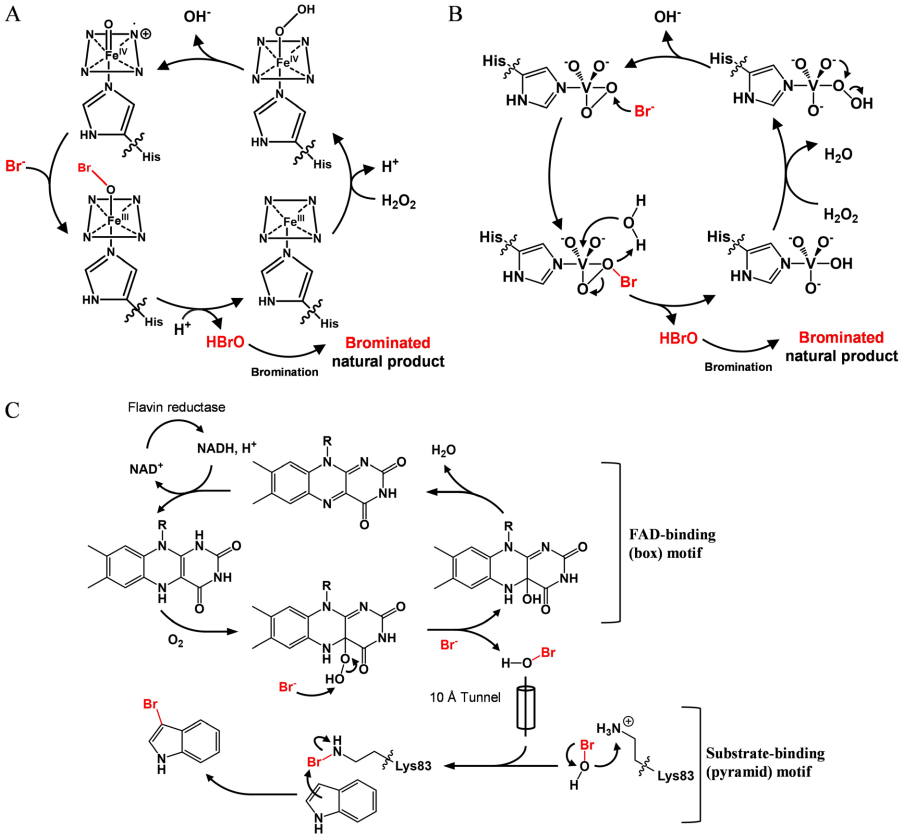


Figure 3. The proposed catalytic mechanisms for three categories of brominases. (A) heme-iron-dependent bromoperoxidase (Heme-Fe BPO); (B) vanadium-dependent bromoperoxidase (V-BPO); (C) flavin-dependent brominase (FDB, indole substrate used as an example)
图 3. 三种溴化酶的催化机制示意图。(A) 血红素铁依赖型溴过氧化酶(Heme-Fe BPO)；(B) 钒依赖型溴过氧化酶(V-BPO)；(C) 黄素依赖型溴化酶(FDB，以吲哚底物为例)

海藻、海绵、蓝细菌等海洋动植物合成的溴酚、溴化吡咯、溴化类萜、溴化吲哚及衍生物具有很高的潜在临床价值。提取自多管藻 *Polysiphonia morrowii* 的 3-溴-4,5-二羟基苯甲醛被多个研究报道具有抗皮肤角质细胞氧化、抗心肌缺氧、抗坏死病毒等功能[35] [36] [37]。其同分异构体 5-溴-3,4-二羟基苯甲醛具有抗皮肤过敏、消炎、促进生发能力[38] [39]。海绵(如 *Dysidea sp.*)、红藻(如 *Vertebrata lanosa*，*Rhodomela*

confervoides)、绿藻(如 *Avrainvillea rawsoni*)、褐藻(如 *Leathesia nana*)等生物产生的多种溴酚类化合物具有程度不一的抗癌症、抗肥胖、抗细菌、抗氧化活性[40] [41] [42] [43]。多种分离自海绵的吡啶类溴化生物碱，如 *Stylissa massa* 来源的 Stylisine 族化合物、*Agelas oroides* 来源的 Agesamine 和 Oroidin，具有抑制革兰氏阴性病原菌生物膜形成的功能[44] [45]。*Axinella brevistyla* 来源的 3-溴马来酰亚胺和 *Callyspongia* sp.来源的 Hymenialdisine，也具有潜在的抗癌能力[46]。海洋尾索动物产生的多种吲哚类溴化物具有潜在的抗炎症、癌症等功能[47]。提取自 *Diazona cf formosa*，*Eudistoma Olivaseum* 等各种海鞘动物的溴化物，如 Tanjugide、蕈状海鞘素(Eudistomins)、Meridianins 都具有明显抗肿瘤等活性[48] [49] [50] [51]。此外，多种海洋动物及微生物产生的溴化代谢物 Tambjamines 具抗癌、抗细菌、免疫抑制等活性：红藻来源的溴化环状倍半萜烯具有广谱抗细菌活性；主要由红藻产生的海乐萌(Halomon)是具有选择性肿瘤细胞抗性的溴化单帖[52] [53] [54]。一些溴化脂肪酸、溴化多肽、溴化氨基酸、溴化嘧啶衍生物也具有极高的药用价值。绝大多数的上述高价值有机物无法人工合成，或化学合成成本极高。

4. 溴化酶分类与作用机理

目前结构与功能完全鉴定的溴化酶不多，借鉴卤化酶分类方式，已知的溴化酶大致可以分为两类：溴过氧化酶和黄素依赖型溴化酶。前者可以继续细分为血红素铁依赖型溴过氧化酶(Heme-Fe BPO)和钒依赖型溴过氧化酶(Vanadium-BPO, V-BPO)；后者细分为直接作用于小分子自由底物的溴化酶和需要底物通过磷酸泛酰巯基乙胺连接酶固定于载体蛋白的溴化酶(图 2) [55] [56]。是否还存在第三类溴化酶有待更多的研究。

4.1. 溴过氧化酶(Bromoperoxidase, BPO)

按照能够氧化电负性最高的卤离子分类，卤过氧化酶家族包含氯过氧化酶(氧化 Cl^-、Br^-、I^-)、溴过氧化酶(氧化 Br^-和 I^-)以及碘过氧化酶(只能氧化 I^-) [57]。自然界中的溴化物合成大多需要 BPO 参与，这类溴化酶普遍依赖 Fe^{III}卟啉或配位钒原子激活溴离子并生成次溴酸(HBrO)，溴化或氧化底物，因此，BPO 又分为血红素铁 BPO 和钒依赖型 BPO。

4.1.1. 血红素铁依赖型溴过氧化酶(Heme-Fe BPO)

真菌 *Calariomyces fumago* 来源的 CPO 是人类发现的第一个天然卤化酶，能够在血红素铁辅基的参与下催化环乙酰基丙酮的二氯化修饰[58]。此后，多个依赖于血红素铁的溴过氧化酶也被陆续报道[59]。Heme-Fe BPO 结构中包含一血红素原卟啉基团，基团中心的铁元素以+3 价态形式与蛋白活性中心的氨基酸(组氨酸或半胱氨酸)相连接(图 3(A)) [60]。过氧化氢(H_2O_2)能够轴向连接于铁离子并将其氧化，形成一个$^+$heme-Fe$_{(4+)}$=O 结构，称作复合物 I 过渡态。复合物 I 有很高的氧化能力，可以进攻溴离子，产生活泼的 HBrO 分子，催化 HBrO 与亲核碳位点的结合。

Heme-Fe BPO 广泛存在于多种原核生物、植物、动物乃至人类。部分 Heme-Fe BPO 具有明显的溴偏好性。茶树菇 *Agrocybe aegerita* 中的 AaP 和 *Coprinus radians* 中的 CrP 皆具有溴化苯酚的活性[61] [62]。蓝细菌 *Lyngbya* sp.来源的 Heme-Fe HPO 被发现具有极高溴化活性和广泛的过氧化能力[63]。哺乳动物中具有卤化能力的过氧化酶的主要包括髓过氧化酶(Myeloperoxidase, MPO)、嗜酸性粒细胞过氧化酶(Eosinophil peroxidase, EPO)、乳过氧化酶(Lactoperoxidase, LPO)、甲状腺过氧化酶(Thyroid peroxidase, TPO)以及目前机制尚不完全明晰的过氧蛋白(Peroxidasin, PXDN) [64]。其中，EPO 主要利用 Br^-，在酸性条件下，其溴化能力明显提升。EPO 溴化所生成的 HBrO 在体内具有广泛的有机底物结合活性，能够溴化半胱氨酸、色氨酸、蛋氨酸、酪氨酸等氨基酸，以及牛磺酸、脱氧胞苷、缩醛磷脂等一系列生物小分子，溴化反应产物普遍具有抗菌效果，且往往随着感染和炎症反应(如嗜酸性细胞增多症)显著上调[19] [65] [66]。3-溴酪氨酸、3,5-二溴酪氨酸及其结构类似物在呼吸道上皮细胞或尿液中的含量可作为哮喘、糖尿病的无创诊断指标，并应用于临床[67] [68]。最早在果蝇体内发现的 PXDN 通过 Br^-氧化介导动物体

内胶原质基底膜的合成[69]。PXDN 具有 BPO 结构域，能够特异性地溴化蛋氨酸形成溴化锍，随后与临近的羟赖氨酸残基相偶联[21]。PXDN 也可合成 3-溴酪氨酸，可能参与免疫响应功能[70]。

4.1.2. 钒依赖型溴过氧化酶(V-BPO)

