

Article

PBP4 is Likely Involved in Cell Division of the Longitudinally Dividing Bacterium *Candidatus Thiosymbion Oneisti*

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Extracted mass chromatogram: +204.100 (0.1000 Da) : TOF MS^E (100-2000) 15-40eV ESI+ - High CE

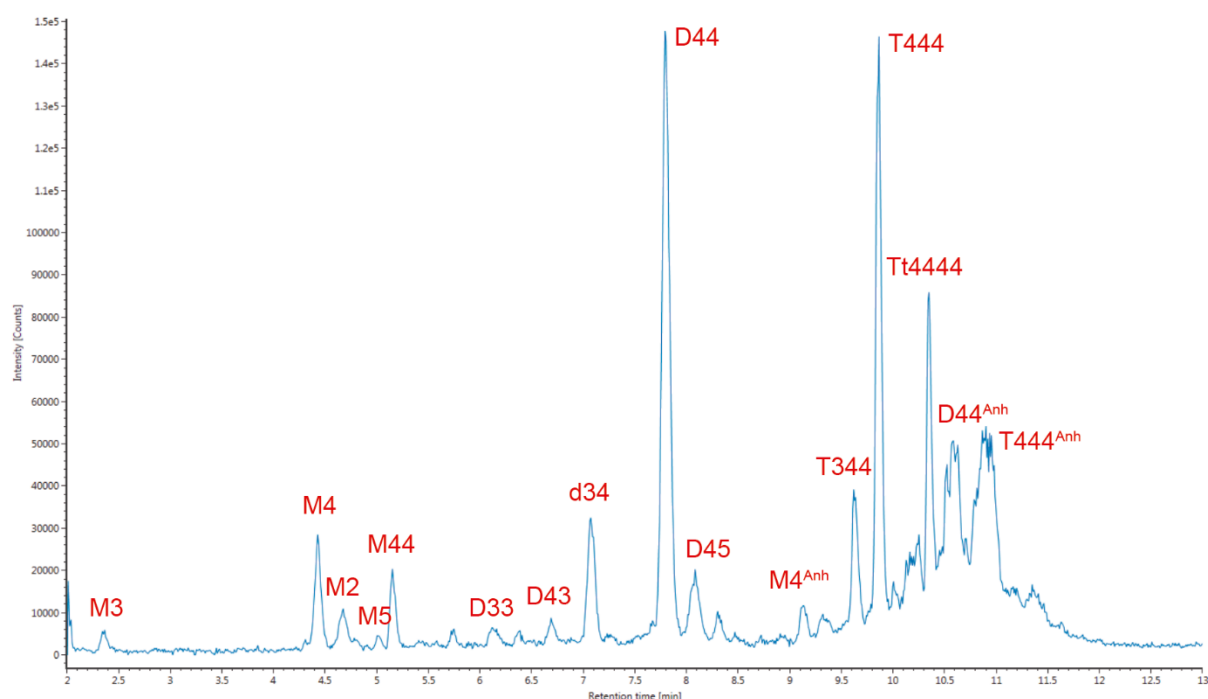
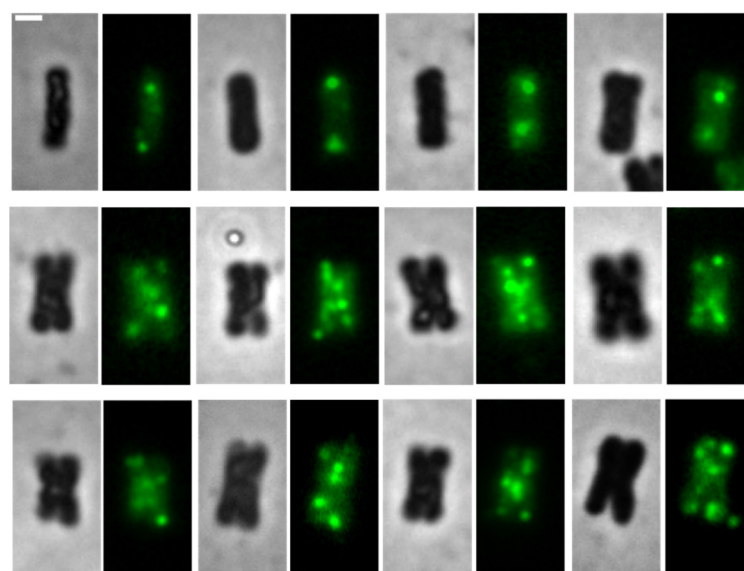


Figure S1. Muropeptide profile of *Ca. T. oneisti* obtained by LC-MS. Masses of indicated peaks are shown in supplementary table S3.

