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Anaerobic biotransformation mechanism of marine toxin domoic acid

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ABSTRACT

Domoic acid (DA) is a major marine neurotoxin, occurs frequently in most of the world's coastlines and seriously threatens ecosystem and public health. However, information on its biotransformation process in coastal anaerobic environments remains unclear. In this study, the underlying mechanism of anaerobic biotransformation of DA by marine consortium GLY was investigated using the combination of liquid chromatography–high-resolution Orbitrap mass spectrometry and comparative metatranscriptomics analysis. The results demonstrated that DA could be cometabolically biotransformed under anaerobic conditions with pseudo-first-order reaction. Anaerobic biotransformation pathway of DA was clarified, including decarboxylation, dehydrogenation, carboxylation activation with CoA and multiple β-oxidation steps occurring at aliphatic side chain, which facilitated DA detoxification. Furthermore, anaerobic cometabolic biotransformation mechanism of glycine-DA by consortium GLY was established for the first time, a number of genes related to the metabolic pathways of glycine fermentation, fatty acid synthesis and β-oxidation were responded in the consortium GLY transcriptome and involved in the anaerobic biotransformation of DA. This study could deepen understanding of interaction mechanism between toxin DA and marine microorganisms, which provides a new insight into the DA fate and its effects on benthic microbial community in marine environments.

1. Introduction

Domoic acid (DA) is a potent neurotoxin, naturally produced by seasonally blooming diatoms belonging to the genus *Pseudo-nitzschia* (Kumar et al., 2009; Teng et al., 2015; Trainer et al., 2012). It is excitotoxic in the central nervous system, myocardium and other glutamate receptor-rich organs (Hampson et al., 1992; Pulido, 2008). Every year, there are large number of marine creatures dying of domoic acid poisoning worldwide (Bates et al., 2018; Lefebvre et al., 2002). The largest risk of DA exposure for humans is linked to the ingestion of DA-contaminated shellfish, it can cause headaches, vomiting, seizures and even death (Perl et al., 1990). More seriously, as the increasing appearance of diatom blooms in the global ocean, DA has already been detected in marine mammals, shellfish, birds, zooplankton, echinoderms, as well as sediments (Anderson et al., 2012; Bargu et al., 2002). DA has obviously become a huge potential threat to the marine ecosystem.

Directly or indirectly (sensitized), DA can be photodegraded in marine euphotic zone (Bandala et al., 2009; Bouillon et al., 2008; Fisher et al., 2006; Jaramillo et al., 2020; Jin et al., 2018; Jones et al., 2009). The main active centers of DA are conjugated double bond, proline ring and carboxyl groups when photodegraded (Jin et al., 2018; Jones et al., 2009). Moreover, based on the reaction with different active species, the half-life of DA could range from a few hours to 9 weeks (Jaramillo et al., 2020). The photodegradation products of DA are mainly isodomoic acids D, E and F, which are usually less toxic than DA (Campbell et al., 2005). Additionally, dehydrogenated, dihydroxylated and decarboxylated products were also detected during DA photodegradation (Jin et al., 2018). However, after algal blooms, the aggregates (marine snow), which are typically comprised of senescent algae, plant debris, fecal particles, are further quickly transferred to the dark area of the sea floor under the action of gravity. The senescent algae will subsequently release a lot of algal organic matter and DA into sediment (Alldredge and Silver, 1988; Schnetzer et al., 2017; Smetacek, 1985). Specifically, the maximum concentration of DA in offshore sediments can reach 1 mg L^{-1} (Sekula-Wood et al., 2009). Thus, we reasoned that the fate of DA in the coastal environment cannot be explained by photolysis alone.

For aphotic zone, microbes are expected to play a major role in the

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degradation of DA in marine environment. However, the current research on the biotransformation of DA is very limited, there are only a few studies recorded that some marine autochthonous bacteria had the ability to transform DA under aerobic conditions (Hagström et al., 2007; Stewart, 2008; Stewart et al., 1998), while the products and mechanisms of DA biotransformation still remain obscure. What's more, anaerobic metabolism of microorganisms usually occur in the anoxic microenvironments within marine snow during the downward journey to seabed (Bristow, 2018). After reaching seabed, the released algal organic matter could be aerobically metabolized, thus resulting in an anaerobic local microenvironment in the sediment–water interface (Mckew et al., 2013). Also, natural fluidity of seawater makes organic matter and microorganisms easily buried into deep anaerobic sediments. Therefore, the anaerobic microbial degradation of DA might be the dominant degradation process. So clarify the pathway and mechanism of anaerobic biotransformation of DA by marine microorganisms could provide crucial information regarding fate of DA in marine environment.