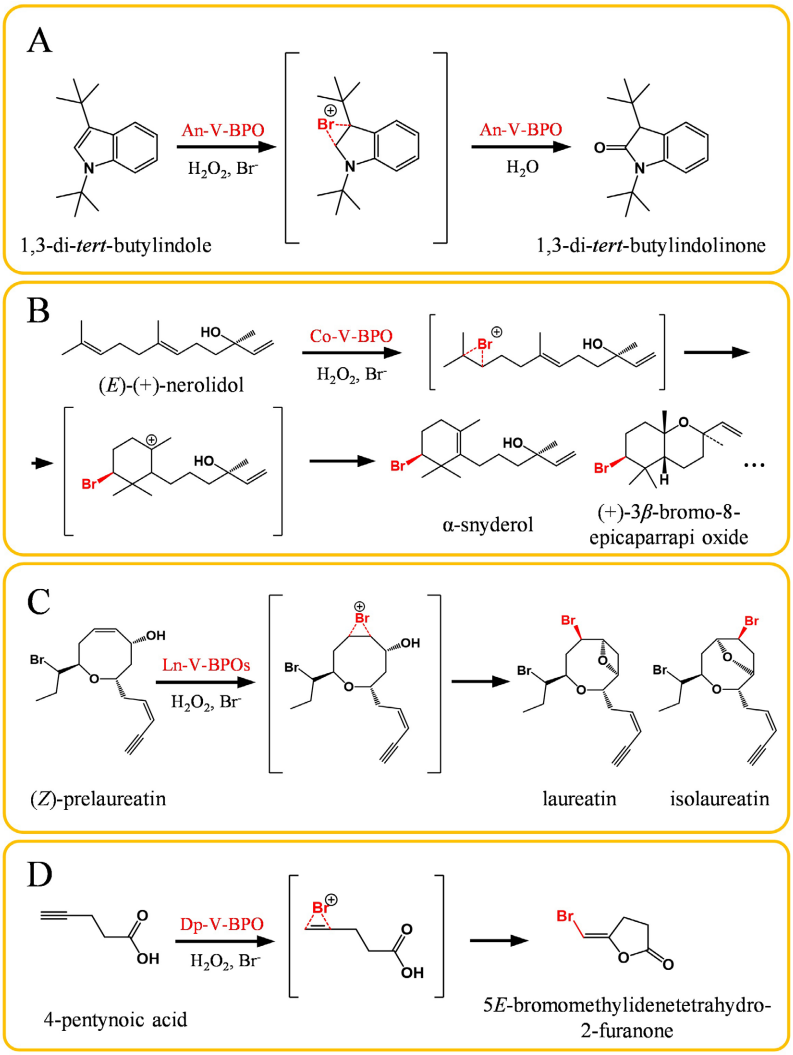


Figure 4. Substrate-specific catalyzation by V-BPOs. (A) Regioselective bromination of 1,3-di-tert-butylindole by the brown algae Ascophyllum nodosum V-BPO (An-V-BPO). (B) Bromination of (E)-(+)-nerolidol by the red algae Corallina officinalis V-BPO (Co-V-BPO). (C) Bromination of (Z)-prelaureatin by the red algae Laurencia nipponica V-BPO (Ln-V-BPO). (D) Bromolactonization of 4-pentynoic acid by the red algae Delisea pulchra V-BPO from (Dp-V-BPO) to form 5E-bromomethylidenetetrahydro-2-furanone

图 4. 溴过氧化酶(V-BPO)参与的底物特异性催化反应。(A) 褐藻 Ascophyllum nodosum 中 V-BPO(An-V-BPO)溴化并氧化 1,3-二叔丁基吲哚，合成 1,3-二叔丁基吲哚酮。(B) 红藻 Corallina officinalis 中 V-BPO(Co-V-BPO)负责溴化并环化底物(E)-(+)-橙花叔醇。(C) 红藻 Laurencia nipponica 来源 V-BPO(Ln-V-BPO)催化 Laureatin 生物合成中的溴化与环化反应。(D) 红藻 Delisea pulchra 来源 V-BPO(Dp-V-BPO)催化 4-戊炔酸的溴化内酯化反应，形成溴化呋喃酮

V-BPO 于 1984 年在褐藻(*Ascophyllum nodosum*)中首次发现，随后，多个来自各类藻类植物的 BPO 被定为钒依赖型[71] [72]。V-BPO 负责自然界中大部分有机溴化物的生物合成，但极少伴随基因簇出现[73]。V-BPO 具有高度的稳定性，能够耐受高温、表面活性剂和强氧化环境[74] [75]。已知的 V-BPO 蛋白结构总体相似程度不高，但活性中心都存在一个保守的组氨酸残基(图 3(B))，该组氨酸被推测用于结

合一个以钒原子辅基为中心的四脚金字塔型结构。受到 H_2O_2 进攻时，钒原子与周边羟基的连接会被打断并替换为过氧基团，转变为一个三角双锥形状的 η^2-氧过氧化钒中间体，随后溴离子进入活性位点被钒金属复合体氧化为 HBrO [76]。钒元素在全反应过程中不经历价态变化。

V-BPO 所产生的 HBrO 被释放至水溶液环境中自由地进攻各类亲核官能团，因此，V-BPO 通常具有较差的底物、区位选择性，其催化的反应往往生成溴化位点与数量不一的混合物[77]。目前为数不多的 V-BPO 晶体结构中也没有发现溴原子或底物结合位点[78]。即便如此，V-BPO 仍能够对小部分底物产生区域与空间的特异性催化。很多 V-BPO 对吲哚及衍生物具有很强的偏好性，且可以催化选择性的溴化和氧化反应(图 4(A)) [79] [80]。褐藻 *A. nodosum* 和红藻 *Corallina officinali* 来源的 An-V-BPO 和 Co-V-BPO 可特异性地催化倍半萜烯(Sesquiterpenes)的溴化和环化反应，且反应呈现出酶催化特有的动力学特性(图 4(B)) [54]。15 碳多聚乙烯(Acetogenins)是一类由海藻生产的非萜类环醚物质，红藻 *Laurencia nipponica* 来源的 Ln-V-BPO 作用于直链底物实施溴化修饰，促进化合物内部醚键的形成，自我环化为多种 8 环多聚乙烯产物，包括 Laureatin [81] (图 4(C))。一些由 V-BPO 催化的反应也体现出手性选择，产生不同比例的对映体产物，而氯过氧化酶催化的反应只产生消旋体[82] [83]。综合各类研究结果，V-BPO 是否结合于生成的 HBrO 并继续进行选择性催化很可能依赖于具体的底物而定。

海洋环境中大量的活泼溴化合物依赖于 V-BPO 参与合成。海藻表面附生菌能合成 3-氧-高丝氨酸内酯、2-庚基-4-喹啉酮等小分子，作为重要的群体感应(Quorum Sensing, QS)信号分子相互交流[84]。多个海藻物种依靠叶片表面的 V-BPO 氧化 Br^-形成大量的 HBrO，溴化修饰细菌的 QS 信号分子，直接干扰细菌的 QS 系统，影响菌落密度和生物膜的形成[85] [86] [87]。*Delisea pulchra* 为代表的一些红藻还能够通过 Dp-V-BPO 合成溴化呋喃酮，干扰革兰氏阴性细菌酰基高丝氨酸内酯介导的群体感应(图 4(D)) [88]。在防御应激条件下，海藻体内的 Dp-V-BPO 表达基因发生上调，利用溴化机制破坏体表附生菌之间的通讯联络，抑制附生菌在海藻叶表的富集，以此干扰附生菌形成生物膜阻碍海藻对光照和养分的吸收[89]。以溴仿、二溴甲烷为代表的溴代甲烷是 V-BPO 在海洋环境中体量最大的催化产物。多个被报道生产大量溴代甲烷的藻类、浮游植物、蓝细菌体内都发现了 V-BPO 积累于胞外，并参与溴代甲烷的合成途径[90] [91]。目前主流的解释认为，由 V-BPO 催化产生的 HBrO 被自由地释放至胞外海水环境中，与海水中的可溶性有机成分(Dissolved Organic Matter，DOM)发生反应[92]。该反应过程非常迅速，迅速形成大量结构不稳定的溴代化合物，随后自然降解为各种单碳原子溴化有机物，主要为 CH_2Br_2 与 $CHBr_3$。环境光照、宿主自身的生存压力等都会促进 H_2O_2 含量提升和 DOM 溴化[93]。另一种理论推测，V-BPO 会针对性地溴化海藻体内的酮类化合物，随后降解形成溴仿[94]。近期一项基于蓝细菌 V-BPO 的体外酶活实验中，以 2,4-戊二酮和 2,4,6-三庚酮为底物反应后观察到了溴仿的形成，从而支持了上述第二种理论推断[24]。

4.2. 黄素依赖型溴化酶(Flavin-Dependent Brominase, FDB)

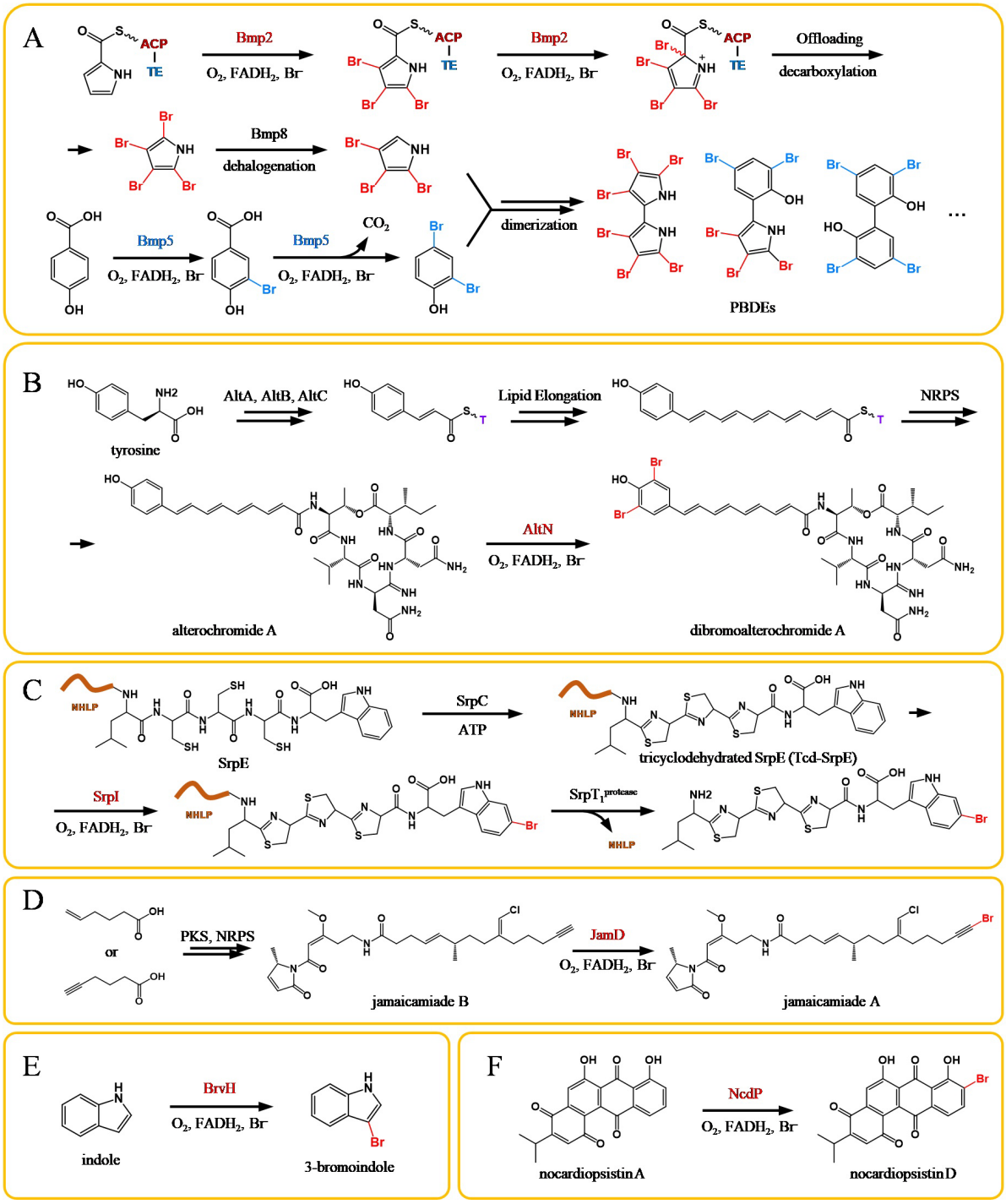
相比于黄素依赖型氯化酶，能够高效利用溴元素的黄素依赖型溴化酶只在近 15 年内才被陆续发现。相比 BPO，每个 FDB 都体现出显著的底物与位点选择性，且时常伴随于天然产物合成基因簇出现[95]。这类蛋白能够通过氧化溴离子生成次溴酸，以特异地亲电取代芳香环、杂环或其他类似结构上的富电子位点。FDB 与黄素依赖型氯化酶具有类似的催化机理和性质，但对卤素的偏好性差异使得两者之间产生清晰的分界[96]。FDB 依赖还原型黄素二核苷酸($FADH_2$)作为辅因子。因此，溴化反应一般需要单独的 FAD 还原酶共同参与，以完成黄素的循环使用。

