

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	TCCTCCATTTTCGCATTTAATGCGTACGTCCATTGGAGTTC GCACATGCACCCGAGCTCGAATTGGGGATCTT	This study
Bamb6053_Trim01R2	CGTCGCGCGGGACGGCATGCGTTGGGACCGCAGCCGGC CCAATTTTCAGCTCGAGCTCGAATTAGCTTCAAA	This study
Bamb6040_02F_	AAGCTTGTCGACAACGCTCGACTACA	This study
Bamb6040_02R	TTTCGAATACATCGTCGAAGC	This study
Bamb6040_03F	ATTTCCGATCTCGAACTTGC	This study
Bamb6040_03R	AAGCTTACGTGCGTAATGGACTGTGA	This study
Bamb6040_01F2	CGTCATGACGACGTTGTGTCGCGCGCGCTGAGCTTCGACGA TGTATTCGAAACCGAGCTCGAATTGGGGATCTT	This study
Bamb6040_01R2	GCCAGATAGATCAGCCGGCTTTCCGTGGCGGCAAGTTCG AGATCCGAAATCGAGCTCGAATTAGCTTCAAA	This study

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1
CepI2 MHOTTIGNAS QLSASNLAL ASYRAIAE E KLGWDLPV-- -VNOQEDDF E RPDTHIFG REDS--GAIC SCARALPTTR FYLLSEIFPG LMGAAPVNA PDWELSRFS
EsaI MLELFDVSYE ELQTRSEEL YKIRKTSFSD RLQWVVIC-- -SQGMESDF E GPGTRYILG ICEG--QLVC -SVRFTSLDR PNMIHTFOH CFSVDLTPAY GT--ESSRF-
LasI -MIVQIGRRE EFDKRLGEM HKIRKQVFE E RKGWVSV-- -IDEMEDDY E DALSPYMLI QEDTPEAVF GCMRFLDTTG FYLLKNTFPE LLHGKEAPCS PHWELSRF-
RhII MIELLESIE GLSAAMIAEL GRVIRKQVFE E KLGWVSVTS VRVDQEDDF E HPQTRYIVA MSRQ--G-IC GCMRFLPTTD AYLLKDVFA LCGETP-PSD PSWELSRF-
LuxI --MIKKSDFL GIPSEYRGI LSIKQVFE E RLEWDLVS-- -EDNLESEDF E HNSAEYIYA CDDA--EEVN GCMRFLPTTG DYMLKTVFPE LLGDQVAPRD FNWELSRF-

111
CepI2 SYILGADSGG LKRAH-ANTR RLLADIVEFA RSNVRLIT VSPLGVERLL MRLNVHAHRA APP--QIIDG KPVFACMIEI DEITCASLGI D-----CPR TPTPS*----
EsaI -FVDKARARA LLGHPYISQ VLFAMVNA QNNAYNGIYT IVSRAMKIL TRSQWQIKVI KEA--FLTEK ERIYLLTLP A GDDDKQQLGG DVVSRTEGCP VAVTHTPLTL
LasI -AIN-SQKQG SLGFS-DCTL EAMRALARYS LQNDIQTLVT VTTVGVEKMM IRAGLDVSRF GPH--LKIGI ERAVALRIEL NAKTQIALYG G-----VLV EQRLAVS---
RhII -AASADDF- ----QLAM KIFWSSLQCA WYLGASSVVA VTTTAMERYF VRNGVILQRL GFP--QKVG ETLVAISFPA YQER---GL E-----MLL RYHPEWLQGV
LuxI -AVGKNSSKI NNSAS-EITM KLFQAIYKHA VSQGITEYVT VTSIAIERFL KRKIVPCHRI GDKEIHLLGN TRSVVLSMPI NDQFRKAVSN -----

221
CepI2 -----
EsaI PV-----
LasI -----
RhII PLSMAV*
LuxI -----

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Supplementary Figure 1: Multiple-sequences alignment of CepI2 and selected AHL Synthases. EsaI (*Pantoea stewartii*), LasI and RhII (*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).