The natural production of DA occurs during harmful algal blooms along with high levels of algal organic matter, such as amino acids, proteins and carbohydrates, which is the most important carbon and energy source for heterotrophic bacteria, thus cometabolic degradation might play a critical role. In this study, an enriched marine consortium named GLY from Dalian Bay sediment (Dalian, China) had capacity to biotransform DA anaerobically. Our objectives were (1) to investigate anaerobic biotransformation characteristics of DA; (2) to establish the DA anaerobic biotransformation pathway combined DA metabolites analysis with catabolic genes involved in DA anaerobic biotransformation; (3) to reveal the global response mechanism of consortium during DA anaerobic biotransformation by metatranscriptomics analysis.

2. Materials and methods

2.1. Materials

The DA certified calibration solution, CRM-DA-g (103.3 μg mL^{-1}), was purchased from the National Research Council Canada. DA (98% purity) used for biochemical experiments was obtained from Enzo life sciences, inc. (USA). A stock solution of DA (10 mg L^{-1}) was prepared in ultrapure water, and stored at 4 °C in the dark prior to use. HPLC grade acetonitrile, methanol, trifluoroacetic acid (TFA) and formic acid (FA) were purchased from Spectrum Chemical Mfg. Corp (USA). All other chemicals were analytical grade or better. Ultrapure water from a Milli-Q water purification system (Millipore, Bedford, USA) was used throughout the experiments. All glassware used was chemically cleaned.

2.2. Anaerobic biotransformation of DA

The goal of the experimental design was to mimic anaerobic cometabolic conditions. Glycine, glucose, alginate, bovine serum albumin (BSA), 2216E liquid medium (a marine culture medium), glycine-glucose and alginate-BSA were used as the co-substrates in cultures, which were model organic compounds for algal organic matter and readily-degradable matter in natural marine environment (Vandamme et al., 2016). All enrichment experiments were conducted under anaerobic conditions as described in Text S1 and Fig. S1 of the Supporting Information (SI). After 8 months of serial cultivation, seven DA-transforming consortia were obtained. Consortia spiked with glycine, glucose, alginate, BSA, glycine-glucose, alginate-BSA and 2216E liquid medium in the presence of 1 mg L^{-1} DA, were denoted as GLY, GLU, GLA, GLB, GLYU, GLAB and 2216E, respectively. Based on biotransformation performance, Consortium GLY cultured in GPY medium was selected to explore the mechanism of anaerobic cometabolic biotransformation of DA. The composition of GPY medium (pH 8.0) was that of the artificial seawater with 4 g L^{-1} glycine and 1 mg L^{-1} DA, which was prepared anaerobically and sterilized prior to use. Specifically, consortium GLY spiked with 1 mg L^{-1} DA was set as the DA group, and a CK group was established consisting of the same communities without DA addition, each in triplicate. All DA biotransformation assays were cultured in anaerobic chamber at 25 °C in darkness to avoid photolysis.

2.3. Molecular biological analysis

High-throughput 16S rRNA gene amplicon sequencing was performed on the 3-and 7-day samples in DA and CK groups; the detailed information on consortia analysis was listed in Text S2. The 16S rRNA sequencing data was deposited at GenBank (see Table S1 for accession number).

To analyze global response mechanism of consortium GLY during DA anaerobic biotransformation, metatranscriptome sequencing was performed on 3-day samples in DA and CK groups. The information on RNA sequencing, annotation, and differential gene expression was given in Text S3 and Fig. S2. The metatranscriptome sequencing data was deposited in the NCBI sequence read archive (SRA) (see Table S1 for accession number).

2.4. Chemical analytical methods

DA was measured by a high-performance liquid chromatography (HPLC, Shimadzu LC-20AT, Kyoto, Japan) (Text S4). DA anaerobic biotransformation metabolites were extracted by solid phase extraction technique and identified by high-resolution HPLC-LTQ-orbitrap MS (Text S5). OD_{600} was measured by UV–vis spectrophotometer. The pH was measured using a digital pH meter (FE20K, Mettler-Toledo, Switzerland). The membrane integrity including flow cytometry (FCM) measurement and the extracellular polymeric substances (EPS) assay were measured according to the method previously described (Gu et al., 2018). Field-emission scanning electron microscopy (FESEM) (FEI NOVA NanoSEM450, America) was used to observe the cell morphology. Total organic carbon (TOC) and total nitrogen (TN) were analyzed using a TOC/TN analyzer (Shimadzu, Japan). The volatile fatty acids (VFAs), CO_2 and CH_4 were measured as previously described (Wang et al., 2019).

