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Archives of Microbiology

Comparative proteome analysis of *Acidaminococcus intestini* supports a relationship between outer membrane biogenesis in Negativicutes and Proteobacteria --Manuscript Draft--

Manuscript Number:	AOMI-D-14-00009
Full Title:	Comparative proteome analysis of <i>Acidaminococcus intestini</i> supports a relationship between outer membrane biogenesis in Negativicutes and Proteobacteria
Article Type:	Short Communication
Abstract:	The presence of bona fide outer membranes in members of the class Negativicutes is anomalous as phylogenetic analyses place this class within the phylum Firmicutes. To explore the relationships of a representative member of Negativicutes, we have performed a whole proteome BLAST analysis of <i>Acidaminococcus intestini</i> , which indicates that a substantial proportion (7%) of the <i>A. intestini</i> proteome is closely related to sequences from members of the phylum Proteobacteria. In addition we have identified key proteins involved in outer membrane biogenesis in <i>A. intestini</i> . This work highlights the need for further studies to define the relationships and evolutionary history of the Negativicutes.
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Author Comments:	Dear Professor Stackebrandt, I am herewith submitting a manuscript entitled "Comparative proteome analysis of <i>Acidaminococcus intestini</i> supports a relationship between outer membrane biogenesis in Negativicutes and Proteobacteria", in the form of a short communication, for your consideration for publication in the Archives of Microbiology. The work described here was carried out in collaboration with Prof. Iain C. Sutcliffe of the Northumbria University (UK) and it report analysis of genome sequence data to understand the origin of outer cell membrane is some atypical Gram-negative bacteria. The results described here provide important insights in this regard. We believe the data presented is well suited to Archives of Microbiology as your journal considers manuscripts that report analysis of 'mining' of data' if new information, interpretation, or hypotheses emerge. The manuscript has been formatted to match the journal's short communication format. We hope that this work will be considered suitable for publication in Archives of Microbiology and look forward to receiving your decision soon. Sincerely yours, Prof Radhey Gupta on behalf of the authors

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	Prof. Brian Hedlund brian.hedlund@unlv.edu expert in genomics who has used genomic data to explore cell envelope characteristics
	Dr. Damien Devos damien.devos@cos.uni-heidelberg.de expert in the use of genomic data to explore cell envelope characteristics

1 **Comparative proteome analysis of *Acidaminococcus intestini* supports a**
2 **relationship between outer membrane biogenesis in *Negativicutes* and**
3 ***Proteobacteria*.**

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18 **Abstract**

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2 19 The presence of *bona fide* outer membranes in members of the class *Negativicutes*
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4 20 is anomalous as phylogenetic analyses place this class within the phylum
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7 21 *Firmicutes*. To explore the relationships of a representative member of
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9 22 *Negativicutes*, we have performed a whole proteome BLAST analysis of
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11 23 *Acidaminococcus intestini*, which indicates that a substantial proportion (7%) of the
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14 24 *A. intestini* proteome is closely related to sequences from members of the phylum
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16 25 *Proteobacteria*. In addition we have identified key proteins involved in outer
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19 26 membrane biogenesis in *A. intestini*. This work highlights the need for further studies
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22 27 to define the relationships and evolutionary history of the *Negativicutes*.
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29 **Keywords:** *Acidaminococcus*; *Clostridia*; lipopolysaccharide; *Negativicutes*;
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33 Bacterial cells exhibit one of two major cell envelope architectures, either monoderm
34 (i.e. a single cytoplasmic membrane (e.g. most *Firmicutes* and *Actinobacteria*) or
35 diderm (i.e. a plasma membrane and a lipid outer membrane e.g. *Proteobacteria*)
36 (Gupta 2011; Sutcliffe 2010). At the phylum level, it appears that most phyla are
37 typically diderm and that within the typically monoderm phyla there are some
38 important diderm exceptions (Sutcliffe 2010). An intriguing example of this is the
39 presence of members of the class *Negativicutes* within the phylum *Firmicutes*
40 (Marchandin et al 2010). Members of this class appear to have typical diderm cell
41 envelopes, notably with an outer membrane based on lipopolysaccharide
42 (Mavromatis et al. 2009; Sutcliffe 2010; Tocheva et al., 2011). In this regard it is
43 notable that some members of the class *Clostridia* (e.g. *Halothermothrix orenii*) also
44 exhibit diderm lipopolysaccharide-based cell envelopes. The relationship between
45 the class *Clostridia* and the class *Negativicutes* has yet to be fully resolved;
46 although the status of the latter class has recently been questioned by Yutin and
47 Galperin (2013), other analyses (Segata et al. 2013; Gupta et al., unpublished)
48 support the integrity of the *Negativicutes* taxon.