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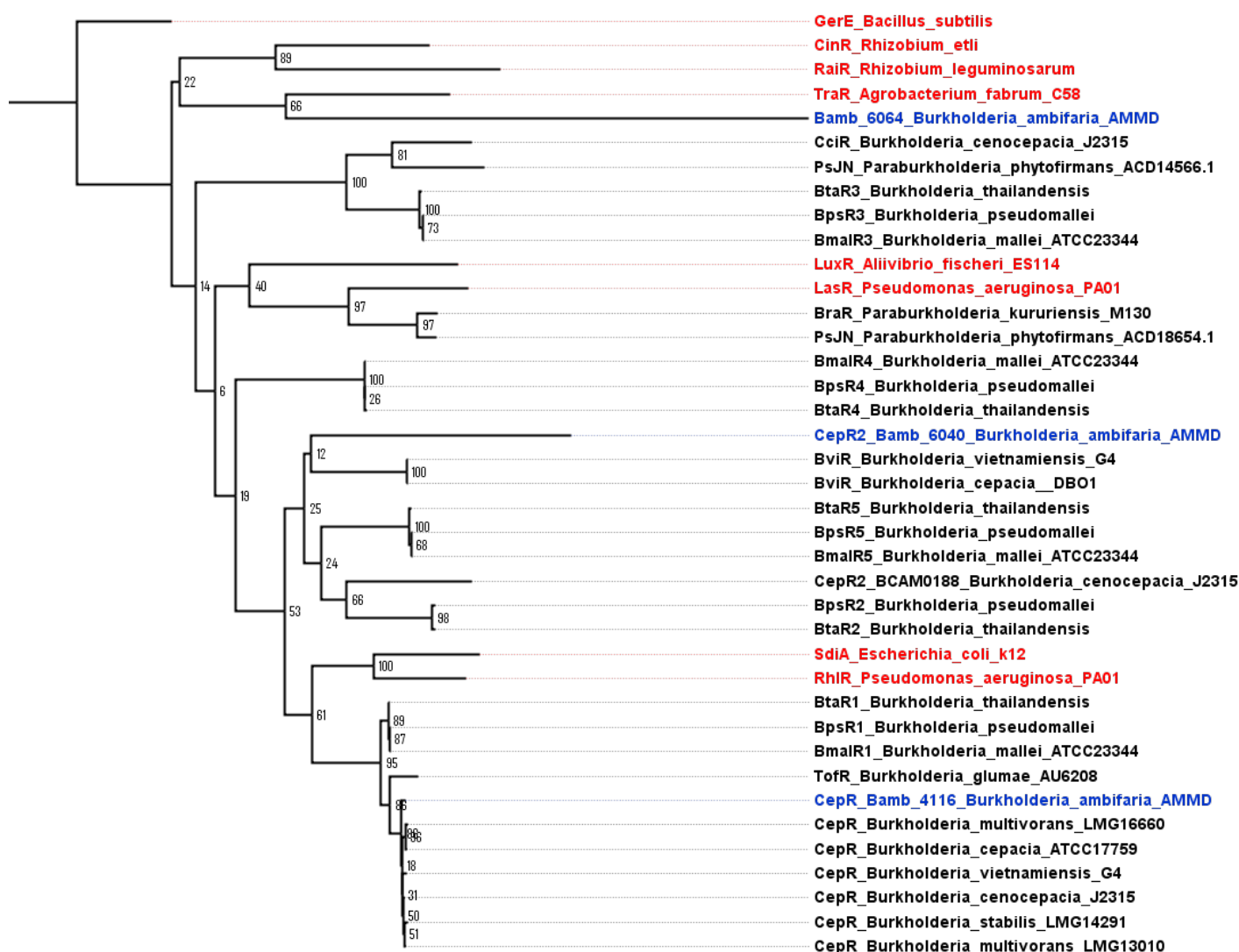
1
CepR2 ----- MTT LSRALSFDVV FEI----- VAAQARA LD-----FEM CAYGAR---D PLPVTPTFR ---TRNTYPE SQQ-----
Bamb_6064 ----- MSAALSIP DLPLSHVAVF TDT----- LGDIAQA IG--TPQFMR AVYDTLVRYV DFDVHLDYE RSAASGRRSV QWEGSPGREP ELVAQVMBY
TraR ----- MQHWLQKLDL LAALQGDCEI LKD----- GLADLAEH FG-----FTG YAYLHI ----- QHK HTIAVTNHYR DRS-----
LuxR ----- MNIRN INANEKIIDK IKTCNNNKDI NQC----- LSEIAKI IH-----CEY YLFAII ---Y PHSIIKPDVS ---IIDNYPE NPK-----
LasR ----- MALVDGFLE LERSSGKLEW SAI----- LQKMSAD LG-----FSK ILFGLL ----- PKDSQDYE NAFIVGNYP AWE-----
RhIR ----- MRNDGGF LLWWDGLRSE MQPIHDSQGV FAV----- LEKEVRR LG-----FDY YAYGVR--H TIPPTRPKTE ---VHGTYPK AWE-----
RaiR MLFSATAKCY HCEQSGKHAT TSPSHAEQFS FFLQFGPNFR IADIAGSRND AGRSPPHLCF IAHGSA--C D-PLSGATAS NPLMLLTYPP AWK-----
CinR ----- MIENTY SDKFEPAFEQ IKAAPNVDA A IRI----- LQAE YG-----LDF VTYHLA ---QTIASKID SPFVRTTYPD AWV-----