3. Results and discussion

3.1. Anaerobic cometabolic biotransformation of DA

It was found that DA could not be utilized as the sole carbon and energy source (Fig. S3), which led us to try degrading DA by cometabolic process. Seven kinds of co-substrates (glycine, glucose, alginate, BSA, 2216E, glycine-glucose and alginate-BSA) were chosen to simulate readily-degradable organic matter in marine environment. There was no apparent decrease of DA in abiotic blank and control experiments, indicating the abiotic influence (i.e., chemical hydrolysis, adsorption) was negligible (data not shown). Moreover, 10–82% of DA could be cometabolically biotransformed in seven enriched consortia after 14 days of cultivation under anaerobic conditions (Fig. 1A). It was shown that addition of glycine, glucose or glycine-glucose had a significantly positive effect on DA biotransformation, but a negative effect was found with high concentrations of alginate, BSA, 2216E and alginate-BSA.

The enriched consortia experiments showed that glycine was the most effective co-substrate for DA anaerobic biotransformation (Fig. 1A-B). Up to 82% of the initial mass of DA was anaerobically transformed by consortium GLY after 7days. The biomass (OD_{600}) greatly increased in the first 7 days and finally kept stable (Fig. 1C). DA anaerobic biotransformation followed pseudo-first-order kinetics (Fig. 1D). The biotransformation rate constant (k) was 0.2333 ± 0.0155 d^{-1}, which corresponded to half-life ($t_{1/2}$) of 3 days. Efficient reduction of DA by indigenous consortium GLY under anaerobic conditions implies that anaerobic biotransformation is an important natural attenuation pathway for DA in the marine aphotic zone (i.e., sediment–water

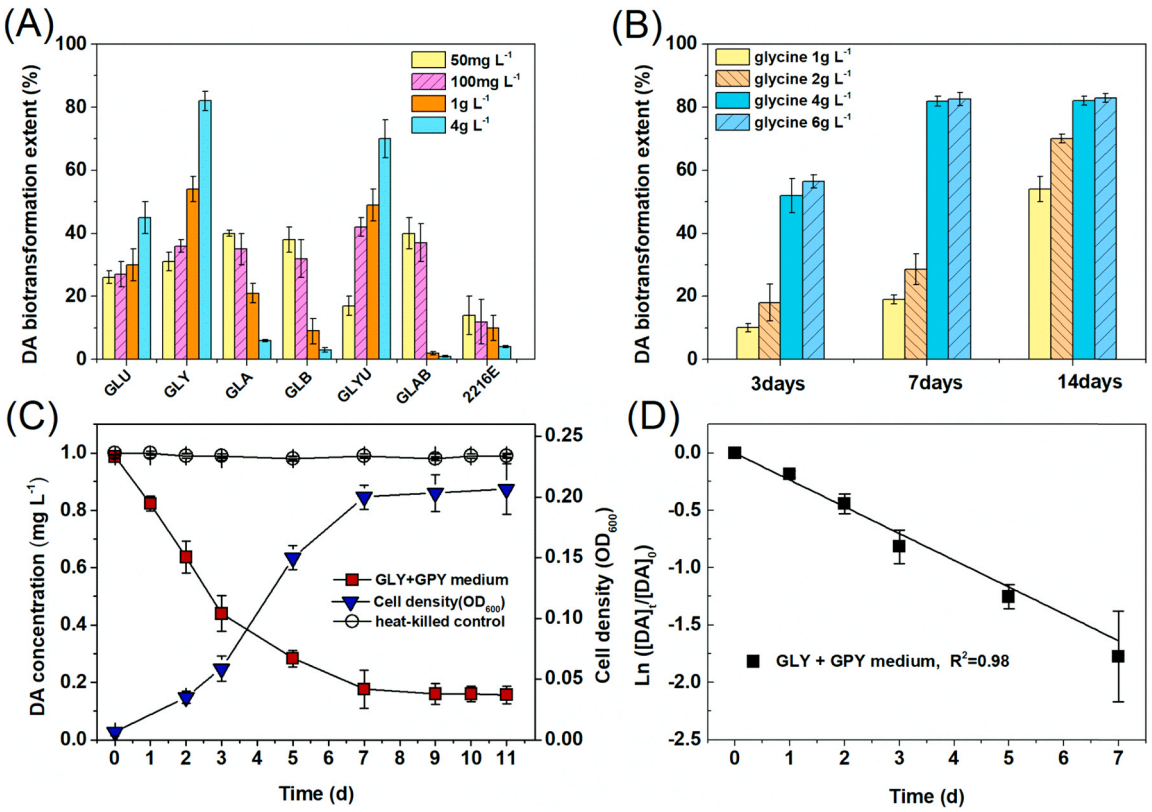

Fig. 1. The cometabolic biotransformation of DA under anaerobic conditions. (A) The anaerobic transformation of 1 mg L^{-1} DA by seven consortia supplied with different co-substrates (50 mg L^{-1} to 4 g L^{-1}) after 14 days cultivation; (B) DA biotransformation by consortium GLY under different initial glycine concentrations; (C) DA biotransformation and cell growth by consortium GLY in GPY medium; (D) The plot of $\ln([DA]_t/[DA]_0)$ versus time.

interface). The relatively constant TOC and TN values were observed during glycine uptake (data not shown). In the anaerobic cometabolic process, propionate and acetate were produced with the pH value decreased accordingly, and trace amounts of carbon dioxide were detected, but there was no methane formation (Fig. S4). Collectively, glycine anaerobic fermentation stayed in the acidification stage. These results suggested that acidogenesis might contribute to some extent to DA biotransformation under anaerobic conditions.

