SUPPLEMENTARY INFORMATION

SUPPLEMENTARY TABLES

Table S1. Orthologs of *A. tumefaciens* UPP biosynthesis cluster in *P. hirschii*

P. hirschii NCBI locus	Ortholog in *P. hirschii*	Ortholog in *A. tumefaciens* (% identity)*	General description of function#
KPL55196‡	C1_5522‡	UppH Atu_0482 (29%)^	Deacetylase
KPL55195	C1_5521	UppA Atu_1240 (31.3%)	Acetyltransferase
KPL55194	C1_5520	UppB Atu_1239 (35.5%)	MPA-1
KPL55193	C1_5519	UppC Atu_1238 (63.4%)	Secretin
KPL55192	C1_5516	UppD Atu_1237 (44.4%)	Glycosyltransferase
KPL55191	C1_5515	UppE Atu_1236 (54.2%)	Glycosyltransferase
KPL55190	C1_5514	UppF Atu_1235 (44.6%)	Polymerase
KPL52370	C1_1957	Atu_0480 (80%)^	Flippase
Not present	Not present	Atu_0481	Polymerase

* % identity of bidirectional best hits to *P. hirschii* orthologs were determined using Clustal Omega except where indicated

general descriptions of function are provided as a reference

‡ Non-syntenic *P. hirschii* locus that has higher sequence homology to *A. tumefaciens uppH.* NCBI locus (KPL54727), Ortholog in *P. hirschii* (C1_4935) is the bidirectional best hit.

^Lower confidence hits due to low sequence similarity with less than 12% overall coverage

Table S2. Orthologs of cell cycle regulators in *P. hirschii*

Regulatory Cell Cycle Protein	*P. hirschii* NCBI locus	Ortholog in *P. hirschii*	Ortholog in *A. tumefaciens* (% identity)*	Ortholog in *C. crescentus* (% identity)*	General description of function#
DivJ	KPL52058	C1_1571	Atu_0921 (46.6%)	CC_1063 CCNA_01116 (49.2%)	Histidine kinase responsible for DivK phosphorylation
DivK	KPL51044	C1_0307	Atu_1296 (80.5%)	CC_2463 CCNA_02547 (80.0%)	Response regulator; phosphorylation state controls cell fate
PleC	KPL54541	C1_4701	Atu_0982 (44.9%)	CC_2482 CCNA_02567 (39.8%)	Histidine kinase dephosphorylates DivK

PdhS1	KPL51802	C1_1248	Atu_0614 (51.5%)	Not present	Histidine kinase involved in polar differentiation
PdhS2	KPL51665	C1_1072	Atu_1888 (48.5%)	Not present	Histidine kinase involved in polar differentiation
CckA	KPL51705	C1_1122	Atu_1362 (53.5%)	CC_1078 CCNA_01132 (49.4%)	Hybrid histidine kinase; along with ChpT regulates phosphorylation state of CtrA
ChpT	KPL54390	C1_4527	Atu_2438 (52.0%)	CC_3470 CCNA_03584 (36.0%)	Hpt phosphotransferase; along with CckA regulates phosphorylation state of CtrA
CtrA	KPL54404	C1_4543	Atu_2434 (94.2%)	CC_3035 CCNA_03130 (82.3%)	Master regulator of cell cycle progression
SciP	KPL54413	C1_4554	Atu_2430 (84.6%)	CC_0903 CCNA_00948 (82.7%)	Accessory protein functioning in regulation of CtrA-dependent promoters
MucR2/ ros^	KPL52063	C1_1579	Atu_0916 (62.3%)	CC_0949 CCNA_00998 (66.9%)	Repressor of CtrA-activated genes
CpdR	KPL55751	C1_1840	Atu_3883 (86.3%)	CC_0744 CCNA_00781 (63.0%)	Response regulator involved in ClpXP localization
RcdA	KPL52102	C1_1635	Atu_4742 (55.8%)	CC_3295 CCNA_03404 (53.3%)	Degradation factor required for ClpXP mediated proteolysis of CtrA
ClpP	KPL55257	C1_5596	Atu_1258 (69.0%)	CC_1963 CCNA_02041 (67.0%)	Essential ATP-dependent ClpXP protease component; responsible for CtrA proteolysis
ClpX	KPL55258	C1_5597	Atu_1259 (81.8%)	CC_1961 CCNA_2039 (81.3%)	Essential ATP-dependent ClpXP protease component; responsible for CtrA proteolysis
CcrM	KPL52394	C1_1988	Atu_0794 (68.7%)	CC_0378 CCNA_0328 (65.7%)	DNA methyltransferase; methylation state impacts cell cycle regulation
GcrA	KPL55789	C1_2139	PC-6^{+} (47.2%)	CC_2245 CCNA_02328 (42.9%)	Transcriptional regulator involved in late cell cycle progression
DnaA	KPL52602	C1_2255	Atu_0324 (52.0%)	CC_0008 CCNA_00008 (37.0%)	Initiates DNA replication and transcriptional activator during early cell cycle progression
DivL	KPL56005	C1_4100	Atu_0027 (43.2%)	CC_3484 CCNA_03598 (35.7%)	Tyrosine kinase required for cell division and growth
PodJ	KPL52461	C1_2083	Atu_0499 (40.0%)	CC_2045 CCNA_2125 (33.8%)	Polar development protein required for localization of several polar organelles