111
CepR2 EGNYPARD P VRYGLIYT SPLVWVVD-- ---ACADES FWEAKAH-- ---GA-H GWSQPIRDKH G-RIAMNSVA RSADPIS-DL ELAATESRLI YLAHAHAVN
Bamb_6064 YRSYASBET P VAIDSEDT QLLQVBSQ-- ---RVASELRH LFFDAGDIHD ECVIAGT-G GTRYISIIAR SRRLPPPSLK ELSLLKQLSQ VVLPMASANK RLLGAVCADE
TraR ENNFDKLD P VVKRAKSRK HVPMBGGEQE RSRLSKEERA FYAHAADF-- ---GR-S GITIPIKTAN G-SMSMFTLA SERPAIDLDR EIDAAAAA-- ---GAVGQLH
LuxR DAGLLEYD P FVDYSKSHH SPINWVFEK K-TIKKESPN VIKEAQES-- ---GLI-T GFSPPHHTAS N-GFGMLSPA HSDKDIYTDS LFLHASTNVP LM---LPSLV
LasR RAGYARVD P FVSHCTQSV LPIDHPEPSYI Q---TRKQHE FFEASAA-- ---GLV-Y GLTMPLHGAR G-ELGALSLS VEAENRAEAN RFMESVLP TL WMLKDYALQS
RhIR MQNYGAVD P PAILNLGRSS EMVWBDLSLF D-----QSRM LWNEARDN-- ---GLC-V GATLPIRAPN N-LLSVLSVA RDQONIS-SF EREEIRLRLR CM---YELLT
RaiR ERDYFSD P FVRLGRGRGF LPVMBGASKM D-----SQAYG FFKAMAF-- ---SGRQ GVTLPVGRPH G-ERSLFTVT SNHPDAYWRQ FRMDSMRDLQ FL---AHLHL
CinR LNSYVKV P FVKQGFERQ LPPFDVFEV P ---TFEAYA MLVDAQKH-- ---LGGN GYSIPVADKA Q-RRALLSMN ARIPADHWAE LVRRRCRNEWI EI---AHLIH

221
CepR2 SRLVDAPAAA MQMGP----- FTTRKEV P KWAADKNTAL EIASILSISE RTITFHMQNI MEKLSNVNKT QAIKAVLLG IYI-----
Bamb_6064 GPRDELDDR VAQWLPQLQE RLTARESHVC ASFIQMTSA AIAQSMGLKT STVDTYAKRA FAKLGVDSRR QLMALVLRNA SRRHDA----
TraR ARISFLQTTT TVEDAAM----- LDPRRATY P RWIAMSMTME EVAADVGVKY NSVRVKLREA MKRFDVRSKA HLTALAIRRK LI-----
LuxR DNYQKINTTR KKSDSI----- LTKRKECE P AWASEKSTW DISKILGCSE RTVTPHLNT QMKLNTNRC QSISKALTG AINCPLYLN
LasR GAGLAFEPH-- -VSKPVV----- LTSREKEV P QWCAISMTSW EISVICNCE ANVNFHMGN I RKKFGVTSRR VAAIMAVNLG LITL-----
RhIR QKLTDLHPM LMSNPVC----- LSHREKEV P QWTAKKSSG EIAIILSISE STVNFHKN I QKKFDAPNKT LAAAYAAALG LI-----
RaiR DRAMVLSGMR KITDFPQ----- LSRREKEV P EMTANSLAK QICARLSISV SAVQLYLASA RRKLTVATTS EAVAKATALE LI-----
CinR QKAVYELYGE NDPVPA----- LSPREKEV P HWTALREYK DISVLGISE HTTRDYLKTA RFKLGCATIS AAASRAVQLR IINP-----

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Supplementary Figure 2: Multiple-sequence alignment of CepR2 and selected LuxR. TraR (*Agrobacterium tumefaciens* C58), LuxR (*Aliivibrio fischeri*), LasR and RhIR (*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).