3.2. Anaerobic biotransformation pathway of DA

DA metabolites were analyzed by HPLC/HRMS/MS (Tables S2 and S3, Text S6, Figs. S5 and S6), and the metabolites were identified based on MS/MS ion fragments and comparison with the degradation products of DA reported in previous studies. As shown in Fig. S5A, isodomoic acid E, one major identifiable DA isomer product (retention time: 11.94 min), was detected by comparing with the retention times of the product mixture and DA isomers in a certified DA standard solution. This isomer was also identified in the DA photolysis process, which is less toxic than DA (Campbell et al., 2005). What is more, concentration of isodomoic acid E continuously increased with time, and the maximum concentration was 130 ug L^{-1} on 7 days (Fig. S5B). This result showed that isomerization is a biotransformation pathway of DA and since the toxicity of the isodomoic acid E was lower than that of DA, which might be important for detoxification taking place during the anaerobic biotransformation of DA. Overall, the anaerobic biotransformation extent of DA by GLY could reach 82% after 7 days, 13% of DA was transformed to isodomoic acid E and the remaining 69% was further biotransformed through other pathways.

In order to explore DA anaerobic biotransformation pathway, several DA metabolites were captured. The less polar product with retention time of 14.00 min was identified as decarboxylation product (P267) with the *m/z* of 268.1535 and its major fragments at *m/z* 250.1433 (loss of H_2O), *m/z* 232.1324 (loss of two H_2O) and *m/z* 222.1478 (loss of CH_2O_2). The hydroxylation product (P283) with retention time of 9.60 min and *m/z* of 284.1486 was detected, its major fragments at *m/z* 266.1377 (loss of H_2O), *m/z* 238.1428 (loss of CH_2O_2) and *m/z* 192.1373 (loss of two CH_2O_2). The methyl ketone product (P281) with retention time of 5.62 min and *m/z* of 282.1329 was identified, its major fragments at *m/z* 264.1220 (loss of H_2O), *m/z* 240.1222(loss of C_2H_2O), *m/z* 236.1272(loss of CH_2O_2) and *m/z* 224.0910 (loss of C_3H_6O). Therefore, based on the above three identified products, the distinct pathway during DA anaerobic biotransformation is proposed in Fig. 2. First, decarboxylation may be initial activation reaction of DA, and intermediate P267 was produced via α decarboxylation reaction at the aliphatic side chain of DA. Second, P267 might be converted to P283 by hydroxylating the side chain at the carbon atom proximal to conjugated diene with water. Moreover, secondary alcohol P283 was further oxidized via dehydrogenation to form the corresponding methyl ketone P281. The decarboxylation from aliphatic chain of DA has also been extensively observed during DA photodegradation (Jin et al., 2018; Campbell et al., 2005). Meanwhile, decarboxylation is an important reaction in amino acids and aromatic substrates anaerobic degradation (Li et al., 2012). Thus, it seems likely that the anaerobic biotransformation of DA involved the decarboxylation at aliphatic side chain via decarboxylase reaction to form P267. Additionally, the hydroxylation product of P267 was detected. This hydroxylation has been observed to occur for benzene, naphthalene and ethylbenzene via a dehydrogenase under anaerobic conditions (Grbic-Galic and Vogel, 1987; Rabus and Widdel, 1995; Ball et al., 1996). Hence, P267 might be further converted via dehydrogenation to form P283 and P281 by analogous anaerobic degradation mechanism. Furthermore, P281 was detected at day 1 with commencement of DA biotransformation, and steady-state plateau in P281 concentration was observed after 3 days, indicating P281

