Chapter

Iron Assimilation Mechanisms in *Aeromonas*

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Abstract

This chapter provides an overview of the current understanding of iron acquisition mechanisms in *Aeromonas*. Two mechanisms, heme utilization and siderophore synthesis, have been extensively studied. All *Aeromonas* species can use heme to get iron, a mechanism facilitated by the production of hemolysins that release heme-containing molecules from host tissues. The predominant siderophore produced by most *Aeromonas* spp. is amonabactin, comprising a family of four different compounds. Amonabactins are internalized via FstC, an outer membrane transporter (OMT) characterized by a broad ligand plasticity. In addition to amonabactin, *A. salmonicida* also produces the siderophore acinetobactin, which is transported through FstB. Notably, both siderophores are concurrently produced, sharing part of the biosynthetic pathways. OMTs involved in iron acquisition hold a potential utility as tools for identification and as antigens for novel vaccines. Furthermore, synthetic derivatives of siderophores could serve as promising candidates for the development of novel antimicrobials, leveraging their specific internalization through OMTs.

Keywords: *Aeromonas*, iron uptake, siderophores, amonabactin, acinetobactin, heme uptake, outer membrane transporters, vaccines

1. Introduction

Within the *Aeromonas* genus, *Aeromonas hydrophila* and *Aeromonas salmonicida* have garnered significant attention for the study of iron acquisition mechanisms. As a result, most of the existing knowledge on iron uptake in *Aeromonas* spp. stems from research conducted on these two species, particularly *A. salmonicida* subsp. *salmonicida*. Thus, since iron acquisition systems have been poorly studied in other *Aeromonas* spp., this chapter will provide a summary of the current understanding of iron acquisition mechanisms in both species.

Iron is a crucial element for the metabolism of most microorganisms, but its assimilable forms are often scarce in biological environments due to its -chemical properties. This scarcity extends to animal tissues, where most iron is tightly bound to iron-containing proteins or heme-containing proteins. Consequently, bacteria have developed sophisticated mechanisms to acquire iron from the environment and from their hosts, particularly in the case of animal pathogens. These mechanisms for iron acquisition are widely recognized as essential for the survival of bacterial pathogens within their

hosts [1]. It is now understood that they play a significant role in bacterial virulence and contribute substantially to the development of infectious diseases, but they also play relevant roles in microbial ecology [2]. Within bacterial cells, iron levels are strictly controlled through diverse regulatory mechanisms since they are crucial for keeping many cell functions, but at the same time, an excess of iron is toxic [1]. Besides the role of iron in the cell metabolism, iron plays a key role in regulating virulence determinants, being at the same time a nutritional and a regulatory element [3]. Regulation usually occurs at the transcriptional level through the global transcriptional regulator Fur (ferric uptake regulator) and iron-responsive small regulatory noncoding RNAs. Fur and these regulatory RNAs can regulate, either directly or indirectly, many virulence determinants of pathogenic bacteria, such as invasion of eukaryotic cells, toxin production, type VI secretion systems (T6SS), motility, quorum sensing, stress resistance or biofilm formation [3]. It has been recently shown that Fur functions as an activator of the T6SS that mediates virulence in *A. hydrophila* [4].

One of the primary strategies employed by microorganisms to acquire iron is through the synthesis and secretion of siderophores. Siderophores are low-molecular-weight Fe(III) chelators with diverse structures that efficiently extract iron from iron-binding proteins or other iron-containing compounds. They then enter the cell through specific transport proteins in the cell envelope, including outer membrane cognate receptors [1, 5]. The synthesis of siderophores is a complex process that typically involves a complete set of dedicated genes encoding the necessary biosynthetic enzymes. Nonribosomal peptide synthetases (NRPS) are often the main type of enzymes involved in the synthesis of many siderophores. NRPS enzymes are multimodule and multicatalytic, assembling the different residues to form the final compound [5–7]. By analyzing the amino acid sequences of these enzymes and comparing them with other known NRPSs, it is possible, to some extent, to predict the residues that will be incorporated into the synthesized siderophore. This provides important insights into the structure of the compound synthesized.