目前已知的 FDB 蛋白单体结构基本都由一个箱型的 FAD 结合结构域和一个塔型的底物结合结构域组成，与主流的 FAD 依赖型单加氧酶、氯化酶类似。其中，箱型结构域中的保守位点可用于固定还原型 $FADH_2$，从而使其中的异咯嗪官能团被氧化，形成 FAD(C4α)-OOH。溴离子与之反应，产生 HBrO 和 FAD(C4α)-OH。经过脱水后，黄素可在还原酶作用下被 NADH 还原并循环利用。生成的 HBrO 则会由经

FDB 内部一个约 10 Å 长的通道进入底物结合域，与通道末端的一个保守赖氨酸残基结合，对芳香环结构底物进行亲核取代，以生成单溴或多溴化有机物[97] [98] (图 3(C))。由于 FDB 的结构数据仍较为稀少、彼此特征迥异，研究人员还无法解释为何溴化酶倾向于利用体积更大、电负性更弱的溴原子[99]。

5. 近期报道的 FDB

5.1. 假交替单胞菌中的溴化酶 Bmp2 和 Bmp5



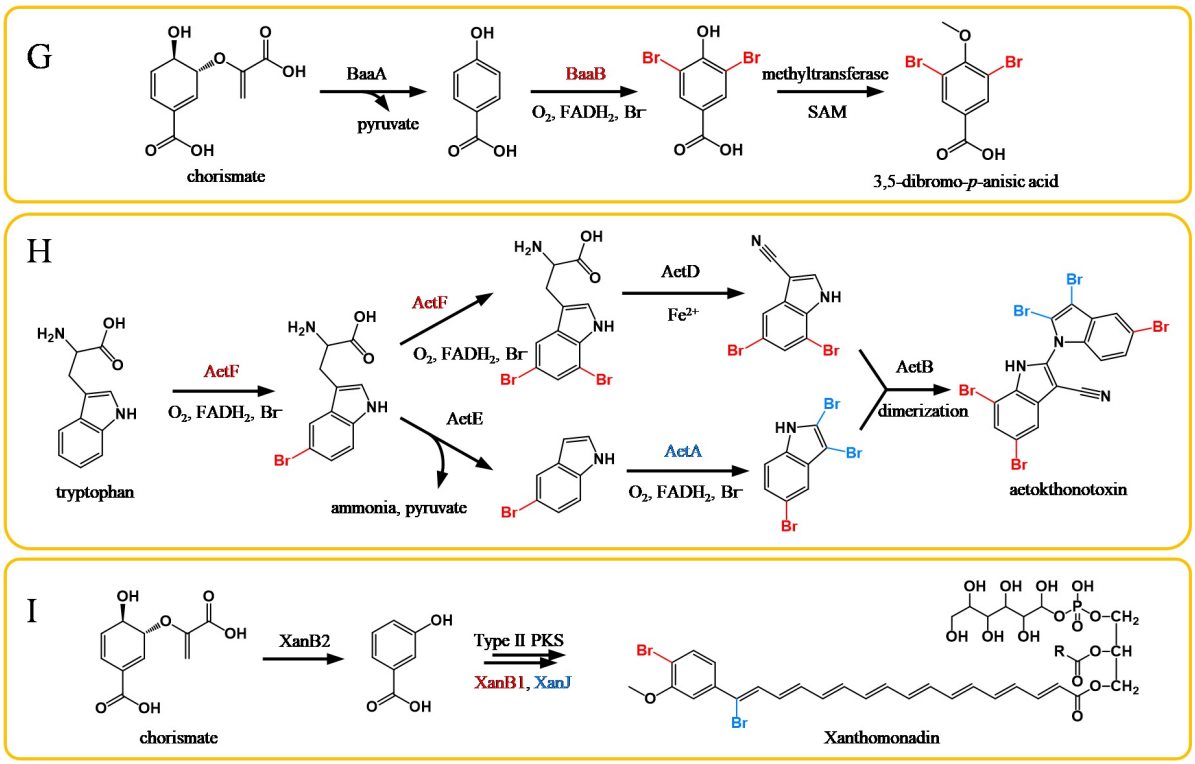


Figure 5. Representative flavin-dependent brominases and their substrates and products. (A) Bromination by Bmp2 and Bmp5 in the postulated biosynthetic scheme of polybrominated diphenyl ethers (PBDEs) in *Pseudoalteromonas* sp. Bmp2 catalyzes tetrabromination on acyl carrier protein (ACP)-thioesterase (TE)-tethered pyrrole. The C-2 bromine atom is subsequently removed by an obligate debrominase Bmp8 to give 2,3,4-tribrominated pyrrole. Bmp5 catalyzes decarboxylation and dibromination of 4-hydroxybenzoic acid (4-HBA). Brominated pyrroles and phenols are coupled to yield different ether hybrids. (B) In the putative biosynthetic pathway of bromoalterochromide in *Pseudoalteromonas* sp., AltN catalyzes bromination on the aryl ring of alterochromide. (C) In the biosynthesis of ribosomally synthesized and post-translationally modified peptide (RiPP) in sponge microbiome, SrpI carries out bromination on the C-6 position of the terminal tryptophanyl sidechain of tricyclodehydrated SrpE (Tcd-SrpE). (D) In the biosynthetic pathway of jamaicamide in the cyanobacterium *Moorea producens*, JamD catalyzes terminal alkyne bromination on jamaicamide B to give jamaicamide A. (E) The *Brevundimonas* BAL3-derived BrvH is able to brominate indole to form 3-bromoindole. (F) In the biosynthetic pathway of nocardiopsistin in *Nocardiopsis* sp. HB-J378, NcdP was proposed to brominate nocardiopsistin A to form nocardiopsistin D. (G) In the biosynthetic pathway of 3,5-dibromo-4-anisic acid in *Planctomycetales* 10988, BaaB was proposed to brominate 4-HBA on the C-3 and C-5 position.. (H) In the proposed aetokthonotoxin biosynthetic pathway in the freshwater cyanobacterium *Aetokthonos hydrillicola*, AetF catalyzes a two-step bromination on tryptophan at the C-5 and subsequently the C-7, forming 5-bromotryptophan and 5,7-dibromotryptophan. 5-bromotryptophan was converted to 5-bromoindole by a tryptophanase AetE, and then dibrominated by AetA on the C-3 and C-2 to give 2,3,5-tribromoindole. (I) In the putative synthetic pathway of xanthomonadin in *Xanthomonas* sp., XanB1 was proposed to catalyze bromination on the C-4' position of the aryl ring while XanJ was proposed to brominate the C-17 of the polyene chain

图 5. 代表性黄素依赖型溴化酶及其其催化底物和合成产物。(A) Bmp2 和 Bmp5 参与假交替单胞菌中多溴二苯醚(PBDE)生物合成的溴化反应。Bmp2 在底物酰基载体蛋白(ACP)-硫酯(TE)-吡咯上溴化 C-2、3、4、5 位点。C-2 上的溴原子被去溴化酶 Bmp8 脱去，形成 2,3,4-三溴吡咯。Bmp5 负责 4-羟基苯甲酸(4-HBA)的脱羧和二溴化。溴代酚与溴代吡咯随后相互连接，形成多种醚类混合物。(B) 在假交替单胞菌溴代 alterochromide 的生物合成途径中，AltN 溴化 alterochromide 的芳香环结构。(C) 在海绵共生菌的核糖体合成-翻译后修饰肽(RiPP)合成途径中，SrpI 溴化脱水环化后的 SrpE(Tcd-SrpE)，溴化位点为 C 末端色氨酸 C-6。(D) 在蓝细菌 *Moorea producens* 的 jamaicamide 合成途径中，JamD 可在底物 jamaicamide B 的末端炔烃基团溴化，形成 jamaicamide A。(E) 来源于短波单胞菌 BAL3 的 BrvH 能够催化吲哚形成 3-溴吡咯。(F) 在诺卡氏菌 HB-J378 nocardiopsistin 生物合成途径中，NcdP 可能溴化底物 nocardiopsistin A 形成 nocardiopsistin D。(G) 在浮霉菌 *Planctomycetales* 10988 3,5-二溴-4-茴香酸合成途径中，BaaB 可能作用于底物 4-HBA，在 C-3 和 C-5 位点溴化。(H) 在淡水蓝细菌 *Aetokthonos hydrillicola* aetokthonotoxin 的合成途径中，AetF 先后溴化色氨酸的 C-5、C-7，形成的 5-溴色氨酸和 5,7-二溴色氨酸。5-溴色氨酸在色氨酸酶 AetE 的作用下转化为 5-溴吲哚，随后被另一个溴化酶 AetA 在 C-3 和 C-2 位点分别溴化，形成 2,3,5-三溴吲哚。(I) 在黄单胞菌菌黄素的生物合成途径中，XanB1 负责芳香环 C-4'位点的溴化，XanJ 负责多烯链 C-17 位点的溴化

自然界中存在的多溴二苯醚(PBDE)类物质包含了溴苯、溴化杂环相互偶联得到的混合产物，多源于生物合成与食物网积累[28]。Agarwal 等人(2014)在假交替单胞菌(*Pseudoalteromonas sp.*)中发现了一个负责合成 PBDE 的 *bmp* 基因簇，该基因簇中的两个基因分别编码黄素依赖型溴化酶 Bmp2 与 Bmp5 [27]。Bmp2 对其底物 ACP(Acyl Carrier Protein)-TE(Thioesterase)-吡咯进行最多 4 个溴原子的取代，生成 2,3,4,5-四溴吡咯[100]。其中，第四个溴原子的修饰(C-5 位点)能够促进底物的脱羧与释放，并随后在专性去溴化酶 Bmp8 催化下脱去，最终形成 2,3,4-三溴吡咯[101] [102] (图 5(A))。对蛋白晶体结构的解析认为，Bmp2 独特的四溴化能力可能源于其侧链存在 3 个不同于其他吡咯卤化酶的氨基酸残基。将这 3 个残基突变为其他卤化酶的保守序列后，Bmp2 结构不会改变，但只能催化一溴取代[100]。