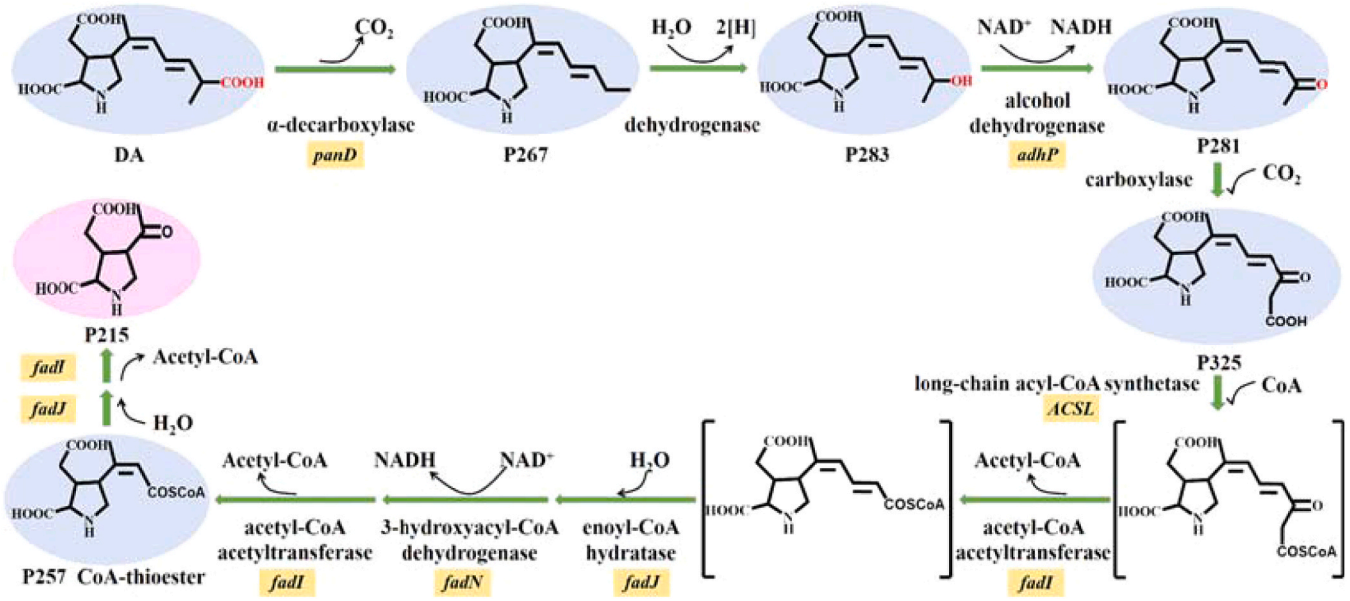

Fig. 2. Proposed anaerobic DA biotransformation pathway.

production rate was balanced with its transformation rate (Fig. S6). Considering anaerobic degradation of ketones required carboxylation-like reactions as primary activation steps (Heider et al., 2016), P281 might be further mineralized to α keto-acid via carboxylation reaction. The corresponding carboxylation product (P325) was captured with retention time of 6.84 min and *m/z* of 326.1228. In addition, P257 (*m/z* 258.0979) and P215 (*m/z* 216.0871) were observed with retention times of 2.54 and 2.47 min, respectively. The MS^2 spectrum of P257 mainly consisted of fragments of *m/z* 240.0871 (loss of H_2O) and *m/z* 212.0919 (loss of CH_2O_2). The MS^2 spectrum of P215 mainly consisted of fragments of *m/z* 170.0810 (loss of CH_2O_2) and *m/z* 198.0758 (loss of H_2O). This result suggested that intermediate P325 might be further anaerobically transformed to downstream products. Taken together, α decarboxylation, dehydrogenation and carboxylation might occur during DA anaerobic biotransformation process.

Comparative metatranscriptomics analysis was further applied to explore catabolic genes and construct complete DA biotransformation pathway. In the α decarboxylation step, the gene *panD* encoding aspartate 1-decarboxylase was significantly upregulated (Fig. S7). It is reported that aspartate 1-decarboxylase catalyzes aspartate to yield CO_2 and β-alanine (Liu et al., 2012). Due to the structural similarity of aspartic acid and DA, this enzyme might be responsible for α decarboxylation reaction of DA. In addition, the gene *adhp* encoding alcohol dehydrogenase was significantly upregulated (Fig. S7). The short-chain alcohol dehydrogenase catalyzes 1-phenylethanol to acetophenone during ethylbenzene anaerobic degradation process (Fuchs et al., 2011). It speculated that alcohol dehydrogenase was involved in the dehydrogenation from P283 to P281. As mentioned above, P281 could be further mineralized to α keto-acid by a carboxylase. In this study, the gene *pyc* encoding pyruvate carboxylase, *accC* encoding acetyl-CoA carboxylase and *pccB* encoding propionyl-CoA carboxylase were significantly upregulated. Among these upregulated carboxylases, pyruvate carboxylase might be the more plausible option for carboxylation of P281. For ketones, such as acetophenone and *p*-hydroxyacetophenone, after carboxylation steps, subsequent reactions commonly involve in activation with CoA and β oxidation (Heider et al., 2016; Durante-Rodríguez et al., 2018). In this study, the gene *ACSL* encoding long-chain acyl-CoA synthetase, *fadI* encoding acetyl-CoA acyltransferase, *fadJ* encoding enoyl-CoA hydratase and *fadN* encoding 3-hydroxyacyl-CoA dehydrogenase were significantly upregulated (Fig. S7). It speculated that further transformation of P281 was proceeded via pyruvate carboxylase (*pyc*) to P325, followed by CoA-activation of P325 to yield the respective CoA thioesters via long-chain acyl-CoA synthetase (*ACSL*), and then thiolytic cleavage to acetyl-CoA and shortened CoA thioester (less two carbon) via acetyl-CoA acyltransferase (*fadI*). Schnell et al. (1991) reported that sorbate is anaerobically degraded by fermenting bacterium AmSol, with the pathway undergoes β-oxidation on double bond of sorbate, including hydration and dehydrogenation reactions. Similarly, the double bonds in the shortened CoA thioester generated from P325 biotransformation were localized in a pattern well suited for further transformation through β-oxidative chain-shortening reactions. Hence, further biotransformation of this shortened CoA thioester resembled a β-oxidation cycle with an addition of water to a double bond (*fadJ*), a dehydrogenation reaction (*fadN*), and thiolytic cleavage (*fadI*) leading to acetyl-CoA and P257 CoA-thioester as intermediates. The cycle is then repeated until yielding intermediate P215, which was nontoxic and also detected during DA photodegradation (Jaramillo et al., 2020).

