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Review

The lipopolysaccharide of *Aeromonas* spp: structure-activity relationships

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ABSTRACT

Marine microorganisms, including *Aeromonas*, are a source of compounds for drug development that have generated great expectations in the last decades. *Aeromonas* infections produce septicaemia, and ulcerative and haemorrhagic diseases in fish. Among the pathogenic factors associated with *Aeromona*s, the lipopolysaccharides (LPS), a surface glyconconjugate unique to Gram-negative bacteria consisting of lipid A (lipid anchor of the molecule), core oligosaccharide and O-specific polysaccharide (O antigen), are key elicitors of innate immune responses. The chemical structure of these three parts has been characterized in *Aeromonas*. Based on the high variability of repeated units of O-polysaccharides, a total of 97 O-serogroups have been described in *Aeromonas* species, of which four of them (O:11; O:16; O:18 and O:34) account for more than 60% of the septicemia cases. The core of LPS is subdivided into two regions, the inner (highly conserved) and the outer core. The inner core of *Aeromonas* LPS is characterized by the presence of 3-deoxy-D-*manno*-*oct*-2-*ulosonic* (ketodeoxyoctonic) acid (Kdo) and L-*glycero*-D-*manno*-Heptoses (L,D-Hep), which are linked to the outer core, characterized by the presence of Glc, GlcN, Gal, and GalNAc (in *Aeromonas salmonicida*), D,D-Hep (in *Aeromonas salmonicida*), and L,D-Hep (in *Aeromonas hydrophila*). The biological relevance of these differences in the distal part of the outer core among these species has not been fully assessed to date. The inner core is attached to the lipid A, a highly conserved structure that confers endotoxic properties to the LPS when the molecule is released in blood from lysed bacteria, thus inducing a major systemic inflammatory response known as septic or endotoxic shock. In *Aeromonas salmonicida* subsp. *salmonicida* the Lipid A components contain three major lipid A molecules, differing in acylation patterns corresponding to tetra-, penta- and hexaacylated lipid A species and comprising of 4′-monophosphorylated β-2-*amino*-2-*deoxy*-D-glucopyranose-(1→6)-2-*amino*-2-*deoxy*-D-glucopyranose disaccharide. In the present review, we discuss the structure-activity relationships of *Aeromonas* LPS, focusing on its role in bacterial pathogenesis and its possible applications.

KEYWORDS: lipopolysaccharide (LPS), chemical structure, structure-activity relationships, *Aeromonas*, virulence factors, O antigen, Lipid A, inner core, outer core, oligosaccharide, polysaccharide

1. INTRODUCTION

Since the golden era of antibiotic discovery in the 1940s to 1960s [1], research on the potential therapeutical uses of bacterial compounds has not ceased to increase [2-4].

Among the different bacterial structures, bacterial lipopolysaccharides (LPS), the major component of the outer membrane of Gram-negative bacteria [5-7],

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have unique structural characteristics [2, 8] and a specific biological activity [8]. LPS is usually seen as a "double-edged sword" [9, 10], endowed with endotoxic activity in most pathogenic bacteria, mainly enterobacteria, or with interesting immunostimulatory activity in other Gram-negative bacteria [2, 5, 10].

In this context, current research is focused on the beneficial activity of LPS from enterobacteria and from other Gram-negative bacteria and looks promising for the development of new drugs for the prevention and therapy of several human diseases [10].

One of the aspects that has generated great expectations in drug development is the characterization and assessement of compounds extracted from marine organisms [11, 12], including marine microorganisms [2, 13]. At present, considerable attention is being given to the elucidation of the chemical structures of LPS of Gram-negative marine bacteria such as *Aeromonas* spp [2, 14].

In this review, we present an update on the structural elucidation of the different parts of LPS (O antigen, core and Lipid A) of the different species of the genus *Aeromonas* and of the main serogroups, focusing on the unique structural characteristics that can be associated with the biological activity from both points of view: pathogenesis and possible beneficial effects for the treatment of human diseases.

2. The bacterial lipopolysaccharide from Gram-negative bacteria

LPS are the major components of the outer membrane of Gram-negative bacteria [5, 7], having a structural role since they contribute to the cellular rigidity by increasing the strength of cell wall and mediating contacts with the external environment that can induce structural changes to allow life in different conditions [5]. Furthermore, the low permeability of the outer membrane acts as a barrier protecting the bacteria from host-derived antimicrobial compounds [5].

Since LPS are microbe-associated molecular glycoconjugates, produced only by Gram-negative bacteria, they are recognized as a molecular hallmark of invading microbes by the host immunological systems [5]. For these reasons, LPS has a very important role in the elicitation of the animal and plant host innate immunity [5, 15].

2.1. General structure of LPS

LPS are amphiphilic macromolecules generally comprising of three defined regions distinguished by their genetics, structures, and function: the lipid A, the core oligosaccharide and a polysaccharide portion, the O-chain [5, 10, 16, 17] (Figure 1).

Similar to most cell envelope components, LPS is made at the cytoplasmic face of the inner membrane and must be transported across the two bilayers and the periplasm to become integrated in the outer membrana [17], using undecaprenyl phosphate (Und-P) as lipid carrier [18]. All regions of LPS display heterogeneity [2]. In fact, Gram-negative organisms have evolved several LPS modification strategies that allow these organisms to adapt to their unpredictable and often hostile surroundings [17].

While the structures of lipid A and core oligosaccharide are highly conserved among bacterial genera, the O-polysaccharide varies and differs in common bacterial species [2, 16]. Although studies of the biological activities of LPS have mainly focused on the lipid A moiety, recent studies are gradually clarifying the importance of core and O antigen to elicit the biological activities [16]. In many bacteria, the O-specific side chain variations give the biological identity at an intraspecific level (serogroups, serotypes or serovars) [2].

Products described as S-type LPS normally consist of the populations of molecular sructures with different degrees of polymerization of the O-specific side chain (smooth strains), including molecules with a few and/or single repeating unit (also called as semi-rough antigen, SR-type), and also contain the species without a side chain (R-type) and perhaps even molecules with an incomplete core oligosaccharide (core-defective R-types).