Bmp5 的底物是 4-羟基苯甲酸(4-HBA)，具溴化及脱羧双功能。首先，Bmp5 在 4-HBA 的 C-3 位点进行单溴取代，生成 3-溴中间产物；随后，第二个溴原子于 C-1 位点取代，并促使羧基脱离，生成 2,4-二溴苯酚(图 5(A))。Bmp5 的氨基酸序列与其他卤化酶同源性低，不具有常见的 WxWxIP 保守基序，反而与单组份黄素依赖型单加氧酶序列特征类似[103]。Bmp5 能够不依赖黄素还原酶自行还原并回收 FAD，因此被称作单组份 FDB。经由 Bmp2 与 Bmp5 合成的多溴代吡咯与多溴代酚，在 P450 连接酶 Bmp7 的作用下组合连接，能够形成各类结构迥异的 PBDE 衍生物。两个卤化酶都在氯元素过量的条件下反而显现出明显的溴元素偏好，且催化效率与常见氯化酶接近。

5.2. 假交替单胞菌中的溴化酶 AltN

假交替单胞菌也可产生一类溴代黄色色素 Bromoalterochromide，这类脂肽类物质由一个环状五肽结构与芳香多烯链连接而成，其肽链长度、氨基酸的选择以及溴化修饰的数量在不同成员之间存在差异[104] [105]。Nguyen 等(2013)通过建立二级质谱分子网络将天然产物和基因簇相匹配的方法，成功在假交替单胞菌基因组中找到一段长度约 34 kb 的 *alt* 基因簇[106]。该基因簇末尾有溴化酶基因 *altN*。AltN 负责产物分子苯环亲核位点上 1~2 个溴原子的修饰[107] (图 5(B))。AltN 表现出严格的溴化偏好，且无法利用氯元素。由于缺少直接的体外酶活证据，AltN 行使溴化的具体底物和分子机理尚不明确[108]。

5.3. 海绵共生菌中的溴化酶 SrpI

2021 年报道的 SrpI 是一个以核糖体肽为底物的卤化酶。借助已知的黄素依赖型氯化酶 MibH 为索引，Nguyen 等在海绵 *Smenospongia aurea* 共生菌宏基因组中找到了一类高度保守的 *srp* 基因簇[109] [110]。该基因簇负责合成一种基于核糖体合成-翻译后修饰(Ribosomally-synthesized and post-translationally modified peptide, RiPP)途径的溴化肽。在 RiPP 合成途径中，溴化酶 SrpI 作用于基因簇自身编码的 SrpE 小肽，在 C 末端色氨酸残基的 C-6 位点进行溴化(图 5(C))。SrpE 的 N 端存在一段腈水合酶类似前导肽(Nitrile Hydratase like leader peptide，NHLP)序列，可引导溴化酶修饰，并在修饰完成后被切除。不含特异性 NHLP 的多肽底物无法被 SrpI 识别，而将 SrpE 中的 NHLP 前导序列替换为其他同源的前导肽时，溴化效率会随着同源性的降低而减弱[111]。SrpI 也能够在自由的吲哚小分子上溴化，但是溴化的位点变为了吲哚 C-3 位。SrpI 只催化溴化，无法利用氯元素。

5.4. 海洋蓝细菌中的溴化酶 JamD

Jamaicamide 是一类最初于海洋蓝细菌 *Lyngbya majuscula* 中发现的脂肽类化合物，具有明显的动物神经毒性[112]。Jamaicamide A 的化学结构上存在一个特殊的乙炔基末端溴化修饰。*jam* 基因簇负责 Jamaicamide 的生物合成，其中，*jamD* 编码一个负责末端溴化的黄素依赖型溴化酶。由于蓝细菌体内同时检测到无溴化修饰的 Jamaicamide B，具有完整的炔烃结构，这说明 JamD 可能直接于 Jamaicamide B 末端炔基溴化(图 5(D))。^{15}N 同位素示踪实验和体外酶活实验随后验证了这一后期修饰假说[113] [114]。

JamD 偏爱溴元素，能极少地利用碘，但不接受氯。JamD 是目前唯一报道的具有末端炔烃修饰能力的溴化酶，除天然底物以外，还能够广泛地溴化其它带有末端炔烃的分子[114]。

5.5. 短波单胞菌中的溴化酶 BrvH

BrvH 的发现依赖于宏基因组系统发育分析和预测，序列来自于短波单胞菌菌株 *Brevundimonas* BAL3，研究人员未能够找到 BrvH 在体内对应的天然底物和参与的代谢途径[98]。即便如此，BrvH 具有鲜明的黄素依赖型卤化酶序列特征，能在体外反应中溴化吲哚底物的 C-3 位点(图 5(E))。虽然与很多典型的色氨酸卤化酶有极高的同源性，BrvH 并不能利用色氨酸、或与色氨酸结构类似的吲哚-3-乙腈、吲哚-3-丙酸[115]。晶体结构解析发现，BrvH 不具有用于固定色氨酸羧基、氨基部分的残基，且底物结合域呈开放结构，可能用于结合更庞大的天然底物分子。BrvH 偏好溴元素，在氯离子含量高于溴离子十倍的反应体系中优先利用溴离子[98]。

5.6. 深海放线菌拟诺卡氏菌中的溴化酶 NcdP

深海放线菌拟诺卡氏菌(*Nocardiopsis* sp.) HB-J378 体内存在一个溴化酶 NcdP，参与其标志性代谢物 Nocardiopsistin 的生物合成[116]。Nocardiopsistin 是一种具抗生素活性的成角四环素(Angucycline)类物质，在末端苯环结构的酚羟基邻位具有一个天然的溴化修饰。Nocardiopsistin 的生物合成依赖于 *ncd* 基因簇，其中 *ncdP* 编码一个潜在的 FDB。借助强启动子在野生型菌株体内过表达 *ncdP* 可以显著提高 Nocardiopsistin 的溴化比例(两种物质比例由 0.15 提高至 1.11)。因此，NcdP 可能于 Nocardiopsistin 完整结构末端实施后期溴化(图 5(F))。溴化修饰后的 Nocardiopsistin D 抗菌活性明显高于无溴产物，对耐甲氧西林金黄色葡萄球菌的抑菌能力提高了 128 倍，但溴化修饰不会提高 Nocardiopsistin 的细胞毒性，因而具有潜在的临床应用价值。

5.7. 海洋浮霉菌中的溴化酶 BaaB

海洋浮霉菌(*Planctomycetes*)产生的小分子次级代谢产物 3,5-二溴-*p*-茴香酸，具有较强的植物细胞毒性[117]。一个 2 个 ORF 构成的小型基因簇 *baaAB* 负责 3,5-二溴-*p*-茴香酸的生物合成。*baaA* 编码一个 4-HBA 合成酶，*baaB* 编码一个假定的 FDB，负责 4-HBA 上 C-3 和 C-5 位点的溴化。由于菌株培养环境中未能发现任何氯化衍生物的存在，BaaB 被认为是以自由 4-HBA 为底物的专性溴化酶(图 5(G))。

5.8. 淡水蓝细菌中的溴化酶 AetA 与 AetF

湖水水体蓝细菌 *Aetokthonos hydrillicola* 合成的动物毒素 Aetokthonotoxin(AETX)是很多鸟类患有脑白质空泡死亡的致病元凶[26]。AETX 分子由两个不对称的吲哚结构在 N1-C2’之间相互连接组成，含有一个腈基和多达 5 个溴原子的修饰，由蓝细菌体内一段 *aet* 基因簇负责合成。其中，*aetA* 与 *aetF* 分别编码两个 FDB。溴化酶 AetF 在 L-色氨酸的 C-5 和 C-7 两个芳香残基位点先后实施单溴、二溴化，开启整个合成途径[26] (图 5(H))。AetF 和底物共晶结构的解析发现，AetF 在进行单溴化后，会将 5-溴-L-色氨酸的结合构象反转，并使得 C-7 位继而对准转运 HBrO 的离子通道，方便第二个溴原子的取代反应，实现高位点特异性的催化[118]。AetF 严格使用色氨酸作为底物，而不接受吲哚，说明 AETX 合成途径中色氨酸分解为吲哚基团的反应步骤必须于 AetF 溴化之后完成。与 Bmp5 一样，AetF 为单组份黄素依赖型溴化酶，能够自我回收 FAD 辅因子而不依赖于黄素还原酶。有趣的是，催化 FAD 还原反应时，AetF 倾向于利用 NADPH 而非 NADH 作为电子供体[119]。AetF 对溴具有明显的偏好性，并能一定程度上利用碘离子[120]。AetF 与现有研究的黄素依赖型卤化酶序列同源性较低，序列更接近于吡咯核苷酸二硫键氧化还原酶和葡萄糖－甲醇－胆碱氧化还原酶[114]。虽然来自淡水细菌，其系统发育关系更靠近海洋

来源蛋白。

在 AETX 生物合成途径中，溴化酶 AetA 负责再次溴化中间产物 5-溴吲哚[119] (图 5(H))。AetA 能够先后在 5-溴吲哚的 C-3 和 C-2 两个位点加溴，生成产物 2,3,5-三溴吲哚。虽然被注释为典型色氨酸 FDH，AetA 无法利用色氨酸或去溴化的 AETX 完整结构，表明其负责的溴化只能够发生在 5-溴-L-色氨酸脱去丙氨酸残基之后、后续吲哚偶联步骤之前。在 AetA 和 AetF 完成溴化后，两个吲哚基团会经过腈化和分子重排，随后由经 P450 酶 AetB 连接形成最终产物 AETX。值得注意的是，AetB 只能接受溴化、腈化修饰全部完成后的吲哚残基，这说明两个溴化酶都是 AETX 生物合成途径中必需的前修饰酶，其催化反应直接影响到后续步骤的进行。

5.9. 植物病原黄单胞菌中的溴化酶

黄单胞菌是一类重要的植物病原细菌，能够侵染至少 400 种单子叶、双子叶植物，包括多种重要的经济作物[121]。Ismail 等(2019)在野油菜黄单胞菌(*Xanthonomas campestris* pv. *campestris*, *Xcc*) b100 菌株基因组中通过发掘黄素依赖型卤化酶的两个特征性基序 GxGxxG 与 WxWxIP，发现了 3 个注释为色氨酸卤化酶的基因 Xcc1333、Xcc4156 和 Xcc4345。虽然未能找到这 3 个卤化酶的天然底物，Xcc1333、Xcc4156 和 Xcc4345 都在体外反应体系中具有溴化活性。虽然 3 个卤化酶都与已报道的色氨酸卤化酶具有很高的序列同源性，它们却不能利用色氨酸作为底物，反而倾向于催化吲哚(和部分衍生物)的 C-3 位点溴化，这一系列特征都与前文描述的溴化酶 BrvH 十分类似(图 5(E))。对 Xcc4156 蛋白结构的解析发现其与 BrvH 高度相近，同样存在较为开放的底物结合区域，可能用于结合更大的天然底物[99]。*Xcc* 中的上述三个溴化酶都表现出严格的溴偏好，无法利用氯元素。

