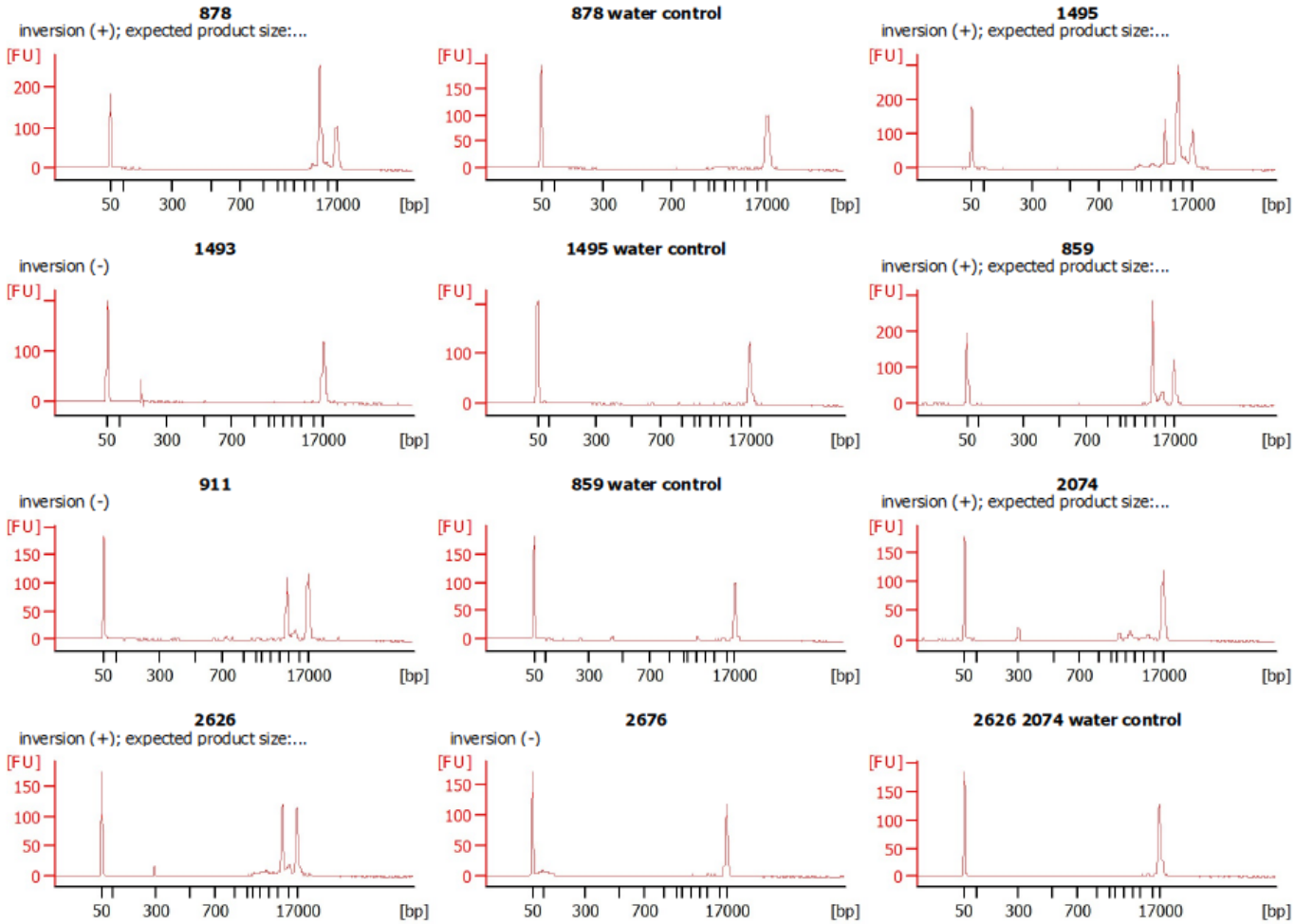
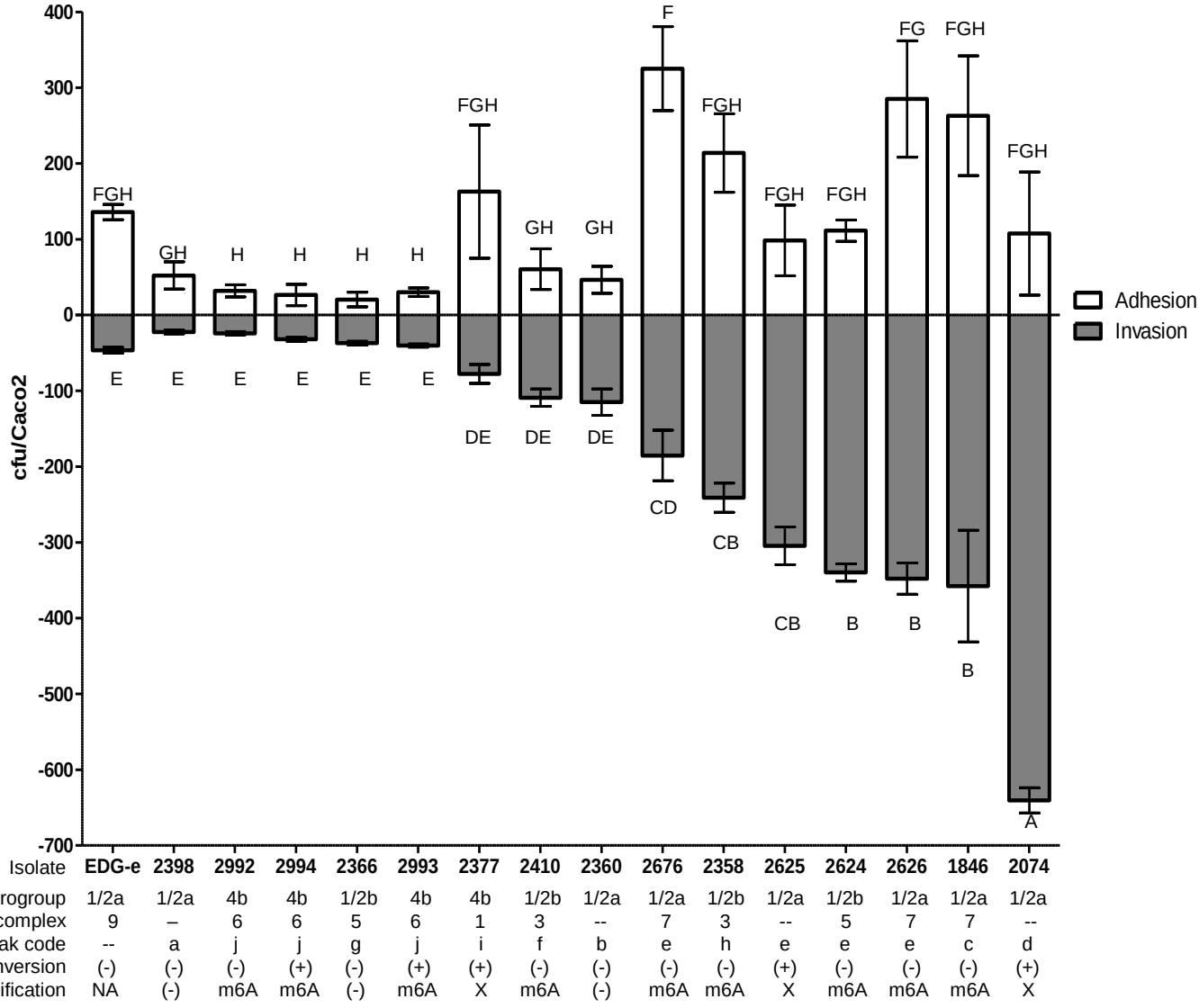