* % identity of bidirectional best hits to *P. hirschii* orthologs were determined using Clustal Omega

general descriptions of function are provided as a reference

^ An additional MucR paralog (C1_2945) is present in the genome

+ GcrA was not annotated in the original *A. tumefaciens* genome sequence but was later identified during re-annotation (1)

SUPPLEMENTARY FIGURES

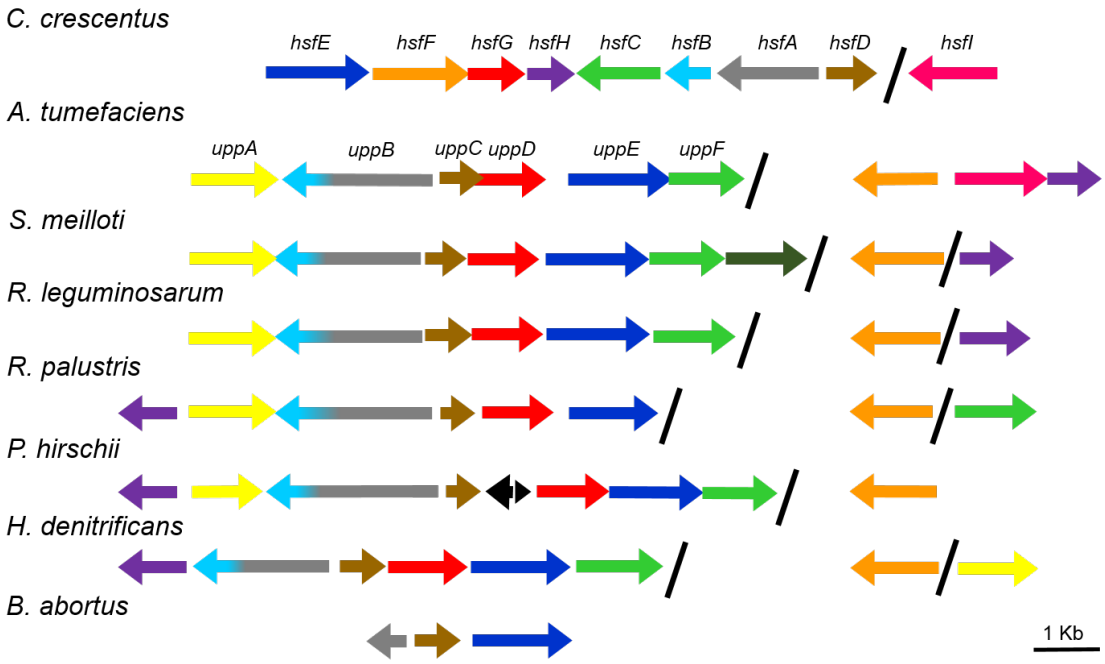

Figure S1. **Conservation of polar polysaccharide synthesis genes**. Genetic organization of the holdfast biosynthesis gene cluster in *C. crescentus* encodes genes that function as a glycosyltransferase (HsfE, dark blue arrow and HfsG, red arrow), flippase (HsfF, orange arrow), deacetylase (HsfH, purple arrow), polymerase (HsfC, light green arrow and HsfI, pink arrow), secretin (HsfD, brown arrow), autokinase (HfsB, light blue arrow), and membrane periplasmic axillary protein (HsfA, grey arrow). All of these gene products contribute to the synthesis and export of the *C. crescentus* holdfast. Gene names for the major *A. tumefaciens* unipolar polysaccharide biosynthesis cluster (*uppA-F*) are listed above each arrow. Conserved coding indicates the predicted function in polysaccharide biosynthesis as described for holdfast. In the *Rhizobiales, uppA* (yellow arrows) is predicted to encode an acetyltransferase, *uppB* (shaded with both light blue and grey) is a fusion of *hfsA* and *hfsB,* and flippase genes are typically found at another genomic loci. Several members of the Rhizobiales, including *P. hirschii,* have a *wzy*-type polysaccharide gene cluster that is similar in cluster structure to *A. tumefaciens*. Black arrows represent hypothetical proteins with no significant homology to known polysaccharide biosynthesis genes. Scale bar is 1Kb.

