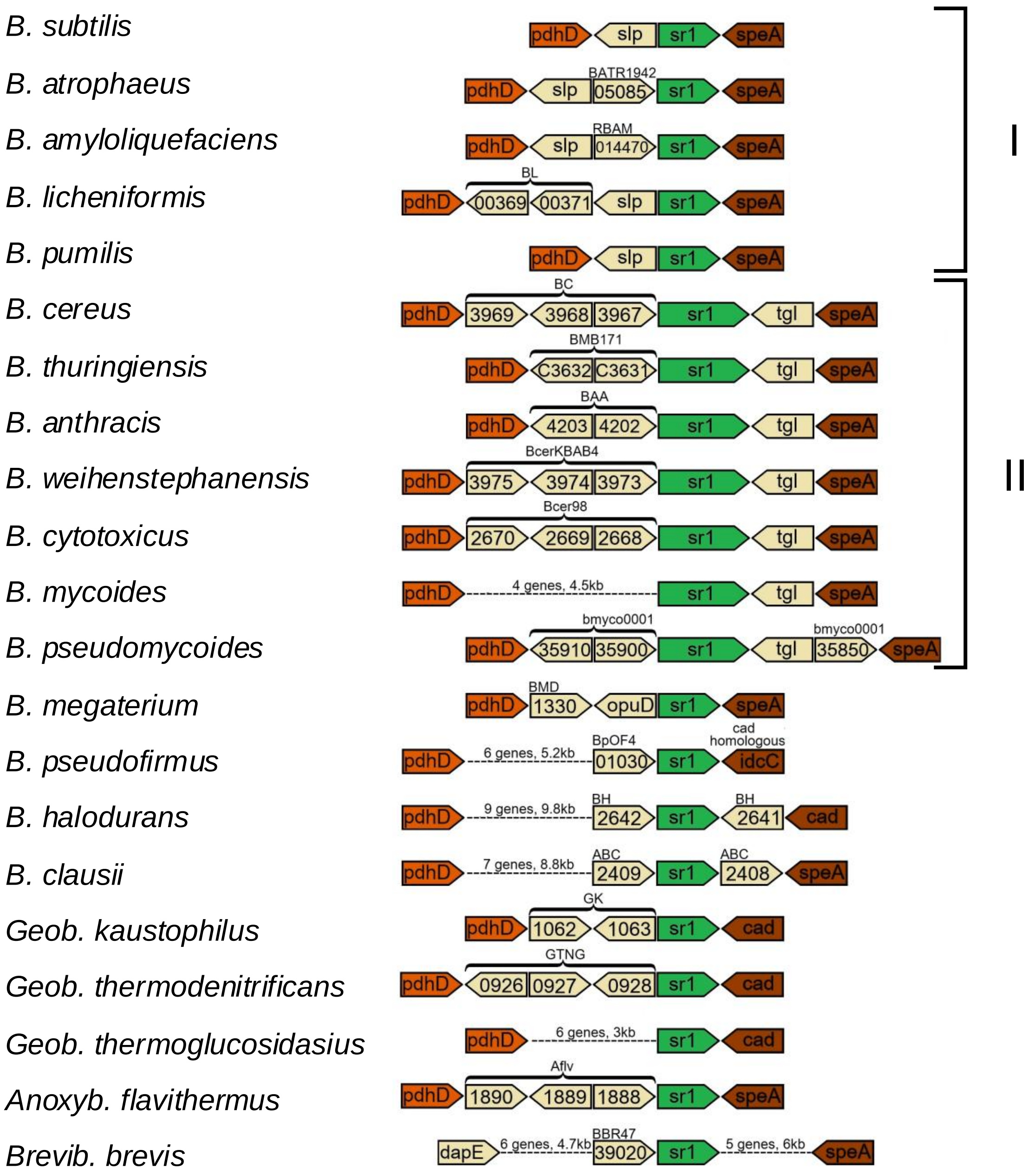


Figure S1. Gene synteny of the region centred on *sr1*

The gene synteny of each specific *sr1* homologue is shown with the homologue in green. The genes encoding PdhD and SpeA/Cad are considered bounderies of the region and shown in red and dark red, respectively. The *sr1p* ORF is present in all cases in comparable locations. In *B. pseudofirmus, B. halodurans, B. clausii, Geob. thermoglucosidasius* and *Brev. brevis*, the gene encoding PdhD or its analogue is located further upstream of *sr1*, shown by the dashed line and stated distance; the additional genes between *sr1* and *pdhD* are not individually represented. The same holds true for *Brev. brevis*, whose *speA* gene is located further downstream from *sr1*. The gene arrangements