Mucosal pathogens often produce lipooligosaccharide (LOS), in which the repeating O antigen domain is absent and is replaced by an extended core region [17]. LOSs are identified in such Gram-negative bacteria as *Bordetella pertussis*,

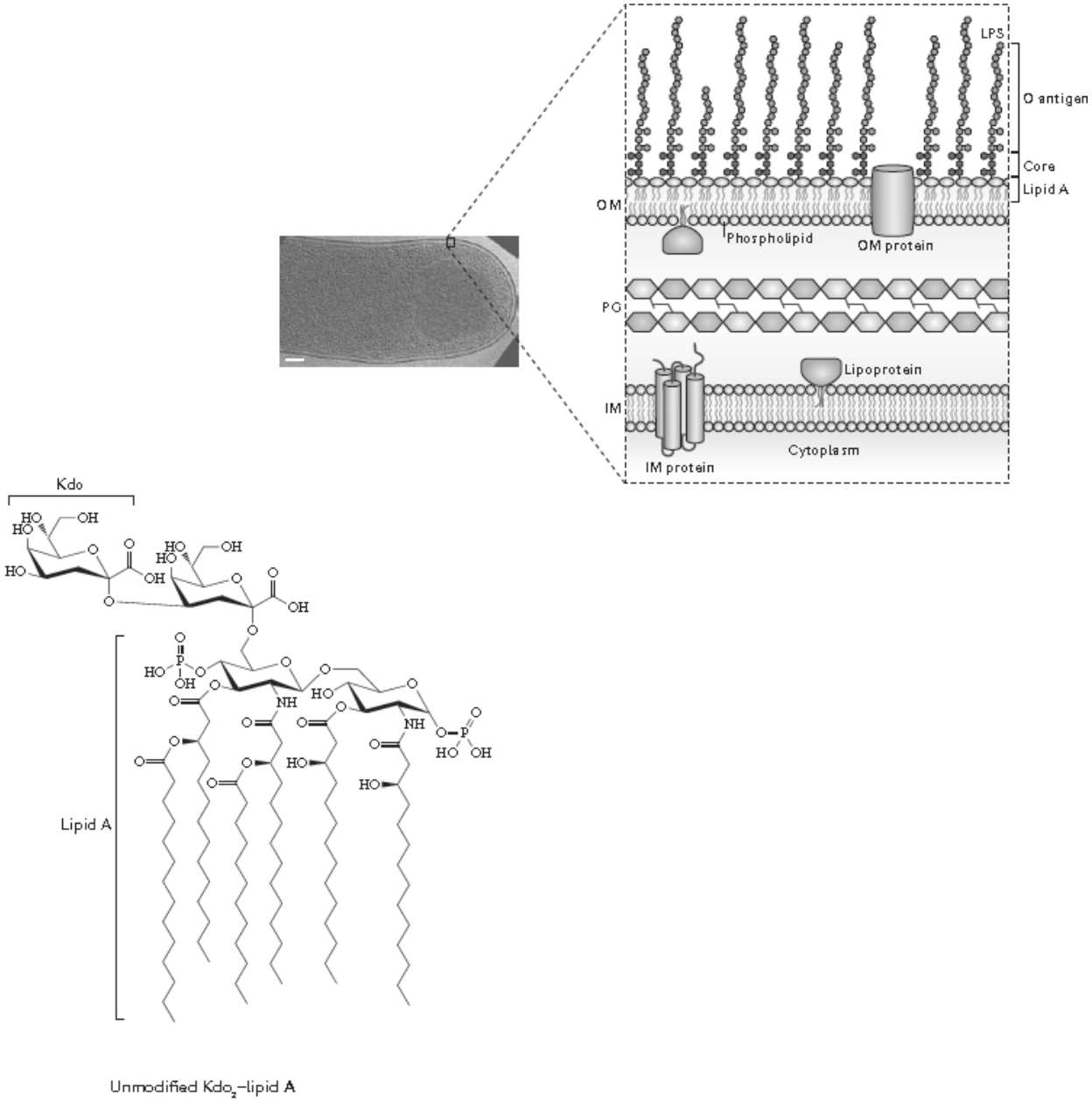


Figure 1. General structure of LPS [17]. Reprinted by permission from Macmillan Publishers Ltd: [Nature Reviews Microbiology] (Needham, B. D. and Trent, M. S. 2013, Nat. Rev. Microbiol., 11, 467), copyright (2013).

Neisseria meningitidis, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, *Haemophilus ducreyi*, *Burkholderia (Pseudomonas) multivorans*, *Burkholderia (Pseudomonas) cenocepacia*, *Alteromonas addita* KMM 3600T, and *Campylobacter jejuni* [10, 19, 20].

A 2-keto-3-deoxyoctonate (Kdo) unit of the inner core links the lipid A to a core oligosaccharide (OS) composed of about 10 sugar residues [10]. The core region of enterobacterial LPS includes an outer portion, distal from lipid A (proximal to the O-polysaccharide chain), and an inner portion directly linked to the lipid A. The complete outer core region (Ra-structure) mainly consists of hexoses and hexosamines, whereas the inner core region is composed of KDO and heptose [10].

A successive truncation of Ra-structure LPS associated with specific alterations of core oligosaccharide biosynthesis in different *Salmonella* strains (R-mutants) results, respectively, in the Rb, Rc, Rd, and Re core structures. The last structure, which contains only lipid A and KDO residues, is a minimal LPS structure [10].

Enterobacterial lipopolysaccharide core regions can be classified into two types: the *Salmonella* type and core region different to the *Salmonella* type. In the first, the common structural element L,D-Hep-(1→7)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo is present, which is substituted at O-3 of the second heptose by glucopyranose (Glcp). Heptose residues I and II are phosphorylated and O-4 of Hep I is not substituted by a saccharide [21].

The core region different to the *Salmonella* type possesses a common partial structure L,D-Hep-(1→7)-L,D-Hep-(1→3)-L,D-Hep-(1→5)-Kdo, which is not substituted at O-3 of Hep II by Glc and in which heptose residues are not generally phosphorylated. Position O-4 of Hep I is substituted by a hexose residue or oligosaccharide [21].