			Signal peptide	→ Predicted α helix of PBP4 ^{TO}	→ Predicted β sheet of PBP4 ^{TO}	
PBP4_Ec	1	MR-----	FSRFIIIGLTSCIAFSVQA	ANVDEYITQLPAGANLALMVQ		41
PBP4_To	1	MQPGISPALFLGALLTLGLILTAGSTPASAGSGDALSRITLKLKPKASLLVT				50
PBP4_Ec	42	KVGASAPAIIDYHSQQMALPASTQKVITALAALIQLGPDFRFTTTLETGKN				91
PBP4_To	51	EHGR--PVISRHPDRAMVPASTLKILTAFAAIERWGLDHRFHTDFYL-GN				97
PBP4_Ec	92	VENGVLKGDIVARFGADPTLKRQDIRNMVATLKKSGVNQIDGNVLIDTSI				141
PBP4_To	98	DDRLWVGK-----YG-DPYLVSEELDIIADALKARGVRKVAG-IGTDDSY				140
PBP4_Ec	142	FASHDKAPGWPWDMTQCFSAAPAAAI VDRNCF SVSLYSAPKPGDMAFIR				191
PBP4_To	141	F-----DPNLAI SGRSS--SDNFPYDAPVTG-----L				164
PBP4_Ec	192	VASYYPVTMFSSQVRTLPRGSAEAQYCELDVVPGD-LNRFTLTGCLPQRSE				240
PBP4_To	165	AANFNTVNVINKGKVSRAEAQTPLTPMARRFGQGLGAGTHRNVLEQREK				214
PBP4_Ec	241	PLPLAFVQDGASYAGAILKDELKQAGITWSGTLRLRQTQVNEPGTVVASK				290
PBP4_To	215	AV-----RYFGELLSAKLEQAGVRVGSNLRNGVPVTRI KKVYRHQ				254
PBP4_Ec	291	QSAPLHDLKIMLKSDNMIADTVFRMIGHARFNVPGTWRA--GSDAVRQ				338
PBP4_To	255	NSHDLRSVITAMLEYSNNFIANALFLKLAD----PGDGRALNMSKAQRA				299
PBP4_Ec	339	ILRQ-QAGVDIGNTIIADGSGLSRHNLIAPATMMQVLQYIAQHDNELNFI				387
PBP4_To	300	FARWVDQTFEWRDYRIEDGAGLSRGNRLSARQLLEVNVAFAPYR-----				343
PBP4_Ec	388	SMLPLAGYDGSGLQYRAGLHQAGVDGKVSATGSLQGVYNLAGFITTSASGQ				437
PBP4_To	344	TLLPKQ-----NAQVRKTGTLRGVSSYAGFVKR-NGR				375
PBP4_Ec	438	RMAFVQYLSGYAVEPADQRNRRIPLVRFESRLYKDIYQNN				477
PBP4_To	376	WEPF-SLLINQPVPHNLRQVANSLANTPDLTRLCPPGGSC				414

Figure S2. Alignment of PBP4^{EC} and PBP4^{TO}.Figure S3. PBP4 localization in *Ca. T. oneisti*. Phase contrast and fluorescence images of representative *Ca. T. oneisti* cells of PBP4 immunolabeling.

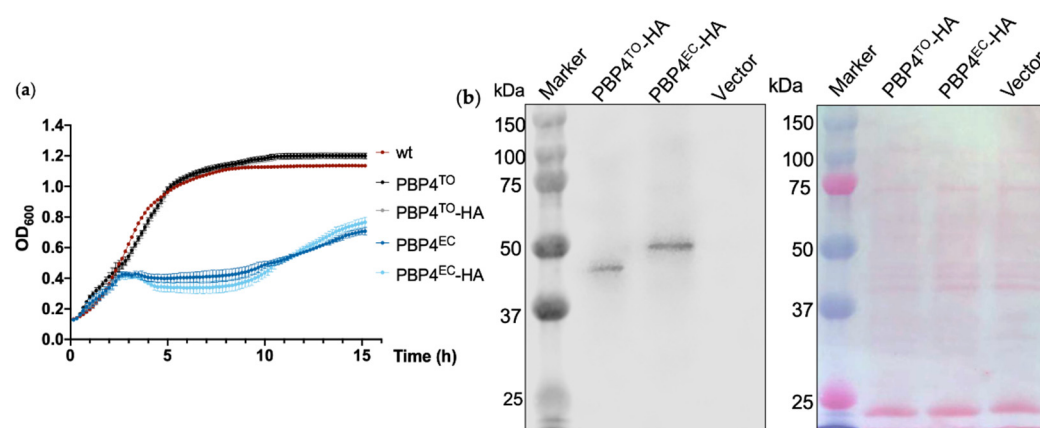


Figure S4. PBP4TO/EC protein expression level. (a) Growth curves of wild type *E. coli* with PBP4EC/TO from plasmid pXL133/pJW05 or PBP4EC/TO-HA overexpression from plasmid pJW08/pJW09 induced with 200 μ M IPTG in TY medium at 28 $^{\circ}$ C. The values are the mean $\pm$ SD from three re-peats. (b) Western blot image and corresponding Ponceau S stain shows the expression level of PBP4TO/EC in wild type *E. coli*. induced with 50 μ M IPTG for 4h cultured in TY medium at 28 $^{\circ}$ C.

Table S1. Muropeptides of *Ca. T. oneisti* analyzed by LC-MS.

