

Additional File 1: Table S1. Characteristics of each bacterium genome

	GC content (%)	Genome size (Mb)	CDS cumulated size (Mb)	CDSs
<i>Acinetobacter baylyi</i> ADP1	40	3.59	3.15	3306
<i>Bacillus subtilis</i> 168	43	4.21	3.71	4256
<i>Escherichia coli</i> MG 1655	50	4.63	4.08	4306
<i>Lactococcus lactis</i> MG1363	35	2.52	2.19	2734
MIX	43	14.98	13.15	14602

Additional File 1: Table S2. Proportions of detected genes and of covered intergenic bases

Library name	% detected genes				% covered intergenic bases			
	replicate		replicate		replicate		replicate	
	1		2		1		2	
	MIX	<i>L. lactis</i> ^a	MIX	<i>L. lactis</i> ^a	MIX	<i>L. lactis</i> ^a	MIX	<i>L. lactis</i> ^a
TS_L.L		86.6		89.2		15.3		17.9
ENC_L.L		84		81		21.2		19.1
OV_L.L		84.6		84.7		12.9		13.1
SMART_L.L		88.4		87.7		18.3		18
TS_L.L control		90.7		91.1		18.4		18
OV_L.L control		92.7		85.4		12.5		11.7
SMART_L.L control		70.9		66.6		19.3		19.5
TS_MIX	76.4	63.4	72.6	73.5	16.8	15.3	17.1	15.4
ENC_MIX	72.8	72.7	72.7	59	18.3	19.3	18.5	19.6
OV_MIX	68.9	61.2	72.3	64.2	13.2	11.8	13.1	11.7
SMART_MIX	76.7	76.1	74.5	73.2	19	21.1	19	21.5
TS_MIX control	85.8	85.4	87.7	85.4	19.3	19.3	18	18
OV_MIX control	68.9	63.5	45	59.4	13.3	11.2	13.6	13.2
SMART_MIX control	64.7	63	54.8	54.6	18.2	20.1	18.2	20.5

_L.L : library prepared from *L. lactis* depleted RNA.

_L.L control : library prepared from *L. lactis* total RNA.

_MIX : library prepared from the MIX depleted RNA.

_MIX control : library prepared from the MIX total RNA.

a: for the MIX libraries, the sequences attributed to *L. lactis* were extracted and the rates were calculated for the MIX and for *L. lactis* separately.

Additional File 1: Table S3. Taxonomic assignment of the MIX libraries reads

Library name	Reads assigned to <i>A. baylyi</i> (%)		Reads assigned to <i>B. subtilis</i> (%)		Reads assigned to <i>E. coli</i> (%)		Reads assigned to <i>L. lactis</i> (%)	
	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2	replicate 1	replicate 2
TS_MIX	38.4	36.5	15.05	14.59	17.24	22.2	27.86	23.65
ENC_MIX	35.16	35.84	14.75	14.67	19.27	20.9	27.6	25.37
OV_MIX	34.7	34.6	16.9	16.9	7.93	7.97	37.78	38.04
SMART_MIX	35.6	35.6	21.7	21.2	17.6	19.3	21.4	20.3
TS_MIX control	32.8	33.1	18.8	18.3	18.9	22.6	25.8	22.5
OV_MIX control	37	35	19.5	16.4	13.2	10.7	27.5	35.5
SMART_MIX control	32.9	33.2	24.5	23.6	21.5	22.4	17.8	17.6

_MIX : library prepared from the MIX depleted RNA.

_MIX control : library prepared from the MIX total RNA.

Additional File 1: Table S4. Sequences statistics for SMART control libraries prepared with 5ng total RNA

Library name	Raw reads (millions)	% rRNA ^a	Cleaned reads ^b (millions)	% mapped reads ^c	% duplication rate ^d	% detected genes ^e
SMART_L.L control 5ng input	18.530	95.5	0.795	63.4	14.4	88.2
SMART_MIX control 5ng input	18.447	93	1.242	64.7	7.21	82 (pool) 83.8 (<i>L.lactis</i>)

_L.L control : library prepared from *L. lactis* total RNA.