Lipid A, the endotoxic portion of LPS and the site for many LPS modifications, is initially synthesized as a β-1,6-linked disaccharide of glucosamine that is both phosphorylated and fatty acylated (Figure 1) [17]. In some organisms, such as *Escherichia coli* K12, this structure represents the typical form of lipid A in the outer membrane. Despite initial studies reporting that lipid A could be modified with polar substituents (such as amino sugars), it was nevertheless viewed as a static structure [17]. Modification of lipid A equips Gram-negative bacteria with an ability to evade immune recognition and survive within a host [17].

While chemical structures of enterobacterial LPS are well stablished, in recent years, the elucidation of LPS structures from other Gram-negative bacteria, such as marine bacteria, has generated great interest [2].

2.2. Biological activity of LPS

LPS exists virtually throughout the environment and can be found in air, food, water, skin and intestine [16, 22, 23]. However, LPS can cause severe damage to the host immune system through systemic inflammation (known as endotoxin shock) due to systemic activation of the innate immune system, which occurs under specific conditions, such as when LPS is administered via either the intraperitoneal or the intravenous route [16, 24] (Figure 2).

Some of the manifestations of acute endotoxemia in mice include systemic arterial hypotension (i.e., shock), lactic acidosis, impaired myocardial contractility, a short-lived monophasic spike in the circulating level of TNF, a more prolonged elevation in the circulating level of IL-6 and a delayed increase in the circulating level of high mobility group box (HMGB)-1. These manifestations are also features of sepsis or septic shock in humans, although the temporal kinetics and the magnitude of these changes from normal physiology are often different from what is observed in acute murine endotoxemia [24].

Activation of systemic inflammation by LPS in mice is largely mediated via interaction of the bacterial product with Toll-like receptor (TLR) 4, which is expressed on the surface of both “professional” immune cells, such as monocytes and macrophages, as well as many other cell types, including alveolar epithelial cells and myocardial cells. The intracellular signaling pathways are triggered by the interaction of LPS with TLR4 [24].

Compared with humans, mice, even “normally responsive” strains, are remarkably less sensitive to the toxic or lethal effects of LPS. The LD50 dose of LPS in mice is about 1000-fold to 10000-fold greater than the dose of LPS that is required to induce severe illness and hypotension in humans [24, 25].

On the other hand, LPS can also regulate enteric immunity by oral administration, without serious side effects [16, 26]. Therefore, LPS has now been re-recognized as exohormone capable of regulating homeostatic balance in relation to the external environment [22, 16].

As many other molecules in biology, LPS appears as a “double-edged sword” [9, 10]. Beneficial activity of both typical and atypical endotoxins looks promising for the development of new drugs for the prevention and therapy of several human diseases [10].

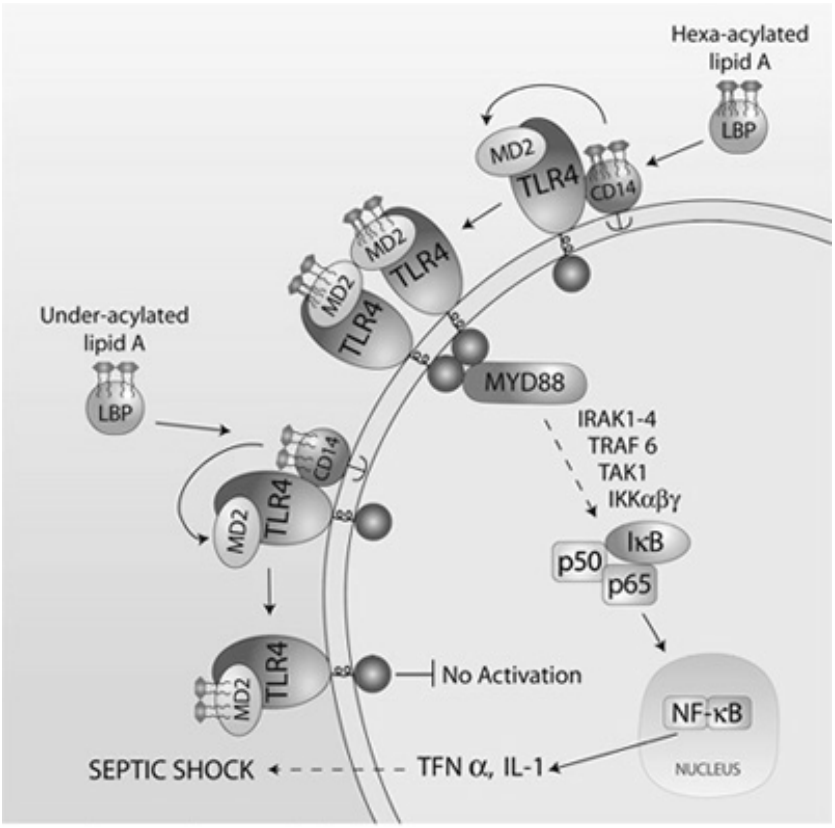


Figure 2. Biological activity of LPS.

In fact, naturally occurring (often typical) LPS modulate the immune system of higher vertebrates in order to keep pathogens away and to avoid the possibility of saprophytes/commensals to become invaders (translocation). Moreover, it has been demonstrated that the immune system is dependent on certain microbial products including LPS for normal development [10, 27].

The complex population of microbes that we harbor within our mucosal cavities is not just passive bystanders, rather these organisms seem to actively shape our immune system responses both along the mucosal surface and in very remote tissues/organs [10, 28]. Therefore, LPS could control broad and increasingly diffused chronic, inflammatory, and degenerative diseases during the human evolution [10, 29]. In this regard, epidemiology studies in young children have found that LPS exposure at home is inversely correlated with the development of atopic diseases, following the "hygiene hypothesis" for allergic disorders [10, 30].

Taking all of this into account, it has been suggested that some atypical LPS, with low endotoxic activity and/or prominent antagonistic effect on LPS from enteric bacteria, could be plausible candidates that could be developed into useful drugs for many diseases such as allergic illness, inflammatory bowel disease, and demyelinizing pathology of CNS [10, 29].