Due to their prevalence in the host, heme and heme-containing proteins can also serve as a viable iron source for invading microorganisms. Consequently, one of the primary mechanisms employed by Gram-negative bacterial pathogens to acquire iron involves the utilization of heme or heme-containing proteins derived from host tissues [8]. Despite the high affinity of siderophores for iron binding, they are unable to extract iron from heme. Thus, Gram-negative bacterial pathogens possess the capability to acquire iron from free heme or heme proteins through mechanisms independent of siderophores. However, *in vivo*, free heme is not typically encountered, leading bacteria to acquire heme from hemoproteins instead. Hemoproteins are recognized by iron-regulated outer membrane protein receptors, through which the heme moiety is unloaded and transported across the outer membrane. The heme receptors are required to extract heme from various carrier proteins before the transport process. However, the precise mechanism by which the heme receptor interacts with the heme-protein complex to release the heme ligand remains incompletely understood.

In the genus *Aeromonas*, both systems, siderophore synthesis and heme utilization, have been described.

2. Utilization of heme by *Aeromonas* spp.

The initial evidence indicating that *A. salmonicida* strains possess the ability to internalize heme was provided by Ishiguro et al. [9]. Their studies revealed that

certain atypical *A. salmonicida* strains rely on heme as a porphyrin source for growth in laboratory media. However, these investigations did not specifically assess the utilization of heme as an iron source, and similarly, there was limited understanding regarding heme uptake in typical *A. salmonicida* subsp. *salmonicida* strains. Subsequently, a proteomic study conducted under iron-limited conditions shed further light on this matter. This study revealed that *A. salmonicida* expresses three iron-regulated outer membrane receptors, with one of these receptors being suggested as a potential heme receptor based on sequence similarity [10].

Our research group demonstrated that *A. salmonicida* subsp. *salmonicida* strains isolated from turbot can utilize heme compounds as the sole iron source. We identified a nine-gene cluster (*hutAZXBCD*) containing some of the genes essential for heme uptake [11]. Six of these genes exhibit significant similarities with heme transport and utilization genes found in other bacteria. HutA, previously identified by Ebanks et al. [10], is considered the putative heme receptor in *A. salmonicida* subsp. *salmonicida*. HutB shares homology with periplasmic heme-binding proteins, while HutC shows similarity to ABC-type permease proteins, and HutD displays homology to the ATP-binding protein component of ABC transporters. Deletion of *hutB* significantly impairs the ability of *A. salmonicida* to utilize hemin as the sole iron source. This mutant exhibits over a fivefold reduction in growth levels compared to the parental strain when cultured in an iron-restricted medium supplemented with hemin [11]. Although *hutB* is necessary for growth with hemin as the sole iron source, it is likely not entirely essential. The residual growth observed in the *hutB* mutant could be attributed to other periplasmic iron transporters encoded in the *A. salmonicida* genome. While the mutation of *hutB* leads to a notable reduction in the capacity to utilize heme as the sole iron source, it is important to note that deletion of *hutA*, which encodes a predicted outer membrane heme receptor, does not have the same effect, suggesting the probable existence of other heme receptors [11]. The functions of HutZ and HutX, along with their homologs in other bacteria, remain unclear.

The heme uptake gene cluster in *A. salmonicida* is organized into five transcriptional units, with three being polycistronic (*orf2-orf3, hutX-hutZ*, and *hutBCD*) and two being monocistronic (*hutA* and *orf1*). This spatial organization differs from heme clusters found in other bacteria. The absence of TonB system genes linked to the heme uptake genes in *A. salmonicida* is noteworthy [11].

In A. *hydrophila*, most isolates have been observed to utilize heme compounds [12]. Heme sources utilized by this species include hemin, hemoglobin, and hemoglobin complexed with human haptoglobin. The production of iron-regulated hemolysins in this species may facilitate the release of heme-containing molecules from host tissues. In the genome of *A. hydrophila* ATCC 7966, a gene cluster (loci AHA_0964 to −0972) has been identified as containing putative genetic determinants for heme utilization, which bears high similarity to the cluster described *in A. salmonicida* [11].

Maltz et al. [13] identified in an *A. veronii* strain isolated from the medicinal leech, *Hirudo verbana*, a putative outer membrane heme receptor, named *hgpB*, along with a downstream transcriptional activator (*hgpR*). In vitro, assays provided evidence that *hgpB* is crucial for utilizing iron bound to heme, and it was observed that both iron acquisition systems, siderophore synthesis and iron utilization from heme, are essential for *A. veronii* to grow in blood. Recently, it has been demonstrated that heme uptake genes were present in 24 genomes of *A. media* strains isolated from different host species and environmental samples [14], reinforcing the idea that heme uptake genes are likely to present in all *Aeromonas* spp., although specific data about other species are not yet available.