黄单胞菌的一个标志性表型特征是能够产生一种黄色色素，学名菌黄素。菌黄素是为一类磷脂类天然化合物，其结构由芳香基多烯链通过酯键连接至甘油骨架形成，定位于细胞外膜[10]。Andrewes 等(1976)鉴定了菌黄素芳香多烯链的化学结构为 17-(4-溴-3-甲氧基苯)-17-溴-2,4,6,8,10,12,14,16-烯-十七酸酯，并发现在苯环 C-4’和多烯链 C-17 位点存在两个溴化修饰[122]。黄单胞菌中的 *xan* 基因簇负责菌黄素的生物合成，其中 *xanB1* 与 *xanJ* 分别编码两个潜在的 FDB。初步研究结果表明：XanJ 在黄单胞菌体内可能负责多烯链上 C-17 的溴化，溴化后的产物继续参与菌黄素的生物合成(图 5(I))。因此，敲除 *xanJ* 导致菌黄素合成显著降低[123]。XanB1 负责芳香多烯苯环上的 C-4’位点的溴化，敲除 *xanB1* 可导致单溴菌黄素生物合成(未发表结果)。

6. 总结

天然溴化物在自然界广泛存在，具有多种生物学功能，影响海洋生态环境，还具有临床应用潜力。天然溴化物的生物合成由溴化酶负责，其成员主要包括溴过氧化物酶和黄素依赖型溴化酶。本文总结了这些溴化酶的特征、催化的底物、参与合成的产物以及可能的作用机理，为未来溴化酶的进一步研究提供科学依据。

目前发现和研究的溴化酶数量十分有限，“为什么溴化”这一科学问题仍然有待回答。借鉴氯化酶领域的研究成果，作者认为溴化酶研究领域未来在如下方面有待进一步加强：(1) 采用生物学、化学和信息系等交叉科学技术，从不同生态环境鉴定更多新型天然溴化产物，为溴化研究提供基础。虽然海洋是有机溴化物主要来源，但也不可忽视陆生生物来源的溴化物，尤其是逆境条件下合成的天然溴化物。(2) 基于现有基因组学和代谢组学，结合生物信息学方法与人工智能算法等手段，深入挖掘溴化酶基因。通过遗传学、分子酶学、结构生物学等手段加以实验验证，深入系统研究溴化酶的作用机理。(3) 重视已知溴化酶的改造与应用。溴化酶是一种绿色催化剂，在农业化工和医药等领域具有很大的应

用潜力。通过遗传改造，可以在催化能力、底物选择、酶稳定性方面改进其催化活性和应用范围，实现商业化应用。

基金项目

国家自然科学基金(31972231、32172355)。

参考文献

[1] Gribble, G.W. (2015) Biological Activity of Recently Discovered Halogenated Marine Natural Products. *Marine Drugs*, **13**, 4044-4136. https://doi.org/10.3390/md13074044

[2] Gribble, G.W. (2023) Naturally Occurring Organohalogen Compounds—A Comprehensive Review. In: Kinghorn, A.D., Falk, H., Gibbons, S., Asakawa, Y., Liu, J.K. and Dirsch, V.M., Eds., *Naturally Occurring Organohalogen Compounds. Progress in the Chemistry of Organic Natural Products*, Vol. 121, Springer, 1-546.

[3] Carvalho, M.F. and Oliveira, R.S. (2017) Natural Production of Fluorinated Compounds and Biotechnological Prospects of the Fluorinase Enzyme. *Critical Reviews in Biotechnology*, **37**, 880-897. https://doi.org/10.1080/07388551.2016.1267109

[4] Fournier, J.-B., Rebuffet, E., Delage, L., Grijol, R., Meslet-Cladière, L., Rzonca, J., *et al*. (2014) The Vanadium Iodoperoxidase from the Marine *Flavobacteriaceae* Species *Zobellia galactanivorans* Reveals Novel Molecular and Evolutionary Features of Halide Specificity in the Vanadium Haloperoxidase Enzyme Family. *Applied and Environmental Microbiology*, **80**, 7561-7573. https://doi.org/10.1128/AEM.02430-14

[5] Büchler, J., Papadopoulou, A. and Buller, R. (2019) Recent Advances in Flavin-Dependent Halogenase Biocatalysis: Sourcing, Engineering, and Application. *Catalysts*, **9**, Article 1030. https://doi.org/10.3390/catal9121030

[6] Fisher, B.F., Snodgrass, H.M., Jones, K.A., Andorfer, M.C. and Lewis, J.C. (2019) Site-Selective C-H Halogenation Using Flavin-Dependent Halogenases Identified via Family-Wide Activity Profiling. *ACS Central Science*, **5**, 1844-1856. https://doi.org/10.1021/acscentsci.9b00835

[7] Gan, J., Yates, S.R., Ohr, H.D. and Sims, J.J. (1998) Production of Methyl Bromide by Terrestrial Higher Plants. *Geophysical Research Letters*, **25**, 3595-3598. https://doi.org/10.1029/98gl52697

[8] Leri, A.C. and Myneni, S.C.B. (2012) Natural Organobromine in Terrestrial Ecosystems. *Geochimica et Cosmochimica Acta*, **77**, 1-10. https://doi.org/10.1016/j.gca.2011.11.012

[9] Starr, M.P. and Stephens, W.L. (1964) Pigmentation and Taxonomy of the Genus *Xanthomonas*. *Journal of Bacteriology*, **87**, 293-302. https://doi.org/10.1128/jb.87.2.293-302.1964

[10] He, Y.-W., Cao, X.-Q. and Poplawsky, A.R. (2020) Chemical Structure, Biological Roles, Biosynthesis and Regulation of the Yellow Xanthomonadin Pigments in the Phytopathogenic Genus *Xanthomonas*. *Molecular Plant-Microbe Interactions*, **33**, 705-714. https://doi.org/10.1094/mpmi-11-19-0326-cr

[11] Beekman, A.M., Wossa, S.W., Kevo, O., Ma, P. and Barrow, R.A. (2015) Discovery and Synthesis of Boletopsins 13 and 14, Brominated Fungal Metabolites of Terrestrial Origin. *Journal of Natural Products*, **78**, 2133-2135. https://doi.org/10.1021/acs.jnatprod.5b00202

[12] Niemann, H., Marmann, A., Lin, W. and Proksch, P. (2015) Sponge Derived Bromotyrosines: Structural Diversity through Natural Combinatorial Chemistry. *Natural Product Communications*, **10**, 219-231. https://doi.org/10.1177/1934578x1501000143

[13] Thoms, C., Ebel, R. and Proksch, P. (2006) Activated Chemical Defense in *Aplysina* Sponges Revisited. *Journal of Chemical Ecology*, **32**, 97-123. https://doi.org/10.1007/s10886-006-9355-x

[14] Horna-Gray, I., Lopez, N.A., Ahn, Y., Saks, B., Girer, N., Hentschel, U., *et al*. (2022) *Desulfoluna* spp. Form a Cosmopolitan Group of Anaerobic Dehalogenating Bacteria Widely Distributed in Marine Sponges. *FEMS Microbiology Ecology*, **98**, Article fiac063. https://doi.org/10.1093/femsec/fiac063

[15] Paul, N., de Nys, R. and Steinberg, P. (2006) Chemical Defence against Bacteria in the Red Alga *Asparagopsis armata*: Linking Structure with Function. *Marine Ecology Progress Series*, **306**, 87-101. https://doi.org/10.3354/meps306087

[16] Thapa, H.R., Lin, Z., Yi, D., Smith, J.E., Schmidt, E.W. and Agarwal, V. (2020) Genetic and Biochemical Reconstitution of Bromoform Biosynthesis in *Asparagopsis* Lends Insights into Seaweed Reactive Oxygen Species Enzymology. *ACS Chemical Biology*, **15**, 1662-1670. https://doi.org/10.1021/acschembio.0c00299

[17] Gribble, G.W. (2000) The Natural Production of Organobromine Compounds. *Environmental Science and Pollution Research*, **7**, 37-49. https://doi.org/10.1065/espr199910.002

[18] Mitra, S.N., Slungaard, A. and Hazen, S.L. (2000) Role of Eosinophil Peroxidase in the Origins of Protein Oxidation in Asthma. *Redox Report*, **5**, 215-224. https://doi.org/10.1179/135100000101535771

[19] Wu, W., Samoszuk, M.K., Comhair, S.A.A., Thomassen, M.J., Farver, C.F., Dweik, R.A., *et al*. (2000) Eosinophils Generate Brominating Oxidants in Allergen-Induced Asthma. *Journal of Clinical Investigation*, **105**, 1455-1463. https://doi.org/10.1172/jci9702

[20] Park, H.B., Lam, Y.C., Gaffney, J.P., Weaver, J.C., Krivoshik, S.R., Hamchand, R., *et al*. (2019) Bright Green Biofluorescence in Sharks Derives from Bromo-Kynurenine Metabolism. *iScience*, **19**, 1291-1336. https://doi.org/10.1016/j.isci.2019.07.019

[21] McCall, A.S., Cummings, C.F., Bhave, G., Vanacore, R., Page-McCaw, A. and Hudson, B.G. (2014) Bromine Is an Essential Trace Element for Assembly of Collagen IV Scaffolds in Tissue Development and Architecture. *Cell*, **157**, 1380-1392. https://doi.org/10.1016/j.cell.2014.05.009

[22] Yanagisawa, I. and Yoshikawa, H. (1973) A Bromine Compound Isolated from Human Cerebrospinal Fluid. *Biochimica et Biophysica Acta* (*BBA*)-*General Subjects*, **329**, 283-294. https://doi.org/10.1016/0304-4165(73)90293-6

[23] Pospíšil, P. (2016) Production of Reactive Oxygen Species by Photosystem II as a Response to Light and Temperature Stress. *Frontiers in Plant Science*, **7**, Article 1950. https://doi.org/10.3389/fpls.2016.01950

[24] Thapa, H.R. and Agarwal, V. (2021) Obligate Brominating Enzymes Underlie Bromoform Production by Marine Cyanobacteria. *Journal of Phycology*, **57**, 1131-1139. https://doi.org/10.1111/jpy.13142

[25] Salawitch, R.J. (2006) Biogenic Bromine. *Nature*, **439**, 275-277. https://doi.org/10.1038/439275a