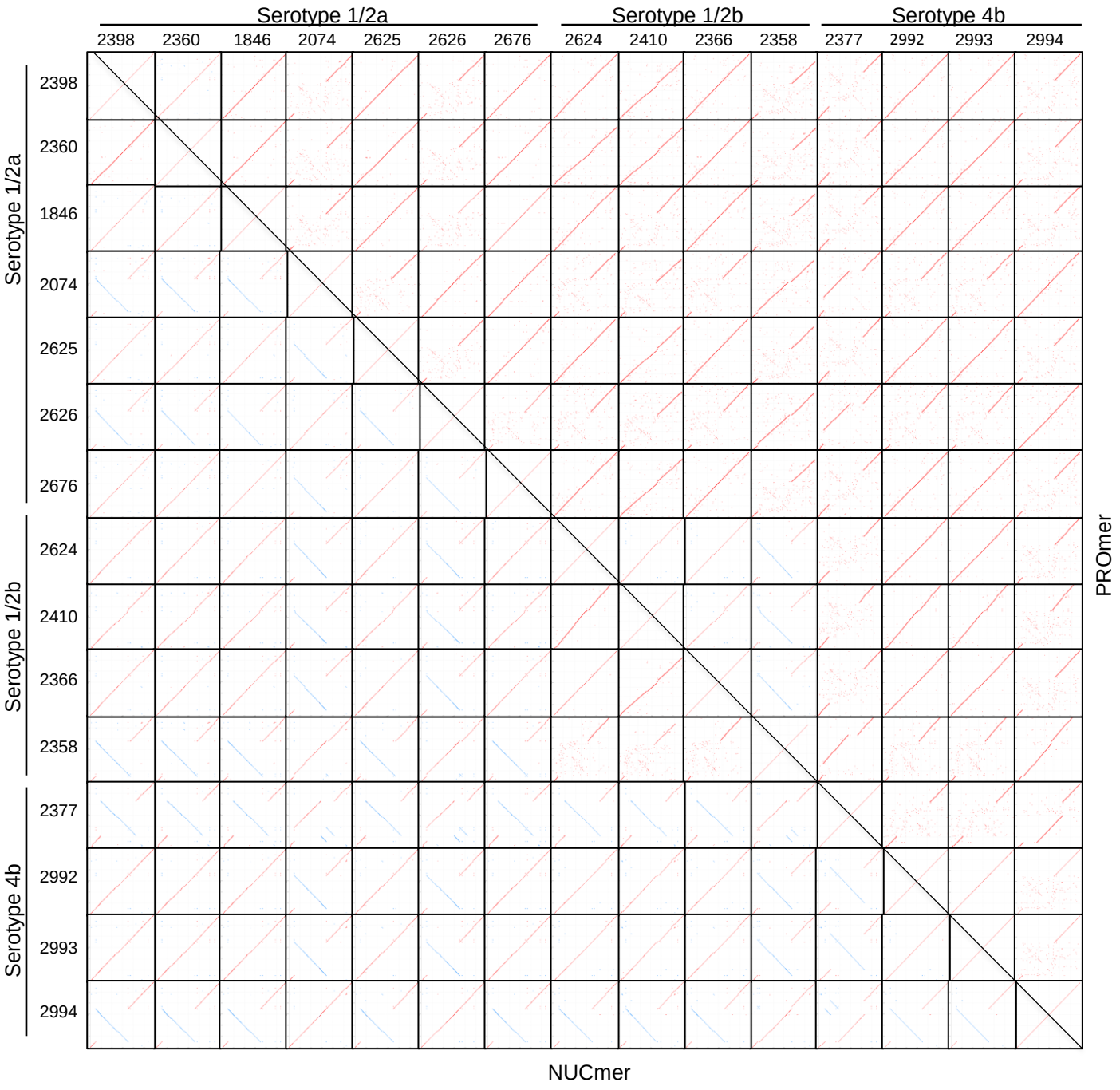