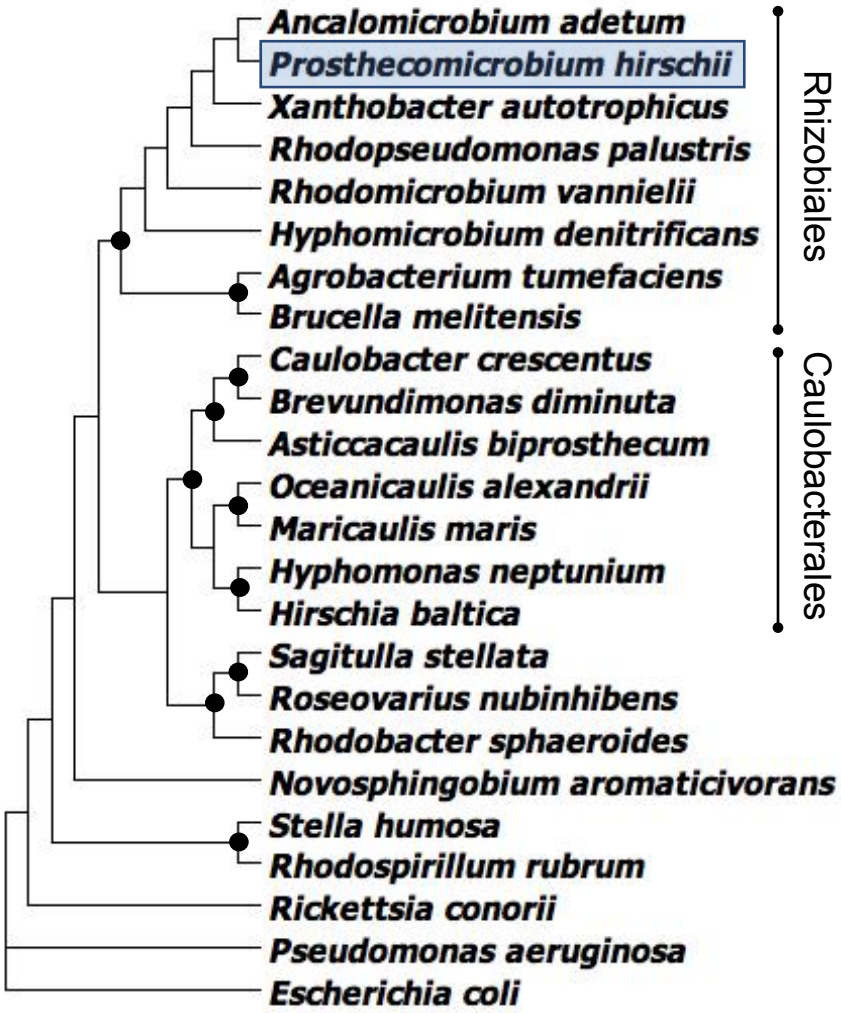

Figure S2. Phylogeny places *P. hirschii* within the *Rhizobiales*. Maximum likelihood phylogeny inferred from *gyrA* sequences. Node values indicate clade frequency among 100 nonparametric bootstrapped datasets. Black nodes represent bootstrap values above 90.