3. The LPS of the genus *Aeromonas*

3.1. The genus *Aeromonas*

The genus *Aeromonas* consists of Gram-negative, rod-shaped, non-spore-forming, facultative anaerobic, chemoorganotrophic bacteria with an optimal growing temperature of about 22 °C to 37 °C.

Generally they are motile by polar flagellation, oxidase- and catalase-positive, resistant to vibriostatic agent O/129, able to reduce nitrates to nitrites, and able to catabolize glucose and several carbohydrates while producing acids and often gases as well.

The taxonomy of the genus *Aeromonas* is complex due to the continuous description of novel species and the rearrangement of strains and species described so far (http://www.bacterio.net). In the first edition of *Bergey´s Manual of Systematic Bacteriology* [31], the genus *Aeromonas* was included in the family *Vibrionaceae* and comprised of four species, a non-motile and psycrophilic species (*A. salmonicida*) and three motile and mesophilic species (*A. hydrophila*, *A. caviae*, and *A. sobria*). Based on 16S rRNA and 5S rRNA gene sequence comparisons, and rRNA–DNA hybridization data, Colwell *et al*. (1986) [32] proposed the creation of the family *Aeromonadaceae* including *Aeromonas* as its type and unique genus. In the current edition of *Bergey´s Manual of Systematic Bacteriology*, three genera (*Aeromonas*, *Oceanimonas* and *Tolumonas*) are listed in this family, although two more genera have been described recently, *Oceanisphaera* [33] and *Zobellella* [34, 35].

In the latest edition of *Bergey's Manual of Systematic Bacteriology* [35], 14 phenospecies that correspond to 17 genomospecies (DNA hybridization groups or HGs) were included within the genus *Aeromonas*: *A. hydrophila* (HG1), *A. bestiarum* (HG2), *A. salmonicida* (HG3), *A. caviae* (HG4), *A. media* (HG5), *A. eucrenophila* (HG6), *A. sobria* (HG7), *A. veronii* (bv. Sobria, HG8, and bv. Veronii, HG10), *A. jandaei* (HG9), *A. schubertii* (HG12), *A. trota* (HG14), *A. allosaccharophila* (HG15), *A. encheleia* (HG16), and *A. popoffii* (HG17). *Aeromonas* spp. HG11 was included in *A. encheleia* [36] and recently the proposal to assign *A. diversa* spp. nov. as a novel species designation for *Aeromonas* spp. HG13 (group 501) has been validated [37]. In recent years, eleven new species have been described: *A. simiae* [38], *A. molluscorum* [39], *A. bivalvium* [40], *A. tecta* [41], *A. piscicola* [42], *A. fluvialis* [43], *A. sanarelli* [44], *A. taiwanensis* [44], *A. rivuli* [45], *A. dhakensis* [46] and *A. australiensis* [47]. Therefore, 26 species have been described in the genus *Aeomonas* until now.

Aeromonads are common inhabitants of aquatic environments and have been described in connection with fish and human diseases [2]. They have been isolated from marine waters, rivers, lakes, swamps, sediments, chlorine water, water distribution systems, drinking water and residual waters, especially during hot months in greater numbers [48]. *Aeromonas* species also can be isolated from a wide range of foods (shellfish, fish, meat, raw milk and dairy products, vegetables, etc.) [49], which include healthy animals, suggesting that *Aeromonas* form part of some animal microbiota, as demonstrated in leechs [48].

Some *Aeromonas* species are pathogens not only of fish and other cold-blooded animals but also of warm-blooded animals and humans. Although psychrophilic, non-motile, and pigmented *A. salmonicida* strains are the principal fish pathogens as the causal agent of furunculosis in salmonids, some authors have shown that marine and freshwater fish species also can be affected by furunculosis, and mesophilic motile *Aeromonas* species (mainly *A. hydrophila*, *A. veronii*, *A. bestiarum* and *A. piscicola*) are also associated with fish diseases [50].

In humans, *Aeromonas* (mainly *A. hydrophila*, *A. caviae*, and *A. veronii* bv. Sobria) is an opportunistic pathogen that can be associated with gastrointestinal and extraintestinal diseases. Despite the demonstration of the enterotoxical potential of some *Aeromonas* strains, there is still a debate on its consideration as an etiological agent in bacterial gastroenteritis, as there are no big epidemical outbreaks described and no adequate animal model is available to reproduce the gastroenteritis caused by *Aeromonas*. Skin and soft tissue infections are the most common extraintestinal infection caused by *Aeromonas*, often acquired not only as a direct consequence of traumatic occupational injuries or unexpected recreational exposures, but also by means of animal bites or the introduction of foreign bodies containing aeromonads or by major traumatic events. *Aeromonas* may be an important pathogen associated with wound infections in natural disasters as occurred in Thailand in 2004. Septicemia, intra-abdominal infections and other extraintestinal *Aeromonas* infections can be a cause of medical complications in immunocompromised persons [48].

The observed clinical manifestations of *Aeromonas* infections suggest that there could be a complex

network of pathogenic mechanisms forming a multifactorial process. Recent studies seem to strengthen this hypothesis as the virulence of this genus depends on the bacterial strain, the infection route and the animal used as model organism [51].

Although there are several *Aeromonas* strains with their draft genome published, to date four complete genomes of genus *Aeromonas* have been published from the following strains: *A. salmonicida* subsp. *salmonicida* A449 (NCBI Reference Sequence: NC_009348) [52], *A. veronii* B565 (NCBI Reference Sequence: NC_015424) [53], *A. hydrophila* ML09-119 (NCBI Reference Sequence: NC_021290) [54] and the type strain, ATCC 7966^T, of *A. hydrophila* (NCBI Reference Sequence: NC_008570) [55]. This information is of great value, although there is a great diversity within the genus and some virulence factors will probably not be present in these strains or these strains show different mechanisms to infect the host than others.

The main pathogenic factors associated with *Aeromona*s are: filamentous (flagella and fimbriae) and non-filamentous (capsule, outer membrane proteins, lipopolysaccharide, glucan, and S-layers) adhesins, exotoxins and other extracellular enzymes (enterotoxins, lipases, proteases, nucleases, etc), secretion systems, iron acquisition mechanisms, and quorum sensing events [50, 56].

