

1 **A putative type V pilus contributes to *Bacteroides thetaiotaomicron* biofilm**
2 **formation capacity**

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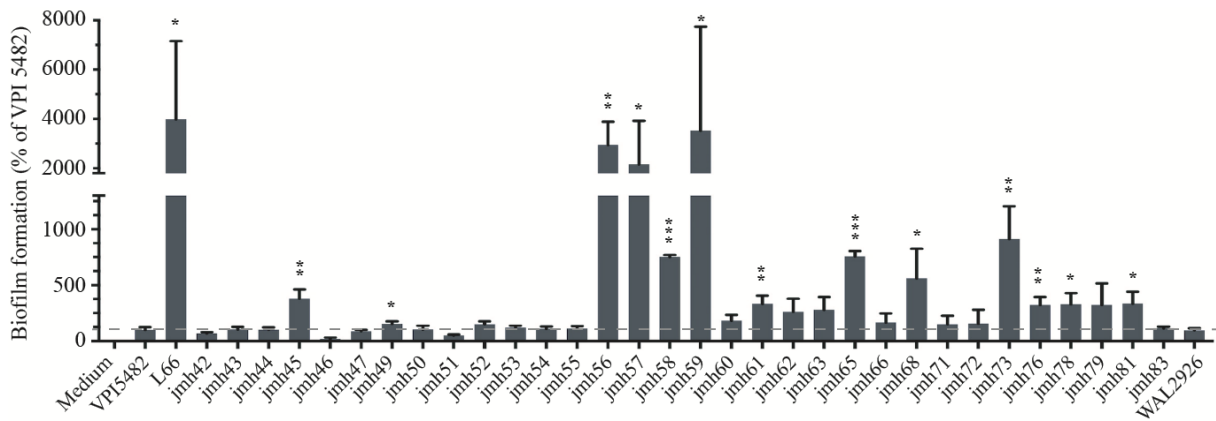
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6 **SUPPLEMENTARY MATERIAL**

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8 **SUPPLEMENTARY FIGURES**

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11 **Supplementary Figure S1. Biofilm formation by 34 *B. thetaiotaomicron* natural isolates.**

12 Comparison of biofilm formation in various *B. thetaiotaomicron* isolates in a 96-well plate
13 biofilm assay followed by crystal violet staining. VPI 5482 biofilm formation has been set to
14 100% as represented by the grey dashed lines and used for reference in the statistical test.
15 These strains were collected from adults by the Microbiology laboratory of Dupuytren
16 (Limoges) and Dron Tourcoing (Tourcoing) hospitals in France. For a complete list of the
17 strains, see supplementary Table S3. Error bars indicate standard deviation of 3 technical
18 replicates. * p<0.05; ** p<0.01; *** p<0.001.

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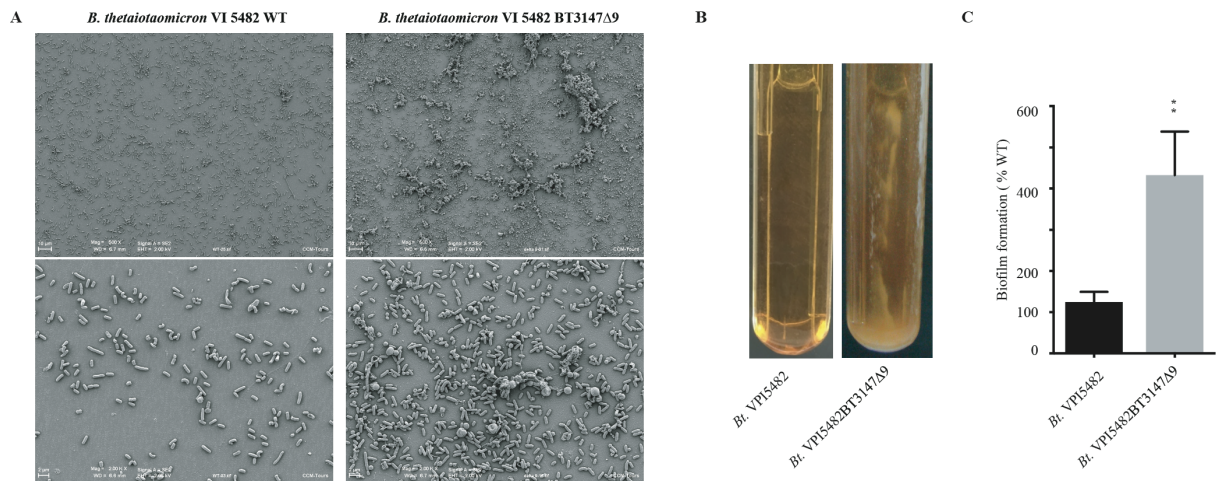
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23 **Supplementary Figure S2. Transcription in *BT3148-5* locus in WT *B. thetaiotaomicron***
 24 **VPI 5482 and the transposon mutant 1H2.** Reads mapping in the *BT3148-5* region are
 25 represented in blue, antisense reads are represented in red. Strand-specific mapping shows no
 26 antisense transcripts arising from the *BT3146-5* towards *BT3147*. *BT3147* transcript is
 27 uninterrupted in the WT indicating that there are no premature stop codons or transcription-
 28 stopping secondary structures. Transcription of the last portion of *BT3147* (position 1047 to
 29 1167 bp) and *BT3146-5* is abolished in 1H2 due to transposon insertion.