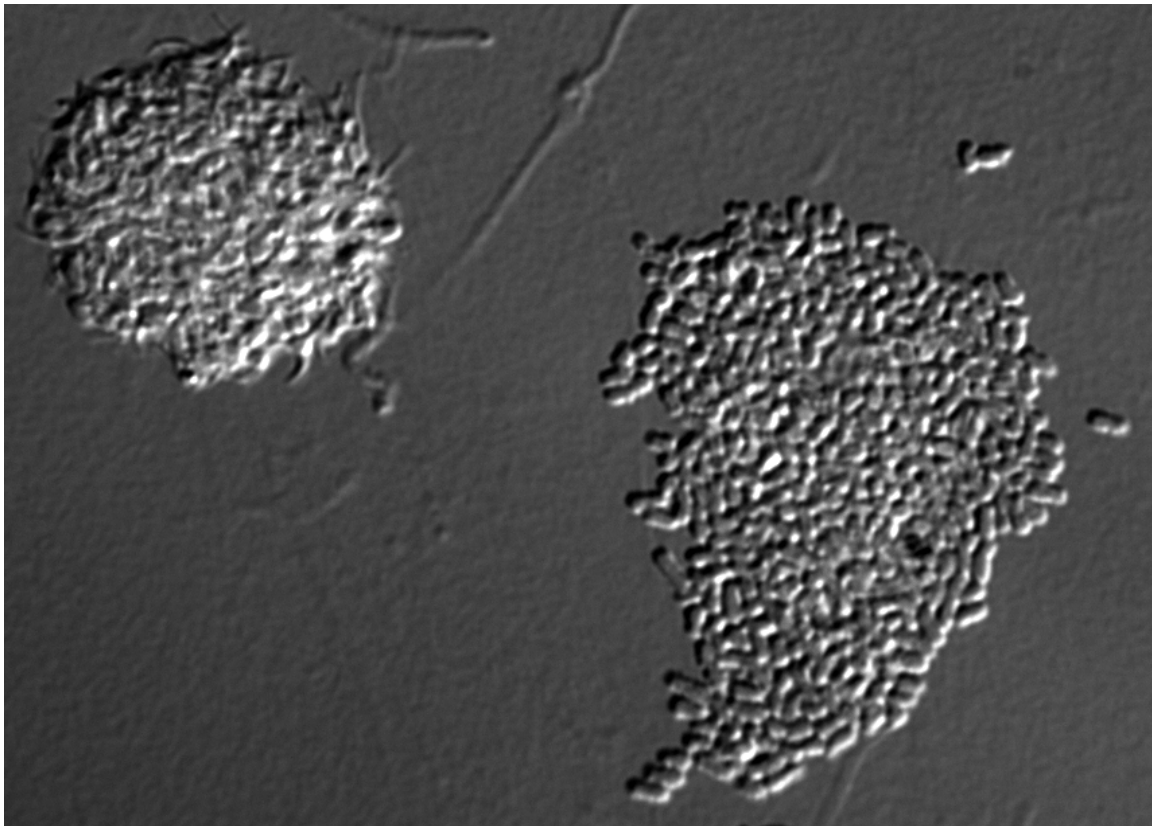

Figure S3. *P. hirschii* forms microcolonies of a single cell type. Differential interference contrast (DIC) microscopy image of *P. hirschii* microcolonies comprised of tightly packed, non-motile long-stalked cells (left) and motile short-stalked cells (right).