for the *sr1* homologues from *B. coagulans* and *B. coahuilensis* are not included as genome sequencing and annotation are not yet complete. I, *B. subtilis* group; II, *B. cereus* group.

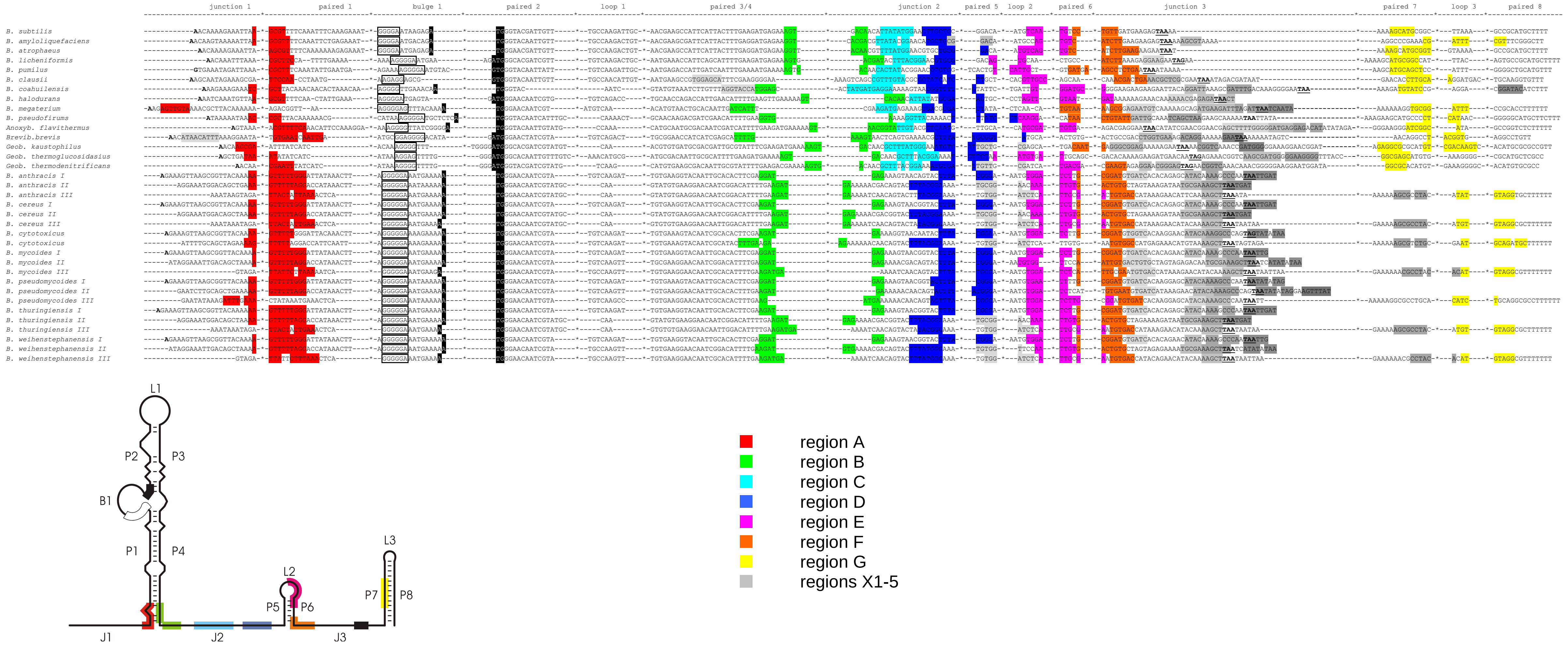


Figure S2. Alignment of SR1 sequences

The nucleotide sequences of the SR1 homologues were aligned based on the predicted RNA secondary structures. Start codons are highlighted in black, SD sequences are boxed, and stop codons are in bold and underlined. Regions complementary to the corresponding *ahrC* homologues are highlighted in colour. Above, paired regions, loops and single-stranded junctions between stem-loops are indicated. Below, the secondary structure of *B. subtilis* SR1 with the accordingly coloured complementary regions is shown.