49 We are interested in further investigating the basis of outer membrane
50 biogenesis in *Negativicutes*. Thus to explore the relationships between a
51 representative *Negativicute* and members of other taxa, BLAST (Altschul et al.1997)
52 searches were conducted on all proteins found in the *Acidaminococcus intestini*
53 RyC-MR95 genome (D'Auria et al. 2011). The sources (species level) of the first
54 three 'hits' from the BLAST search that were not members of *Negativicutes* and had
55 expect values of less than 10^{-5} were recorded. The phylum of each top hit (or in the
56 case of *Firmicutes*, the class for each top hit) was also recorded. The frequency of
57 each top hit phylum/class was tallied to determine which phyla/classes were most

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58 related to the *Negativicutes* with respect to the proteins analysed. Proteins that did
59 not have a non-*Negativicutes* hit or that had an insignificant top hit (i.e. expect [E]
60 values $>10^{-5}$) were excluded from the tally. As a control, the analysis was repeated
61 using all proteins encoded in the *Erysipelothrix rhusiopathiae* genome (Ogawa et al.
62 2011) as this monoderm species is representative of an independent class
63 (*Erysipelotrichia*) within the *Firmicutes*.

64 Only the top hit from each BLAST search was taken into account when
65 determining the closest relatives to the *A. intestini* proteins (although the 2nd and 3rd
66 hits typically showed similar patterns). 2027 out of the 2400 proteins were used due
67 to the fact that 373 of the proteins did not have significant first hits ($E > 10^{-5}$) or did not
68 have any hits that were from non-*Negativicutes*. Hits from members of the class
69 *Clostridia* represented approximately 68% of top relatives to the proteins, with
70 members of the class *Bacilli* the second most frequent top hit, representing
71 approximately 11.5% of the top relatives (Fig. 1). Notably, the third most frequent top
72 hit (7%, 142 proteins) was to sequences from members of the Gram-negative
73 phylum *Proteobacteria* (Fig. 1). Overall, 8.6% of the *A. intestini* proteins have closest
74 homologues encoded by members of diderm phyla. In contrast, for the control
75 analysis with 1257 *E. rhusiopathiae* proteins, only 1.4 % of the top hits were from
76 members of *Proteobacteria* and a total of 2.9% hits from members of diderm phyla.
77 Thus, hits to *Proteobacteria* sequences are 5-times more frequent for an *A. intestini*
78 query than for the *Erysipelothrix* control.

79 Of the 142 *A. intestini* proteins for which sequences from *Proteobacteria* were
80 the top hits outside of *Negativicutes*, 14 (10%) corresponded to outer membrane
81 function and 10 others (7%) can be linked to LPS biosynthesis (Supplementary
82 Table 1). In addition, 21 (15%) of the 142 proteins are of unknown function. To

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83 further explore the nature of the outer membrane biogenesis pathway in *A. intestini*,
84 we therefore looked for orthologues of key proteins involved in biogenesis and
85 functioning of the *Escherichia coli* outer membrane (Table 1). Clear homologues of
86 all proteins were found encoded in the *A. intestini* genome, with six exceptions.
87 Notably, the outer membrane biogenesis proteins were localised into two loci in the
88 *A. intestini* genome, Acin_0625- Acin_0636 and Acin_1764-Acin_1776 (Table 1).
89 The proteins lacking clear homologues by BLAST analysis include LpxH, a UDP-
90 sugar hydrolase. However, this step in lipid A biosynthesis is bypassed by an
91 alternative step catalysed by LpxI in α -Proteobacteria, many δ -Proteobacteria and
92 some other diderm phyla (Metzget IV and Raetz 2010; Opiyo et al. 2010). Notably an
93 LpxI homologue is encoded by Acin_1764 in the *A. intestini* genome (Table 1).
94 Mavromatis et al. (2009) reported that both *Thermosinus carboxydivorans*
95 (*Negativicutes*) and *H. orenii* (Class Clostridia, order *Halanaerobiales*) also have a
96 complete lipid A biosynthesis path except for LpxH (Mavromatis et al. 2009) and an
97 LpxI homologue is also encoded in each of these genomes (data not shown).
98 Notably, almost all (11/12) of the *A. intestini* proteins that function in the lipid A
99 pathway (Table 1) have a closest proteobacterial homologue from δ -Proteobacteria
100 (data not shown).

