Supporting Materials

Detection of Intracellular Transposition, and Capture of Multiple Mobile Genetic Elements, Following Intercellular Conjugation of Multidrug Resistance Conjugative Plasmids from Clinical Enterobacteriaceae Isolates.

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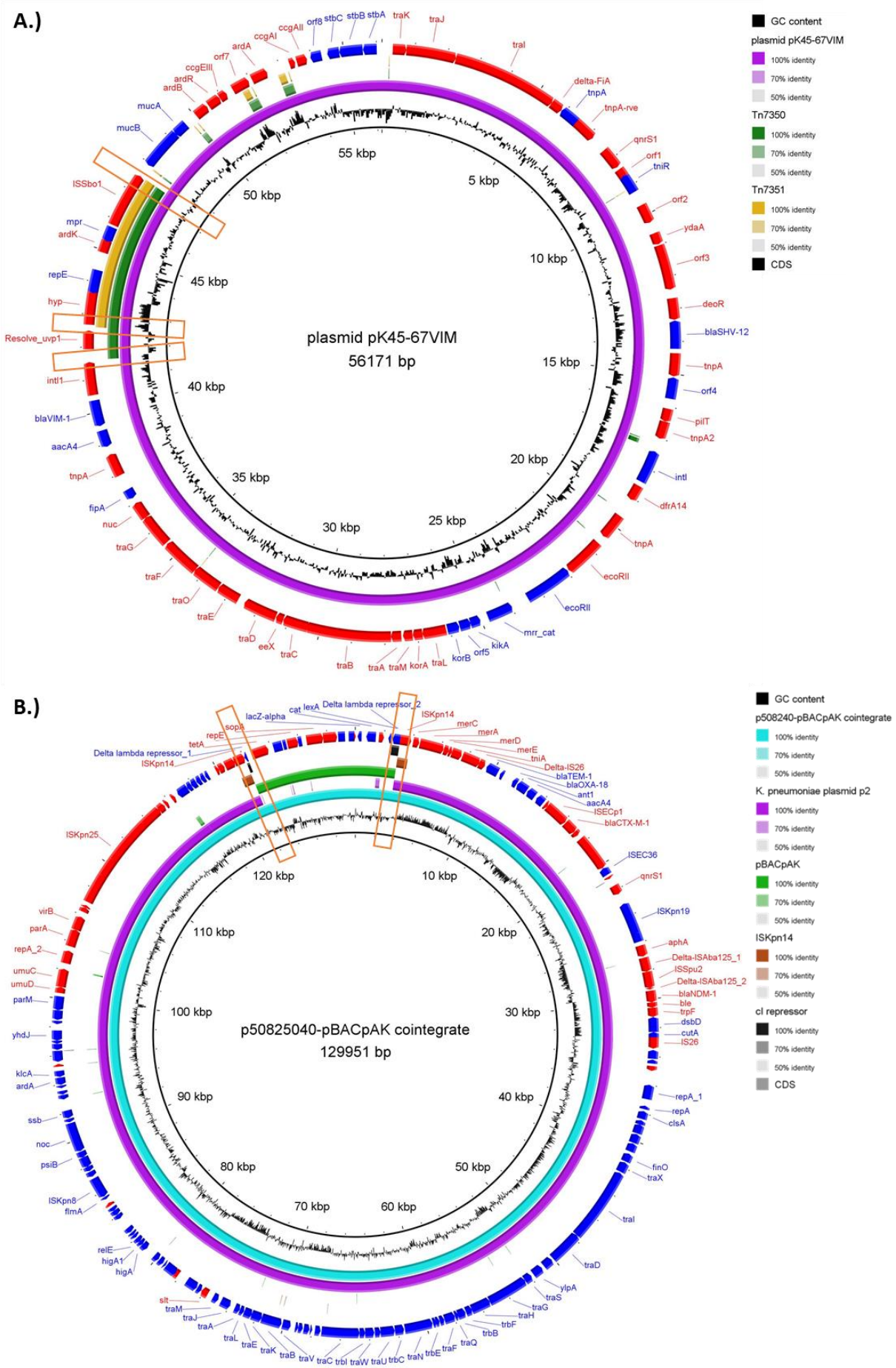
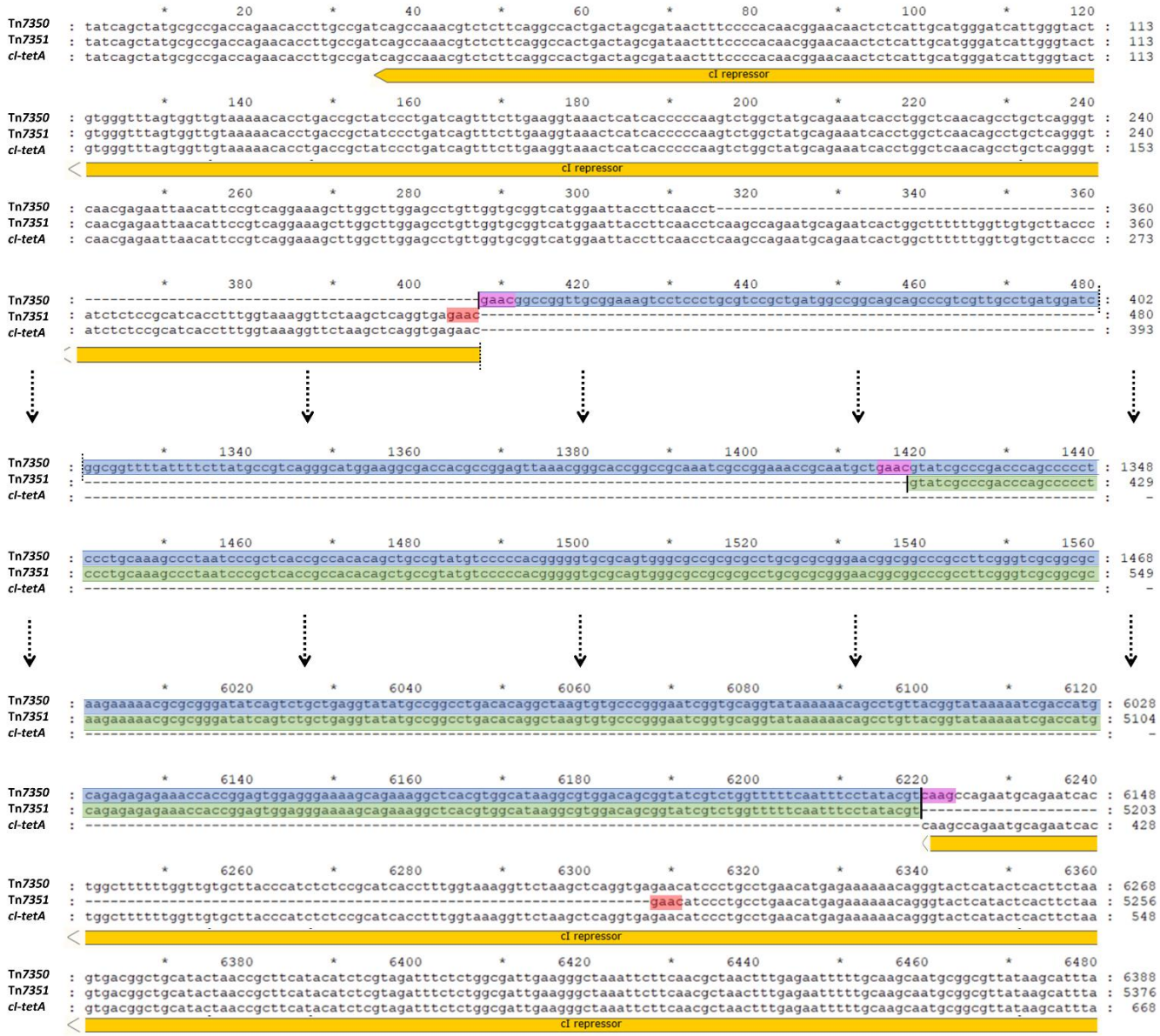
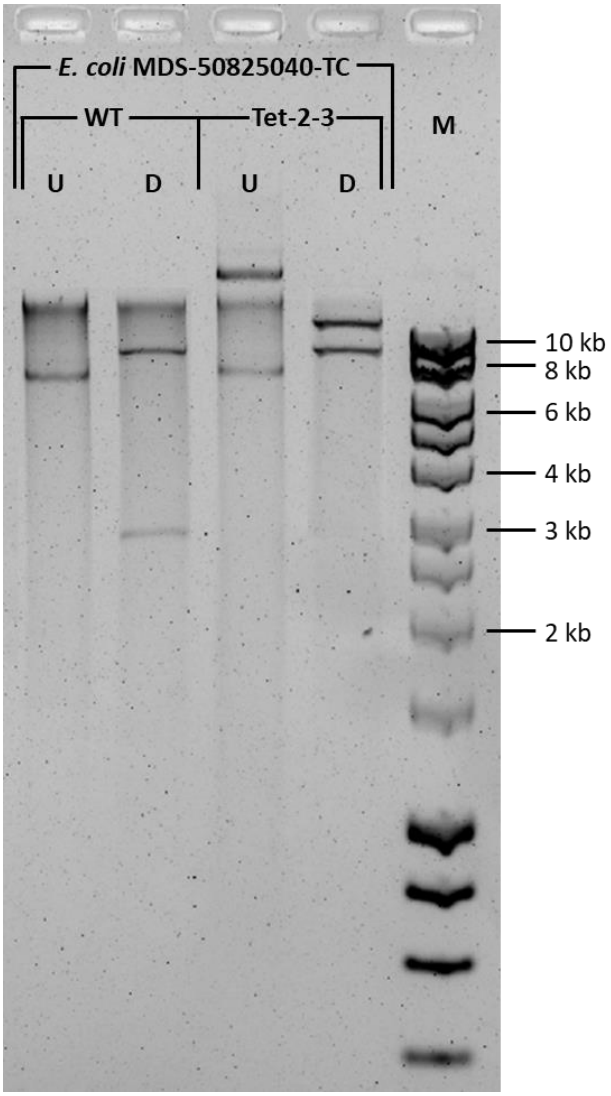
Supplementary Figure S1. Comparative analysis of the captured transposons with their best match from BlastN analysis. A.) Analysis between Tn*7350*, Tn*7351* and plasmid pK45-67VIM from *K. pneumoniae* (Accession number HF955507). B.) Analysis between Tn*7359*-containing pBACpAK (p50825040-pBACpAK cointegrate) with plasmid p2 from *K. pneumoniae* (Accession number CP009115). They were performed with the BLAST Ring Image Generator (BRIG) (1). The blue and red open arrowed boxes represent counterclockwise and clockwise coding sequences (CDSs), respectively. Note, there is actually only one copy of IS*Kpn14* in plasmid p2. The borders of all insertions are indicated with orange boxes.