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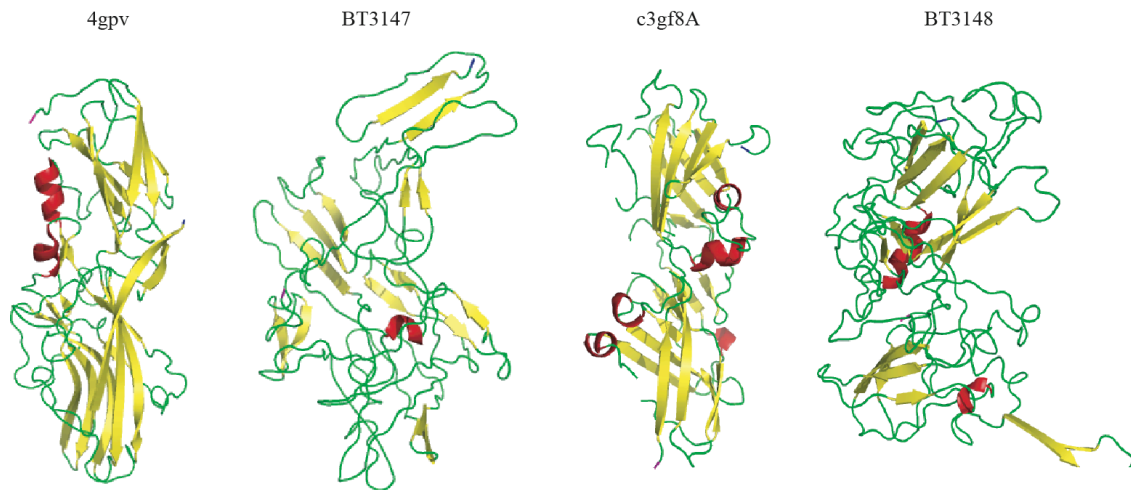
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34 **Supplementary Figure S3. C-terminal truncation of BT3147 increases *in vitro* adhesion**
35 **and biofilm formation.** **A** Scanning electron microscopy observation of *B. thetaiotaomicron*
36 WT and *BT3147Δ9* grown in continuous flow biofilm-microfermenter (x500 and x2000
37 magnification) **B** Biofilm formation *B. thetaiotaomicron* WT and *BT3147Δ9* on a glass slide
38 inserted in continuous flow biofilm microfermenters. The images were taken 48h after
39 inoculation. **B** Quantification after resuspension and measure at OD 600 of *B.*
40 *thetaiotaomicron* WT and *BT3147Δ9* in continuous flow biofilm microfermenters after 48h.

