Supplementary Material

Material and Methods

Phenotypic assays

Phenotypic assays were performed as previously described (Sokol et al., 1979;Vial et al., 2010;Chapalain et al., 2013). Protease activity was evaluated on BHI-milk agar (Sokol et al., 1979). Competition assays against human monocytes/macrophages THP-1 cell line were conducted as previously described (Vial et al., 2010) except that a MOI = 5 was used instead of 10. Competition assays in pea rhizosphere (*Pisum sativum*) were conducted as previously described (Vial et al., 2010) with the following modifications: roots were sampled 8 days after inoculation and bacteria were collected by 2 min vortexing. Cytotoxicity against human monocytes/macrophages THP-1 cells line was estimated using cells differentiated for 48h with Phorbol 12-myristate 13-acetate (PMA, Sigma). The supernatants were collected after 24h infection at a MOI = 1 and cytotoxicity was determined by measuring the lactate dehydrogenase (LDH) activity using the CytoTox 96® Non-Radioactive Cytotoxicity Assay kit (Promega). Values were calculated as % compared to a positive lysis control.

Phylogenetic analysis

The sequences of LuxR proteins with predicted autoinducer binding domain and LuxI proteins of *B. ambifaria* strain AMMD, were obtained from the InterPro database (entry IPR005143 and IPR001690 respectively). Sequences of highly similar *luxR* and *luxI* genes were obtained by using blast tool (Kent, 2002) and aligned against TraR (*A. tumefaciens* C58) and EsaI (*Pantoea stewartii*) with Muscle (Edgar, 2004). Detection of all key residues previously reported to be invariant in several functional LuxR proteins have been sought with respect to TraR of *Agrobacterium tumefaciens* (W57, Y61, D70, P71, W85, G113, E178, L182, G188) and LuxI proteins with respect to EsaI of *P. stewartii* (Arg24, Phe28, Trp34, Asp45, Asp48, Arg68, Glu97, Arg100) (Egland and Greenberg, 2001;Watson et al., 2002;Koch et al., 2005). Phylogenetic analyses were performed by using the Maximum Likelihood method based on the LG matrix-based model (Le and Gascuel, 2008), which generated trees with the highest log likelihood. Significance was estimated by using Bootstrap analysis.

Supplementary Table 1: Primers used in this study

Primers	Description	Reference
hmqAA-L	AAGAATTCATCCCGACTAGCTGGTGATG	This study
hmqAA-R	CGCGGATCCGTTCTTCGACTGCGGTATCC	This study
ndhF	GCGATCGGGCTGTACAAGTT	Subsin, 2007
ndhR	AGTGGCTCAGCGACTGGAA	Subsin, 2007
hmqARTF	GCCGCTACCTGAATGACACG	This study
hmqARTR	ACCGTGTAGCCGGAGCTGAT	This study
qBafIF	TTTCTCGCGAACACGTAGACGGTA	This study
qBafIR	GTGTATTCGTCGAACAGCTCGGTTG	This study
SLG_qRTcepI2_F	GACCGGACACGATCCATATC	This study
SLG_qRTcepI2_R	CCGAGGATGTAGGACGAAAA	This study
Bamb6053_02F	AAGCTTCGAAATCCAGCCATTCATGGC	This study
Bamb6053_02R	GTGCATGTGCGAACTCCAATG	This study
Bamb6053_03F	AGCTGAAATTGGGCCGGCTG	This study
Bamb6053_03R2	AAGCTTCGGTACGCGATGTAGTTCAG	This study
Bamb6053_Trim01F2	TCCTCCATTTCGCATTTAATGCGTACGTCCATTGGAGTTC GCACATGCACCCGAGCTCGAATTGGGGATCTT	This study
Bamb6053_Trim01R2	CGTCGCGCGGGACGGCATGCGTTGGGACCGCAGCCGGC CCAATTTCAGCTCGAGCTCGAATTAGCTTCAAA	This study
Bamb6040_02F_	AAGCTTGTCGACAACGCTCGACTACA	This study
Bamb6040_02R	TTTCGAATACATCGTCGAAGC	This study
Bamb6040_03F	ATTTCGGATCTCGAACTTGC	This study
Bamb6040_03R	AAGCTTACGTGCGTAATGGACTGTGA	This study
Bamb6040_01F2	CGTCATGACGACGTTGTCGCGCGCGCTGAGCTTCGACGA TGTATTCGAAACCGAGCTCGAATTGGGGATCTT	This study
Bamb6040_01R2	GCCAGATAGATCAGCCGGCTTTCCGTGGCGGCAAGTTCG AGATCCGAAATCGAGCTCGAATTAGCTTCAAA	This study