Supplementary Figure 1. PCR verification of chromosome inversions.
Large scale inversions were verified by PCR amplification across inversion junctions. PCR primers are listed in Supplementary Table 2. PCR product sizes were analyzed using Agilent DNA1000 Bioanalyzer kits.

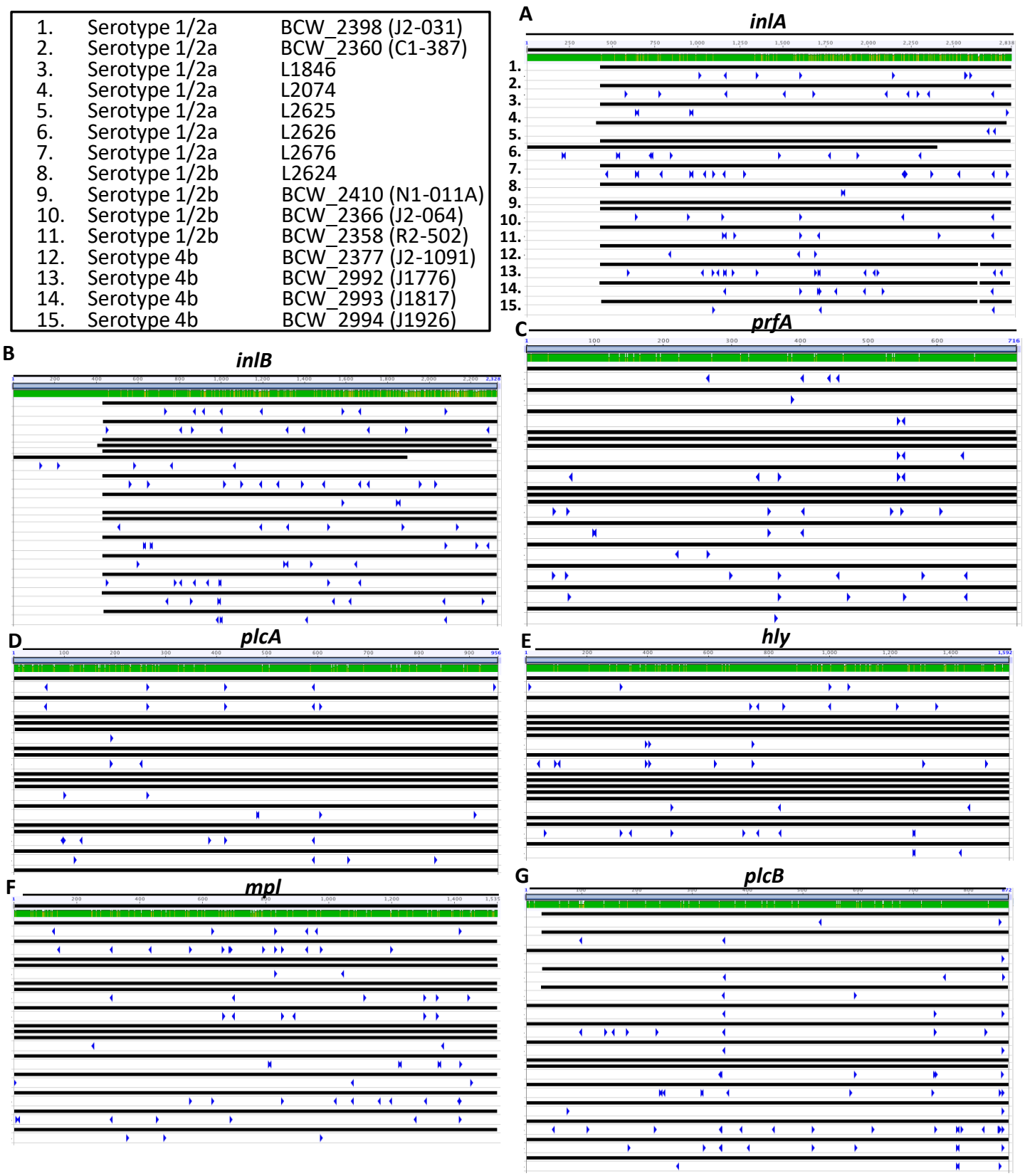


Supplementary Figure 2. Variation of *L. monocytogenes* to associate and localize during in vitro adhesion and invasion assays. *L. monocytogenes* invasion was quantified using a modified gentamicin protection assay (Elsinghorst EA. 1994. Methods Enzymol 236:405-420). Isolates were grown to early stationary phase prior to infection of differentiated Caco-2 monolayers. Adhesion and Invasion was quantified as colony forming units (cfu)/Caco-2 cell (n = 3) and organized by invasiveness using GraphPad InStat 3 (GraphPad Software, Inc., La Jolla, CA). $P<0.05$ was considered to be statistically significant. Positive Y-axis values represent the number of cfu adhered to a single host cell (Caco-2) surface. Negative Y-axis values represent the absolute number of cfu invaded into a single Caco-2 cell. Details of each isolate are listed below the strain designation. Unspecified clonal complexes are denoted by the dashed lines. Isolates found to carry the unknown DNA modification are denoted as X.



Supplementary Figure 3. Multipanel MUMmerplots of 15 *L. monocytogenes* genomes.
Whole genome alignments of L. monocytogenes isolates were carried out using MUMmer. Isolates are arranged by serotype and results of both NUCmer and PROmer alignments are displayed.