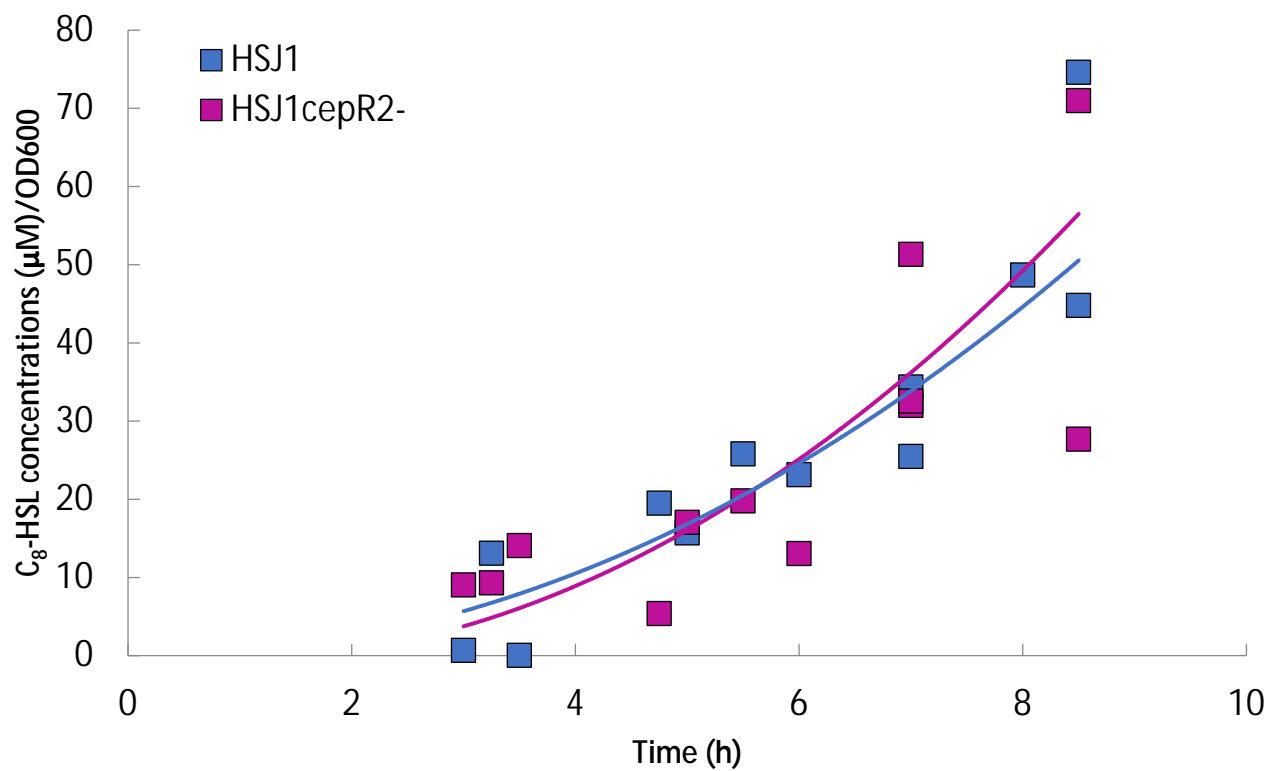


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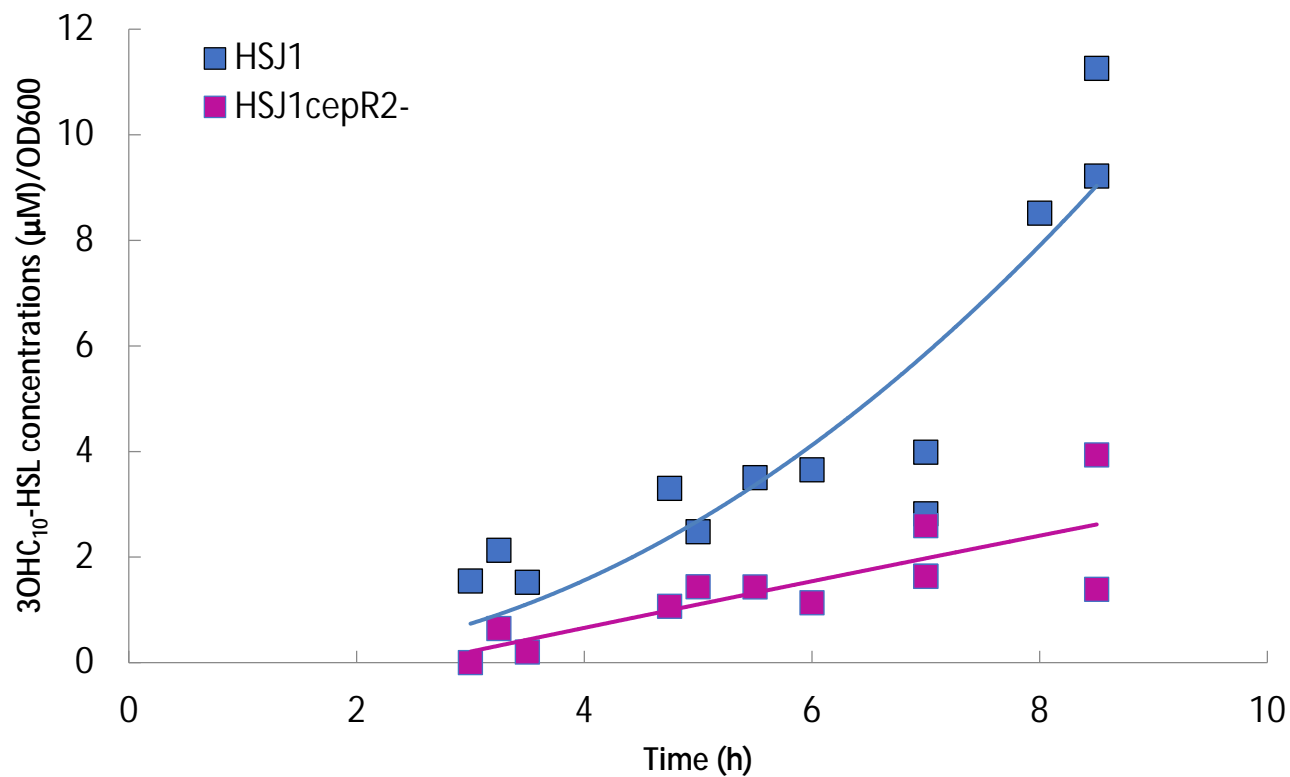
Supplementary Figure 3 : Phylogenetic tree of described *Burkholderia* LuxR

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 bootstrap values, and tree is rooted by GerE (*Bacillus subtilis*).