3. Siderophores produced by *Aeromonas*

The first data about siderophore production by *Aeromonas* was provided by the seminal work of Chart and Trust [15]. These authors demonstrated the capacity of typical strains of *A. salmonicida* to capture Fe3+ from high-affinity iron chelators such as ethylenediaminedihydroxy-phenylacetic acid, transferrin, or lactoferrin by a mechanism that seemed to be inducible and that was linked to the generation of low-molecular-weight soluble siderophore(s). A few years later it was demonstrated that *A. hydrophila* excreted two biologically active variants of a novel siderophore called amonabactin, a biscatecholate siderophore made from residues of 2,3-dihydroxybenzoic acid (DHBA), lysine, glycine, and either tryptophan (amonabactin T) or phenylalanine (amonabactin P) [16]. In fact, there are two different amonabactins T (named T789 and T732 according to specific molecular weight), and two amonabactins P (P750 and P693) (**Figure 1**) [17].

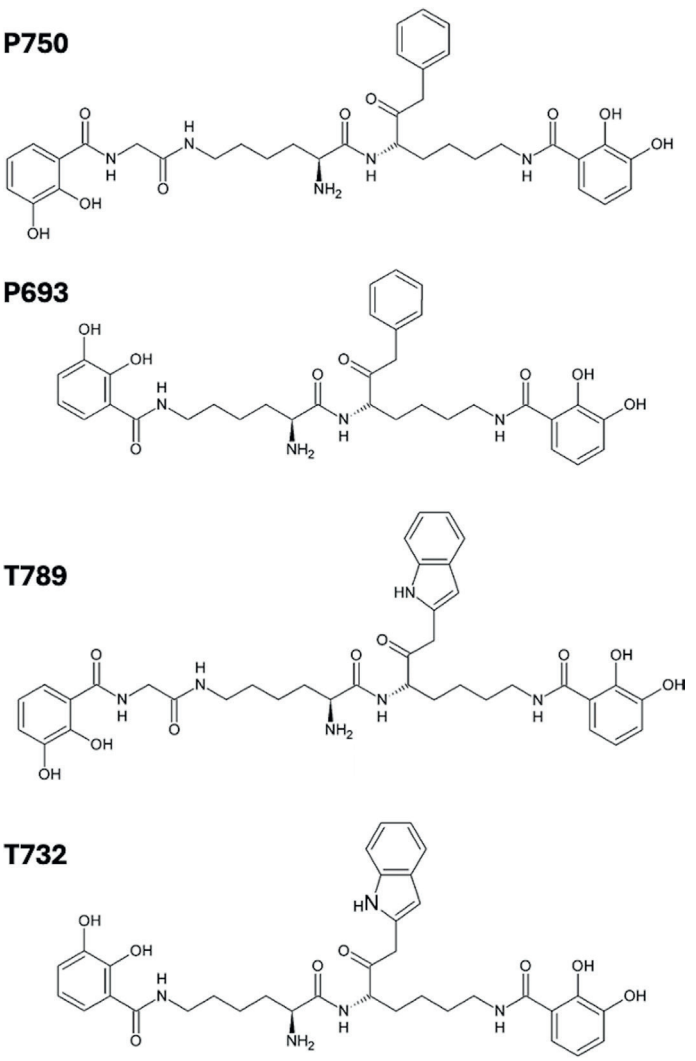

Figure 1.
Structure of the amonabactins produced as siderophores by most species of Aeromonas.

The difference among each one is in the presence or absence of a glycine residue in the chain between both catechol groups. It is believed that a single assembly pathway could potentially synthesize both the tryptophan and phenylalanine forms of amonabactin [18]. This pathway would harbor an unusual NRPS enzyme capable of incorporating either tryptophan or phenylalanine into amonabactin by an atypical mode of synthesis that is iterative, alternative, and optional [19]. According to this, the main siderophore being produced is amonabactin P750, which would represent up to 75% of all amonabactins produced by a single strain, although the specific proportion of each amonabactin would also depend on the amino acids present in the cell surroundings [19].