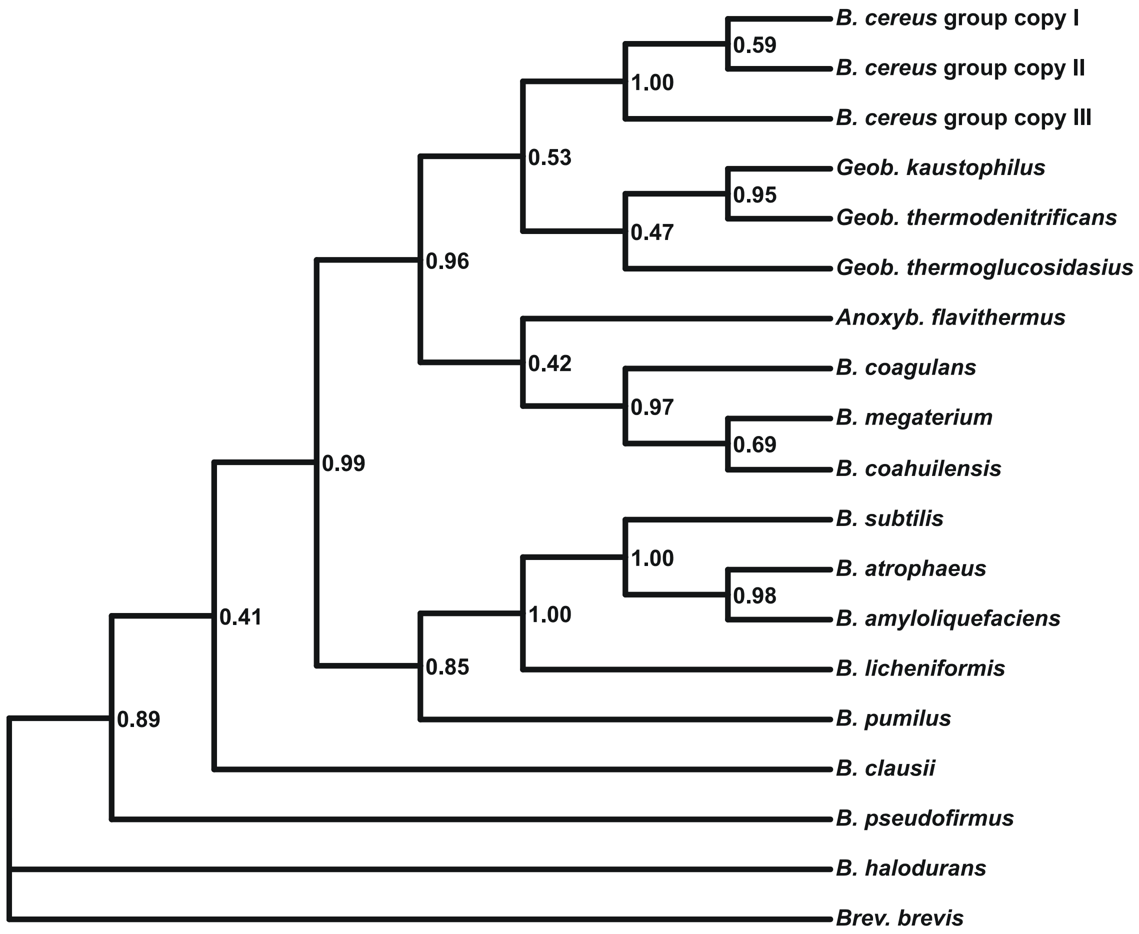


Figure S3. Phylogenetic tree based on the nt sequence of the SR1 homologues.
The phylogenetic tree was generated as described in Materials and Methods. A consensus sequence for each SR1 copy of the *B. cereus* group members was derived and used as representative of the entire *B. cereus* group.

