

Supplementary materials

Applied Microbiology and Biotechnology

Surface proteins involved in the adhesion of *Streptococcus salivarius* to human intestinal epithelial cells

Fanny Chaffanel¹, Florence Charron-Bourgoin¹, Claire Soligot², Mounira Kebouchi², Stéphane Bertin¹, Sophie Payot¹, Yves Le Roux², Nathalie Leblond-Bourget¹ #

¹ DynAMic, Université de Lorraine, INRA, 54506, Vandoeuvre-lès-Nancy, France

² UR AFPA, équipe PB2P, Université de Lorraine, INRA, 54506, Vandoeuvre-lès-Nancy, France

Corresponding author

Tel: +33 3 72 74 51 46

Fax: +33 3 72 74 53 56

E-mail address: nathalie.leblond@univ-lorraine.fr

Table S1: Primers used for the construction of *S. salivarius* F6-1 mutants.

^a primers used for amplification of the spectinomycin resistance gene of the pSL1180 spec lox plasmid

^b primers used for amplification of the *SpeI-SpeI* spectinomycin resistance cassette from the pSET4s plasmid

Fig. S1: Strategy of *S. salivarius* F6-1 mutants construction by allelic replacement.

The spectinomycin (*spec*) or erythromycin (*ery*) resistance genes were amplified from plasmids by PCR using specific primers represented by black arrows. In parallel, two DNA fragments overlapping the upstream (1) and downstream (2) regions of the chromosomal target gene were amplified by PCR using specific primers that present an extended sequence matching with the 3' and 5' ends of the resistance gene. A second PCR amplification was carried out using these two PCR fragments and the resistance gene as template to synthesize an overlap PCR product carrying *spec* or *ery* flanked by the chromosomal regions 1 and 2. This final PCR product was used to transform the *S. salivarius* F6-1 strain. The crossover events represented by crosses, upstream and downstream from the target gene, were positively selected by the newly acquired antibiotic resistance (Spec^R or Ery^R) of the transformed clones.

Table S1: Primers used for the construction of *S. salivarius* F6-1 mutants

Primers	Nucleotide sequences (5'-3')	Product size (bp)
F6-1 sortase_1	GCATGTTTCGATACCAAGTG	975
F6-1 sortase_2	TAAGAAAGGGGCCCTCGG	
F6-1 sortase_3	TAGTTGTCACCACTTTCTTGA	1027
F6-1 sortase_4	GCACAGAGCTTATGGTCG	
gyr-eryF	TCAAGAAAGTGGTGACAATAATGAACAAAAATATAAA ATATTCTCA	774
eryRlyase	CCGAGGGCCCCCTTTCTTATTTCTCCCGTTAAATAATAG	
F6-1_SALIVA_0442.1	ACAGCTGGAGTAAATCAAGG	1000
F6-1_SALIVA_0442.2	TTGGGAAATATTCATTCTAATTGGGGCTGTTTGATAAAA ATTGG	
F6-1_SALIVA_0442.3	CCAGTCACGTTACGATAAATTGCGGACGTTATGTAGCTG TG	1016
F6-1_SALIVA_0442.4	TCTACATAGCGTCTTGAC	
SALIVA_0576.1	TTCGGAAGTTAAGGTTACCA	1038
SALIVA_0576.2	TATTTAATATTTGGGAAATATTCATTCTAATTGGGGTTT CCCCTAAGTCAACAG	
SALIVA_0576.3	AATTTAGTTTATTTATAGATTTTATTGGCTTCTAGCTGCT GTTTCTATCACGGT	1038
SALIVA_0576.4	GCGACGATGGCTATATGCT	
SALIVA_1457.1	GAATCCGTCTGACTTACTGAT	996
SALIVA_1457.2	TATTTAATATTTGGGAAATATTCATTCTAATTGGCCAAG CGCTTCCTAAGACT	
SALIVA_1457.3	AATTTAGTTTATTTATAGATTTTATTGGCTTCTACCCGTC ATTTGTCACTTTCA	1020
SALIVA_1457.4	GAACTGATCGCGTGACTCG	
SALIVA_1472.1	CTGTTACTGGCAATTGTTTAT	1017
SALIVA_1472.2	TATTTAATATTTGGGAAATATTCATTCTAATTGGAACCA GGAACACCAATCTA	
SALIVA_1472.3	AATTTAGTTTATTTATAGATTTTATTGGCTTCTACCACTT GTTTCATCACGGTA	1274
SALIVA_1472.4	GAACTTCCACCAGTACCAGC	
F6-1 SALIVA_1473.1	CTTCGGCTACTGCTGTTGAG	996
F6-1 SALIVA_1473.2	CCAGTCACGTTACGATAAATTGCGGTGTCAACAAGCCAT ACG	
F6-1 SALIVA_1473.3	TTGGGAAATATTCATTCTAATTGGGGAGCACAGCCATA GTCG	1013
F6-1 SALIVA_1473.4	GAGGAGATTTTACGGGAGCG	
SALIVA_1475.1	TCAGCTGAATCGTCGTCATC	1071
SALIVA_1475.2	TATTTAATATTTGGGAAATATTCATTCTAATTGGCGTTCT TAGCGATGGTAACG	
SALIVA_1475.3	AATTTAGTTTATTTATAGATTTTATTGGCTTCTACAACG ATTGCTTTAGCTGT	1051
SALIVA_1475.4	ACAGTTGAAGTTGCTAAGAA	
Spec Fwd ^a	TAGAAGCCAATGAAATCTAT	936
Spec Fwd_bis ^a	CGAAGTTATCGTAACGTGAC	1083
SpecRev ^a	CCAATTAGAATGAATATTTCCC	
Spec-lox71-SpeI F ^b	TTTTTACTAGTTCGTACCGTTCGTATAGCATACATTATAC GAAGTTATCGTAACGTGACTGGCAAGA	1169
Spec-lox66-SpeI R ^b	TTTTTACTAGTTCGTACCGTTCGTATAATGTATGCTATAC GAAGTTATCCAATTAGAATGAATATTTCCC	

^a primers used for amplification of the spectinomycin resistance gene of the pSL1180 spec lox plasmid

^b primers used for amplification of the *SpeI-SpeI* spectinomycin resistance cassette from the pSET4s plasmid

Fig. S1