Analyzing additional siderophore-producing isolates of *Aeromonas*, most of the *A. hydrophila* isolates were found to produce these amonabactins, which were also produced by some *A. sobria* and *A. caviae* isolates, indicating a prevalent synthesis of amonabactins in several *Aeromonas* species [16].

Amonabactins are also produced by virulent strains of *A. salmonicida* subsp. *salmonicida* [20]. The genome of *A. salmonicida* A449 contains a gene cluster with high similarity to that involved in the synthesis of amonabactins in *A. hydrophila* and that would include the genes necessary for DHBA biosynthesis (*entCEBA*). It also includes the genes necessary for siderophore transport. Closed homologs of these genes are extensively present in all *Aeromonas* species for which genomic data are available, including *A. dhakensis* [21]. The main genes putatively involved in siderophore assembly are *amoF*, *amoG*, *amoH* (encoding nonribosomal peptide synthetases), and *amoD*, which would encode a phospho–pantetheinyl transferase. The percentage of similarity of the corresponding *A. salmonicida* proteins with the *A. hydrophila* homologs is above 90% in most of the cases (**Table 1**). The only noticeable

***Aeromonas salmonicida* subsp. *salmonicida* A449**		**Homology to Amonabactin cluster from *A. hydrophila* ATCC 7966 (%aa identity, %aa similarity)**	
Locus (No aa)	**Encoded protein, description**		
ASA_1838 (392)	EntC, Isochorismate synthase	WP_011706310.1	(87, 92)
ASA_1839 (556)	EntE, Siderophore synthase component E	WP_011706309.1	(88, 92)
ASA_1840 (302)	EntB, Isochorismatase	WP_011706308.1	(93, 95)
ASA_1841 (1029)	AmoF, Nonribosomal peptide synthetase	WP_011706307.1	(91, 94)
ASA_1842 (259)	EntA, 2,3-dihydroxybenzoate-2,3-dehydrogenase	WP_011706306.1	(94, 95)
ASA_1843 (2078)	AmoG, Nonribosomal peptide synthetase	WP_011706305.1	(85, 89)
ASA_1844 (536)	AmoH, Nonribosomal peptide synthetase	WP_011706304.1	(86, 90)
ASA_1845 (314)	Periplasmic binding protein	WP_011705834.1	(94, 96)
ASA_1846 (392)	AmoD, Phosphopantetheinyl transferase	WP_011705835.1	(71, 78)
ASA_1847 (271)	ABC transporter, ATP-binding protein	WP_011705836.1	(93, 95)
ASA_1848 (351)	ABC transporter, permease	WP_011705837.1	(92, 96)
ASA_1849 (338)	ABC transporter, permease	WP_011705838.1	(97, 98)
ASA_1850 (657)	TonB-dependent siderophore receptor	WP_011705839.1	(94, 98)
ASA_1851 (395)	Major facilitator family transporter	WP_011705840.1	(92, 95)

Table 1.
Description of the proteins involved in amonabactins biosynthesis that are encoded by the genes of the amonabactin cluster in A. salmonicida subsp. salmonicida and homologies to the corresponding loci in A. hydrophila ATCC 7966. Adapted with permission from [20]. Copyright 2015 American Chemical Society.

difference between the two species is that in *A. hydrophila* the genes encoding the transport proteins, including the outer membrane receptor, are in a different location in the chromosome.

Although most *Aeromonas* species seem to produce amonabactins, *A. salmonicida* also produces a second siderophore with high relevance in the virulence of this species. The genome of *A. salmonicida* A449 contains a gene cluster spanning from ORF ASA_4368 to ASA_4380 containing the *asbGFDCBI* genes [22] that encode the synthesis of acinetobactin, another catechol siderophore (**Figure 2**) [20]. This cluster also contains the genes involved in siderophore transport, including the outer membrane receptor FstB (locus ASA_4368). It is interesting to note that although acinetobactin also contains a DHBA moiety, the *asb* cluster of *A. salmonicida* does not contain genes encoding the synthesis of DHBA. Thus, the synthesis of acinetobactin depends on the unique DHBA genes harbored within the amonabactin gene cluster.