A.)
plasmid pK45-67VIM
56171 bp
GC content
plasmid pK45-67VIM
100% identity
70% identity
50% identity
Tn7350
100% identity
70% identity
50% identity
Tn7351
100% identity
70% identity
50% identity
CDS
B.)
p50825040-pBACpAK cointegrate
129951 bp
GC content
p508240-pBACpAK cointegrate
100% identity
70% identity
50% identity
K. pneumoniae plasmid p2
100% identity
70% identity
50% identity
pBACpAK
100% identity
70% identity
50% identity
ISKpn14
100% identity
70% identity
50% identity
cI repressor
100% identity
70% identity
50% identity
CDS

Supplementary Figure S2. Insertion sites of Tn*7350* and Tn*7351* in the *cI* repressor gene on pBACpAK entrapment vector. An alignment between a wild-type *cI* repressor gene and the *cI* regions from *E. coli* MDS-K46-62-TC-Tet-11 and Tet-21 containing Tn*7350* and Tn*7351*, respectively. The blue and green boxes represent Tn*7350* and Tn*7351*, respectively. The dashed arrows indicate parts of sequence alignment that were not shown in the figure. The GAAC direct and inverted repeats on Tn*7350* and Tn*7351* are indicated with purple and red boxes, respectively.



Supplementary Figure S3. XhoI digestion of plasmids extracted from *E. coli* MDS-50825040-TC-Tet-2-3, compared to the wild-type (WT). Lane M, HyperLadder™ 1kb. U: Undigested plasmid. D: Digested plasmid.