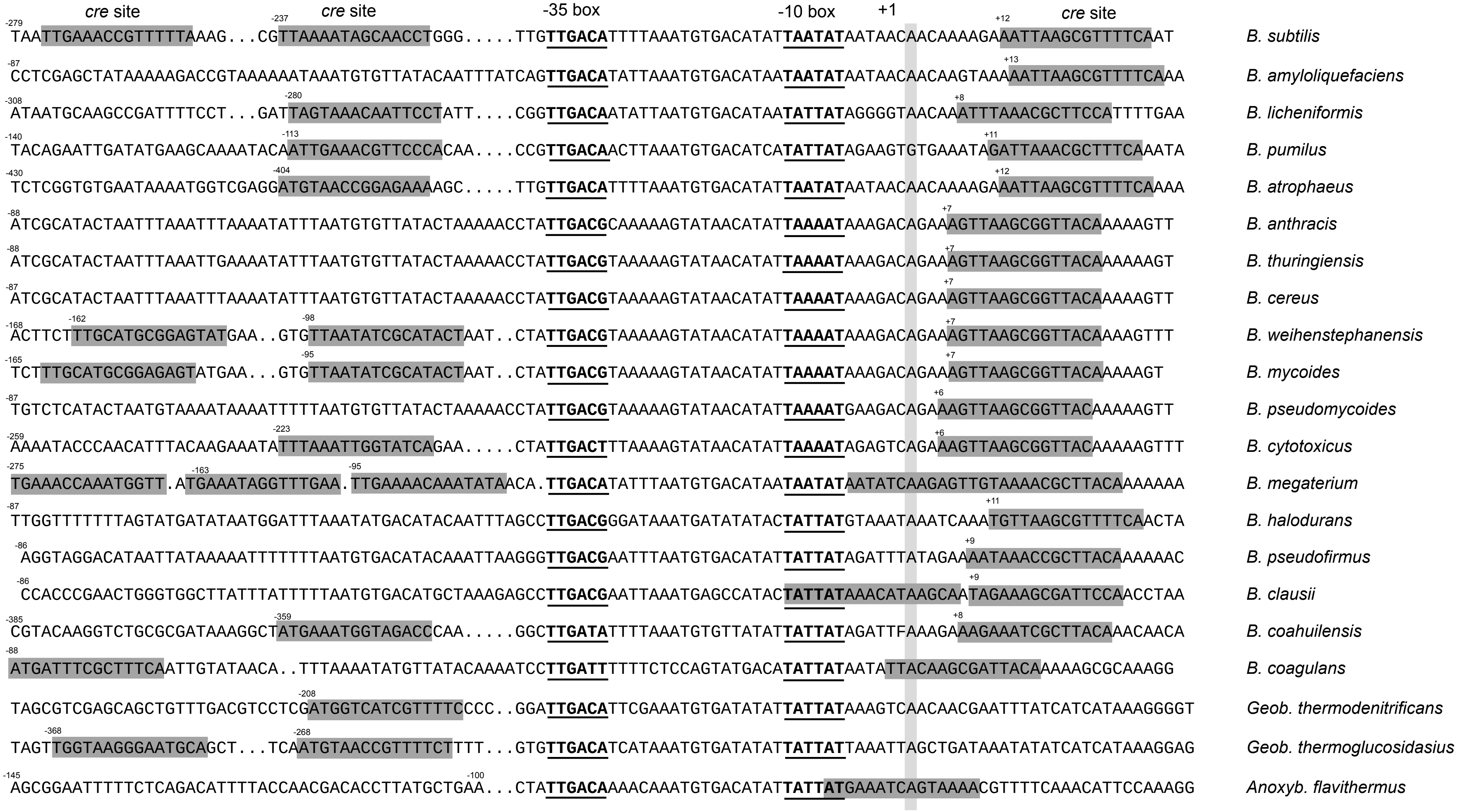


Figure S4. Binding sites for CcpA in the promoter regions of the *sr1* homologues.
Putative CcpA binding sites (*cre* sites). Hypothetical *cre* sites are shown in grey. The consensus sequence for the *B. subtilis cre* site is WTGNAANCGNWNNCW, thereby W = A or T, N = A, C, G or T. In the vicinity of or in the *sr1* promoters of *Geobacillus kaustophilus* and *Brevibacillus brevis*, no *cre* sites were found.