101 A homologue of LptD (OstA), part of the LPS transfer machinery, was not
102 found in the *A. intestini* genome. However, Acin_0634 is noted to contain OstA
103 domains and resides within an *A. intestine* LPS biosynthesis locus and so may
104 replace LptD; similarly, an LptC homologue was not detected by BLAST analysis but
105 Acin_0633 encodes an LptC (PF06835) family member. Our analysis did not identify
106 a homologue of BamD, an accessory part of the outer membrane assembly
107 machinery, although this component is not uniformly conserved in diderm bacteria

108 (Webb et al. 2012). Homologues of LolA and LolB, which function in the *E. coli*
109 pathway by which lipoproteins are moved to the outer membrane, were not identified
110 but, again, this pathway is not well conserved even within *Proteobacteria* (Sutcliffe et
111 al. 2013).

112 The above data are consistent with a close relationship between a significant
113 proportion of the proteome (7%) of a representative of *Negativicutes* and the
114 *Proteobacteria*, particularly with regard to cell envelope biogenesis. Importantly, the
115 other phyla of diderm prokaryotes (e.g. *Fusobacteria*, *Synergistetes*) or even diderm
116 members of the class *Clostridia* (i.e. members of the order *Halanaerobiales* such as
117 *H. orenii*), did not show significant numbers of top BLAST hits to the protein queries
118 from the representative *Negativicutes* (Fig. 1; Supplementary Table 2). With regard
119 to the *Negativicutes*, while our results suggest that a large number of genes,
120 particularly those involved in cell envelope biogenesis, are probably laterally
121 acquired from *Proteobacteria*, and δ -*Proteobacteria* in particular, it is important to
122 recognize that the results of BLAST hits are influenced by numerous factors and
123 they are not always the closest relatives (Koski and Golding, 2001). Hence, to gain
124 further understanding of the origin of the outer membrane in the *Negativicutes*, it will
125 be helpful to carry out additional studies on members of these groups to determine
126 the origin of the proteins related to outer membrane biogenesis.

128 **Acknowledgements**

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195 **Figure legend:**

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197 Figure 1. Top BLAST hits summarising the closest relative (phyla; class within
198 *Firmicutes*) of 2027 signature proteins from the *A. intestinalis* genome (A) or 1257
199 proteins from the *E. rhusiopathiae* genome (B). Phyla/classes that represented less
200 than 1.5% of the hits were placed cumulatively into the 'Others' category.

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Table 1. Homologue of key outer membrane (OM) biogenesis proteins and representative OM proteins identified in the *A. intestini* genome by BLAST analysis with *E. coli* proteins as query, except for LpxI (for *Caulobacter crescentus*).

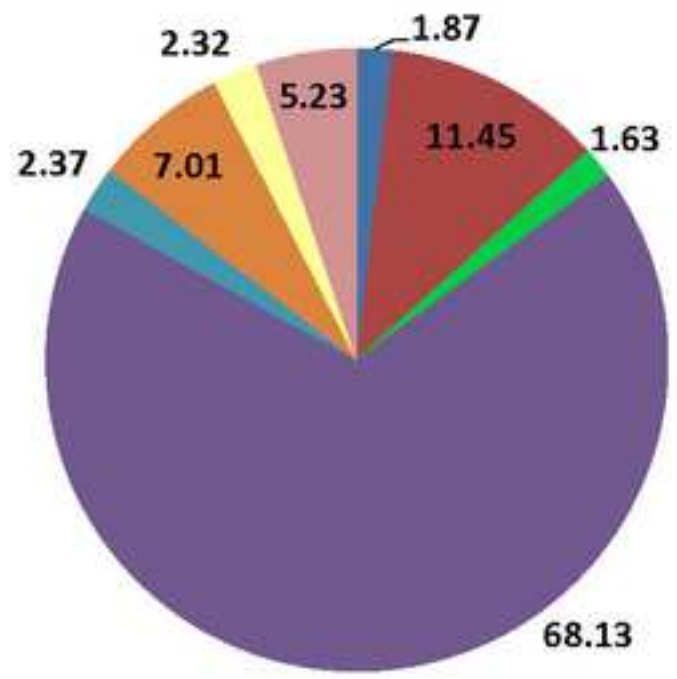
<i>E. coli</i> Protein	UniProt code	Function	<i>A. intestini</i> homologue	Amino acid identity (%); E number
LpxA	P0A722	Lipid A biosynthesis	Acin_1765	120/262 (46%); 1×10^{-75}
LpxB	P10441	Lipid A biosynthesis	Acin_0625	121/379 (32%); 3×10^{-60}
LpxC	P0A725	Lipid A biosynthesis	Acin_1767	107/284 (38%); 1×10^{-52}
LpxD	P21645	Lipid A biosynthesis	Acin_1770	109/334 (33%); 4×10^{-57}
LpxH	P43341	Lipid A biosynthesis	No significant homologue	
LpxI	B8GWR0	Lipid A biosynthesis	Acin_1764	92/283 (33%); 6×10^{-35}
LpxK	P27300	Lipid A biosynthesis	Acin_0627 (aa 505-840)	83/341 (25%); 4×10^{-22}
KdtA (WaaA)	P0AC75	Lipid A biosynthesis	Acin_0627(aa 13-430)	129/425 (30%); 6×10^{-62}
HtrB (LpxL)	P0ACV0	Lipid A biosynthesis	Acin_0632	70/285 (25%); 1×10^{-15}
LpxM	C4ZZL2	Lipid A biosynthesis	Acin_0632	61/272 (22%); 7×10^{-12}
KdsA	P0A715	Lipid A biosynthesis	Acin_0629	126/268 (47%); 7×10^{-81}
KdsB	P04951	Lipid A biosynthesis	Acin_0628	117/239 (49%); 3×10^{-66}
KdsC	P0ABZ4	Lipid A biosynthesis	Acin_0631	72/157 (46%); 3×10^{-39}
KdsD	P45395	Lipid A biosynthesis	Acin_0630	164/321 (51%); 1×10^{-103}