1.	Serotype 1/2a	BCW_2398 (J2-031)
2.	Serotype 1/2a	BCW_2360 (C1-387)
3.	Serotype 1/2a	L1846
4.	Serotype 1/2a	L2074
5.	Serotype 1/2a	L2625
6.	Serotype 1/2a	L2626
7.	Serotype 1/2a	L2676
8.	Serotype 1/2b	L2624
9.	Serotype 1/2b	BCW_2410 (N1-011A)
10.	Serotype 1/2b	BCW_2366 (J2-064)
11.	Serotype 1/2b	BCW_2358 (R2-502)
12.	Serotype 4b	BCW_2377 (J2-1091)
13.	Serotype 4b	BCW_2992 (J1776)
14.	Serotype 4b	BCW_2993 (J1817)
15.	Serotype 4b	BCW_2994 (J1926)



Supplementary Figure 4. Virulence gene methylation patterns.
Internalin and LIPI-1 genes were analyzed for gene-specific methylation patterns . **A)** *inlA* **B)** *inlB* **C)** *prfA* **D)** *plcA* **E)** *hly* **F)** *mpl* **G)** *plcB.* Isolate arrangement is specified in the legend (upper left) with alleles of each gene appearing in order of 1-15. Alignment consensus sequences are represented as green bars; individual genes are represented by black bars. Methylation events are represented by blue arrows below black bar (which represents the gene that they are found in) with the direction of arrows indicating strandedness of methylation.

Supplementary Figure 5. *actA* sequence alignment across all strains.
actA genes were extracted and aligned using Geneious 7.0. *actA* sequences are displayed as color blocks with overall sequence identity displayed as the green bar at the top of each section. Disparities in the sequence identity are displayed as dips in the bar. Nonexistent regions of *actA* in BCW_002366, L2624, and BCW_002377 are displayed as black lines through the sequence region.