When comparing the genomes of *A. salmonicida* and *A. hydrophila*, it is evident that the acinetobactin gene cluster represents a clear-cut insertion between *fhuE* gene (encoding a permease for ferrichrome uptake) and *trmE* gene (encoding a putative tRNA modification GTPase), both common to all species of the genus *Aeromonas*. Thus, the acinetobactin gene cluster was likely acquired through an ancient gene transfer event at some point during the evolution of *A. salmonicida*. It is noteworthy that acinetobactin was first described as the siderophore produced by the human pathogen *Acinetobacter baumannii*. The horizontal transfer of gene clusters encoding the synthesis and transport of siderophores seems to be a common feature in many bacteria. We showcase that the majority of *A. salmonicida* subsp. *salmonicida* strains concomitantly produce both, acinetobactin and amonabactin. Intriguingly, the synthesis of these siderophores hinges on a unique set of genes responsible for the synthesis of the catechol moiety (2,3–dihydroxybenzoic acid) and a phosphopantetheinyl transferase, only present within the amonabactin cluster. A singular mutation in any of these genes nullifies the production of both siderophores [20]. Hence, most strains of *A. salmonicida* subsp. *salmonicida* synthetize two siderophores simultaneously, amonabactin and acinetobactin, that share part of the biosynthetic pathway (**Figure 3**). It has been also reported that certain virulent *A. salmonicida* subsp. *salmonicida* strains, isolated from marine-reared fish, produce only acinetobactin due to the presence of a deletion in the amonabactin biosynthesis gene *amoG*, suggesting that acinetobactin alone is enough to allow multiplication of *A. salmonicida* within the host. From the genomic data available at GenBank, seems that the amonabactin synthesis gene cluster is widespread among all species of *Aeromonas*, while acinetobactin synthesis is restricted to *A. salmonicida*, being present in all strains and subspecies for which genomic data are available: *salmonicida, achromogenes, pectinolytica, smithia,* and *masoucida*.

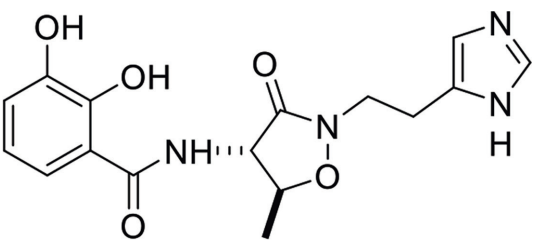

Figure 2.
Structure of acinetobactin, a siderophore produced by A. salmonicida subsp. salmonicida.

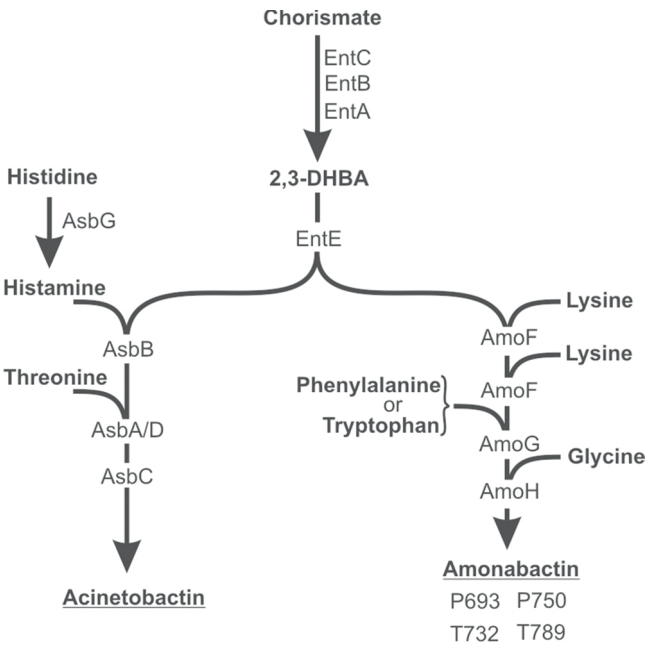

Figure 3.
Schematic pathway for the biosynthesis of siderophores acinetobactin and amonabactin in A. salmonicida subsp. salmonicida. The enzymes putatively involved in each step and the main precursors for each siderophore are indicated. Reprinted with permission from [20]. Copyright 2015 American Chemical Society.

