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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--

<b>Manuscript Number:</b>	AOMI-D-14-00009
<b>Full Title:</b>	Comparative proteome analysis of <i>Acidaminococcus intestini</i> supports a relationship between outer membrane biogenesis in Negativicutes and Proteobacteria
<b>Article Type:</b>	Short Communication
<b>Abstract:</b>	<p>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.</p>
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<b>Author Comments:</b>	<p>Dear Professor Stackebrandt,</p> <p>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.</p> <p>Sincerely yours,</p> <p>Prof Radhey Gupta on behalf of the authors</p>

<b>Suggested Reviewers:</b>	Dr. Paul Lawson paul.lawson@ou.edu expert in the taxonomy of clostridia and relatives
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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 2<sup>nd</sup> and 3<sup>rd</sup>  
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  $\alpha$ -Proteobacteria, many  $\delta$ -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  $\delta$ -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  $\delta$ -*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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133 **References**

134

135 Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ  
136 (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database  
137 search programs. *Nucleic Acids Res* 25: 3389-3402.

138

139 D'Auria G, Galan J-C, Rodriguez-Alcayna M, Moya A, Baquero F, Latorre A (2011)  
140 Complete genome sequence of *Acidaminococcus intestini* RYC-MR95, a Gram-  
141 negative bacterium from the phylum *Firmicutes*. *J Bacteriol* 193: 7008-9

142

143 Gupta RS (2011) Origin of diderm (Gram-negative) bacteria: antibiotic selection  
144 pressure rather than endosymbiosis likely led to the evolution of bacterial cells with  
145 two membranes. *Anton van Leeuwen* 100: 171-182.

146

147 Koski LB, Golding GB (2001) The closest BLAST hit is often not the nearest  
148 neighbor. *J Mol Evol* 52: 540-542.

149

150 Metzget IV LE, Raetz CRH (2010) An alternative route for UDP-diacylglucosamine  
151 hydrolysis in bacterial lipid A synthesis. *Biochemistry* 49: 6715-6726

152

153 Marchandin H, Teyssier C, Campos J, Jean-Pierre H, Roger F, Gay B, Carlier J-P,  
154 Jumas-Bilak E (2010) *Negativicoccus succinicivorans* gen. nov., sp nov., isolated  
155 from human clinical samples, emended description of the family *Veillonellaceae* and  
156 description of *Negativicutes* classis nov., *Selenomonadales* ord. nov and  
157 *Acidaminococcaceae* fam. nov in the bacterial phylum *Firmicutes*. *Int J Syst Evol*  
158 *Microbiol* 60:1271-1279

159

160 Mavromatis K, Ivanova N, Anderson I, Lykidis A, Hooper SD, Sun H, Kunin V,  
161 Lapidus A, Hugenholtz P, Patel B, Kyrpides, NC (2009) Genome analysis of the  
162 anaerobic thermohalophilic bacterium *Halothermothrix orenii*. *PLOS One* 4: e4192

163

164 Ogawa Y, Ooka T, Shi F, Ogura Y, Nakayama K, Hayashi T, Shimoji Y (2011) The  
165 Genome of *Erysipelothrix rhusiopathiae*, the causative agent of swine erysipelas,

166 reveals new insights into the evolution of *Firmicutes* and the organism's intracellular  
1 167 adaptations. J Bacteriol 193: 2959-2971  
2  
3 168  
4  
5 169 Opiyo SO, Pardy RL, Moriyama H, Moriyama EN (2010) Evolution of the Kdo<sub>2</sub>-lipid A  
6 biosynthesis in bacteria. BMC Evol Biol 10:362  
7 170  
8  
9 171  
10  
11 172 Segata N, Boernigen D, Morgan XC, Huttenhower C (2013) PhyloPhlAn is a new  
12 method for improved phylogenetic and taxonomic placement of microbes. Nature  
13 173 Comm 4: 2304 (doi:10.1038/ncomms3304)  
14 174  
15  
16 175  
17  
18 176 Sutcliffe IC (2010) A phylum level perspective on bacterial cell envelope architecture.  
19 Trends Microbiol 18: 464-470  
20 177  
21  
22 178  
23 179 Sutcliffe IC, Harrington DJ, Hutchings MI (2012) A phylum level analysis reveals  
24 lipoprotein biosynthesis to be a fundamental property of bacteria. Protein & Cell 3:  
25 180 163-170  
26  
27 181  
28  
29 182  
30  
31 183 Tocheva E, Matson EG, Morris DM, Moussavi F, Leadbetter JR, Jensen GJ (2011)  
32 Peptidoglycan remodeling and conversion of an inner membrane into an outer  
33 184 membrane during sporulation. Cell 146: 799-812  
34  
35 185  
36 186  
37  
38 187 Yutin N, Galperin MY (2013) A genomic update on clostridial phylogeny: Gram-  
39 negative spore formers and other misplaced clostridia. Env Microbiol 15: 2631-2641  
40 188  
41  
42 189  
43 190 Webb CT, Heinz E, Lithgow T (2013) Evolution of the  $\beta$ -barrel assembly machinery.  
44 Trends Microbiol 20: 612-620  
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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.