Considering active genes and intermediates observed in DA anaerobic biotransformation, the anaerobic pathway for DA is constructed in Fig. 2. DA is decarboxylated to P267, which is further transformed via dehydrogenation to form P283 and P281. P281 could be carboxylated to produce P325 and subsequent reactions involve in activation with CoA and β-oxidation steps, finally yielding nontoxic P215. Because downstream product of P215 was not captured, the possibility of further transformation of P215 was unknown and remained to be investigated. The result indicated that anaerobic biotransformation could be an important natural detoxification process for DA.

3.3. *Anaerobic cometabolic biotransformation mechanism of DA by consortium GLY*

During DA anaerobic biotransformation, the *Bacilli* were the predominant active species, with a relative abundance increasing from 77.5% (3 days) to 96.8% (7 days) (Fig. 3A, S8). These *Bacilli* are mainly consisting of the genus *Bacillus* (Fig. S9). These data suggested that the *Bacilli* (*Bacillus*) were tolerate to DA and might be potentially DA-transforming bacteria. It is known that the *Bacillus* species are ubiquitous and diverse in the marine ecosystem (Oguntoyinbo, 2007), which is a type of hydrolytic, acetogenic bacteria in marine environment (Lei et al., 2017). Hence, the increased abundance of *Bacillus* would enhance the hydrolysis and acidogenesis processes in DA group. These results indicated that anaerobic communities (i.e., hydrolytic and acidogenic)