In this review, we focus on the structure and biological activity of the LPS molecule from *Aeromonas* spp.

3.2. LPS of *Aeromonas* spp

The structural diversity of LPS from *Aeromonas* spp is thought to be a reflection of the ability of these bacteria to adapt to an array of habitats, protecting the cell from being compromised by exposure to harsh environmental stress factors [2]. Although a possible correlation between particular chemical features and the environmental adaptations are suggested [2], no extensive research have been done on the possible industrial and therapeutic applications of these LPS molecules, which are endowed with low toxic activity.

3.2.1. O Antigen

The *Aeromonas* genus includes a total of 97 serogroups, serotyped from reference strains of *A. hydrophila*, *A. caviae* and *A. sobria* [57, 58], although some of these strains could be misidentified because of the increasing complexity in the identification of *Aeromonas* strains at the species level [59]. However, only some of them such as O3, O6, O11, O14, O16, O18, O21, O29, O33, O34 and O41 seem to be associated with virulence for specific fish species [60] and more than 60% of the septicemia cases are related to four of these serogroups: (O:11; O:16; O:18 and O:34) [48].

Serogroup O:11 is associated with severe infections in humans, like septicemia, meningitis and peritonitis while serogroup O:34, the most common in mesophilic *Aeromonas*, is associated with wound infections in humans and outbreaks of septicemia in fishes [61].

LPS from *Aeromonas* are mainly high heterogeneous mixtures of S-form LPS molecules containing 1 to over 50 repeating oligosaccharide units and contain ubiquitously, a varying proportion of R-form molecules lacking the O-specific chain. Many clinically relevant Gram-negative bacteria synthesize this type of LPS. LPS are amphipathic molecules whose hydrophobicity decreases with increasing length of the sugar part [62]. Based on these differences, S- and R-form LPS show marked differences in the kinetics of their blood clearance and cellular uptake as well as in the ability to induce oxidative burst in human granulocytes [63] and to activate the host complement system [64].

It is known that the S form of LPS protects the bacteria from the bactericide effects of the non-immune serum, since the complement component C3b binds to the long O antigen chains being far away from the membrane and unable to form the complement attack complex, and therefore avoids cell lysis [65]. In this regard, it has been demonstrated that the long O:34 antigen chains increase hemolytic activity, virulence in fishes and mice [66] and adherence to human epithelial cells [67] and can be considered an important *in vivo* colonization factor [68].

Of note, the LPS of serogroups O:13, O:33, O:34 and O:44 shows thermoadaptation. Thus, high growth temperatures (37 °C) increase the levels of hydroxilated and saturated fatty acids in the lipid A

of serogroup O:34 [66] and in serogroups O:13, O:33, O:34 and O:44, the S forms of LPS predominate in growth conditions of 20 °C or 37 °C at higher osmolarity, while R forms predominate at 37 °C at lower osmolarity [67, 68]. The smooth strains were resistant to the bactericidal activity of non-immune serum, while the rough strains were sensitive and showed better adhesion to Hep-2 cells than the smooth strains. Furthermore, the smooth strains were more virulent for fish and mice than the rough strains. For mesophilic *Aeromonas* spp. strains from serogroups O:1 to O:44, these changes were not observed, except for serogroups O:13, O:33, O:34 and O:44 [69].

It has also been shown that some strains of *A. salmonicida* lose their virulence at temperatures above 21 °C, due to the thermolability of a large pVirA virulence plasmid [70, 71]. Fish can be used as hosts to evaluate virulence of *A. salmonicida* at low temperatures, although this requires specific installations and poses significant practical problems, such as disposal of contaminated water [71].

Based on these findings, temperature and osmolority should always be considered for the growth of *Aeromonas* spp. The possible applications of these thermoadaptative changes deserve further studies, with both whole cells (generation of attenuated strains, etc.) or as purified LPS (as immunomodulatory molecule, etc.).

The chemical structure of the O-antigens of *A. hydrophila* O:34 [72] and *A. salmonicida* subsp. *Salmonicida* [73] have been characterized, together with the chemical structure of the O:11 antigen of *A. hydrophila* LL1 with S-layer [74] and *Aeromonas caviae* ATCC15468 [75] (Figure 3).

The *O*-polysaccharide of *Aeromonas hydrophila* O:34 was obtained by mild-acid degradation of the LPS and studied by chemical methods and NMR spectroscopy before and after O-deacetylation. The polysaccharide was found to contain D-Man, D-GalNAc and 6-deoxy-L-talose (L-6dTal) [2, 72]. Although less common than L-rhamnose and L-fucose, 6-deoxy-L-talose occurs in a number of bacterial polysaccharides and is often present in an O-acetylated form. However, to date, random O-acetylation has not been reported for either this or any other monosaccharide component of the LPS [2] (Figure 3). The biological significance of 6-deoxy-L-talose remains unknown.

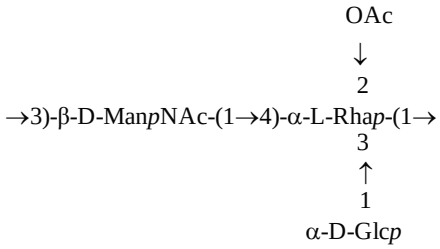
Of note, it has been found that when grown *in vitro*, *A. salmonicida* strain 80204-1 produced a capsular polysaccharide with a structure identical to that of the lipopolysaccharide O-chain polysaccharide. Both polymers were shown to be composed of linear trisaccharide repeating units consisting of 2-acetamido-2-deoxy-D-galacturonic acid (GalNAcA), 3-[(N-acetyl-L-alanyl)amido]-3,6-dideoxy-Dglucose {3-[(N-acetyl-L-alanyl)amido]-3-deoxy-D-quinovose, Qui3NAlaNAc} and 2-aceacetamido-2,6-dideoxy-D-glucose (2-acetamido-2-deoxy-D-quinovose, QuiNAc) [2, 76]. It was also shown that the structures of both capsule and O-chain polysaccharide were distinct from the previously reported O-chain polysaccharide of *A. salmonicida* produced in Tryptic Soy Broth (TSB) and consisting of L-rhamnose, D-mannosamine and D-glucose [76, 77], which was also detected in the bacterial inoculum TSB culture used to prepare the *in vivo* growth chambers [76].