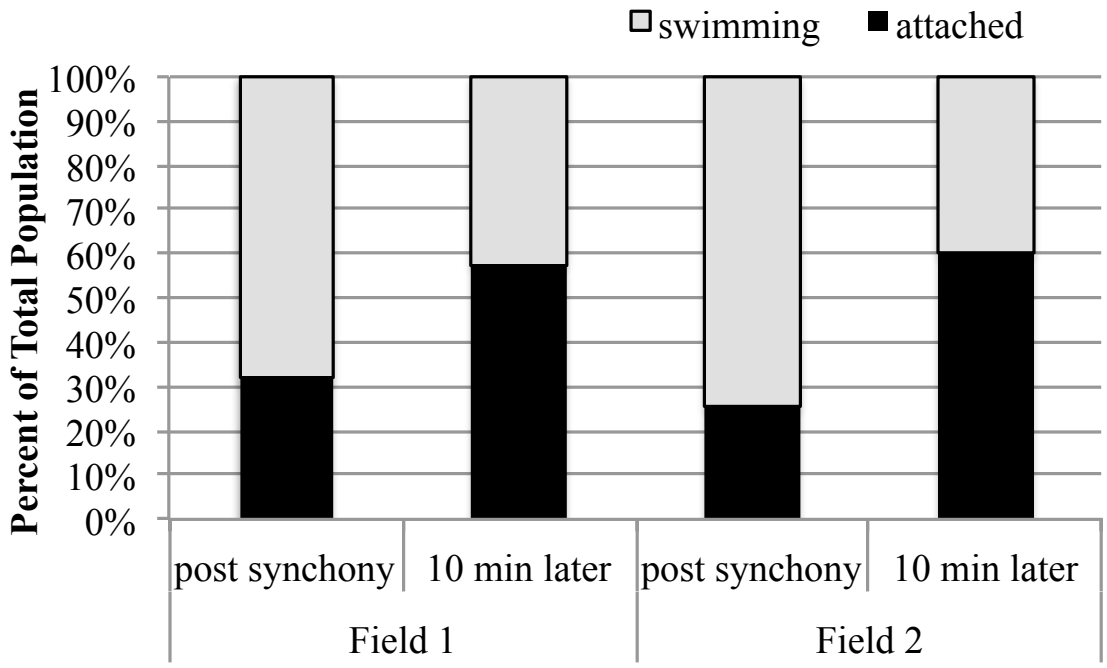

Figure S4. **Rapid attachment of motile *P. hirschii* cells.** Quantification of the proportion of swimming and attached cells following cell synchrony. Synchronized cells were collected in the analysis channel and videos were taken within 5 minutes of the synchrony and again 10 minutes later. Each field represents data from an independent synchrony. A total of 40 – 65 cells were classified as swimming or attached in each field at the indicated times. The proportion of attached cells increases from ~30% to 55% within 10 min, in two independent experiments.

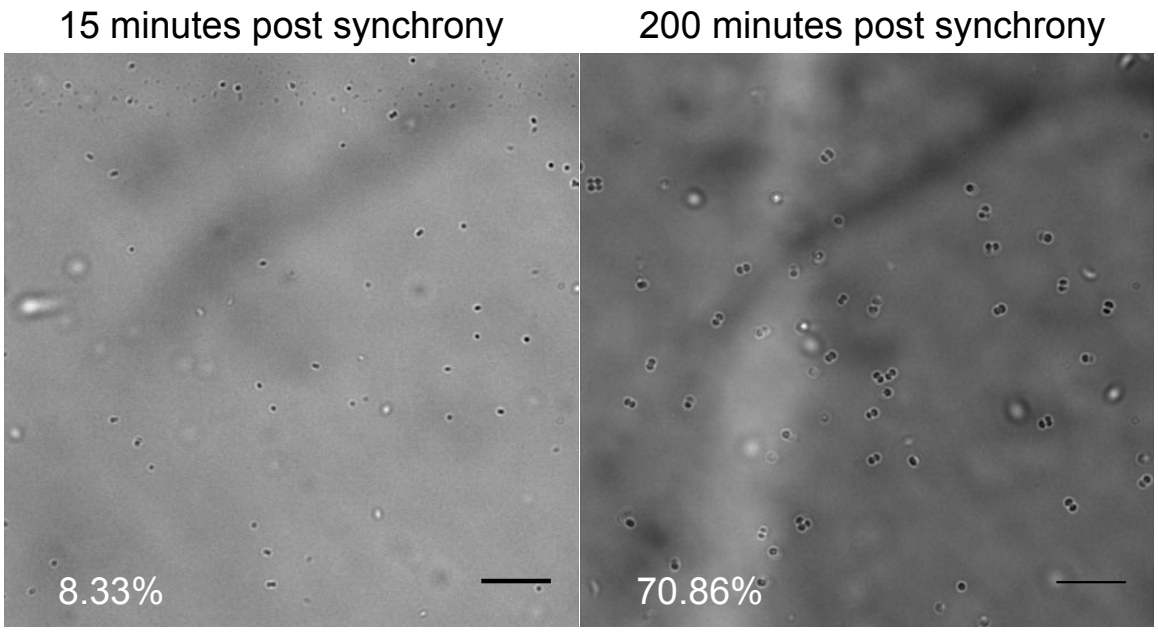

Figure S5. ***P. hirschii*** **cells are synchronized in the microfluidic device.** Representative white light images of cells attached to the analysis channel 15 and 200 min post synchronization are shown. Quantitation of multiple fields reveals that 15 min post synchrony 8.33% of cells (n=108) have pre-divisional septa, whereas 200 min post synchrony 70.86% of cells (n=127) have pre-divisional septa. Scale bar is 5 μm.