_MIX control : library prepared from the MIX total RNA.

a: proportion of rRNA reads detected in the raw reads.

b: number of sequences remaining after the data quality control pipeline applied on raw reads.

c: proportion of cleaned reads uniquely mapped on CDS sequences.

d: duplication rate estimated on 100 000 cleaned reads.

e: for the MIX libraries, the sequences attributed to *L. lactis* were extracted and the rates were calculated for the MIX and for *L. lactis* separately.

Additional File 1: Table S5. Sequences statistics for *A. baylyi*, *B. subtilis*, *E. coli* libraries

Library name	Raw reads (millions)	% rRNA ^a	Cleaned reads ^b (millions)	% mapped reads ^c	% duplication rate ^d	
TS_ <i>A. baylyi</i>	1.770	0.15	1.762	73.7	2.59	
ENC_ <i>A. baylyi</i>	0.698	42.17	0.402	68.3	6.52	
OV_ <i>A. baylyi</i>	0.804	0.45	0.797	67.8	1.78	
SMART_ <i>A. baylyi</i>	0.707	1.16	0.693	70.3	10.28	
TS_ <i>B. subtilis</i>	0.814	0.59	0.801	61.4	8.6	
ENC_ <i>B. subtilis</i>	0.671	17.47	0.552	64.1	7.45	
OV_ <i>B. subtilis</i>	0.773	0.8	0.762	39.1	16.71	
SMART_ <i>B. subtilis</i>	0.661	0.94	0.649	60.9	18.38	
TS_ <i>E. coli</i>	2.164	0.53	2.146	69.8	4.71	
ENC_ <i>E. coli</i>	0.585	56.58	0.253	67.6	6.39	
OV_ <i>E. coli</i>	0.635	0.7	0.626	56	3.25	
SMART_ <i>E. coli</i>	0.947	2.5	0.893	58.2	14.37	
control ^e	TS_ <i>A. baylyi</i>	21.495	91.25	1.849	72.7	4.72
	OV_ <i>A. baylyi</i>	31.253	66.6	10.145	74.8	2.96
	SMART_ <i>A. baylyi</i>	21.755	94.07	1.251	76.4	21.2
	TS_ <i>B. subtilis</i>	27.213	90.56	2.524	63.5	9.45
	OV_ <i>B. subtilis</i>	25.434	54.8	11.267	73.6	2.7
	SMART_ <i>B. subtilis</i>	25.431	88.3	2.865	56.7	20.12
	TS_ <i>E. coli</i>	28.543	93.3	1.862	70.7	4.84
	OV_ <i>E. coli</i>	22.536	87.6	2.696	68.9	2
	SMART_ <i>E. coli</i>	26.207	93.7	1.591	61.8	25.7

a: proportion of rRNA reads detected in the raw reads.

b: number of sequences remaining after the data quality control pipeline applied on raw reads.

c: proportion of cleaned reads uniquely mapped on CDS sequences.

d: duplication rate estimated on 100 000 cleaned reads.

e: library prepared from total RNA.

Additional File 1: Table S6. Proportions of detected genes and of CDS covered bases for other bacteria species libraries