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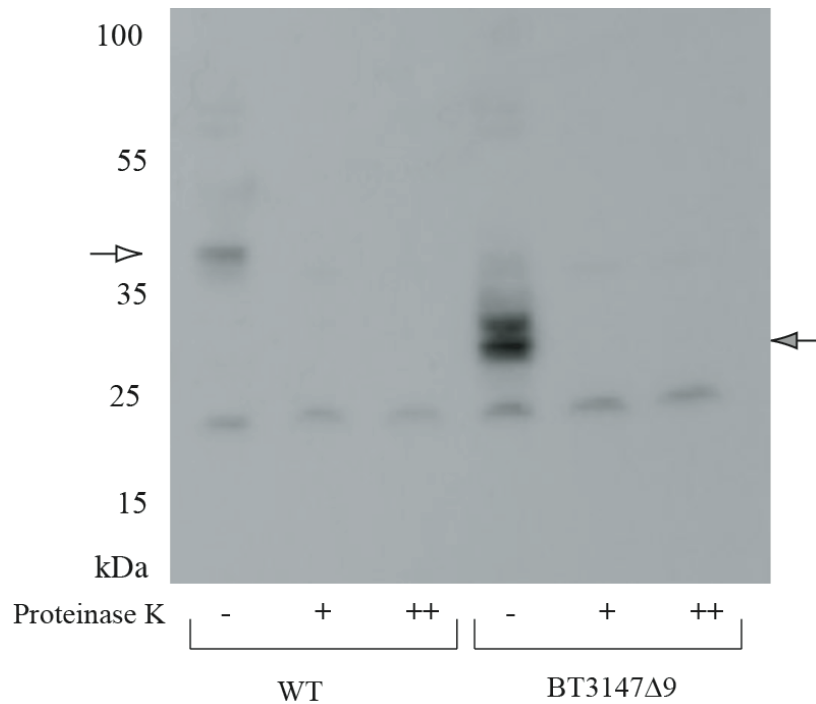


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45 **Supplementary Figure S4. Comparison of structural models of BT3147 and BT3148.**

46 Structural modeling for BT3147 and BT3148 has been performed using Phyre2 web interface
47 with the intensive mode (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>) and
48 compared to 3D-structure of Mfa1-type structural model (PDB code 4gpv:) and Fim2B/Mfa2
49 structural model (PDB code c3gf8A).

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51 WT BT3147 Δ 9
52 **Supplementary Figure S5. BT3147 sensitivity to proteinaseK treatment on whole-cells**
53 Whole cells were washed with Tris 50mM pH 8.8 washed and treated with 10 μ g/ml or 20
54 μ g/ml proteinase K for 30 minutes at 37°C. This treatment leads to degradation of BT3147
55 and disappearance of BT3147 signal. White arrows indicates WT BT3147, black arrows indicate
56 BT3147 Δ 9 variant.
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59 SUPPLEMENTARY TABLES

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61 Table S1. Wild type *B. thetaiotaomicron* collection used in this study

Strains and plasmids	Relevant characteristics	References
<i>Bacteroides thetaiotaomicron</i> VPI 5482	Healthy adult human feces	(1)
L66	Healthy adult human feces, INRA	INRA
Jmh 42	Perforated ulcer	CHU Limoges
Jmh 43	Blood	CHU Limoges
Jmh 44	Blood	CHU Limoges
Jmh 45	Liquid from redon drain	CHU Limoges
Jmh 46	Blood	CHU Limoges
Jmh 47	Hepatic abscess	CHU Limoges
Jmh 48	Abscess	CHU Limoges
Jmh 49	Blood	CHU Limoges
Jmh 50	Liquid ascite	CHU Limoges
Jmh 51	Blood	CHU Limoges
Jmh 52	Blood	CHU Limoges
Jmh 53	Peritoneal liquid	CHU Limoges
Jmh 54	Bone biopsy	CHU Limoges
Jmh 55	Skin biopsy	CHU Limoges
Jmh 56	Blood	CHU Limoges
Jmh 57	Bone biopsy	CHU Limoges
Jmh 58	Blood	CHU Limoges
Jmh 59	Blood	CHU Limoges
Jmh 60	Hip implant	CHU Limoges
Jmh 61	DPCA	CHU Limoges
Jmh 62	Peritoneal liquid	CHRU Lille
Jmh 63	Blood	CHRU Lille

Jmh 65	Abdomen	CHRU Lille
Jmh 66	Maxilar pus	CHRU Lille
Jmh 68	Blood	CHRU Lille
Jmh 71	Blood	CHRU Lille
Jmh 72	Anal abscess	CHRU Lille
Jmh 73	Blood	CHRU Lille
Jmh 76	Fat tissue	CHRU Lille
Jmh 78	Abdominal abscess	CHRU Lille
Jmh 79	Intestinal cyste	CHRU Lille
Jmh 81	Blood	CHU Limoges
Jmh 83	Peritoneal liquid	CHRU Lille