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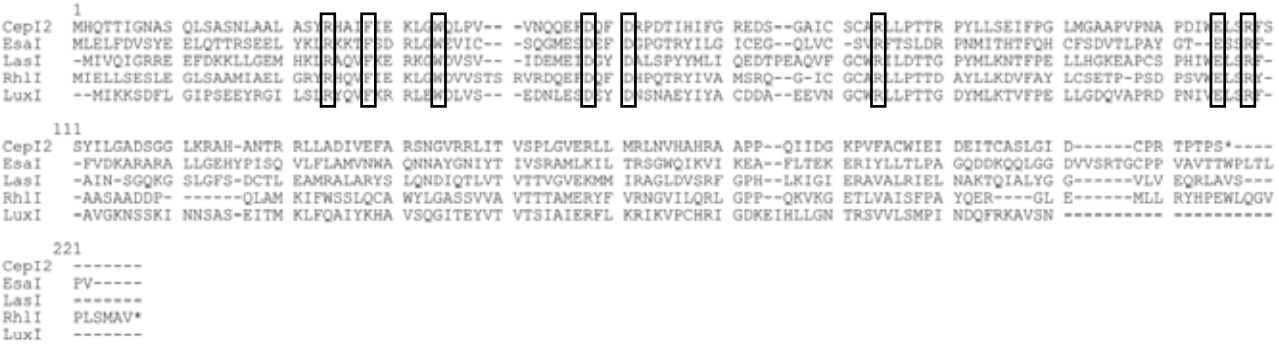
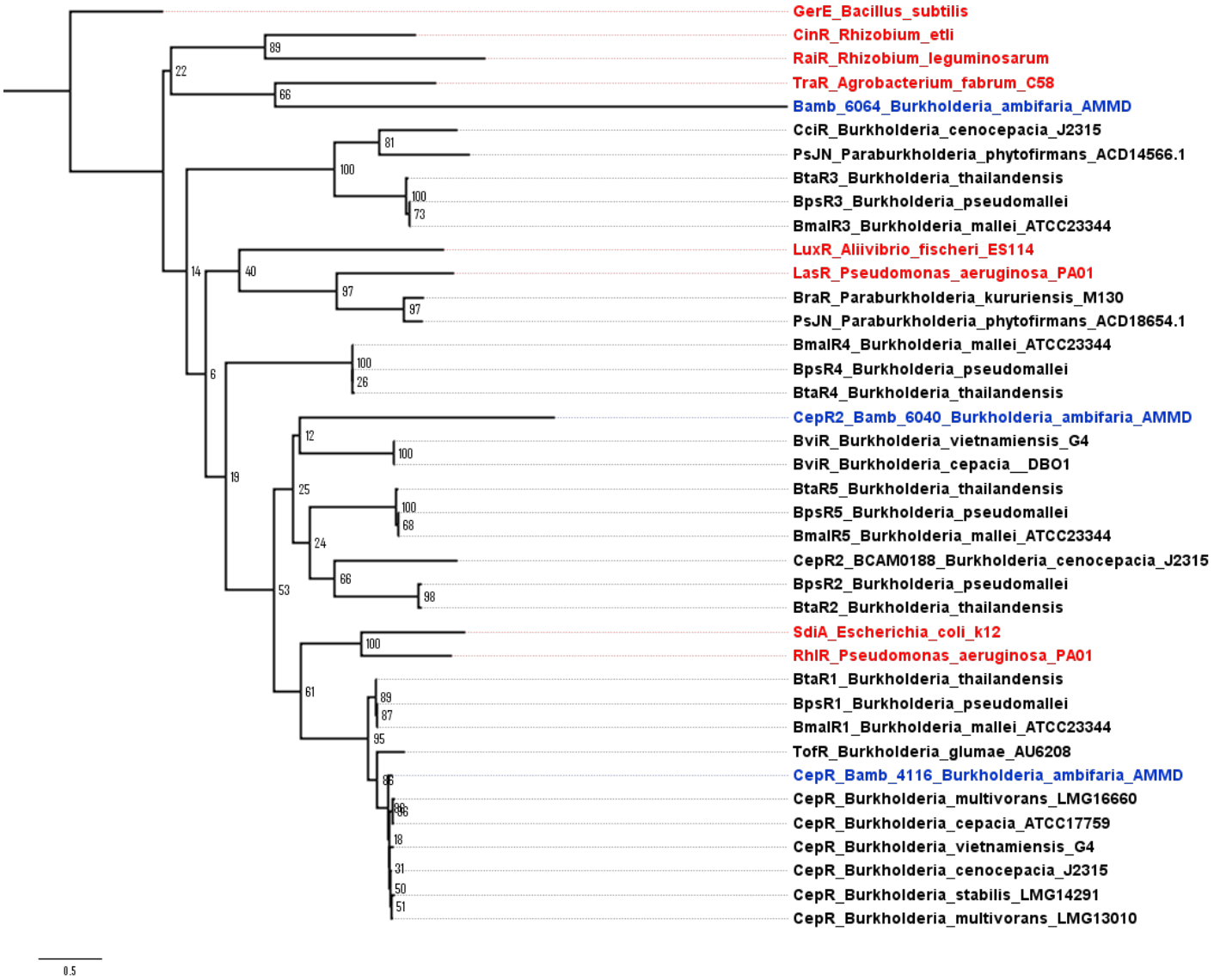