**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*).**

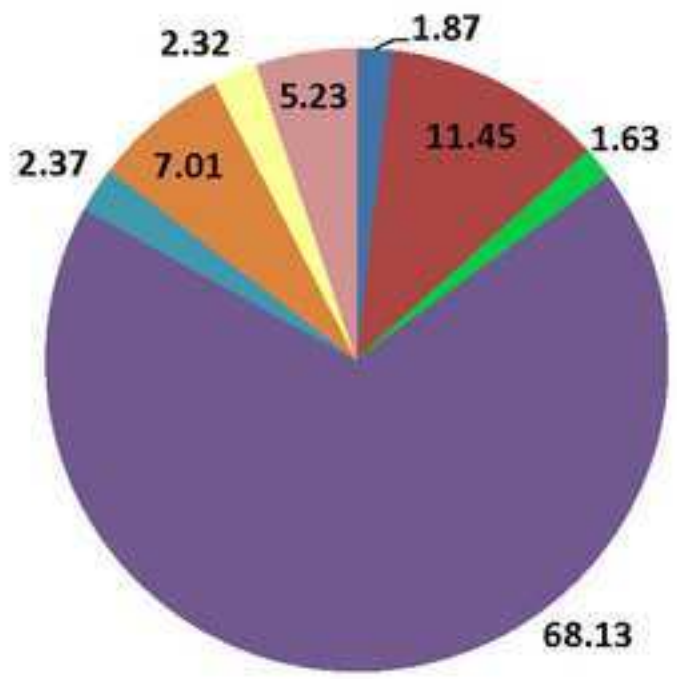
<i>E. coli</i> Protein	UniProt code	Function	<i>A. intestini</i> homologue	Amino acid identity (%); E number
LpxA	P0A722	Lipid A biosynthesis	Acin_1765	120/262 (46%); $1 \times 10^{-75}$
LpxB	P10441	Lipid A biosynthesis	Acin_0625	121/379 (32%); $3 \times 10^{-60}$
LpxC	P0A725	Lipid A biosynthesis	Acin_1767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

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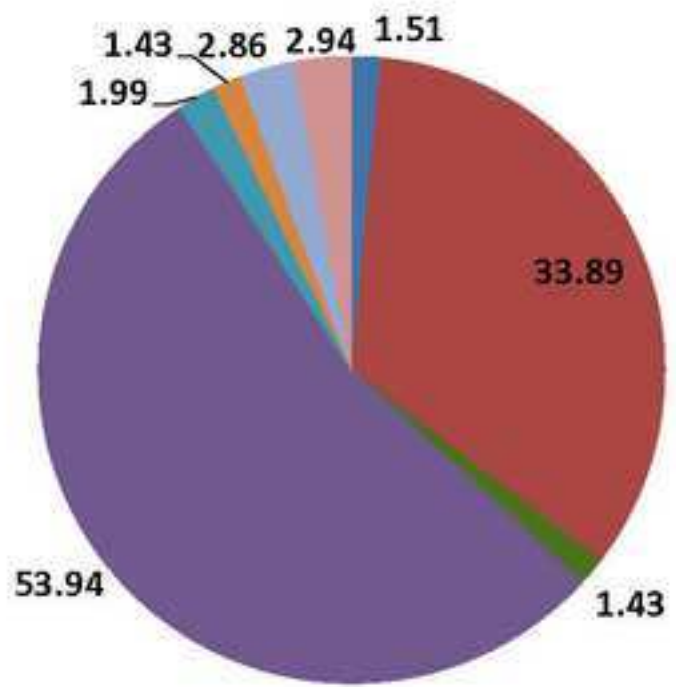
LptA	P0ADV1	LPS export (periplasmic Lipid A shuttle)	Acin_2165	39/166 (23%); 0.046
LptB	P0A9V1	LPS export	Acin_0635	130/237 (55%); $1 \times 10^{-90}$
LptC	P0ADV9	LPS export	No significant homologue*	
LptD	P31554	LPS export (insertion of LPS into OM)	No significant homologue*	
MsbA	P60752	Lipid A flippase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

\* See main text

A) Negativicutes Top Hits



B) Control Top Hits



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

Supplementary Table -1

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Supplementary Table -2

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