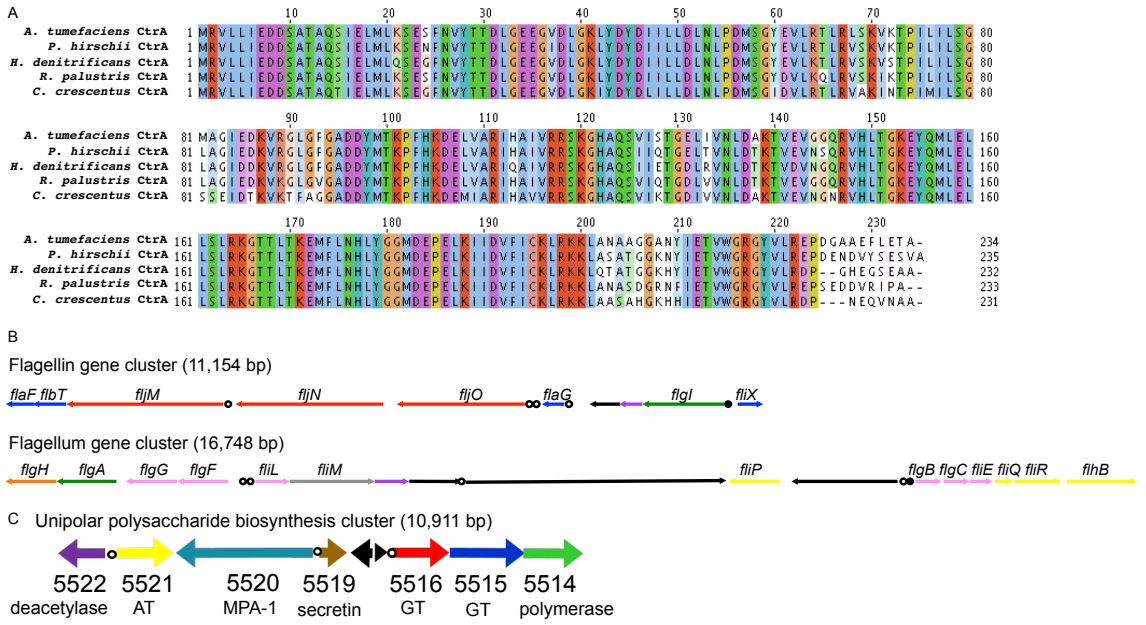

Figure S6. CtrA may function as a global regulator in *P hirschii*. A. Alignment of the CtrA protein from *P. hirschii* to orthologs from other Alphaproteobacteria reveals a high degree of protein identity. *P hirschii* CtrA is 94.2% identical to CtrA from *A. tumefaciens* (Atu_2334). Protein alignment was generated with the Clustal Omega program. B. Multiple putative CtrA binding sites are found in gene clusters encoding flagellum genes. The flagellin gene cluster (top panel) includes regulators of flagellin biosynthesis (blue arrows) and the flagellins (red arrows). The gene encoding FlgI, a protein found in the P-ring, is just downstream and has a CtrA binding site in the intergenic region. A flagellum biosynthesis and export cluster (bottom panel) contains multiple CtrA binding sites. This region of genome encodes proteins found in the L-ring (FlgH; orange arrow), P-ring (FlgA; green arrow), rod (FlgG, FlgF, FliL, FlgB, FlgC, and FliE; pink arrows), export apparatus (FliP, FliQ, FliR, FlhB; yellow arrows), and C-ring (FliM; grey arrow). CtrA binding sites are indicated by closed black circles (exact match to consensus) and open black circles (one substitution). Purple arrows indicate genes encoding chemotaxis proteins whereas black arrows indicate genes encoding hypothetical proteins. C. Multiple putative CtrA binding sites are observed in the unipolar polysaccharide biosynthesis gene cluster. Colored arrows indicate genes with homology to the *upp* biosynthesis cluster in *A. tumefaciens* as described in the legend for Figure S1. Black genes indicate hypothetical proteins. Putative CtrA binding sites with one substitution are indicated by open black circles.

SUPPLEMENTARY MOVIE FIGURE LEGENDS

Movie S1. Cell growth of predivisional *P. hirschii* cell is observed on an agarose pad. Following cell division, the stationary mother cell releases a motile daughter cell.

Movie S2. *P. hirschii* cells form a stable biofilm within the microfluidic device. The biofilm remains surface associated during media perfusion.

Movie S3. *P. hirschii* cells observed in the analysis channel 5 minutes post-synchrony highly motile.

SUPPLEMENTARY REFERENCES

1. **Wang Q, Lei Y, Xu X, Wang G, Chen LL.** 2012. Theoretical prediction and experimental verification of protein-coding genes in plant pathogen genome *Agrobacterium tumefaciens* strain C58. PLoS One **7:**e43176.