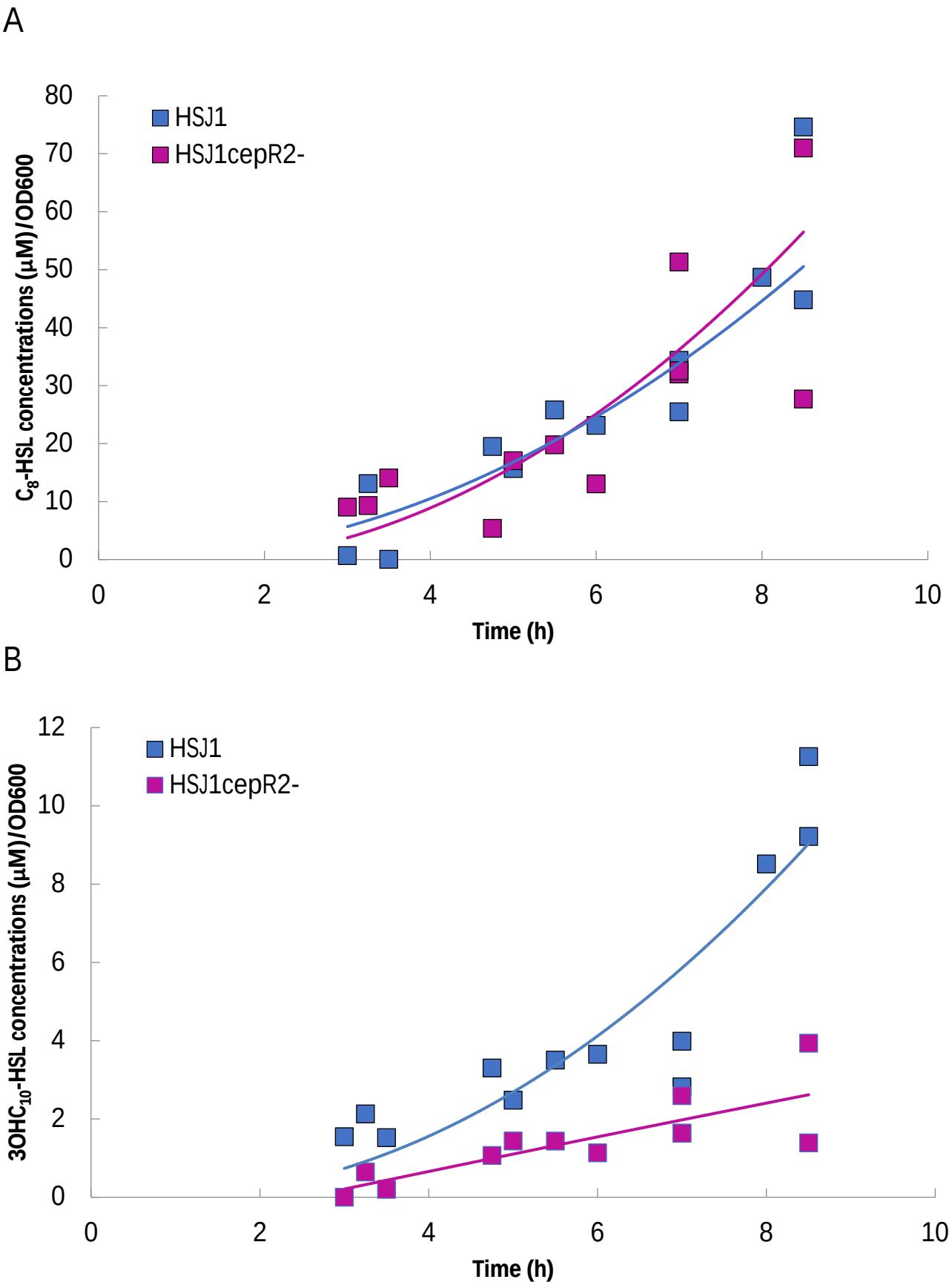
Supplementary Figure 1: Multiple-sequences alignment of CepI2 and selected AHL Synthases. EsaI (*Pantoea stewartii*), LasI and RhlI (*Pseudomonas aeruginosa* PAO1), LuxI (*Aliivibrio fischeri*) were used to align CepI2 (Bamb_6053) from *B. ambifaria* AMMD. Conserved residues are boxed in **black**, using EsaI as a reference (Arg24, Phe28, Trp34, Asp45, Asp48, Arg68, Glu97, Arg100).