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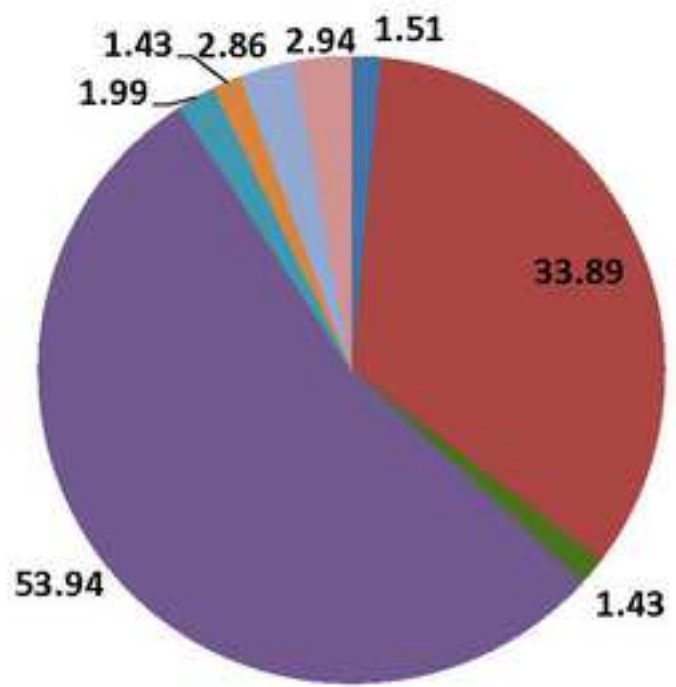
LptA	P0ADV1	LPS export (periplasmic Lipid A shuttle)	Acin_2165	39/166 (23%); 0.046
LptB	P0A9V1	LPS export	Acin_0635	130/237 (55%); 1×10^{-90}
LptC	P0ADV9	LPS export	No significant homologue*	
LptD	P31554	LPS export (insertion of LPS into OM)	No significant homologue*	
MsbA	P60752	Lipid A flippase	Acin_0626	208/572 (36%); 5×10^{-121}
BamA	P0A940	Signature protein for OM biogenesis	Acin_1774	137/560 (24%); 1×10^{-28}
BamD	P0AC02	OM biogenesis	No significant homologue	
LolA	P61316	OM lipoprotein shuttle	No significant homologue	
LolB	P61320	OM lipoprotein insertion	No significant homologue	
TolC	P02930	Canonical OM protein (type 1 secretion systems)	Acin_1776	103/409 (25%); 5×10^{-20}
GspD	P45758	Canonical OM protein (type 2 secretion system)	Acin_0088	74/284 (26%); 1×10^{-23}

* See main text

A) Negativicutes Top Hits



B) Control Top Hits



- Actinobacteria
- Bacilli
- Bacteroidetes
- Clostridia
- Fusobacteria
- Proteobacteria
- Synergistetes
- Tenericutes
- Spirochaetes
- Others

Supplementary Table -1

[Click here to download Supplementary Material: Campbell et al Suppl Table 1.xlsx](#)

Supplementary Table -2

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