[26] Breinlinger, S., Phillips, T.J., Haram, B.N., Mareš, J., Martínez Yerena, J.A., Hrouzek, P., *et al*. (2021) Hunting the Eagle Killer: A Cyanobacterial Neurotoxin Causes Vacuolar Myelinopathy. *Science*, **371**, eaax9050. https://doi.org/10.1126/science.aax9050

[27] Agarwal, V., El Gamal, A.A., Yamanaka, K., Poth, D., Kersten, R.D., Schorn, M., *et al*. (2014) Biosynthesis of Polybrominated Aromatic Organic Compounds by Marine Bacteria. *Nature Chemical Biology*, **10**, 640-647. https://doi.org/10.1038/nchembio.1564

[28] Teuten, E.L., Xu, L. and Reddy, C.M. (2005) Two Abundant Bioaccumulated Halogenated Compounds Are Natural Products. *Science*, **307**, 917-920. https://doi.org/10.1126/science.1106882

[29] Alonso, M.B., Maruya, K.A., Dodder, N.G., Lailson-Brito, J., Azevedo, A., Santos-Neto, E., *et al*. (2017) Nontargeted Screening of Halogenated Organic Compounds in Bottlenose Dolphins (*Tursiops truncatus*) from Rio de Janeiro, Brazil. *Environmental Science & Technology*, **51**, 1176-1185. https://doi.org/10.1021/acs.est.6b04186

[30] Losada, S., Roach, A., Roosens, L., Santos, F.J., Galceran, M.T., Vetter, W., *et al*. (2009) Biomagnification of Anthropogenic and Naturally-Produced Organobrominated Compounds in a Marine Food Web from Sydney Harbour, Australia. *Environment International*, **35**, 1142-1149. https://doi.org/10.1016/j.envint.2009.07.008

[31] Zhao, T., Tang, X., Li, D., Zhao, J., Zhou, R., Shu, F., *et al*. (2022) Prenatal Exposure to Environmentally Relevant Levels of PBDE-99 Leads to Testicular Dysgenesis with Steroidogenesis Disorders. *Journal of Hazardous Materials*, **424**, Article 127547. https://doi.org/10.1016/j.jhazmat.2021.127547

[32] Wu, Z., Han, W., Yang, X., Li, Y. and Wang, Y. (2019) The Occurrence of Polybrominated Diphenyl Ether (PBDE) Contamination in Soil, Water/Sediment, and Air. *Environmental Science and Pollution Research*, **26**, 23219-23241. https://doi.org/10.1007/s11356-019-05768-w

[33] McDonald, T.A. (2005) Polybrominated Diphenylether Levels among United States Residents: Daily Intake and Risk of Harm to the Developing Brain and Reproductive Organs. *Integrated Environmental Assessment and Management*, **1**, 343-354. https://doi.org/10.1002/ieam.5630010404

[34] Chokwe, T.B., Magubane, M.N., Abafe, O.A., Okonkwo, J.O. and Sibiya, I.V. (2019) Levels, Distributions, and Ecological Risk Assessments of Polybrominated Diphenyl Ethers and Alternative Flame Retardants in River Sediments from Vaal River, South Africa. *Environmental Science and Pollution Research*, **26**, 7156-7163. https://doi.org/10.1007/s11356-018-04063-4

[35] Kim, K., Hyun, Y., Hewage, S.R., Piao, M., Kang, K., Kang, H., *et al*. (2017) 3-Bromo-4,5-Dihydroxybenzaldehyde Enhances the Level of Reduced Glutathione via the Nrf2-Mediated Pathway in Human Keratinocytes. *Marine Drugs*, **15**, Article 291. https://doi.org/10.3390/md15090291

[36] Qin, S.-G., Tian, H.-Y., Wei, J., Han, Z.-H., Zhang, M.-J., *et al*. (2018) 3-Bromo-4,5-Dihydroxybenzaldehyde Protects against Myocardial Ischemia and Reperfusion Injury through the Akt-PGC1α-Sirt3 Pathway. *Frontiers in Pharmacology*, **9**, Article 722. https://doi.org/10.3389/fphar.2018.00722

[37] Kang, N., Han, S., Kang, H., Ko, G., *et al*. (2017) Anti-Inflammatory Effect of 3-Bromo-4,5-Dihydroxybenzaldehyde, a Component of *Polysiphonia morrowii*, *in vivo* and *in vitro*. *Toxicological Research*, **33**, 325-332. https://doi.org/10.5487/tr.2017.33.4.325

[38] Wang, Q., Ou, K., Zeng, C. and Fang, Y. (2022) 5-Bromo-3,4-Dihydroxybenzaldehyde Attenuates Endothelial Cells

Injury from High Glucose-Induced Damage. *Biomedicine & Pharmacotherapy*, **155**, Article 113793. https://doi.org/10.1016/j.biopha.2022.113793

[39] Kang, J.-I, Choi, Y.K., Han, S.-C., Nam, H., Lee, G., Kang, J.-H., *et al*. (2022) 5-Bromo-3,4-Dihydroxybenzaldehyde Promotes Hair Growth through Activation of Wnt/β-catenin and Autophagy Pathways and Inhibition of TGF-β Pathways in Dermal Papilla Cells. *Molecules*, **27**, Article 2176. https://doi.org/10.3390/molecules27072176

[40] Liu, M., Hansen, P.E. and Lin, X. (2011) Bromophenols in Marine Algae and Their Bioactivities. *Marine Drugs*, **9**, 1273-1292. https://doi.org/10.3390/md9071273

[41] Arai, M., Shin, D., Kamiya, K., Ishida, R., Setiawan, A., Kotoku, N., *et al*. (2016) Marine Spongean Polybrominated Diphenyl Ethers, Selective Growth Inhibitors against the Cancer Cells Adapted to Glucose Starvation, Inhibits Mitochondrial Complex II. *Journal of Natural Medicines*, **71**, 44-49. https://doi.org/10.1007/s11418-016-1025-x

[42] Hofer, S., Hartmann, A., Orfanoudaki, M., Nguyen Ngoc, H., Nagl, M., Karsten, U., *et al*. (2019) Development and Validation of an HPLC Method for the Quantitative Analysis of Bromophenolic Compounds in the Red Alga *Vertebrata lanosa*. *Marine Drugs*, **17**, Article 675. https://doi.org/10.3390/md17120675

[43] Dong, H., Liu, M., Wang, L., Liu, Y., *et al*. (2021) Bromophenol Bis (2,3,6-Tribromo-4,5-Dihydroxybenzyl) Ether Protects HaCaT Skin Cells from Oxidative Damage via Nrf2-Mediated Pathways. *Antioxidants*, **10**, Article 1436. https://doi.org/10.3390/antiox10091436

[44] Sun, J., Wu, J., An, B., de Voogd, N.J., Cheng, W. and Lin, W. (2018) Bromopyrrole Alkaloids with the Inhibitory Effects against the Biofilm Formation of Gram Negative Bacteria. *Marine Drugs*, **16**, Article 9. https://doi.org/10.3390/md16010009

[45] Kovalerchik, D., Singh, R.P., Schlesinger, P., Mahajni, A., Shefer, S., Fridman, M., *et al*. (2020) Bromopyrrole Alkaloids of the Sponge *Agelas oroides* Collected Near the Israeli Mediterranean Coastline. *Journal of Natural Products*, **83**, 374-384. https://doi.org/10.1021/acs.jnatprod.9b00863

[46] Mahamed, S., Motal, R., Govender, T., Dlamini, N., Khuboni, K., Hadeb, Z., *et al*. (2023) A Concise Review on Marine Bromopyrrole Alkaloids as Anticancer Agents. *Bioorganic & Medicinal Chemistry Letters*, **80**, Article 129102. https://doi.org/10.1016/j.bmcl.2022.129102

[47] Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H.G. and Prinsep, M.R. (2016) Marine Natural Products. *Natural Product Reports*, **33**, 382-431. https://doi.org/10.1039/c5np00156k

[48] Nakamura, Y., Kobayashi, J., Gilmore, J., Mascal, M., Rinehart, K.L., Nakamura, H., *et al*. (1986) Bromo-Eudistomin D, a Novel Inducer of Calcium Release from Fragmented Sarcoplasmic Reticulum that Causes Contractions of Skinned Muscle Fibers. *Journal of Biological Chemistry*, **261**, 4139-4142. https://doi.org/10.1016/s0021-9258(17)35636-3

[49] Murcia, C., Coello, L., Fernández, R., Martín, M., Reyes, F., Francesch, A., *et al*. (2014) Tanjungides A and B: New Antitumoral Bromoindole Derived Compounds from *Diazona cf formosa*. Isolation and Total Synthesis. *Marine Drugs*, **12**, 1116-1130. https://doi.org/10.3390/md12021116

[50] Ota, Y., Chinen, T., Yoshida, K., Kudo, S., Nagumo, Y., Shiwa, Y., *et al*. (2016) Eudistomin C, an Antitumor and Antiviral Natural Product, Targets 40s Ribosome and Inhibits Protein Translation. *ChemBioChem*, **17**, 1616-1620. https://doi.org/10.1002/cbic.201600075

[51] Xiao, L. (2022) A Review: Meridianins and Meridianins Derivatives. *Molecules*, **27**, Article 8714. https://doi.org/10.3390/molecules27248714

[52] Barros-Nepomuceno, F.W.A., *et al*. (2021) The Effects of the Alkaloid Tambjamine J on Mice Implanted with Sarcoma 180 Tumor Cells. *ChemMedChem*, **16**, 420-428. https://doi.org/10.1002/cmdc.202000387

[53] Bucher, C., Deans, R.M. and Burns, N.Z. (2015) Highly Selective Synthesis of Halomon, Plocamenone, and Isoplocamenone. *Journal of the American Chemical Society*, **137**, 12784-12787. https://doi.org/10.1021/jacs.5b08398

[54] Carter-Franklin, J.N. and Butler, A. (2004) Vanadium bromoperoxidase-Catalyzed Biosynthesis of Halogenated Marine Natural Products. *Journal of the American Chemical Society*, **126**, 15060-15066. https://doi.org/10.1021/ja047925p

[55] Crowe, C., *et al*. (2021) Halogenases: A Palette of Emerging Opportunities for Synthetic Biology-Synthetic Chemistry and C-H Functionalisation. *Chemical Society Reviews*, **50**, 9443-9481. https://doi.org/10.1039/D0CS01551B

[56] 郑哲麟, 胡文达, 何亚文. 微生物卤化酶及其应用研究进展[J]. 微生物前沿, 2022, 9(4): 141-155.