Supplementary Figure 2: Multiple-sequence alignment of CepR2 and selected LuxR. TraR (*Agrobacterium tumefaciens* C58), LuxR (*Aliivibrio fischeri*), LasR and RhlR (*Pseudomonas aeruginosa* PAO1), CinR (*Rhizobium etli*) were used to align CepR2 (Bamb_6040) and Bamb_6064 from *B. ambifaria* AMMD. Conserved residues are boxed, in **black** residues needed for AHL fixation (W57, Y61, D70, P71, W85, G113) and in **red** residues needed for DNA-binding (E178, L182, G188 with respect to TraR).

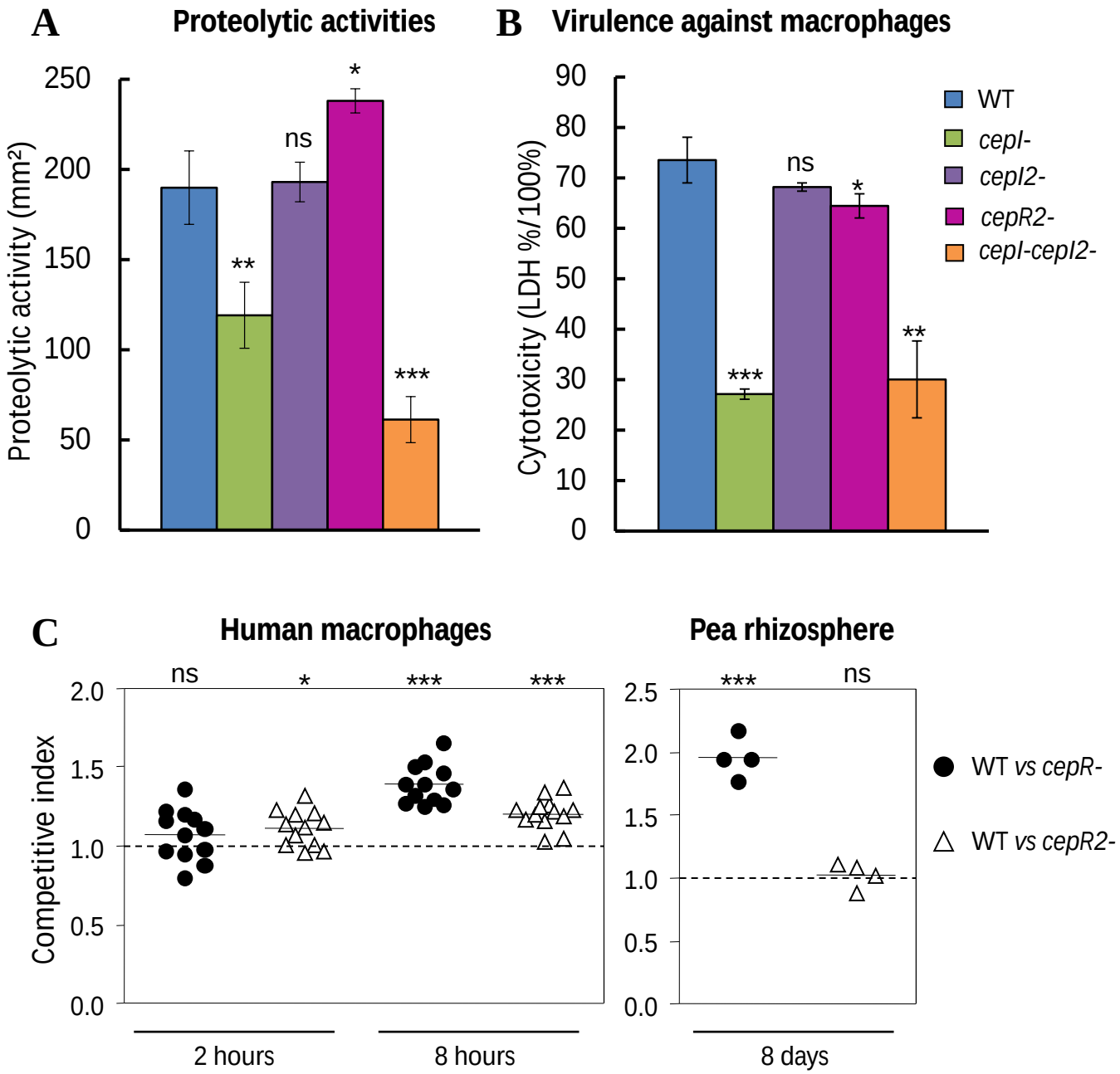


Supplementary Figure 3 : Phylogenetic tree of described *Burkholderia* LuxR

