Prolonged culture and long lasting infections select for poorly transmitted bacterial variants— Supplementary materials S3

November 30, 2018

Integration of $P_{lac} - RBS - lrp$ in the chromosome of V2#25 and full phenotype restoration

1 Materials and methods

First, an *lrp* PCR fragment (including its own RBS but not promoter) is generated using primers L-PstI-RBS-lrp and R-BamHI-lrp and genomic DNA of *X. nematophila* F1 wild type as template. This amplicon was digested with PstI and BamHI and finally cloned under the control of P_{lac} promoter of the pBBR1-MCS1 vector (Kovach et al., 1994) by digestion with PstI and BamHI generating the pBB-P_{lac}-RBS-*lrp* plasmid. After digestion of pJQ200SK suicide vector with SalI and SpeI, three DNA fragments (i) PCR amplicon of glmS

from F1 (SpeI-AatII digested) (ii) PCR amplicon of the ATPase site from F1 (SacI –SalI digested) and (iii) the AatII- SacI fragment of the pBB-P_{lac}-RBS-lrp plasmid containing Cm-P_{lac}-RBS-lrp were cloned generating the pJQ-glmS-Cm-P_{lac}-RBS-lrp-ATPase. After transferring this construction by mating experiment in V2#25 as previously described (Givaudan and Lanois, 2000) using *E. coli* WM3064 as donor strain, the Cm-P_{lac}-RBS-lrp is transferred on V2#25 chromosome at the *glmS* site using allelic exchange and sucrose-resistant selection as previously described (Givaudan and Lanois, 2000). The obtained clones were then checked for GmS, SacR, CamR and by phenotypic tests. Sequence controls were performed on PCR using primers L-GlmS and R-ATPase and genomic DNA of the exconjugants as templates and they confirmed the insertion of the Cm-P_{lac}-RBS-lrp at the glmS site in the chromosome of the obtained clones. Phenotypic assays (haemolysin activity towards sheep blood, lecithinase activity, swimming motility, antibiotic production and dye binding assay) were performed as previously described (Givaudan et al., 1995; Thaler et al., 1998).

Primer name	Primer sequence	Use
L-PstI-RBS-lrp	cgctgcaGGGAAAATGTTATGGGTGTAGG	cloning of *lrp* gene from *X. nematophila*
R-BamHI-lrp	cgggatccTTAACGAGTCTTAATCACCAGACG	F1 wild type with its own RBS
L-GlmS-SpeI	GGACTAGTCCTTCACGACCCAGCTAACA	cloning of *glmS* site from *X. nematophila* F1 wild type
R-GlmS-AatII	GGCGACGTCAACCTTATTCCACCGTCACCG	
L-ATPase-SacI	GGCGAGCTCCAATGTGATTGATTGATTTTTAATC	cloning of *ATPase* site from *X. nematophila* F1 wild type
R-ATPase-SalI	GCAGTCGACAATGAAACGCCCTGATGTTC	
L-GlmS	ACGTTGTGGGTTCCTCTCTG	checking chromosomal insertion at *glmS* site
R-ATPase	TTTCTTGTTCAGACAAGGGTTG	

Table S2-1: Primers used in this study

2 Results

As shown in Table S2-2, the insertion of the Cm-P_{lac}-RBS-lrp at the *glmS* site in the chromosome of V2#25 strain belonging to the group 2 allowed a full restoration of all phenotypes (dye binding, motility, lecithinase et haemolysis activities) to achieve phenotypic pattern similar to the group 1 variant represented by V1#23.

3 References

Kovach ME, Phillips RW, Elzer PH, Roop II R.M, Peterson KM (1994). pBBR1MCS: a broad-host-range cloning vector. BioTechniques 16: 800-802

Givaudan A, Lanois A (2000). flhDC, the flagellar master operon of *Xenorhabdus nematophilus*: requirement for motility, lipolysis, extracellular hemolysis, and full virulence in insects. J Bacteriol 182: 107–115

Givaudan A, Baghdiguian S, Lanois A, Boemare N (1995). Swarming and swimming changes concomitant with phase variation in *Xenorhabdus nematophilus*. Appl Environ Microbiol 61:1408-1413

Thaler JO, Duvic B, Givaudan A, Boemare N (1998). Isolation and entomotoxic properties of the *Xenorhabdus nematophilus* F1 lecithinase. Appl Environ Microbiol 64: 2367–2373

Strains[a]	Dye binding [b]	Motility [c]	Antibiotic production [d]	Lecithinase [e]	Haemolysis activity [f]
V1#23	Blue	60	55	++	T
V2#25	Red	18	35	-	w
V2#25 glmS::P_{lac}-RBS-lrp	Blue	50	40	++	T

Table S2-2: Phenotypic assays in variants and lrp-complemented variants.

[a] All strains were cultured for 2 days at 28°Cbefore assays were interpreted unless indicated otherwise. Experiments were done 3 times, only one is shown

[b] Bromothymol blue

[c] Motility in swim agar (LB medium, 0.35% agar) plates; numbers are halo sizes (mm)

[d] Zone sizes (mm) of growth inhibition of *Micrococcus luteus*

[e] Activity measured as the production of a white halo of precipitation on plate with medium containing 0.01% lecithin; ++, halo up to 10 mm in diameter; - no precipitation observed

[f] T, total haemolysis; W weak haemolysis