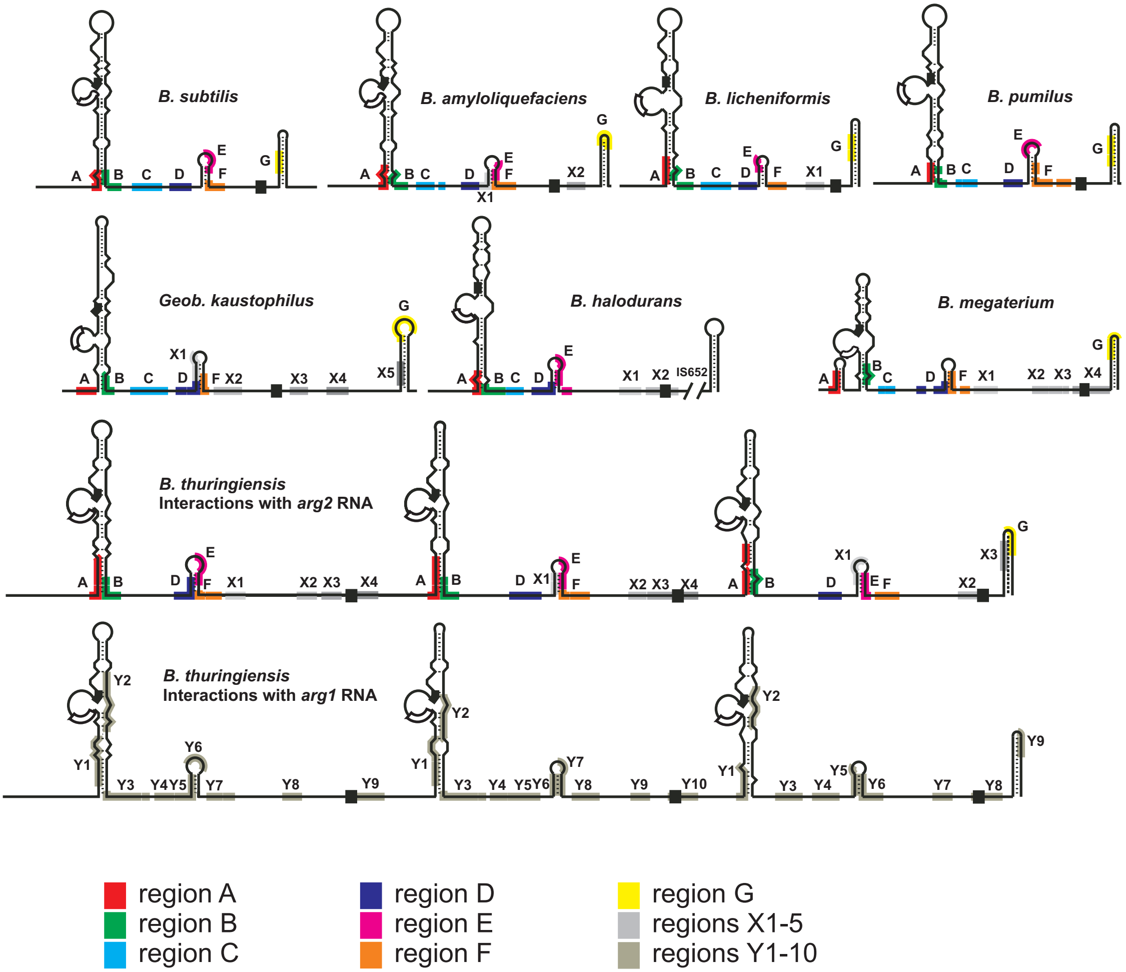