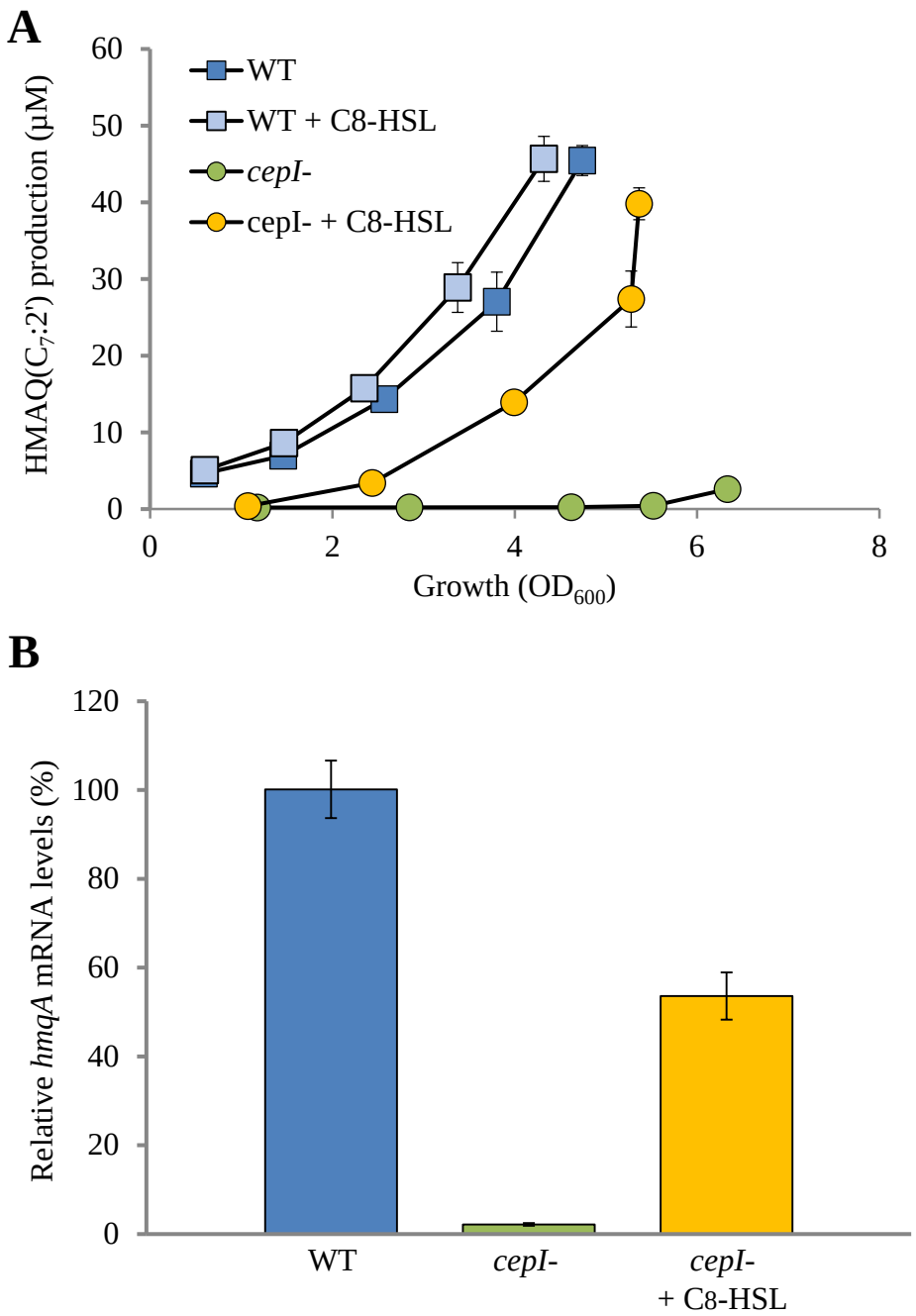
Names in **red** correspond to various LuxR, used as reference (CinR from *Rhizobium etli*, RaiR from *Rhizobium leguminosarum*, TraR from *Agrobacterium tumefaciens* C58, LuxR from *Aliivibrio fischeri*, LasR and RhlR from *Pseudomonas aeruginosa*, SdiA from *Escherichia coli* K12). In **black**, published and described LuxR of *Burkholderia* genus. In **blue**, *Burkholderia ambifaria* AMMD LuxR. The tree was inferred by using the Maximum Likelihood method and node labels correspond to boostrap values, and tree is rooted by GerE (*Bacillus subtilis*).