[57] Butler, A. and Sandy, M. (2009) Mechanistic Considerations of Halogenating Enzymes. *Nature*, **460**, 848-854. https://doi.org/10.1038/nature08303

[58] Shaw, P.D. and Hager, L.P. (1959) Biological Chlorination. IV. Peroxidative Nature of Enzymatic Chlorination. *Journal of the American Chemical Society*, **81**, 6527-6528. https://doi.org/10.1021/ja01533a056

[59] Dunford, H.B., Lambeir, A., Kashem, M.A. and Pickard, M. (1987) On the Mechanism of Chlorination by Chloroperoxidase. *Archives of Biochemistry and Biophysics*, **252**, 292-302. https://doi.org/10.1016/0003-9861(87)90034-8

[60] Hofrichter, M. and Ullrich, R. (2006) Heme-Thiolate Haloperoxidases: Versatile Biocatalysts with Biotechnological and Environmental Significance. *Applied Microbiology and Biotechnology*, **71**, 276-288. https://doi.org/10.1007/s00253-006-0417-3

[61] Ullrich, R., Nüske, J., Scheibner, K., Spantzel, J. and Hofrichter, M. (2004) Novel Haloperoxidase from the Agaric Basidiomycete *Agrocybe aegerita* Oxidizes Aryl Alcohols and Aldehydes. *Applied and Environmental Microbiology*, **70**, 4575-4581. https://doi.org/10.1128/aem.70.8.4575-4581.2004

[62] Anh, D.H., Ullrich, R., Benndorf, D., Svatoš, A., Muck, A. and Hofrichter, M. (2007) The Coprophilous Mushroom *Coprinus radians* Secretes a Haloperoxidase that Catalyzes Aromatic Peroxygenation. *Applied and Environmental Microbiology*, **73**, 5477-5485. https://doi.org/10.1128/aem.00026-07

[63] Auer, M., Gruber, C., Bellei, M., Pirker, K.F., Zamocky, M., Kroiss, D., *et al*. (2013) A Stable Bacterial Peroxidase with Novel Halogenating Activity and an Autocatalytically Linked Heme Prosthetic Group. *Journal of Biological Chemistry*, **288**, 27181-27199. https://doi.org/10.1074/jbc.m113.477067

[64] Arnhold, J. and Malle, E. (2022) Halogenation Activity of Mammalian Heme Peroxidases. *Antioxidants*, **11**, Article 890. https://doi.org/10.3390/antiox11050890

[65] Marcinkiewicz, J. and Kontny, E. (2012) Taurine and Inflammatory Diseases. *Amino Acids*, **46**, 7-20. https://doi.org/10.1007/s00726-012-1361-4

[66] Davies, M.J. and Hawkins, C.L. (2020) The Role of Myeloperoxidase in Biomolecule Modification, Chronic Inflammation, and Disease. *Antioxidants & Redox Signaling*, **32**, 957-981. https://doi.org/10.1089/ars.2020.8030

[67] Wedes, S.H., Wu, W., Comhair, S.A.A., McDowell, K.M., DiDonato, J.A., Erzurum, S.C., *et al*. (2011) Urinary Bromotyrosine Measures Asthma Control and Predicts Asthma Exacerbations in Children. *The Journal of Pediatrics*, **159**, 248-255. https://doi.org/10.1016/j.jpeds.2011.01.029

[68] Asahi, T., Kondo, H., Masuda, M., Nishino, H., Aratani, Y., Naito, Y., *et al*. (2010) Chemical and Immunochemical Detection of 8-Halogenated Deoxyguanosines at Early Stage Inflammation. *Journal of Biological Chemistry*, **285**, 9282-9291. https://doi.org/10.1074/jbc.m109.054213

[69] Péterfi, Z. and Geiszt, M. (2014) Peroxidasins: Novel Players in Tissue Genesis. *Trends in Biochemical Sciences*, **39**, 305-307. https://doi.org/10.1016/j.tibs.2014.05.005

[70] Bathish, B., Paumann-Page, M., Paton, L.N., Kettle, A.J. and Winterbourn, C.C. (2020) Peroxidasin Mediates Bromination of Tyrosine Residues in the Extracellular Matrix. *Journal of Biological Chemistry*, **295**, 12697-12705. https://doi.org/10.1074/jbc.ra120.014504

[71] Butler, A. and Carter-Franklin, J.N. (2004) The Role of Vanadium Bromoperoxidase in the Biosynthesis of Halogenated Marine Natural Products. *Natural Product Reports*, **21**, 180-188. https://doi.org/10.1039/b302337k

[72] Vilter, H. (1984) Peroxidases from Phaeophyceae: A Vanadium(V)-Dependent Peroxidase from *Ascophyllum nodosum*. *Phytochemistry*, **23**, 1387-1390. https://doi.org/10.1016/s0031-9422(00)80471-9

[73] Johnson, T.L., Palenik, B. and Brahamsha, B. (2011) Characterization of a Functional Vanadium-Dependent Bromoperoxidase in the Marine Cyanobacterium *Synechococcus* sp. CC93111. *Journal of Phycology*, **47**, 792-801. https://doi.org/10.1111/j.1529-8817.2011.01007.x

[74] Zhang, B., Cao, X., Cheng, X., Wu, P., Xiao, T. and Zhang, W. (2010) Efficient Purification with High Recovery of Vanadium Bromoperoxidase from *Corallina officinalis*. *Biotechnology Letters*, **33**, 545-548. https://doi.org/10.1007/s10529-010-0454-y

[75] Renirie, R., Pierlot, C., Aubry, J., Hartog, A.F., Schoemaker, H.E., Alsters, P.L., *et al*. (2003) Vanadium Chloroperoxidase as a Catalyst for Hydrogen Peroxide Disproportionation to Singlet Oxygen in Mildly Acidic Aqueous Environment. *Advanced Synthesis & Catalysis*, **345**, 849-858. https://doi.org/10.1002/adsc.200303008

[76] McLauchlan, C.C., Murakami, H.A., Wallace, C.A. and Crans, D.C. (2018) Coordination Environment Changes of the Vanadium in Vanadium-Dependent Haloperoxidase Enzymes. *Journal of Inorganic Biochemistry*, **186**, 267-279. https://doi.org/10.1016/j.jinorgbio.2018.06.011

[77] Martínez, V.M., Cremer, G.D., Roeffaers, M.B.J., Sliwa, M., Baruah, M., De Vos, D.E., *et al*. (2008) Exploration of Single Molecule Events in a Haloperoxidase and Its Biomimic: Localization of Halogenation Activity. *Journal of the American Chemical Society*, **130**, 13192-13193. https://doi.org/10.1021/ja804606m

[78] Agarwal, V., Miles, Z.D., Winter, J.M., Eustáquio, A.S., El Gamal, A.A. and Moore, B.S. (2017) Enzymatic Halogenation and Dehalogenation Reactions: Pervasive and Mechanistically Diverse. *Chemical Reviews*, **117**, 5619-5674. https://doi.org/10.1021/acs.chemrev.6b00571

[79] Tschirret-Guth, R.A. and Butler, A. (1994) Evidence for Organic Substrate Binding to Vanadium Bromoperoxidase. *Journal of the American Chemical Society*, **116**, 411-412. https://doi.org/10.1021/ja00080a063

[80] Martinez, J.S., Carroll, G.L., Tschirret-Guth, R.A., Altenhoff, G., Little, R.D. and Butler, A. (2001) On the Regiospeci-

ficity of Vanadium Bromoperoxidase. *Journal of the American Chemical Society*, **123**, 3289-3294. https://doi.org/10.1021/ja004176c

[81] Kaneko, K., Washio, K., Umezawa, T., Matsuda, F., Morikawa, M. and Okino, T. (2014) Cdna Cloning and Characterization of Vanadium-Dependent Bromoperoxidases from the Red Alga *Laurencia nipponica*. *Bioscience, Biotechnology, and Biochemistry*, **78**, 1310-1319. https://doi.org/10.1080/09168451.2014.918482

[82] Andersson, M.A. and Allenmark, S.G. (1998) Asymmetric Sulfoxidation Catalyzed by a Vanadium Bromoperoxidase: Substrate Requirements of the Catalyst. *Tetrahedron*, **54**, 15293-15304. https://doi.org/10.1016/s0040-4020(98)00956-9

[83] Coughlin, P., Roberts, S., Rush, C. and Willetts, A. (1993) Biotransformation of Alkenes by Haloperoxidases: Regiospecific Bromohydrin Formation from Cinnamyl Substrates. *Biotechnology Letters*, **15**, 907-912. https://doi.org/10.1007/bf00131755

[84] Camilli, A. and Bassler, B.L. (2006) Bacterial Small-Molecule Signaling Pathways. *Science*, **311**, 1113-1116. https://doi.org/10.1126/science.1121357

[85] Michels, J.J., Allain, E.J., Borchardt, S.A., Hu, P. and McCoy, W.F. (2000) Degradation Pathway of Homoserine Lactone Bacterial Signal Molecules by Halogen Antimicrobials Identified by Liquid Chromatography with Photodiode Array and Mass Spectrometric Detection. *Journal of Chromatography A*, **898**, 153-165. https://doi.org/10.1016/s0021-9673(00)00849-9

[86] Keltsch, N.G., Pütz, E., Dietrich, C., Wick, A., Tremel, W. and Ternes, T.A. (2023) Bromination of Quorum Sensing Molecules: Vanadium Bromoperoxidase and Cerium Dioxide Nanocrystals via Free Active Bromine Transform Bacterial Communication. *Environmental Science & Technology*, **57**, 18491-18498. https://doi.org/10.1021/acs.est.3c00459

[87] Syrpas, M., Ruysbergh, E., Blommaert, L., Vanelslander, B., Sabbe, K., Vyverman, W., *et al*. (2014) Haloperoxidase Mediated Quorum Quenching by *Nitzschia cf Pellucida*: Study of the Metabolization of N-Acyl Homoserine Lactones by a Benthic Diatom. *Marine Drugs*, **12**, 352-367. https://doi.org/10.3390/md12010352

[88] Sandy, M., Carter-Franklin, J.N., Martin, J.D. and Butler, A. (2011) Vanadium Bromoperoxidase from *Delisea pulchra*: Enzyme-Catalyzed Formation of Bromofuranone and Attendant Disruption of Quorum Sensing. *Chemical Communications*, **47**, 12086-12088. https://doi.org/10.1039/c1cc15605e

[89] Cosse, A., Potin, P. and Leblanc, C. (2009) Patterns of Gene Expression Induced by Oligoguluronates Reveal Conserved and Environment-Specific Molecular Defense Responses in the Brown Alga *Laminaria digitata*. *New Phytologist*, **182**, 239-250. https://doi.org/10.1111/j.1469-8137.2008.02745.x

[90] Almeida, M., Filipe, S., Humanes, M., Maia, M.F., Melo, R., Severino, N., *et al.* (2001) Vanadium Haloperoxidases from Brown Algae of the Laminariaceae Family. *Phytochemistry*, **57**, 633-642. https://doi.org/10.1016/s0031-9422(01)00094-2

[91] Johnson, T.L., Brahamsha, B., Palenik, B. and Mühle, J. (2015) Halomethane Production by Vanadium-Dependent Bromoperoxidase in Marine *Synechococcus*. *Limnology and Oceanography*, **60**, 1823-1835. https://doi.org/10.1002/lno.10135