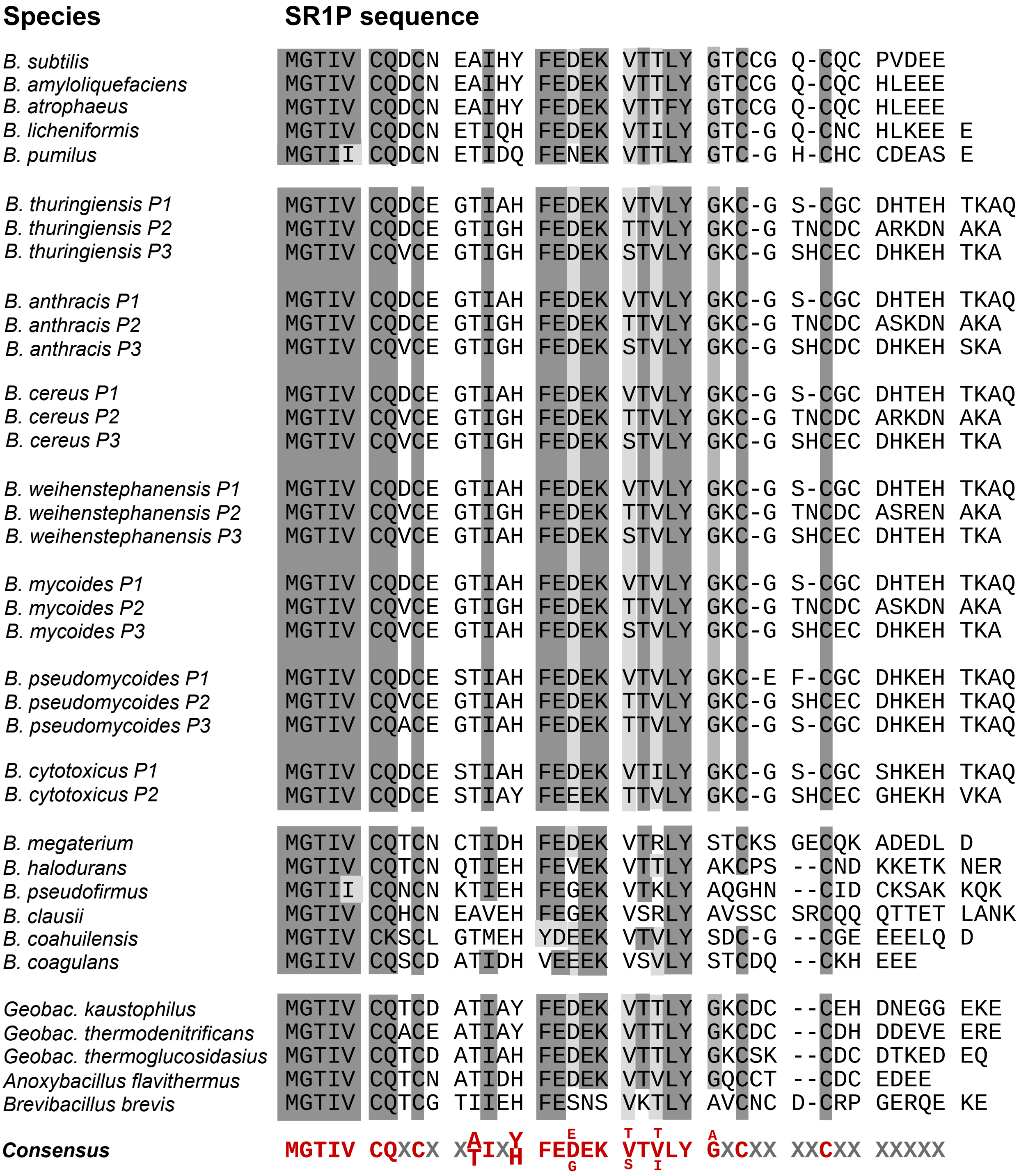