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63 genomic view of the human-Bacteroides thetaiotaomicron symbiosis. Science (New York, NY)
64 299:2074-2076.
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70 **TABLE S2. Primers used in this study.**

Strain construction and analysis	Name	Sequence
<i>ΔBT3147</i>	<i>BT3147</i> -up-F	5'-GCGGTCGACAATGTGACGGAG GACGGCATCAGC-3'
	<i>BT3147</i> -up-R	5'-CAAAAAAAGCTTTTTTAATCATTCTTAATTG-3'
	<i>BT3147</i> -down-F	5'-CGAATGGAGCGGAATATACAATGTGTGGATA ATCATGCGTGGACGGAGTATCC-3'
	<i>BT3147</i> -down-R	5'-GCGTCTAGATGAGCCGATGCAACTGTTG GTTGC-3'
<i>ΔBT3146-5</i>	<i>BT3146</i> -up-F	5'-GCGGTCGACTTTCGAAAACAAGCTTTACC-3'
	<i>BT3146</i> -up-R	5'-AATTGTTTACGTTTAAAATTATTGAATGTGTC-3'
	<i>BT3145</i> -down-F	5'-GACACATTCAATAATTTTAAACGTAAACA ATTATTGAAAAAAAACAAGCTAGG-3'
	<i>BT3145</i> -down-R	5'-CCC CGCGCCCTACGGCACAGTTGA GAGATTGGAG-3'
<i>BT3147Δ9</i>	<i>BT3147Δ9</i> -up-F	5'-GATAACATTCGAGTCGACAGAACGA GGAGTTGTTTACC-3'
	<i>BT3147Δ9</i> -up-R	5'-TTAATCTAGATTTTTTCTACTAAACCG-3'
	<i>BT3147Δ9</i> -down-F	5'-CGGTTTAGTAGAAAAAATCTAGATTAATGA TTTTCGTCTCCACAAAACGATTG-3'
	<i>BT3147Δ9</i> -down-R	5'-CTTATCGATACCCTACGGCACAGTTGA GAGATTGGAG-3'
<i>ΔBT3148</i>	<i>BT3148</i> -up-F	5'- GAAAGAAGATAACATTCGAGGGAAAAACGA TGCCTTGGTG -3'
	<i>BT3148</i> -up-R	5'- ATATTTACTTGTACCGTATTCATCGTCTTTGGT TCTAC - 3'
	<i>BT3148</i> -down-F	5'- AGACGATGAATACGGTAACAAGTAAATATACA ATTAGAA -3'
	<i>BT3148</i> -down-R	5'- ACCGCGGTGGCGCCGCTTAAACCGTCATGACC ACTTTGA -3'
<i>ΔBT3148-5</i>	<i>BT3148</i> -up-F	5'- GAAAGAAGATAACATTCGAGGGAAAAACGA TGCCTTGGTG -3'
	<i>BT3148</i> -up-R	5'-GTTTTGTGGAAGACGCGTATTCATCGTCTTTGG TTCTAC-3'
	<i>BT3145</i> -down-F	5'-AGACGATGAATACGCGTCTCCACAAAACGATTG-3'
	<i>BT3145</i> -down-R	5'-ACCGCGGTGGCGCCGCTGAAACGCAGAAGAGG CTTT-3'
<i>ΔBT3148-7</i>	<i>BT3148</i> -up-F	5'- GAAAGAAGATAACATTCGAGGGAAAAACGA TGCCTTGGTG -3'
	<i>BT3148</i> -up-R	5'-GTTTTGTGGAAGACGCGTATTCATCGTCTTTGG TTCTAC-3'
	<i>BT3147</i> -down-F	5'-AGACGATGAATACGTGACACATTCAATAATTTT AAAC-3'
	<i>BT3147</i> -down-R	5'-ACCGCGGTGGCGCCGCTTTCGGCATTGA GGTCATTTA-3'
Insertion of <i>BT1311</i>	<i>BT1311</i> promoter-F	5'-GGGTCTAGATAGTGCCATATGTTAAAAACA