Structural analysis of capsule and O-chain polysaccharide confirmed that 40% of GalNAcA was present in the amide form. Structural analysis of *in vivo* cultured cells confirmed the formation of a novel polysaccharide, a structure also formed *in vitro*, which was previously undetectable in bacterial cells grown within implants in fish, and in which GalNAcA was fully amidated [2].

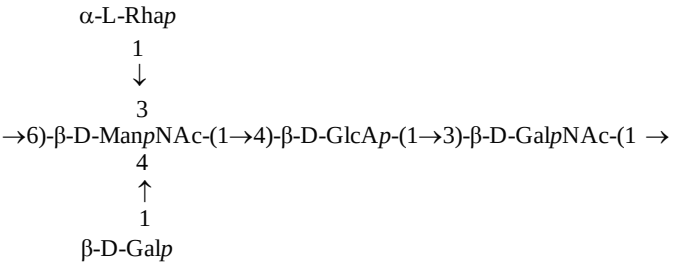
To date, a limited number of bacteria have been reported to produce capsular and O-chain polysaccharides with identical structures. It appears that this property is not uncommon for fish pathogens and similar findings for *Listonella* (formerly *Vibrio*) *anguillarum* and *V. ordalii* [78, 79] have previously been reported. It should be noted that the structures of the CPS and O-chain polysaccharide of *L. anguillarum* and *V. ordalii* have recently been re-examined and that the *galacto* configuration of the 2,3-diacetamido-2,3-dideoxy-hexuronic acid in both structures should be revised in favor of the *gulo* configuration [2].

These studies suggest that caution should be exercised when *in vitro*-cultured cells are used for isolation and structural analysis of bacterial polysaccharides as the resultant structural information may not be

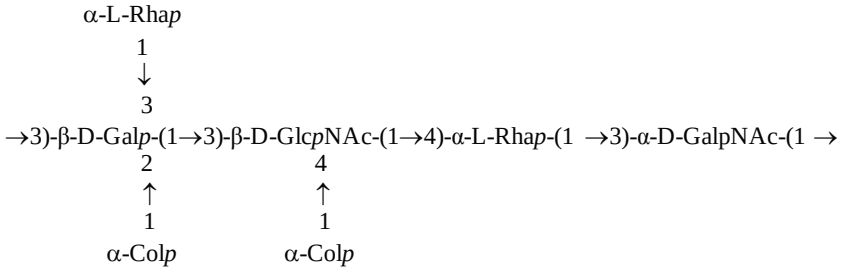
***A. salmonicida* A449, 80204, 80204-1**



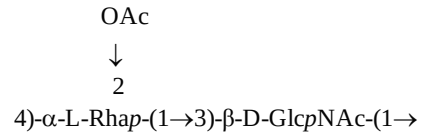
***A. caviae* 11212**



***A. trota* 1354**



***A. hydrophila* LL1**



***A. Hydrophila* AH-3 (O:34)**

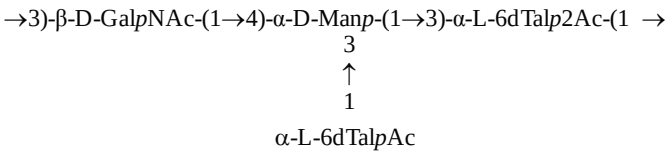


Figure 3. Structure of O-polysaccharide of *Aeromonas* spp.

biologically relevant to *in vivo* conditions [76]. These findings also suggest that additional virulence factors such as capsule and novel LPS O-chain polysaccharide contribute to the pathogenesis of *A. salmonicida in vivo* and emphasize a critical role of a host in host–pathogen interactions [76], although we think the overall biological significance of these findings deserve further research.

While searching for bacteria that cross-react with the recently discovered second causative agent of cholera, *V. cholerae* O139 Bengal [80], six strains of *Aeromonas trota*, have been found to agglutinate with specific antiserum to *V. cholerae* O139 in a slide-agglutination test [2, 81]. All *A. trota* strains were cytotoxic for HeLa cells, positive for adherence to HEp-2 cells, and weakly invasive for HEp-2 cells. One strain was heat-stable toxin positive in the suckling mouse assay; however, all strains were negative for cholera toxin-like enterotoxin [2, 81].

Polyclonal antiserum to a cross-reactive *A. trota* strain cross-protected infant mice against cholera on challenge with virulent *V. cholerae* O139. Serological and genetic studies suggested that the capsular polysaccharide of *V. cholerae* O139 has the same repeating unit as the O-antigen chain [82] and, thus, carries determinants of O-specificity [2, 83, 84].

The known cross-reactivity between the strain studied and *Vibrio cholerae* O139 Bengal is substantiated by the presence of a common colitose-containing epitope shared by the O-specific polysaccharide of *A. trota* and the capsular polysaccharide of *V.cholerae*, which is thought to carry determinants of O-specificity [2].

A 6-deoxyhexose residue, 6-deoxy-L-talose was identified as a dominant component of the *A. hydrophila* O:34 O-specific polysaccharide. Interestingly, strains of this serogroup are most common among mesophilic *Aeromonas* species, accounting for 26.4% of all isolates, and have been documented as an important cause of human infections. It is unknown whether this group of isolates is homogeneous with respect to their O-specific polysaccharide composition. If some departures from the typical O-antigen structure were found, this could suggest the presence of an immunochemical heterogeneity of the isolates, similar to that observed among *A. salmonicida* strains [2, 72].

Interestingly, the phosphoglycerol moiety identified in the structure of the O-chain polysaccharide of *A. caviae* ATCC 15468 was previously found in the O-chain polysaccharides of *Citrobacter* 016 [85], *Hafnia alvei* strain PCM1207 [86] and *Proteus* species [87], as well as in the exo-polysaccharide produced by *Lactobacillus sake* O-1 and the specific capsular polysaccharide of *Streptococcus pneumoniae* type 45 [88]. It is recognized as an immunodominant epitope, and the cross-reactions between the LPS of *Citrobacter* O16 and *H. alvei* strain PCM 1207 could be attributed to the presence of this shared epitope in their respective O-specific polysaccharide structures [2].