Figure S5. Secondary structures of 8 SR1 homologues

The hypothetical secondary structures of the 8 SR1 homologues analysed in this work are shown. The secondary structure of *B. anthracis* SR1 corresponds to that of *B. thuringiensis*, and is, therefore, not shown. These structures were derived from the primary nt sequences on the basis of the experimentally determined secondary structure of *B. subtilis* SR1. SD sequences of SR1P are shown s white boxes, start and stop codons as black squares. The regions complementary to the respective *ahrC/arg2*-mRNAs are coloured as indicated below and designated with letters A to G and X1 to X5 according to Figs. S2 and S8. In *B. thuringiensis*, two *ahrC* homologues, *arg1* and *arg2*, are present. The upper structure shows the putative interactions for all three *sr1* subsequences with *arg2* mRNA which is homologous to *B. subtilis ahrC* mRNA, whereas the structure below shows its putative interactions with *arg1* mRNA. Here, the complementary regions are designated Y1 to Y10. In *B. halodurans*, the *sr1* gene is interrupted by IS652 that is inserted between the SR1P ORF and the transcription terminator. (For more details of the interactions see Table S3 and Fig. S8).



Species	SR1P sequence
B. subtilis	MGTIV CQDCN EAIHY FEDEK VTTLY GTCCG Q-CQC PVDEE
B. amyloliquefaciens	MGTIV CQDCN EAIHY FEDEK VTTLY GTCCG Q-CQC HLEEE
B. atrophaeus	MGTIV CQDCN EAIHY FEDEK VTTFY GTCCG Q-CQC HLEEE
B. licheniformis	MGTIV CQDCN ETIQH FEDEK VTILY GTC-G Q-CNC HLKEE E
B. pumilus	MGTII CQDCN ETIDQ FENEK VTTLY GTC-G H-CHC CDEAS E
B. thuringiensis P1	MGTIV CQDCE GTIAH FEDEK VTVLY GKC-G S-CGC DHTEH TKAQ
B. thuringiensis P2	MGTIV CQDCE GTIGH FEDEK TTVLY GKC-G TNCDC ARKDN AKA
B. thuringiensis P3	MGTIV CQVCE GTIGH FEDEK STVLY GKC-G SHCEC DHKEH TKA
B. anthracis P1	MGTIV CQDCE GTIAH FEDEK VTVLY GKC-G S-CGC DHTEH TKAQ
B. anthracis P2	MGTIV CQDCE GTIGH FEDEK TTVLY GKC-G TNCDC ASKDN AKA
B. anthracis P3	MGTIV CQVCE GTIGH FEDEK STVLY GKC-G SHCDC DHKEH SKA
B. cereus P1	MGTIV CQDCE GTIAH FEDEK VTVLY GKC-G S-CGC DHTEH TKAQ
B. cereus P2	MGTIV CQVCE GTIGH FEDEK TTVLY GKC-G TNCDC ARKDN AKA
B. cereus P3	MGTIV CQVCE GTIGH FEDEK STVLY GKC-G SHCEC DHKEH TKA
B. weihenstephanensis P1	MGTIV CQDCE GTIAH FEDEK VTVLY GKC-G S-CGC DHTEH TKAQ
B. weihenstephanensis P2	MGTIV CQVCE GTIGH FEDEK TTVLY GKC-G TNCDC ASREN AKA
B. weihenstephanensis P3	MGTIV CQVCE GTIAH FEDEK STVLY GKC-G SHCEC DHTEH TKA
B. mycoides P1	MGTIV CQDCE GTIAH FEDEK VTVLY GKC-G S-CGC DHTEH TKAQ
B. mycoides P2	MGTIV CQVCE GTIGH FEDEK TTVLY GKC-G TNCDC ASKDN AKA
B. mycoides P3	MGTIV CQVCE GTIAH FEDEK STVLY GKC-G SHCEC DHKEH TKA
B. pseudomycoides P1	MGTIV CQDCE STIAH FEDEK VTVLY GKC-E F-CGC DHKEH TKAQ
B. pseudomycoides P2	MGTIV CQVCE GTIAH FEDEK TTVLY GKC-G SHCEC DHKEH TKAQ
B. pseudomycoides P3	MGTIV CQACE GTIAH FEDEK TTVLY GKC-G S-CGC DHKEH TKAQ
B. cytotoxicus P1	MGTIV CQDCE STIAH FEDEK VTILY GKC-G S-CGC SHKEH TKAQ
B. cytotoxicus P2	MGTIV CQDCE STIAY FEEEK TTVLY GKC-G SHCEC GHEKH VKA
B. megaterium	MGTIV CQTCN CTIDH FEDEK VTRLY STCKS GECQK ADEDL D
B. halodurans	MGTIV CQTCN QTIEH FEVEK VTTLY AKCPS --CND KKETK NER
B. pseudofirmus	MGTII CQNCN KTIEH FEGEK VTKLY AQGHN --CID CKSAK KQK
B. clausii	MGTIV CQHCN EAVEH FEGEK VSRLY AVSSC SRCQQ QTTET LANK
B. coahuilensis	MGTIV CKSCL GTMEH YDEEK VTVLY SDC-G --CGE EEELQ D
B. coagulans	MGIIV CQSCD ATIDH VEEEK VSVLY STCDQ --CKH EEE
Geobac. kaustophilus	MGTIV CQTCD ATIAY FEDEK VTTLY GKCDC --CEH DNEGG EKE
Geobac. thermodenitrificans	MGTIV CQACE ATIAY FEDEK VTTLY GKCDC --CDH DDEVE ERE
Geobac. thermoglucosidasius	MGTIV CQTCD ATIAH FEDEK VTTLY GKCSK --CDC DTKED EQ
Anoxybacillus flavithermus	MGTIV CQTCN ATIDH FEDEK VTVLY GQCCT --CDC EDEE
Brevibacillus brevis	MGTIV CQTCG TIIEH FESNS VKTLY AVCNC D-CRP GERQE KE
Consensus	MGTIV CQXCX X$\underset{T}{A}$IX$\underset{H}{Y}$ FE$\overset{E}{\underset{G}{D}}$EK $\overset{T}{\underset{S}{V}}T\overset{T}{\underset{I}{V}}$LY $\overset{A}{G}$XCXX XXCXX XXXXX