promoter in pNBU2- <i>bla-erm</i>	<i>BT3131</i> promoter-R	GATTTGGAGTGC-3' 5'-CCCGCGCCGCTGATCTGGAAGAAGCA ATGAAAGC-3'
Cloning <i>BT3148-7</i> in pNBU1311	<i>BT3148-F</i>	5'-CCAAATCTGTTTTTAACATATGCTGGTCAT GTTCGTGTGG-3'
	<i>BT3147-R</i>	5'-GTTCTAGATAGTGCTTATTTTGTATAT CAAAAGC-3'
Cloning of <i>BT3146-5</i> in pNBU1311	<i>BT3146-F</i>	5'-CCCCATATGAATGATTTCAAGAATCAATGG-3'
	<i>BT3146-R</i>	5'-CCGTCTAGATCAGCCTTTCTTGTCCACG-3'
Cloning <i>BT3148-7Δ9</i> in pNBU1311	<i>BT3148-7Δ9-F</i>	5'- GTTTTTAACATATGGCACTATATGCTGGTCATG TTCGTGTG -3'
	<i>BT3148-7Δ9-R</i>	5'-AGCCCGGGGATCCACTAGTTTTAATCTAG ATTTTTTCTACTAAACCGTCA-3'
72 Cloning <i>tetQ</i> into pExchange	<i>tetQ-F</i>	5'-GGCCATATGAATATTATAAATTTAGGAAT TCTTGCTCAC-3'
	<i>tetQ-R</i>	5'-CCCTCTAGATCGTCTATTTTTTTATTGCCA AGTCTAATGC-3'
Deletion of <i>BT3147</i> upstream of Tn	<i>BT3148-up-F</i>	5'-GCGGTCGACAGCAGACCTCTGCTGATAGC-3'
	<i>BT3148-up-R</i>	5'-ACATATCTTCAGATAGATGTATC-3'
	<i>BT3147*-down-F</i>	5'-GATACATCTATCTGAAGATATGTTTCTACC GGCACTTCTGC-3'
	<i>BT3147*-down-R</i>	5'-GCGTCTAGACTCTTTGTTAACCC-3'
Localization of Tn	ARB-bt1	5'-GGCCACGCGTCTGACTAGTAC NNNNNNNNNGATAT-3'
	ARB-bt-bis	5'-GGCCACGCGTCTGACTAGTAC-3'
	pSCIRerm R	5'-GATTTGAACGTTGCGAAGC-3'
	pSCIRerm R-bis	5'-TTTTTGCGTTTCTACCTGC-3'
	pSCIRerm L	5'-CGTATCGGTCTGTATATCAGC-3'
	pSCIRerm L-bis	5'-CTCATCTTTCTGAGTCCACC-3'
DNA contamination check by RT-qPCR	<i>DNApol3-F</i>	5'-TGTCGCCCCGTCTGGTAGATA-3'
	<i>DNApol3-R</i>	5'-CCGCCTCCTTATCCTCACAC-3'

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75 **Table S3. List of strains used in this study**

Strains and plasmids	Relevant characteristics	References ^a
<i>Escherichia coli</i>		
MG1655	K-12 wild-type strain	(2)
S17-1 λ pir	(F ⁻) RP4-2-Tc::Mu aphA::Tn7 recA λ pir lysogen. Sm ^R , Tp ^R	
S17-1 λ pir_pSAM-bt	S17-1 λ pir derivative carrying pSAM-bt	This study
S17-1 λ pir_pExchange-tdk	S17-1 λ pir derivative carrying pExchange-tdk	This study
S17-1 λ pir_pNBU2-bla-erm	S17-1 λ pir carrying pNBU2-bla-erm	This study
S17-1 λ pir_pExchange-BT3147	S17-1 λ pir carrying pExchange-BT3147	This study
S17-1 λ pir_pExchange-BT3146-5	S17-1 λ pir carrying pExchange-BT3146-3145	This study
S17-1 λ pir_pExchange-BT3147 Δ 9	S17-1 λ pir carrying pExchange-BT3147 Δ 9	This study
S17-1 λ pir_pExchange-BT3148	S17-1 λ pir carrying pExchange-BT3148	This study
S17-1 λ pir_pExchange-BT3148-5	S17-1 λ pir carrying pExchange-BT3148-3145	This study
S17-1 λ pir_pExchange-BT3148-7	S17-1 λ pir carrying pExchange-BT3148-3147	This study
S17-1 λ pir_pNBU1311	S17-1 λ pir carrying pNBU1311	This study
S17-1 λ pir_pNBU1311-BT3146-5	S17-1 λ pir carrying pNBU1311-BT3146-3145	This study
S17-1 λ pir_pNBU1311-BT3148-7	S17-1 λ pir carrying pNBU1311-BT3148-3147	This study
S17-1 λ pir_pNBU1311-BT3148-7 Δ 9	S17-1 λ pir carrying pNBU1311- BT3148-3147 Δ 9	This study
S17-1 λ pir_pExchange-tetQ-BT3147*	S17-1 λ pir carrying pExchange-tetQ-BT3147*	This study
S17-1 λ pir_pExchange-tetQ	S17-1 λ pir carrying pExchange-tetQ	This study
<i>Bacteroides thetaiotaomicron</i>		
VPI 5482	Healthy adult human feces	(1)
VPI 5482_Atdk	Deletion of thymidine kinase in the wild type strain ATCC29148	(3)
VPI 5482_ Δ BT3147	Deletion of BT3147	This study
VPI 5482_ Δ BT3146-5	Deletion of BT3146-3145	This study
VPI 5482 Δ BT3148-5	Deletion of BT3148-3145	This study
VPI 5482 Δ BT3148-7	Deletion of BT3148-3147	This study
VPI 5482 BT3147 Δ 9	Deletion of BT3146-3145 and the last 9 C-terminal amino acids of BT3147	This study
VPI 5482 Δ BT3148 BT3147 Δ 9	Deletion of BT3148 in VPI 5482_ BT3147 Δ 9 strain	This study