Supplementary Table S1 Primers used in this study

Primer name	**Sequence (5'-3')**	**Gene target**	**Reference**
Primers to check the insertion within cI-*tet(A)* selection cartridge			
ERIS	GCAAGACTGGCATGATAAGG	*cI-tetA* (reverse primer)	(2)
***cI-tetA*-F1**	CAGCCAGCAGAGAATTAAGG	*cI-tetA* (forward primer)	This study
Primers for sequencing			
MKT-11-F1	GGAGAACTGCCTGAAAGCAT	Tn*7350* extension primer	This study
MKT-21-F1	CGCTGTCGTGTGGAAATC	Tn*7351* extension primer	This study
MKT-11-R1	GATACATTCAGCCCCGCAAT	Tn*7350* and Tn*7351* extension primer	This study
MKT-11-R2	GTCGCAGTCAGGAAGACGAT	Tn*7350* and Tn*7351* extension primer	This study
MKT-11-R3	GAACAGGAGGTGGAGGAAGG	Tn*7350* and Tn*7351* extension primer	This study
MKT-11-R4	AACTTGTTTTCGCGGTTCTG	Tn*7350* and Tn*7351* extension primer	This study
MKT-11-R5	ACATTTACGGCCTGACGAAC	Tn*7350* and Tn*7351* extension primer	This study
M5T-4-F1	ACGAAACTGAAGCGGAGATG	IS*Sbo1* extension primer	This study
M5T-4-R1	CAGACGATACCGCTGTCCAC	IS*Sbo1* extension primer	This study

Supplementary Table S3 Estimation of gene copy numbers based on WGS data of tetracycline resistance *E. coli* MDS-50825040-TC

		***E. coli* MDS-50825040-TC-Strains**						
		Tet-2-1	**Tet-2-3**	**Tet-3-1**	**Tet-3-7**	**Tet-4-13**	**Tet-4-38**	**Tet-4-52**
Reads	*tet*(A)	438	404	791	578	288	540	239
	Chl^R	263	255	530	347	188	310	115
	IS*Kpn14*	538	176	1076	210	267	149	197
	IS*Kpn25*	2388	4139	4662	9035	3933	7470	3242
	bla_{NDM-1}	262	177	524	240	265	118	219
	repE(pBACpAK)	340	283	592	456	183	350	124
	*repA*_1_conjP	299	234	495	262	282	140	248
	*repA*_2_conjP	327	198	609	301	294	137	288
Reads/ gene size	*tet*(A)	0.363	0.335	0.656	0.479	0.239	0.448	0.198
	Chl^R	0.398	0.386	0.803	0.526	0.285	0.470	0.174
	IS*Kpn14*	0.701	0.229	1.401	0.273	0.348	0.194	0.257
	IS*Kpn25*	0.293	0.508	0.572	1.108	0.482	0.916	0.398
	bla_{NDM-1}	0.322	0.218	0.645	0.295	0.326	0.145	0.269
	*repE*_pBACpAK	0.450	0.374	0.783	0.603	0.242	0.463	0.164
	*repA*_1_conjP	0.295	0.231	0.488	0.258	0.278	0.138	0.245
	*repA*_2_conjP	0.365	0.221	0.679	0.336	0.328	0.153	0.321
Copy number per Chl^R	*tet*(A)/Chl^R	0.911	0.867	0.817	0.912	0.838	0.953	1.137
	Chl^R/Chl^R	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	IS*Kpn14*/Chl^R	1.758	0.593	1.745	0.520	1.220	0.413	1.472
	IS*Kpn25*/Chl^R	0.735	1.314	0.712	2.108	1.693	1.950	2.282
	bla_{NDM-1}/Chl^R	0.809	0.563	0.803	0.561	1.144	0.309	1.546
	repE/Chl^R	1.129	0.969	0.975	1.147	0.850	0.986	0.941
	*repA*_1/Chl^R	0.740	0.597	0.608	0.491	0.976	0.294	1.404
	*repA*_2/Chl^R	0.915	0.571	0.845	0.638	1.151	0.325	1.843
Copy number per bla_{NDM-1}	*tet*(A)/bla_{NDM-1}	1.127	1.539	1.018	1.624	0.733	3.085	0.736
	Chl^R/bla_{NDM-1}	1.237	1.775	1.246	1.781	0.874	3.236	0.647
	IS*Kpn14*/bla_{NDM-1}	2.174	1.053	2.174	0.926	1.067	1.337	0.952
	IS*Kpn25*/bla_{NDM-1}	0.909	2.332	0.887	3.754	1.480	6.312	1.476
	bla_{NDM-1}/bla_{NDM-1}	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	repE/bla_{NDM-1}	1.396	1.719	1.215	2.043	0.743	3.190	0.609
	*repA*_1/bla_{NDM-1}	0.915	1.060	0.757	0.875	0.853	0.951	0.908
	*repA*_2/bla_{NDM-1}	1.131	1.014	1.053	1.137	1.006	1.052	1.192
Copy number per *repE* (pBACpAK)	*tet*(A)/*repE*	0.808	0.895	0.838	0.795	0.987	0.967	1.208
	Chl^R/*repE*	0.886	1.032	1.025	0.872	1.177	1.015	1.062
	IS*Kpn14*/*repE*	1.558	0.612	1.789	0.453	1.436	0.419	1.564
	IS*Kpn25*/*repE*	0.651	1.356	0.730	1.837	1.993	1.979	2.424
	bla_{NDM-1}/*repE*	0.717	0.582	0.823	0.489	1.347	0.314	1.642
	repE/*repE*	1.000	1.000	1.000	1.000	1.000	1.000	1.000