3.2.2. Core oligosaccharide

The core of LPS is subdivided into two regions, the inner (highly conserved) and the outer core. The inner core of *Aeromonas* LPS is characterized by the presence of 3-deoxy-D-*manno*-*oct*-2-*ulosonic* (ketodeoxyoctonic) acid (Kdo) and L-*glycero*-D-*manno*-Heptoses (L,D-Hep), which are linked to the outer core, characterized by the presence of Glc, GlcN, Gal, and GalNAc (in *Aeromonas salmonicida*), D,D-Hep (in *Aeromonas salmonicida*), and L,D-Hep (in *Aeromonas hydrophila*) [89, 90] (Figure 4).

The biological relevance of these differences in the distal part of the outer core among these species has not been fully assessed to date.

The core oligosaccharide of *A. hydrophila* (Chemotype III) lipopolysaccharide is unusual in having 3-acetamido-3,6-dideoxy-L-glucose as a constituent [2, 91].

Comparative structural analysis of *A. salmonicida* subsp. *salmonicida* core oligosaccharides from strains A449, 80204-1 and an *in vivo* rough isolate confirmed that the structure of the core oligosaccharide was conserved among different isolates of *A. salmonicida* [2].

Comparative studies with isolated complete LPS and R-type LPS can give clues for the biological activity of the oligosaccharide core structures in different strains of *Aeromonas*.

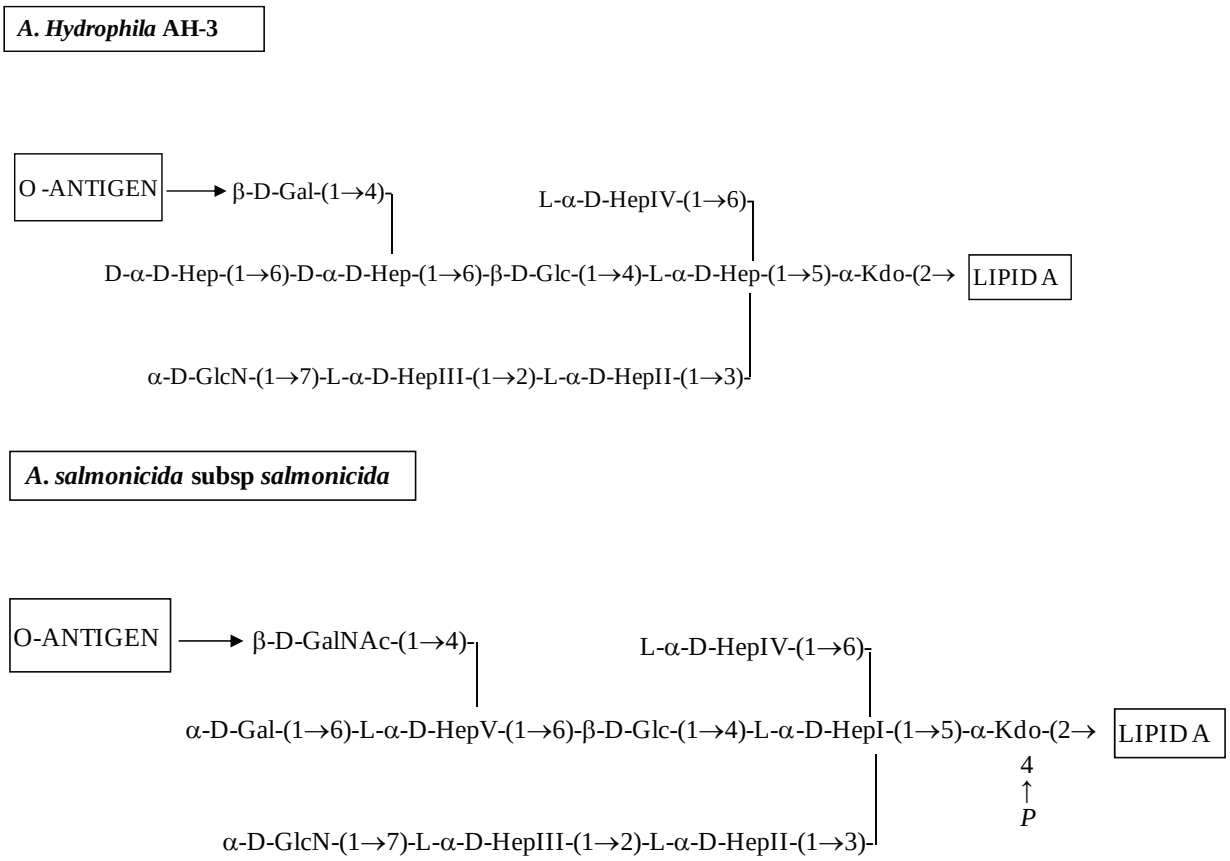


Figure 4. Core oligosaccharide structures of *Aeromonas* species [89, 90].

3.2.3. Lipid A

The lipid A components of *Aeromonas salmonicida* subsp. *salmonicida* contains three major lipid A molecules differing in acylation patterns corresponding to tetra-, penta- and hexaacylated lipid A species and comprising of 4′-monophosphorylated β-2-amino-2-deoxy-D-glucopyranose-(1→6)-2-amino-2-deoxy-D-glucopyranose disaccharide, where the reducing end 2-amino-2-deoxy-D-glucose is present primarily in the α-pyranose form [92]. The tetraacylated lipid A structure contains 3-(dodecanoyloxy) tetradecanoic acid and N-2′,3-hydroxytetradecanoic acid at N-2 and 3-hydroxytetradecanoic acid at O-3, respectively. The pentaacyl lipid A molecule has a similar fatty acid distribution pattern and, additionally, carries 3-hydroxytetradecanoic acid at O-3′. In the hexaacylated lipid A structure, 3-hydroxytetradecanoic acid at O-3′ is esterified with a secondary 9-hexadecenoic acid. Interestingly, lipid A of the *in vivo* rough isolate contains predominantly tetra- and pentaacylated lipid A species suggesting that the presence of the hexaacyl lipid A is associated with the smooth-form LPS [92] (Figure 5).