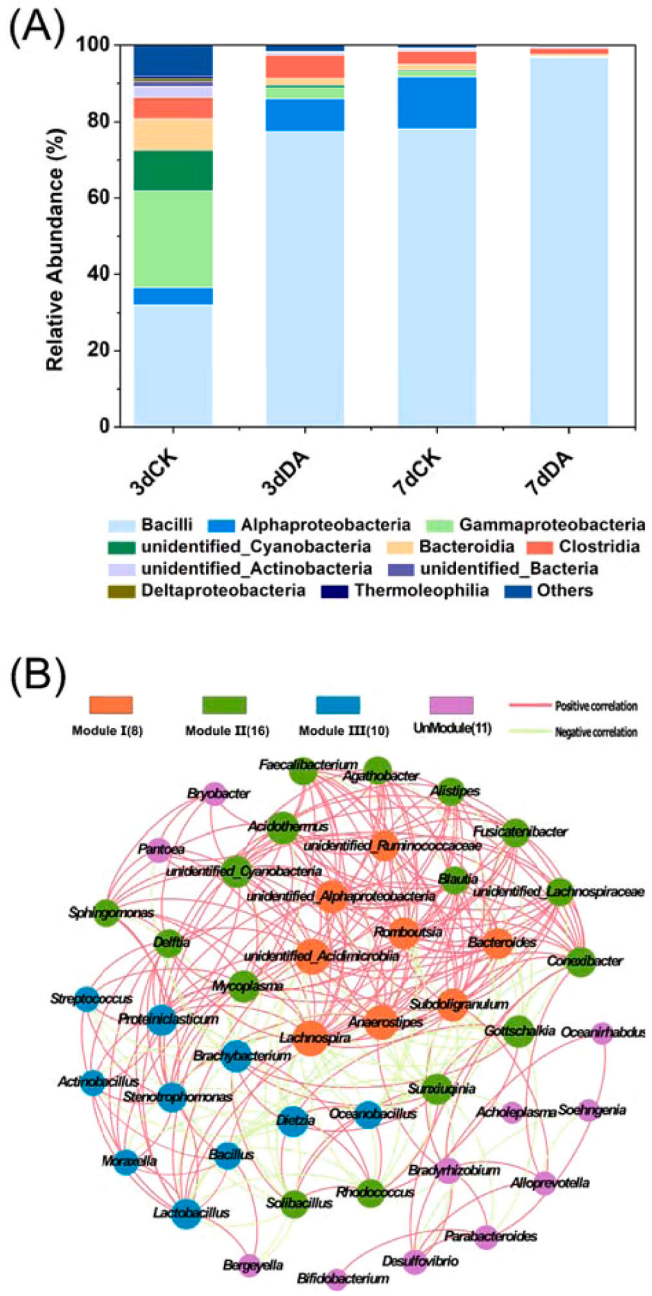

Fig. 3. (A) Mean relative abundance of species taxonomy of CK and DA groups derived from 16S rRNA gene sequencing analysis at class level. (B) Co-occurrence network analysis at genus level in DA groups. Nodes are colored by modularity. A connection represents a strong (Spearman'ρ $\geq$ 0.8) and significant ($p \leq 0.05$) correlation. The size of each node is proportional to the number of connections.

could play a part in biotransformation of DA. Co-occurrence patterns were explored to offer insight into microbial interactions. The modular structure of DA groups was classified into four major modules (Fig. 3B). In module III of the network, some bacteria were involved with hydrolysis and acidogenesis. *Lactobacillu* is typical acetic acid-producing anaerobic bacteria (Xu et al., 2017), and *Proteiniclasticum* is also an anaerobic amino acid-utilizing bacteria with acetic acid and propionate as the main fermentation product (Zhang et al., 2020). *Moraxella* has been reported to be able to transform DA (Stewart et al., 1998). These bacteria co-occurred with *Bacillus*, suggesting that they were co-selected. These results indicated that the anaerobic cometabolic biotransformation of DA is contributed by *Bacillus* in synergy with various microorganisms.

Microbial tolerance to organic pollutants is the key to their efficient biotransformation (Murínová and Dercová, 2014). However, the response mechanisms of microorganisms to micro-pollutants in cometabolism remain obscure. In this study, some of the up-regulated transcripts linked with resistance such as antioxidant, DNA repair and antibiotic resistance genes (i.e., elfamycin (*EF-Tu*) and tetracycline (*tetT*)) were induced during DA biotransformation process (Table S4, Fig. S10), indicating that trace amounts of DA could cause physiological stress in bacteria. Resistance mechanisms (antioxidant system, DNA/protein repair and toxic organics efflux) were shown to be co-regulated at the transcriptional level in consortium GLY (Fig. S11). Moreover, the signature of outer protection was upregulation of glycosyltransferase (GTF), which supports for exopolysaccharide production. Additionally, FCM data showed that oxidative stresses exerted negligible effects (less than 2% cell damage) on the cell membrane (Fig. S12). Amount of EPS, especially protein-like substrates, were significantly increased after DA addition, which might account for the alleviation of oxidative damage (Figs. S13–15). In conclusion, consortium GLY enhanced a series of antioxidant system, repair capacities and toxic organics efflux to deal with DA stress, might strengthen inner and outer strategies of protection and repair under DA addition. Additionally, trace amounts of DA could cause enrichment of antibiotic resistance genes in bacteria, so that potential risk of DA in benthic ecosystems is alarming.

After consortium GLY has been equipped with adaptive capacity to DA, the potential degraders (i.e., *Bacillus*) were allowed to apply their degradation ability in anaerobic cometabolic process. Based on metatranscriptomics information (Table S5), anaerobic cometabolic biotransformation mechanism of glycine-DA by consortium GLY were established (Fig. 4). The genes *TC.AGCS* encoding AGCS family and *TC.SSS* encoding solute:Na^+ symporter, SSS family were significantly over-expressed after DA addition. Therefore, it was considered that the uptake of glycine by consortium GLY was achieved via AGCS family in symport with Na^+ (Ma et al., 2019). Complete genes set for glycine fermentation via the glycine-serine-pyruvate interconversion were found in all transcripts of consortium GLY and most of them were upregulated after DA addition. The results showed that the conversion of glycine to serine was achieved via the condensation of 5,10-methylene-THF with glycine by glycine hydroxymethyltransferase (*glyA*). Then obtained serine could be converted into pyruvate via L-serine/ threonine dehydratase (*tdcG/ tdcB*). The pyruvate pool might be linked to acetyl-CoA and oxaloacetate production, subsequently entered the citrate cycle (TCA cycle). Aspartate could be produced from oxaloacetate via transamination by aspartate aminotransferase (*aspB*), and further converted to β-alanine via aspartate 1-decarboxylase (*panD*). Meanwhile, The gene *panD* was possibly responsible for DA decarboxylation and upregulated ($\log_2^{(\text{fold change})} = 5.99$) after DA addition, suggesting an enhancement for the production of P267. Furthermore, we explored the anaerobic biotransformation of DA with aspartate as co-substrate by consortium GLY. The results showed that biotransformation extent of DA reached 60% after 10 days, and P267 was also captured (Fig. S16). This confirmed that decarboxylation was initial activation reaction of DA during anaerobic biotransformation, and the important source for aspartate 1-decarboxylase (*panD*) was transformation of L-Aspartate to β-Alanine. The key gene *adhp* required for dehydrogenation from P283 to P281 was over expressed ($\log_2^{(\text{fold change})} = 5.68$) after DA addition, which could be produced during acetyl-CoA metabolism. In anaerobic biotransformation of DA, β-oxidation was the crucial step for DA detoxification. It is known that β-Oxidation is the ubiquitous metabolic strategy during metabolization of fatty acids. Major genes involved in fatty acid synthesis pathway such as *accC*, *fabF*, *fabG* and *fabK* were remarkably over-expressed. In fatty acid degradation pathway, the genes *ACSL*, *fadJ*, *fadN* and *fadI* were 5-$\log_2^{(\text{fold change})}$ higher in DA group. It was reported that fatty acid synthesis was activated by acetyl-CoA carboxylase (*accC*), and subsequently entered β-oxidation