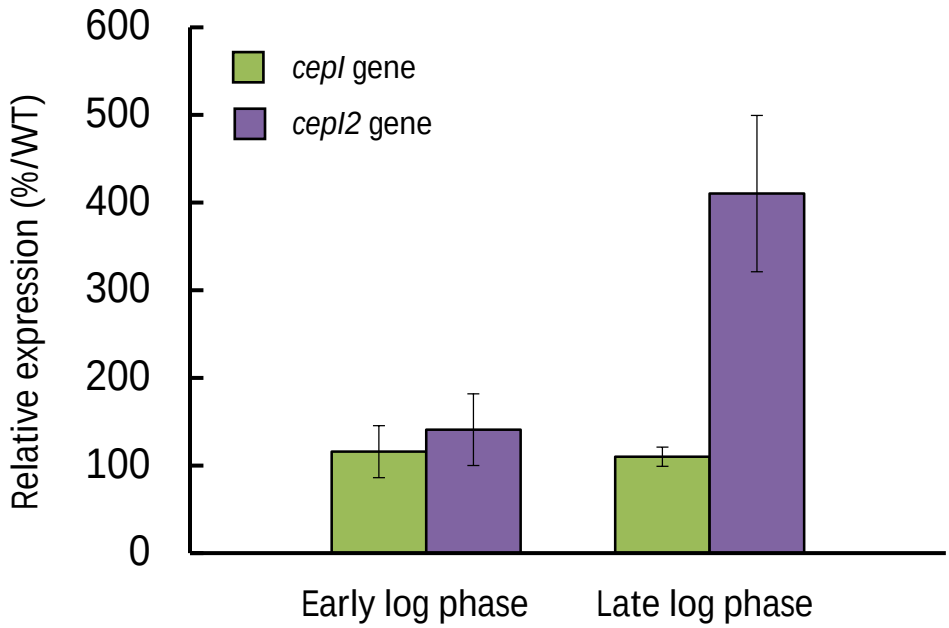
Supplementary Figure 4 : C_8-HSL (A) and $3OHC_{10}$-HSL (B) production in HSJ1 *cepR2*- and HSJ1 WT. Each point represents an extraction performed on one culture at a given time point.



Supplementary Figure 5 : Phenotypic characterization of quorum sensing mutants in *B. ambifaria* HSJ1. (A) Proteolytic activity was estimated by measuring the degradation halo on milk agar plate after 24 h. **(B)** Virulence against human monocytes/macrophages THP1 cell line was estimated by measuring LDH activity in supernatants after 24h post infection at a MOI = 1. Results are expressed as percentage compared to a positive control (100% lysis). Data are expressed as means of at least three values +/- SD. **(C)** Competitive index (CI) analyses of *B. ambifaria* WT, *cepR*- and *cepR2*- mutants in human monocytes/macrophages THP1 cell line and in pea rhizosphere. CI for the entry in macrophages is defined as the ratio between the two strains in the input (intracellular bacteria after 2h treatment with antibiotics) divided by their ratio in the inoculum (t = 0h). CI for the proliferation in macrophages is defined as the ratio between the two strains in the output (intracellular bacteria after 8h treatment with antibiotics) divided by their ratio at the input (t = 2h). CI for colonization of the pea rhizosphere is defined as the CFU output (day 8 after inoculation) ratio of the two strains divided by the CFU input (inoculum) ratio. The wild-type is more competitive than *cepR*- or *cepR2*- when the CI is greater than 1. The bars represent the median value for each group. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, non-significant.



Supplementary Figure 6: Impact of C_8-HSL supplementation on HMAQ production and on *hmqA* gene expression in *B. ambifaria* HSJ1 WT and *cepI*- mutant strains. WT and *cepI*- were analyzed for **(A)** HMAQ-C_7:2' production using LC/MS, in presence or absence of exogenously added C_8-HSL. Values are presented as relative concentration compared to the internal standard HHQ-d4. Results are expressed as means ± SD of three replicates. **(B)** Relative expression of the *hmqA* gene was determined by quantitative reverse transcription PCR (qRT-PCR) in HSJ1 WT, *cepI*- mutant and *cepI*- mutant supplemented with C_8-HSL. The results are presented as relative quantification of gene expression compared to the WT normalized to 100%. The results are expressed as means ± SD of three replicates.



Supplementary Figure 7: Expression of *cepI* and *cepI2* genes in the *hmqA*- mutant at the early and late log phase.

Relative expression of *cepI* and *cepI2* genes in *hmqA*- mutant compared to the WT at the beginning and the end of log phase in TSB liquid cultures. Values are expressed as means ± SD of three replicates.