4. Siderophore outer membrane transporters in *Aeromonas*

The first works which described iron uptake mechanisms in *Aeromonas*, observed that Fe3+ limitation resulted in the increased synthesis of several high-molecular-weight outer membrane proteins in *A. salmonicida* [15]. When it was cultured under iron-restricted conditions in vitro, there was an observable increase in the expression of outer membrane proteins of 73, 76, and 85 kDa, which were absent during growth under iron-replete conditions. When cultured in vivo, in the peritoneal cavity of salmon, *A. salmonicida* up-regulated the identical 73, 76, and 85 kDa proteins. Mass spectrometry analysis identified these proteins as a colicin receptor, an outer membrane heme receptor, and a ferric-siderophore receptor [10]. Furthermore, a proteomics analysis of *A. hydrophila* showed that iron starvation induces the expression of more than 100 proteins in its cell membranes. Bioinformatics analysis suggested that many of these proteins correspond to outer membrane transporters (OMTs) of siderophores, heme, and iron receptors, periplasmic iron-binding proteins and inner membrane ABC transporters also involved in siderophore and heme transport through cytoplasmic membrane [23].

As above mentioned, *A. hydrophila* and many strains of *A. salmonicida* subsp. *salmonicida* produce a family of four amonabactins. Growth promotion assays on *A. salmonicida* subsp. *salmonicida* showed that each amonabactin form has quite different growth promotion activity, with P750 and T789 being the most active. Mutagenesis experiments showed that in *A. salmonicida* the *fstC* gene encodes an outer membrane protein that acts as transporter for amonabactins, but it displays a wide ligand plasticity [24]. FstC recognizes more efficiently biscatecholate siderophores in which the length of the linker between the two iron-binding catecholamide

units is 15 atoms (P750 and T789) instead of 12 atoms (P693 and T732). Analysis of the siderophore activity of synthetic analogs indicated that the presence of Phe or Trp residues is not required for siderophore recognition. In silico analysis showed that the FstC versions deposited in GenBank are highly conserved not only among *A. salmonicida* strains (amino acid identity of 99.2–100%), but they also share high identity (83.3–98.6%) with homologs from most *Aeromonas* species. The only exception is the FstC from *A. veronii* which shows a scarce 61.2% identity at the amino acid level. The phylogenetic analysis shows that *A. salmonicida* FstC clustered on a clade with bootstrap of 98 and displays a closer relationship with *A. piscicola*, *A. bestiarum*, *A. popoffii*, *A. dhakensis*, and *A. hydrophila*. By contrast, FstC showed distant relationships with other *Aeromonas* species like *A. diversa*, *A. schubertii*, and *A. veronii*. This presence of this highly conserved OMT in many different *Aeromonas* species could be interesting for the development of novel amonabactin-based compounds, which could be transported through FstC. This may lead to novel antimicrobials effective against animal and human pathogens of the genus *Aeromonas*, or novel biotechnological tools to selectively introduce synthetic compounds into bacterial cells.

A. salmonicida subsp. *salmonicida* produces the siderophore acinetobactin. The first sequence of a ferric-siderophore transporter reported in *A. salmonicida* subsp. *salmonicida* was deposited in GenBank in 1995 and was named *fstA* (GI:1143382). Since then, other versions (alleles) of this gene, which were called either *fstA* or *fstB*, have been sequenced and deposited in GenBank. The two alleles have a nucleotide identity of 98%; however, the protein similarity is only 86% due to the existence of certain regions (not affecting the protein functionality) that do not align with each other [25]. Most A. *salmonicida* subsp. *salmonicida* genomes deposited in GenBank harbor the *fstB* version of this gene, so this is the name used in this chapter. By means of mutant construction, it was demonstrated that *fstB* gene encodes the outer membrane receptor necessary for the internalization of ferric-acinetobactin in *A. salmonicida* subsp. *salmonicida* [25]. In order to establish some structure-activity relationships, several isoxazolidinone analogs of acinetobactin were synthesized, including its enantiomer, and their biological activity was evaluated by growth promotion assays on *fstB*(+) and *fstB*(−) strains. There is no discernible preference for enantiomers in the siderophore activity of acinetobactin against *A. salmonicida*, nor in its molecular interaction with the FstB protein receptor. Additionally, it was noted that *A. salmonicida* fails to utilize acinetobactin analogs lacking an imidazole or a related heterocyclic ring in their structure (**Figure 4**). Elimination of the methyl group at the isoxazolidinone ring led to increased biological activity, indicating potential alternative pathways for cellular entry. It has been suggested that some of these synthetic analogs of acinetobactin could be potential candidates as vectors in a Trojan horse strategy to develop new antimicrobials against furunculosis in fish [25].