As in the case of O antigen, growth of *Aeromonas hydrophila* strains from serotype O:34 at 20 ºC and 37 ºC in TSB results in changes in LPS and virulence of the strains, due to lipid changes. Cells grown at 20 ºC contains, relative to those cultured at 37 ºC, increased levels of the phospholipid fatty acids hexadecanoate and octadecanoate and reduced levels of the corresponding saturated fatty acids. Furthermore, the lipid A fatty acids also shows thermoadaptation [66].

In relation to the *Aeromonas* spp biological activities, like in other Gram-negative bacteria, the lipid A induce the B cell polyclonal activation and the response to immunoglobulin M, both by a T mitogen independent mechanism. Furthermore, different effects were observed after injection into

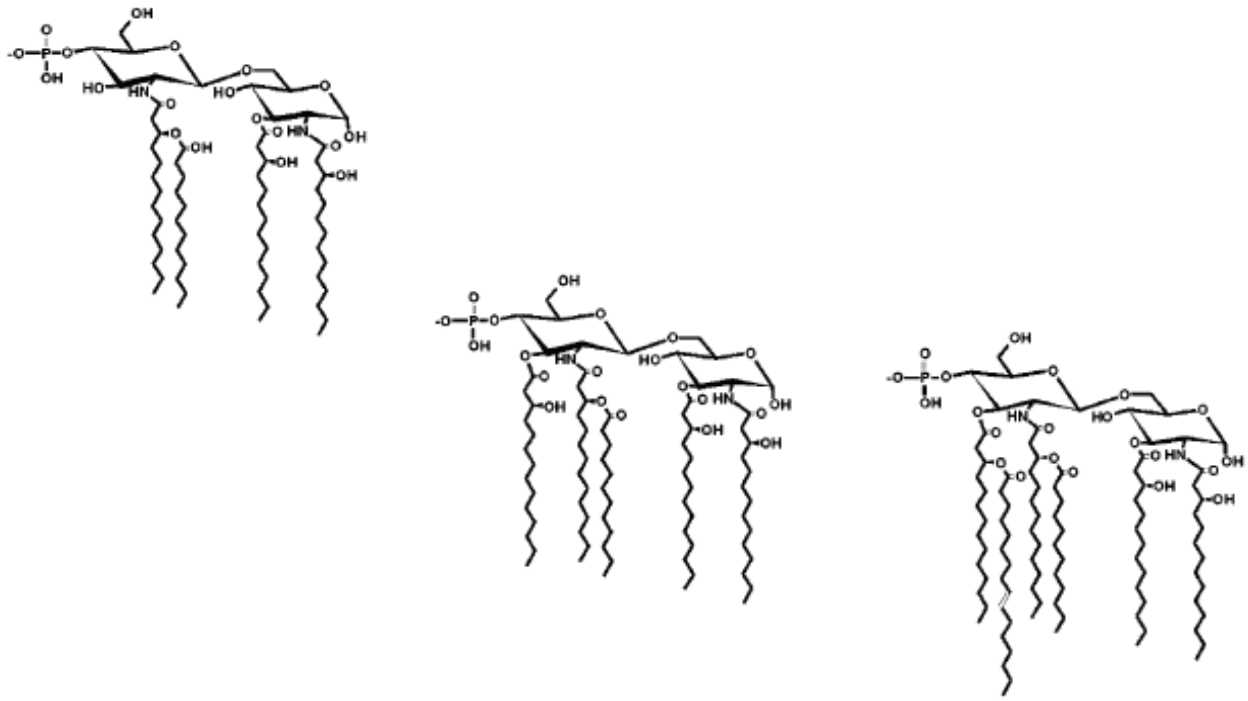


Figure 5. Lipid A structures of *Aeromonas* species [92].

animals: pyrogenicity, leucopenia followed by leucocytosis, septic shock, haemorrhagic necrosis of tumors, diarrhoea and also death [93, 94].

4. CONCLUSIONS AND FUTURE PROSPECTS

In the last decades, compounds extracted from marine organisms have generated great interest [2, 11-13].

At present, a major part of the research on *Aeromonas* bacteria is focused on epidemiology [95] and immunology [96]. However, since the polysaccharides obtained from many different bacteria are important in the manufacture, distribution, storage and consumption of food products [97], cosmetics and paints, we consider that *Aeromonas* LPS could also receive attention for similar applications [2]. Futher research focused on the properties of LPS that could lead to these industrial applications should be performed.

As expected, a higher structural diversity has been found for the O-specific polysaccharide, while more conserved structural features have been described for the core oligosaccharide and Lipid A. In this regard, interesting structure-activity relationships have been found for the variety of O antigen structures, particularly regarding interaction between the bacterial and the frequent host, fishes.

From our view, however, based on the low level of virulence associated with these LPS molecules, we consider that there is still a lack of research regarding the interaction of this battery of LPS molecules as possible therapeutical agents, specifically focusing on the interaction of these molecules with human immunological system.

Several experiments have reported that oral administration of LPS from other bacteria had preventative and curative properties against various diseases, including allergic, and lifestyle-related diseases. In general, in order to expand the usage of the administration of LPS for preventing lifestyle and allergic diseases, it will be necessary to have clarity on the mechanisms that arouse immune responses after administration of LPS [26].

In this regard, we consider that several molecular and cellular *in vitro* and *in vivo* assays could be done with S- or R-types LPS extracted from the different strains of *Aeromonas* or obtained from synthesis of analogous molecules of the LPS active region. Interaction of LPS with the different cellular

types, particularly involved in innate immunity, as different types of antigen presenting cells, could be studied, in order to assess the possible immunoregulatory properties of these molecules.

These advances could facilitate the application of diverse LPS-based molecules in relevant areas such as vaccine technology, allergen immunotherapy, treatment of immune-related diseases/disorders, and LPS-related inflammatory processes or sepsis, as already proposed [98].

Overall, based on the data reviewed in this article, we consider that *Aeromonas* LPS could be suitable candidates for studies in biological assays for drug development.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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