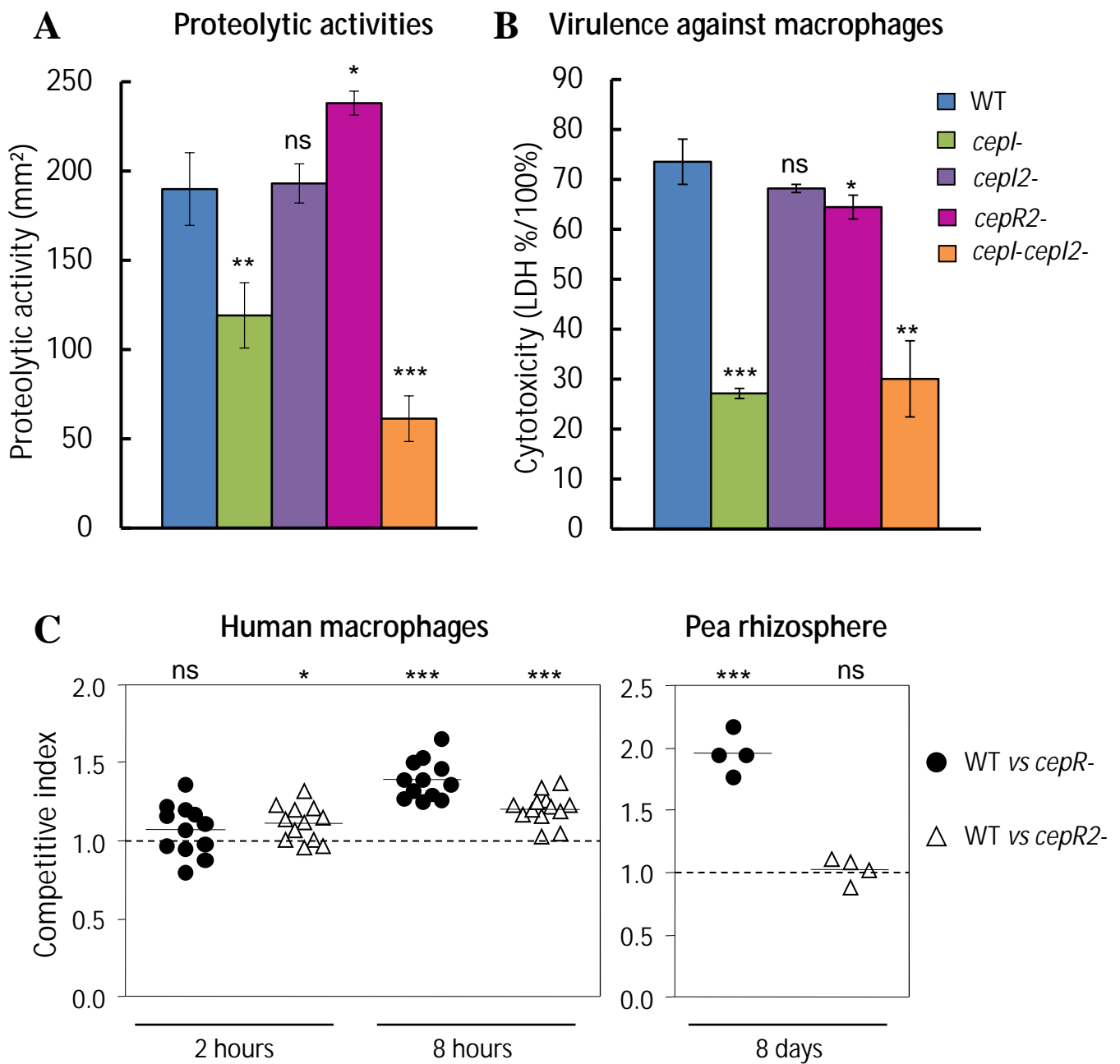
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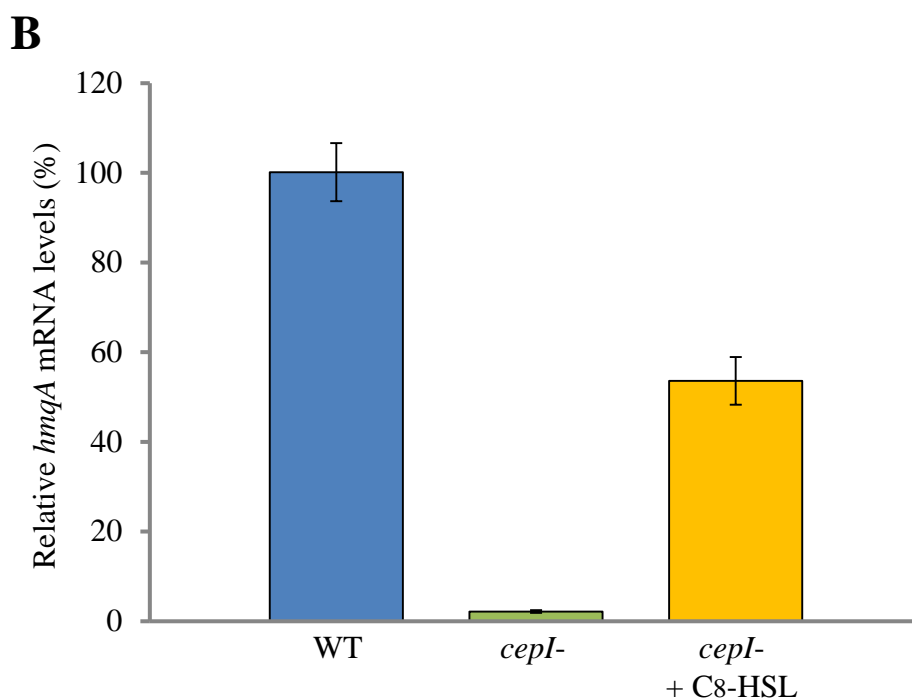
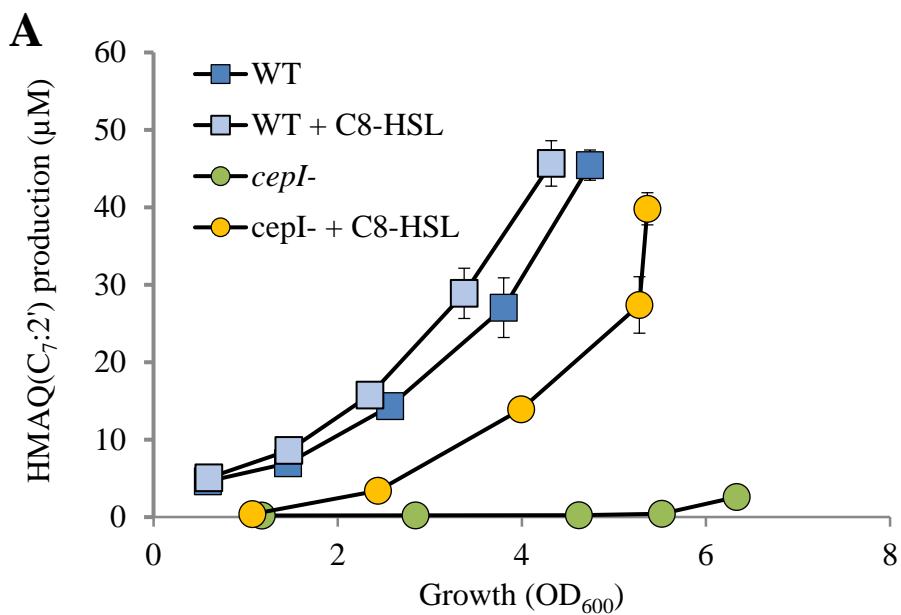
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Supplementary Figure 4 : C₈-HSL (A) and 3OHC₁₀-HSL (B) production in HSJ1 *cepR2*- and HSJ1 WT. Each point represents an extraction performed on one culture at a given time point.