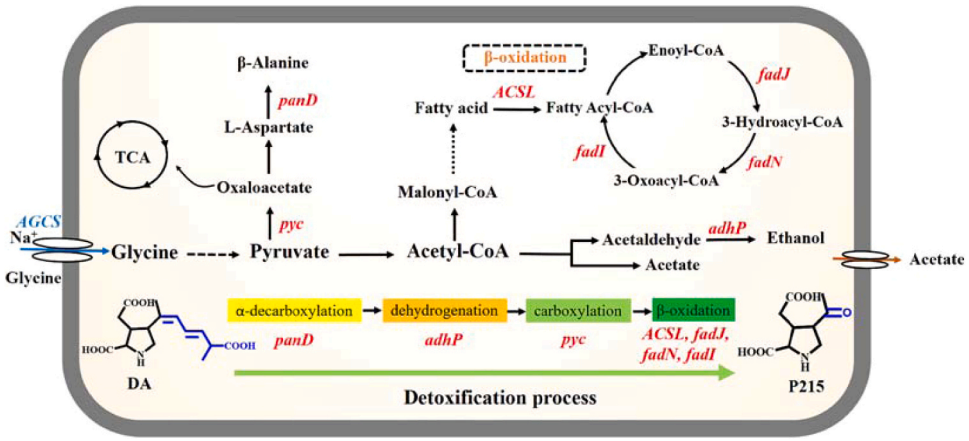

Fig. 4. Anaerobic cometabolic biotransformation mechanism of glycine-DA by consortium GLY.

process for catabolism of fatty acid (Krivoruchko et al., 2015). The mechanism of β-oxidation for the purpose of carbon chain-shortening is quite common in nature, not only in the context of monocarboxylic (fatty) acid catabolism, but also for the degradation of aliphatic and aromatic compounds (Kallscheuer et al., 2017). Since the diene functionality plays a critical role in the toxicity of DA (Jaramillo et al., 2020), β-oxidative chain-shortening reactions may represent the crucial step for DA detoxification. Therefore, the over expression of genes involved in β-oxidation after DA addition could enhance the generation of P215. As outlined above, a number of genes related to the metabolic pathways of glycine fermentation, fatty acid synthesis and β-oxidation were responded in the consortium GLY transcriptome and involved in the anaerobic biotransformation of DA.

4. Conclusions

The underlying mechanism of anaerobic cometabolic biotransformation of DA by marine consortium GLY was clarified for the first time. The results demonstrated that DA could be cometabolically biotransformed under anaerobic conditions. Anaerobic biotransformation was an important detoxification process for DA, underwent decarboxylation, dehydrogenation, carboxylation, activation with CoA and multiple β-oxidation steps. Furthermore, anaerobic cometabolic biotransformation mechanism of glycine-DA by consortium GLY was established. Some genes related to the metabolic pathways of glycine fermentation, fatty acid synthesis and β-oxidation (such as *panD*, *adhP*, *ACSL*, *fadI*, *fadJ* and *fadN*) were responded in the consortium GLY transcriptome and involved in the anaerobic biotransformation of DA. This study deepens knowledge of transcriptomic responses of marine microorganisms to toxin DA as well as highlighted a potential mechanism of biotransforming DA by marine microbes, which provides a new insight into the DA fate and its potential impact on the function of benthic microorganisms in marine environments.

CRediT authorship contribution statement

Miaomiao Du conducted all the experiments, and prepared the first draft of the manuscript. **Miaomiao Du** and **Zelong Li** involved in data analyses, discussion of results and wrote the manuscript. **Jing Wang** planned research, involved in supervised all analyses, data interpretation and discussion as a Project Leader. **Fengbo Wang** assisted in conducting some experiments and sample analyses. **Shuaijun Zan** sampled in the field and assisted in metatranscriptomics analyses. **Chen Gu** assisted in metabolite analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.126798.

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