Library name		% detected genes ^a	% CDS covered bases ^a
control ^b	TS_ <i>A. baylyi</i>	98.3	82.3
	ENC_ <i>A. baylyi</i>	92.8	76.1
	OV_ <i>A. baylyi</i>	95.7	73.2
	SMART_ <i>A. baylyi</i>	96.3	73.5
	TS_ <i>B. subtilis</i>	86	65.9
	ENC_ <i>B. subtilis</i>	84.4	64.9
	OV_ <i>B. subtilis</i>	71.4	35.2
	SMART_ <i>B. subtilis</i>	84.5	53.7
	TS_ <i>E. coli</i>	95.4	83.1
	ENC_ <i>E. coli</i>	80	54.7
	OV_ <i>E. coli</i>	85.6	56.7
	SMART_ <i>E. coli</i>	88.2	63.8
control ^b	TS_ <i>A. baylyi</i>	98.4	94.7
	OV_ <i>A. baylyi</i>	93.5	80.8
	SMART_ <i>A. baylyi</i>	94.5	76.4
	TS_ <i>B. subtilis</i>	95.3	73.7
	OV_ <i>B. subtilis</i>	82	54.1
	SMART_ <i>B. subtilis</i>	86	56.2
	TS_ <i>E. coli</i>	95.2	82.5
	OV_ <i>E. coli</i>	67.4	36.1
	SMART_ <i>E. coli</i>	81.9	55.1

a: proportions calculated by mapping the cleaned reads on the annotations.

b: library prepared from total RNA.

Additional File 1: Table S7. Pearson correlation coefficients related to *A. baylyi*, *B. subtilis*, *E. coli* libraries comparisons

		depleted RNA vs total RNA ^a	depleted vs depleted TS ^b	total RNA vs total RNA TS ^c
<i>A. baylyi</i>	TS	0.901		
	ENC		0.459	
	OV	0.959	0.687	0.683
	SMART	0.987	0.851	0.864
<i>B. subtilis</i>	TS	0.906		
	ENC		0.394	
	OV	0.663	0.635	0.555
	SMART	0.973	0.679	0.785
<i>E. coli</i>	TS	0.993		
	ENC		0.57	
	OV	0.797	0.673	0.682
	SMART	0.961	0.829	0.828

Pearson correlation coefficients between:

a: the depleted RNA library and the total RNA library for each experiment.

b: ENC, OV or SMART library prepared with depleted RNA versus TS library prepared with depleted RNA.

c: ENC, OV or SMART library prepared with total RNA versus TS library prepared with total RNA.

Additional File 1: Table S8. Oligonucleotides used for qRT-PCR analysis

Highly expressed genes ^a	
<i>lmg_0617</i> <i>eno</i> Enolase	CTTGAAACAGCTGTCGGTGA CGTCTTCAGTTCCGTCGAAT
<i>lmg_2050</i> <i>tuf</i> Elongation factor Tu	ATACGACTTCCCAGGTGACG AAAGCACCAAGTGCTGAACC
moderately expressed genes ^a	
<i>lmg_1543</i> <i>nrdE</i> ribonucleotide-diphosphate reductase subunit alpha	GATGGTGGCTAACCCAGAAA GTTCAAGCTCACGGGCATTA
<i>lmg_0124</i> <i>secA</i> Protein translocase subunit SecA	GCTGGTCAACAAGGAGCAGT TGATATCTGTCCCACGACCA
low expressed genes ^a	
<i>lmg_1183</i> <i>gltB</i> glutamate synthase, large subunit	ATACGGACACCGAGGACAAG TCGGTACTGGAAGGAACCAC
<i>lmg_1217</i> <i>thiD1</i> phosphomethylpyrimidine kinase	GTTGAAGGGGTGATTCAAGG GAATAGCCCCACCTTTAGCC
Differentially expressed genes ^b	
<i>lmg_0318</i> <i>tpx</i> thiol peroxidase	GAGGGGCAAATCGTCTATAGTG TTAGGCTCATCCGAAAGCTC
<i>lmg_0355</i> <i>gpmA</i> 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	CGTCGTTATGCTGGTCTTGA AGAGCGCGTTCCAAAGTAAC
<i>lmg_0195</i> putative NADH dehydrogenase	GAGCGGTCATTGTCCTTTTC TCTTGCAATCTCCCGAGAAC
<i>lmg_2023</i> universal stress protein A	TCGTTCTTGGAGCTACTGGTC CCACGGAGCCGATAAAGATA

a: genes selected on the basis of TS *L.lactis* experiments.

b: genes detected as differentially expressed between TS and OV or ENC experiments.