VPI 5482_p. <i>BT3146-5</i>	Chromosomal-based expression of <i>BT3146-5</i> from pNBU1311	This study
VPI 5482_p. <i>BT3148-7</i>	Chromosomal-based expression of <i>BT3148-7</i> from pNBU1311	This study
VPI 5482_p. <i>BT3148-7</i> Δ9	Chromosomal-based expression of <i>BT3148</i> and the 9 amino acid truncated version of <i>BT3147</i> from pNBU1311	This study
VPI 5482_pNBU2- <i>bla-erm</i>	Erythromycin resistant strain	This study
1H2 Δ <i>BT3147</i> *	1H2 mutant with deletion of <i>BT3147</i> portion upstream of the Tn-insertion	This study

Plasmids

pSAM-bt	Random transposon mutagenesis	(Goodman <i>et al.</i> , 2009)
pExchange- <i>tdk</i>	Site-directed unmarked gene deletion	(Martens <i>et al.</i> , 2008)
pNBU2- <i>bla-erm</i>	Chromosomal-based gene expression	(Martens <i>et al.</i> , 2008)
pNBU1311	Derivative of pNBU2- <i>bla-erm</i> with cloned constitutive promoter of <i>BT1311</i> for gene expression	This study
pExchange- <i>BT3147</i>	pExchange derivative for unmarked deletion of <i>BT3147</i>	This study
pExchange- <i>BT3146-5</i>	pExchange derivative for unmarked deletion of <i>BT3146</i> and <i>BT3145</i>	This study
pExchange- <i>BT3147</i> Δ9	pExchange derivative for unmarked deletion of <i>BT3146-5</i> and last 9 amino acids of <i>BT3147</i> C-terminus	This study
pExchange- <i>BT3148</i>	pExchange derivative for unmarked deletion of <i>BT3148</i>	This study
pExchange- <i>BT3148-5</i>	pExchange derivative for unmarked deletion of <i>BT3148</i> to <i>BT3145</i>	This study
pExchange- <i>BT3148-7</i>	pExchange derivative for unmarked deletion of <i>BT3148</i> and <i>BT3147</i>	This study
pNBU1311- <i>BT3146-5</i>	pNBU1311 derivative for expression of <i>BT3146-5</i>	This study
pNBU1311- <i>BT3148-7</i>	pNBU1311 derivative for expression of <i>BT3148-7</i>	This study
pNBU1311- <i>BT3148-7</i> ^{Δ9}	pNBU1311 derivative for expression of <i>BT3148-7</i> ^{Δ9}	This study
pExchange- <i>tetQ</i>	pExchange derivative with <i>erm</i> resistance exchanged for tetracycline	This study
pExchange- <i>tetQ</i> - <i>BT3147</i> *	pExchange- <i>tetQ</i> derivative for deletion of <i>BT3147</i> portion laying upstream of the Tn insertion	This study

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77 ^a Bacterial strains from our laboratory collection unless otherwise specified.

78 CRBIP: *Institut Pasteur, Centre de Ressources Biologiques de l'Institut Pasteur.*

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