Transport of ferri-siderophores and heme to the cytoplasm requires other components besides OMTs. Usually, an intermediate periplasmic binding protein (PBP) and ABC transporters located in the cytoplasmic membrane are essential components. Besides, transport from outer membrane to periplasm requires energy transduction from the cytoplasm through the TonB system. All siderophore and heme OMTs are TonB-dependent transporters (TBDTs) [1, 5]. Genes encoding PBPs and ABC transporters are usually part of the gene clusters involved in siderophore synthesis and transport. Nevertheless, the precise mechanisms governing the operation of these components remain inadequately understood across all species of *Aeromonas*. Further works are necessary to complete our knowledge in this matter.

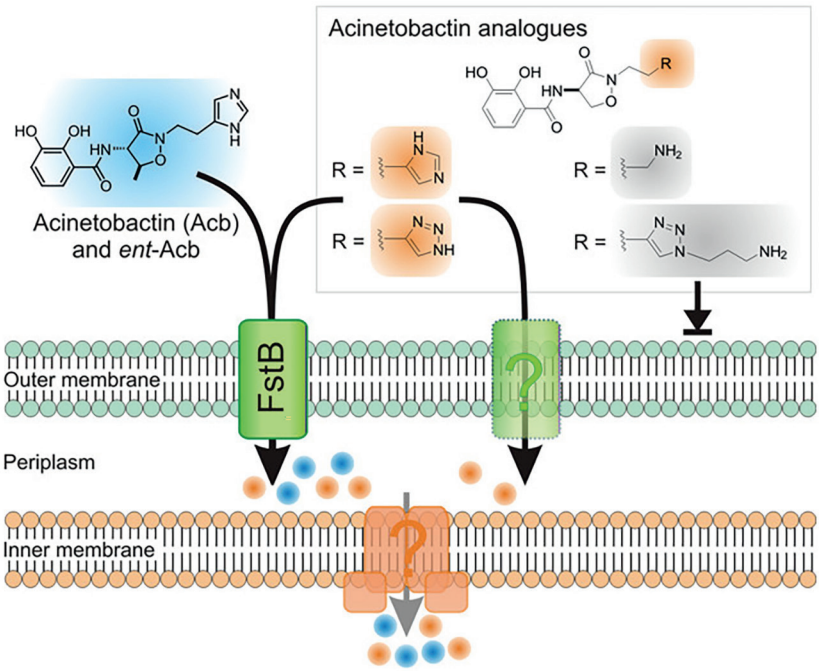

Figure 4.
Transport of acinetobactin and several derivatives through the outer membrane transporter FstB in A. salmonicida subsp. salmonicida. Putative unknown transporters are also indicated. Reprinted with permission from [25]. Copyright 2016 American Chemical Society.

Transporters for other exogenous siderophores like enterobactin, ferrichrome, or desferrioxamine are also present in *A. hydrophila* [26, 27].

5. Biotechnological applications of iron uptake components

5.1 *Aeromonas* detection by PCR

Since OMTs involved in siderophore acquisition are usually specific, they have proved to be good candidates for specific PCR-based detection of *Aeromonas* species. A PCR protocol based on amplification of *fstA* (same gene as *fstB*) gene encoding the acinetobactin transporter in *A. salmonicida* was developed to specifically detect this species. This study demonstrated that the *fstA*-amplification PCR protocol is specific for detecting *A. salmonicida*, showing no cross-reactions with other closely related species like *A. bestiarum*, *A. piscicola* or any other *Aeromonas* species [28].

5.2 Vaccines

OMTs of siderophores can be also used as efficient candidates for the development of subunit vaccines [29]. Recent works with *A. salmonicida* subsp. *salmonicida*, suggest that both, acinetobactin transporter FstB and amonabactin transporter FstC, could serve as potential vaccines against fish forunculosis. Recombinant proteins rFstB and rFstC, are immunogenic for sole (*Solea senegalensis*) and both proteins

could be used as antigens in novel vaccine formulations against *A. salmonicida*. Interestingly, antibody response against rFstB was notably higher than against rFstC and, consequently, FstB must possess some unknown characteristics that make it more reactive to the immune system than FstC [30].