No.	Name	Structure	RT (min)	Neutral Mass (Da)		Difference	RA (%)
				Theoretical	Observed		
1	M3	GlcNAc-Mur-Nac-L-Ala-D-Glu-DAP	2.36	870.3706	870.3665	0.0041	1.57
2	M4	GlcNAc-Mur-Nac-L-Ala-D-Glu-DAP-D-Ala	4.43	941.4077	941.4066	0.0011	24.77
3	M2	GlcNAc-Mur-Nac-L-Ala-D-Glu	4.68	698.2858	698.2856	0.0002	8.33
4	M5	GlcNAc-Mur-Nac-L-Ala-D-Glu-DAP-D-Ala-D-Ala	5.00	1012.4448	1012.4487	-0.0039	1.32
5	M44	M4-M4 (cross-linked at D-Ala-DAP) with loss of a disaccharide	5.15	1384.6093	1384.6172	-0.0079	7.68
6	D33	M3-M3 (cross-linked at DAP-DAP)	6.12	1722.7306	1722.7372	-0.0066	0.35
7	D43	M4-M3 (cross-linked at D-Ala-DAP)	6.69	1793.7677	1793.7773	-0.0096	1.09
8	D34	M3-M4 (cross-linked at DAP-DAP)	7.07	1793.7677	1793.7773	-0.0096	5.77
9	D44	M4-M4 (cross-linked at D-Ala-DAP)	7.79	1864.8048	1864.8019	0.0029	29.72
10	D45	M4-M5 (cross-linked at D-Ala-DAP)	8.08	1935.8420	1935.8412	0.0007	0.04

11	M4 ^{Anh}	GlcNAc-(1-6an- hydro)Mur- NAc-L-Ala-D- Glu-DAP-D-Ala M3-M4-M4	9.14	921.3815	921.3794	0.0021	1.48
12	T344	(crosslinked at DAP-DAP and D-Ala-DAP) M4-M4-M4	9.62	2717.1649	2717.0087	0.1562	0.10
13	T444	(crosslinked at D-Ala-DAP) M4-M4-M4-M4	9.87	2788.2020	2788.2043	-0.0023	10.29
14	Tt4444	(crosslinked at D-Ala-DAP) M4-M4A (cross- linked at D-Ala- DAP)	10.35	3711.5991	3711.3680	0.2311	1.01
15	D44 ^{Anh}	M4-M4-M4A (crosslinked at D-Ala-DAP)	10.62	1844.7786	1844.7692	0.0094	6.32
16	T444 ^{Anh}	M4-M4-M4A (crosslinked at D-Ala-DAP)	10.90	2768.1758	2768.1799	-0.0041	0.16

RA = Relative molar abundance.

Table S2. E. coli strains and plasmids used in this study.

E. coli strain	Relevant Genotype	Reference
LMC500	MC4100 <i>lysA</i>	[1]
ΔPBP4	BW25113Δ <i>dacB</i>	[2]
SF100	KS272 Δ <i>ompT</i>	[3]
D456	<i>his supF ΔdacA::Km dacB::Spc ΔdacC1</i>	[4]
Plasmids	Property	Reference or source
pJW05	pTrc 99A down expressing PBP4 ^{TO} , p15A ori, CmR	This work
pJW06	pTrc 99A expressing PBP4 ^{TO} -8His, ColE1 ori, CmR	This work
pJW07	pTrc 99A expressing PBP4 ^{TO} S69A-8His, ColE1 ori, CmR	This work
pJW08	pTrc 99A down expressing PBP4 ^{TO} -HA, p15A ori, CmR	This work
pXL133	pTrc 99A down expressing PBP4 ^{EC} , p15A ori, CmR	[5]
pJW09	pTrc 99A down expressing PBP4 ^{EC} -HA, p15A ori, CmR	This work

Table S3. Primers used in this study.

Primer	Sequence 5'-3'	use
priJW84	ATGCAACCCGGAATATCCCCAGC	pJW06 construction
priJW85	TCAACAACCTCCCGCCCGGCACA	pJW06 construction
priJW86	TGGGGATATTCGGGTTGCATGGCTT- GTCATGTCATCG	pJW06 construction
priJW87	TGCCCCGGGCGGGAGTTGTT- GATGGTCGACCTGCAGGCATGC	pJW06 construction
priJW88	TCACACAGGAAACAGACCATGTTT- GAACCAATGGAAC	pJW05 construction
priJW89	GTTCCGGGCCCAA- GCTCATTATTAATCAGCTTGCTTACGC	pJW05 construction

priJW105	TTCCGGCGGCAACGCTGAA- GATCCTGACCGCCTTCG	pJW07 construction
priJW106	ATCTTCAGCGTTGCCGCCGGAAC- CATGGCCCCGGTCC	pJW07 construction
priJW111	GGTGGTTCTTACCCATACGATGTTCCA GATTACGCTTGAA AGCTTGGGCCCCGAACA	pJW08 and pJW09 construction
priJW112	ATCGTATGGGTAAGAACCAC- CAGAACCACCACAACCTCC CGCCCCGGGCACA	pJW08 construction
priJW114	ATCGTATGGGTAAGAACCAC- CAGAACCACCATTGTTCTG ATAAATATCTTTATACAA	pJW09 construction

Reference

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