		E. coli MDS-50825040-TC-Strains						
		Tet-2-1	**Tet-2-3**	**Tet-3-1**	**Tet-3-7**	**Tet-4-13**	**Tet-4-38**	**Tet-4-52**
	*repA*_1/*repE*	0.656	0.616	0.623	0.428	1.149	0.298	1.491
	*repA*_2/*repE*	0.811	0.590	0.867	0.556	1.354	0.330	1.957
Copy number per *repA*_1 (Conjugative plasmid)	*tet*(A)/*repA*_1	1.232	1.452	1.344	1.855	0.859	3.243	0.810
	Chl^R/*repA*_1	1.351	1.674	1.645	2.035	1.024	3.402	0.712
	IS*Kpn14*/*repA*_1	2.376	0.993	2.870	1.058	1.250	1.405	1.049
	IS*Kpn25*/*repA*_1	0.993	2.200	1.171	4.288	1.734	6.635	1.626
	bla_{NDM-1}/*repA*_1	1.093	0.943	1.320	1.143	1.172	1.051	1.101
	repE/*repA*_1	1.525	1.622	1.604	2.334	0.870	3.353	0.671
	*repA*_1/*repA*_1	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	*repA*_2/*repA*_1	1.236	0.957	1.391	1.299	1.179	1.106	1.313
Copy number per *repA*_2 (Conjugative plasmid)	*tet*(A)/*repA*_2	0.996	1.518	0.966	1.428	0.729	2.932	0.617
	Chl^R/*repA*_2	1.093	1.750	1.183	1.567	0.869	3.075	0.543
	IS*Kpn14*/*repA*_2	1.922	1.038	2.064	0.815	1.061	1.270	0.799
	IS*Kpn25*/*repA*_2	0.803	2.300	0.842	3.302	1.472	5.998	1.238
	bla_{NDM-1}/*repA*_2	0.884	0.986	0.949	0.880	0.994	0.950	0.839
	repE/*repA*_2	1.234	1.696	1.153	1.798	0.739	3.031	0.511
	*repA*_1/*repA*_2	0.809	1.045	0.719	0.770	0.849	0.904	0.762
	*repA*_2/*repA*_2	1.000	1.000	1.000	1.000	1.000	1.000	1.000

References

1. Alikhan N-F, Petty NK, Ben Zakour NL, Beatson SA. 2011. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402.
2. Bartosik D, Sochacka M, Baj J. 2003. Identification and characterization of transposable elements of *Paracoccus pantotrophus*. Journal of Bacteriology 185:3753-3763.