[92] Lin, C.Y. and Manley, S.L. (2012) Bromoform Production from Seawater Treated with Bromoperoxidase. *Limnology and Oceanography*, **57**, 1857-1866. https://doi.org/10.4319/lo.2012.57.6.1857

[93] Wever, R. and Van der Horst, M.A. (2013) The Role of Vanadium Haloperoxidases in the Formation of Volatile Brominated Compounds and Their Impact on the Environment. *Dalton Transactions*, **42**, 11778-11786. https://doi.org/10.1039/c3dt50525a

[94] Theiler, R., Cook, J.C., Hager, L.P. and Siuda, J.F. (1978) Halohydrocarbon Synthesis by Bromoperoxidase. *Science*, **202**, 1094-1096. https://doi.org/10.1126/science.202.4372.1094

[95] Gkotsi, D.S., Dhaliwal, J., McLachlan, M.M., Mulholand, K.R. and Goss, R.J. (2018) Halogenases: Powerful Tools for Biocatalysis (Mechanisms Applications and Scope). *Current Opinion in Chemical Biology*, **43**, 119-126. https://doi.org/10.1016/j.cbpa.2018.01.002

[96] Thapa, H.R., *et al*. (2018) Chemoenzymatic Synthesis of Starting Materials and Characterization of Halogenases Requiring Acyl Carrier Protein-Tethered Substrates. *Methods in Enzymology*, **604**, 333-366. https://doi.org/10.1016/bs.mie.2018.01.028

[97] Dong, C., Flecks, S., Unversucht, S., Haupt, C., Van Pée, K. and Naismith, J.H. (2005) Tryptophan 7-Halogenase (PrnA) Structure Suggests a Mechanism for Regioselective Chlorination. *Science*, **309**, 2216-2219. https://doi.org/10.1126/science.1116510

[98] Neubauer, P.R., Widmann, C., Wibberg, D., Schröder, L., Frese, M., Kottke, T., *et al*. (2018) A Flavin-Dependent Halogenase from Metagenomic Analysis Prefers Bromination over Chlorination. *PLOS ONE*, **13**, e0196797. https://doi.org/10.1371/journal.pone.0196797

[99] Widmann, C., Ismail, M., Sewald, N. and Niemann, H.H. (2020) Structure of Apo Flavin-Dependent Halogenase Xcc4156 Hints at a Reason for Cofactor-Soaking Difficulties. *Acta Crystallographica Section D Structural Biology*, **76**, 687-697. https://doi.org/10.1107/s2059798320007731

[100] El Gamal, A., Agarwal, V., Diethelm, S., Rahman, I., Schorn, M.A., Sneed, J.M., *et al*. (2016) Biosynthesis of Coral Settlement Cue Tetrabromopyrrole in Marine Bacteria by a Uniquely Adapted Brominase-Thioesterase Enzyme Pair. *Proceedings of the National Academy of Sciences*, **113**, 3797-3802. https://doi.org/10.1073/pnas.1519695113

[101] El Gamal, A., Agarwal, V., Rahman, I. and Moore, B.S. (2016) Enzymatic Reductive Dehalogenation Controls the Biosynthesis of Marine Bacterial Pyrroles. *Journal of the American Chemical Society*, **138**, 13167-13170. https://doi.org/10.1021/jacs.6b08512

[102] Adak, S. and Moore, B.S. (2021) Cryptic Halogenation Reactions in Natural Product Biosynthesis. *Natural Product Reports*, **38**, 1760-1774. https://doi.org/10.1039/d1np00010a

[103] Gkotsi, D.S., Ludewig, H., Sharma, S.V., Connolly, J.A., Dhaliwal, J., Wang, Y., *et al*. (2019) A Marine Viral Halogenase that Iodinates Diverse Substrates. *Nature Chemistry*, **11**, 1091-1097. https://doi.org/10.1038/s41557-019-0349-z

[104] Kalinovskaya, N.I., Dmitrenok, A.S., Kuznetsova, T.A., Frolova, G.M., Christen, R., Laatsch, H., *et al*. (2008) "*Pseudoalteromonas januaria*" SUT 11 as the Source of Rare Lipodepsipeptides. *Current Microbiology*, **56**, 199-207. https://doi.org/10.1007/s00284-007-9023-6

[105] Chau, R., Pearson, L.A., Cain, J., Kalaitzis, J.A. and Neilan, B.A. (2021) A *Pseudoalteromonas* Clade with Remarkable Biosynthetic Potential. *Applied and Environmental Microbiology*, **87**, e02604-20. https://doi.org/10.1128/aem.02604-20

[106] Nguyen, D.D., Wu, C.-H., Moree, W.J., Lamsa, A., Medema, M.H., Zhao, X., *et al*. (2013) Ms/Ms Networking Guided Analysis of Molecule and Gene Cluster Families. *Proceedings of the National Academy of Sciences*, **110**, E2611-E2620. https://doi.org/10.1073/pnas.1303471110

[107] Ross, A.C., Gulland, L.E.S., Dorrestein, P.C. and Moore, B.S. (2014) Targeted Capture and Heterologous Expression of the *Pseudoalteromonas* Alterochromide Gene Cluster in *Escherichia coli* Represents a Promising Natural Product Exploratory Platform. *ACS Synthetic Biology*, **4**, 414-420. https://doi.org/10.1021/sb500280q

[108] Ren, Y., Liu, R., Zheng, Y., Wang, H., Meng, Q., Zhu, T., *et al*. (2024) Biosynthetic Mechanism of the Yellow Pigments in the Marine Bacterium *Pseudoalteromonas* sp. Strain T1lg65. *Applied and Environmental Microbiology*, **90**, e01779-23. https://doi.org/10.1128/aem.01779-23

[109] Foulston, L.C. and Bibb, M.J. (2010) Microbisporicin Gene Cluster Reveals Unusual Features of Lantibiotic Biosynthesis in Actinomycetes. *Proceedings of the National Academy of Sciences*, **107**, 13461-13466. https://doi.org/10.1073/pnas.1008285107

[110] Nguyen, N.A., Lin, Z., Mohanty, I., Garg, N., Schmidt, E.W. and Agarwal, V. (2021) An Obligate Peptidyl Brominase Underlies the Discovery of Highly Distributed Biosynthetic Gene Clusters in Marine Sponge Microbiomes. *Journal of the American Chemical Society*, **143**, 10221-10231. https://doi.org/10.1021/jacs.1c03474

[111] Nguyen, N.A. and Agarwal, V. (2023) A Leader-Guided Substrate Tolerant RiPP Brominase Allows Suzuki-Miyaura Cross-Coupling Reactions for Peptides and Proteins. *Biochemistry*, **62**, 1838-1843. https://doi.org/10.1021/acs.biochem.3c00222

[112] Edwards, D.J., Marquez, B.L., Nogle, L.M., McPhail, K., Goeger, D.E., Roberts, M.A., *et al*. (2004) Structure and Biosynthesis of the Jamaicamides, New Mixed Polyketide-Peptide Neurotoxins from the Marine Cyanobacterium *Lyngbya majuscula*. *Chemistry & Biology*, **11**, 817-833. https://doi.org/10.1016/j.chembiol.2004.03.030

[113] Esquenazi, E., Jones, A.C., Byrum, T., Dorrestein, P.C. and Gerwick, W.H. (2011) Temporal Dynamics of Natural Product Biosynthesis in Marine Cyanobacteria. *Proceedings of the National Academy of Sciences*, **108**, 5226-5231. https://doi.org/10.1073/pnas.1012813108

[114] Lukowski, A.L., Hubert, F.M., Ngo, T., Avalon, N.E., Gerwick, W.H. and Moore, B.S. (2023) Enzymatic Halogenation of Terminal Alkynes. *Journal of the American Chemical Society*, **145**, 18716-18721. https://doi.org/10.1021/jacs.3c05750

[115] Neubauer, P.R., Pienkny, S., Wessjohann, L., Brandt, W. and Sewald, N. (2020) Predicting the Substrate Scope of the Flavin-Dependent Halogenase BrvH. *ChemBioChem*, **21**, 3282-3288. https://doi.org/10.1002/cbic.202000444

[116] Xu, D., Metz, J., Harmody, D., Peterson, T., Winder, P., Guzmán, E.A., *et al*. (2022) Brominated and Sulfur-Containing Angucyclines Derived from a Single Pathway: Identification of Nocardiopsistins D-F. *Organic Letters*, **24**, 7900-7904. https://doi.org/10.1021/acs.orglett.2c02879

[117] Panter, F., Garcia, R., Thewes, A., Zaburannyi, N., Bunk, B., Overmann, J., *et al*. (2019) Production of a Dibrominated Aromatic Secondary Metabolite by a Planctomycete Implies Complex Interaction with a Macroalgal Host. *ACS Chemical Biology*, **14**, 2713-2719. https://doi.org/10.1021/acschembio.9b00641

[118] Gäfe, S. and Niemann, H.H. (2023) Structural Basis of Regioselective Tryptophan Dibromination by the Sin-

gle-Component Flavin-Dependent Halogenase AetF. *Acta Crystallographica Section D Structural Biology*, **79**, 596-609. https://doi.org/10.1107/s2059798323004254

[119] Adak, S., Lukowski, A.L., Schäfer, R.J.B. and Moore, B.S. (2022) From Tryptophan to Toxin: Nature's Convergent Biosynthetic Strategy to Aetokthonotoxin. *Journal of the American Chemical Society*, **144**, 2861-2866. https://doi.org/10.1021/jacs.1c12778

[120] Jiang, Y., Snodgrass, H.M., Zubi, Y.S., Roof, C.V., Guan, Y., Mondal, D., *et al*. (2022) The Single-Component Flavin Reductase/Flavin-Dependent Halogenase AetF Is a Versatile Catalyst for Selective Bromination and Iodination of Arenes and Olefins. *Angewandte Chemie International Edition*, **61**, e202214610. https://doi.org/10.1002/anie.202214610

[121] Timilsina, S., Potnis, N., Newberry, E.A., Liyanapathiranage, P., Iruegas-Bocardo, F., White, F.F., *et al*. (2020) *Xanthomonas* Diversity, Virulence and Plant-Pathogen Interactions. *Nature Reviews Microbiology*, **18**, 415-427. https://doi.org/10.1038/s41579-020-0361-8

[122] Andrewes, A.G., Jenkins, C.L., Starr, M.P., Shepherd, J. and Hope, H. (1976) Structure of Xanthomonadin I, a Novel Dibrominated Aryl-Polyene Pigment Produced by the Bacterium. *Tetrahedron Letters*, **17**, 4023-4024. https://doi.org/10.1016/s0040-4039(00)92565-6

[123] 郑哲麟, 等. 黄素依赖型溴化酶 XanJ 参与黄单胞菌菌黄素的生物合成[J]. 植物病理学报, 2023, 53(2): 229-244. https://doi.org/10.13926/j.cnki.apps.000639