5.3 Fluorescent probes

Amonabactins, produced by *Aeromonas* spp., offer a means for designing conjugates targeted for cellular import through specific transport mechanisms. The synthesis and design of a novel amonabactin-based fluorescent probe, termed AMB-SRB, achieved by conjugating an appropriate amonabactin analog with sulforhodamine B revealed that the AMB-SRB probe effectively labeled *A. salmonicida* subsp. *salmonicida* cells by binding to the amonabactin OMT FstC (**Figure 5**). The labeling of other *Aeromonas* species containing FstC, including *A. hydrophila*, suggests the potential utility of this probe as a valuable molecular tool for investigating the amonabactin-dependent iron uptake mechanism. Furthermore, the selective labeling of several *Aeromonas* species in the presence of other pathogenic bacteria indicates the potential application of this probe for detecting *Aeromonas* spp. in water or fish farming samples through fluorescence assays [31].

Additionally, the AMB-SRB fluorescent probe was successfully used to detect and follow an *A. salmonicida* infection in vivo using zebrafish as a model [32]. This was the first work where a siderophore-based fluorescent probe was used to follow a bacterial infection in vivo. Observation of zebrafish larvae exposed to AMB-SRB-labeled *A. salmonicida* revealed fluorescence predominantly within the digestive tract, albeit less intense compared to strains labeled with the green fluorescent protein (GFP). This discrepancy was explained by the differing emission wavelengths of the SRB fluorophore in the AMB-SRB probe versus GFP. The utilization of siderophore-based fluorescent probes for bacterial labeling offers the advantage of obviating the need for introducing reporter genes, such as GFP, into the bacteria. Consequently, these probes hold promise for studying other *Aeromonas* spp., including atypical *A. salmonicida*, which may be refractory to genetic manipulation. Given the intracellular multiplication of *A. salmonicida*, these probes also offer a promising avenue for investigating the fate of *A. salmonicida* cells within host cells.

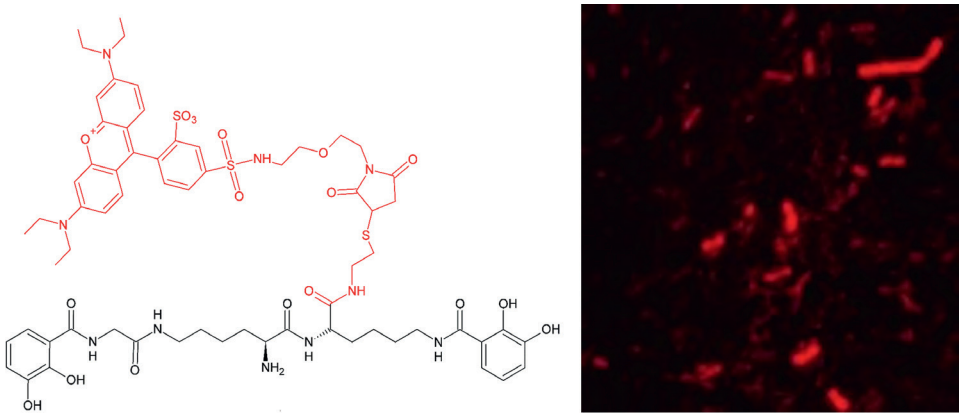

Figure 5.
Fluorescent probe AMB-SRB based on amonabactin conjugated with sulforhodamine B (left) and A. salmonicida subsp. salmonicida cells labeled with AMB-SRB (right) and observed under fluorescence microscopy. See ref. [31] for details.

6. Conclusions

In the genus *Aeromonas*, heme utilization and siderophore synthesis are the two main iron acquisition mechanisms that were extensively studied. Both systems have been mainly analyzed in *A. hydrophila* and *A. salmonicida*. All *Aeromonas* species can use heme to get iron, a mechanism facilitated by the production of iron-regulated hemolysins that release heme-containing molecules from host tissues. The predominant siderophore produced by most *Aeromonas* spp. is amonabactin, comprising a family of four different compounds. Amonabactins are internalized via FstC, an OMT characterized by broad ligand plasticity. In addition to amonabactin, *A. salmonicida* also produces the siderophore acinetobactin, which is transported through FstB. Notably, both siderophores are concurrently produced, sharing part of the biosynthetic pathways. OMTs involved in iron acquisition hold potential utility as tools for bacterial identification and as antigens for novel vaccine formulations, especially against fish diseases caused by *Aeromonas* spp. Furthermore, synthetic derivatives of siderophores could serve as promising candidates for the development of novel antimicrobials, leveraging their specific internalization through the OMTs [33].

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