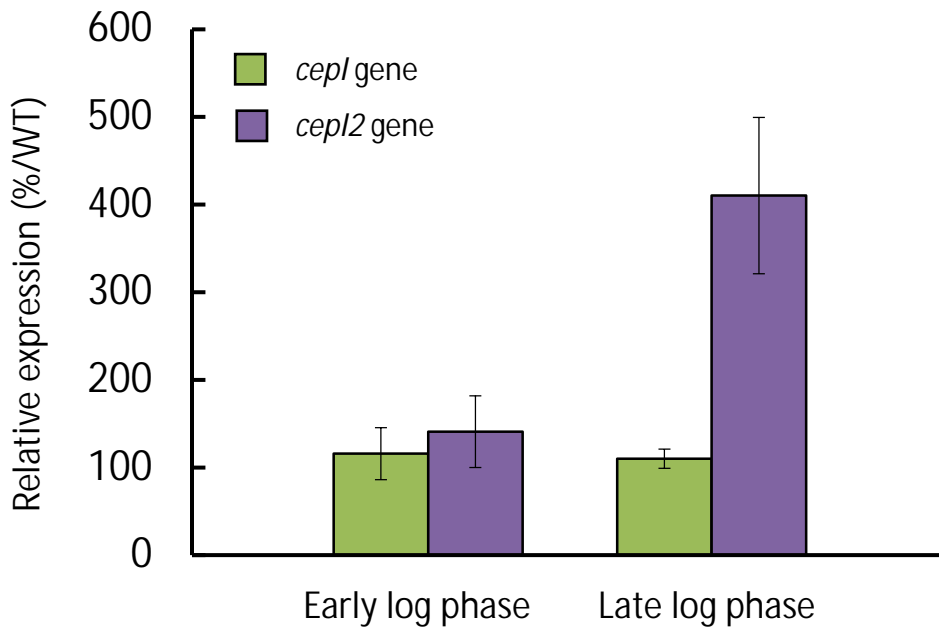


Supplementary Figure 5 : Phenotypic characterization of quorum sensing mutants in *B. ambifaria* HSJ1. (A) Proteolytic activity was estimated by measuring the degradation halo on milk agar plate after 24 h. (B) Virulence against human monocytes/macrophages THP1 cell line was estimated by measuring LDH 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 C₈-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-C₇:2' production using LC/MS, in presence or absence of exogenously added C₈-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 C₈-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 \pm SD of three replicates.