Figure S6. Alignment of SR1P homologues.
ClustalW alignment of SR1P homologues. The SR1P sequences from 23 species were aligned. In the case of the *B. cereus* group, three or two (*B. cytotoxicus*) aa sequences have to be considered, which were designated with P1, P2 and P3 in the order in which they are encoded on the *sr1* genes. Identical aa are highlighted in dark grey, less conserved aa in light grey. Below, a consensus sequence derived from all sequences is presented. Red, conserved aa, dark grey X indicates any aa.

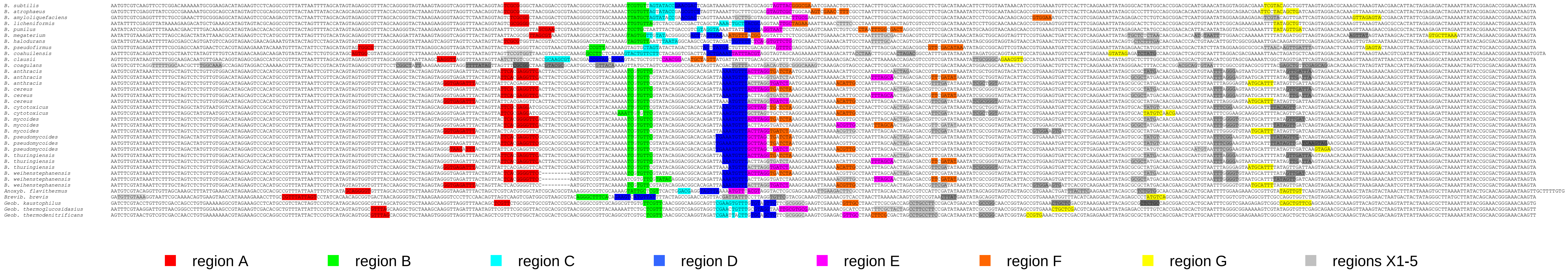
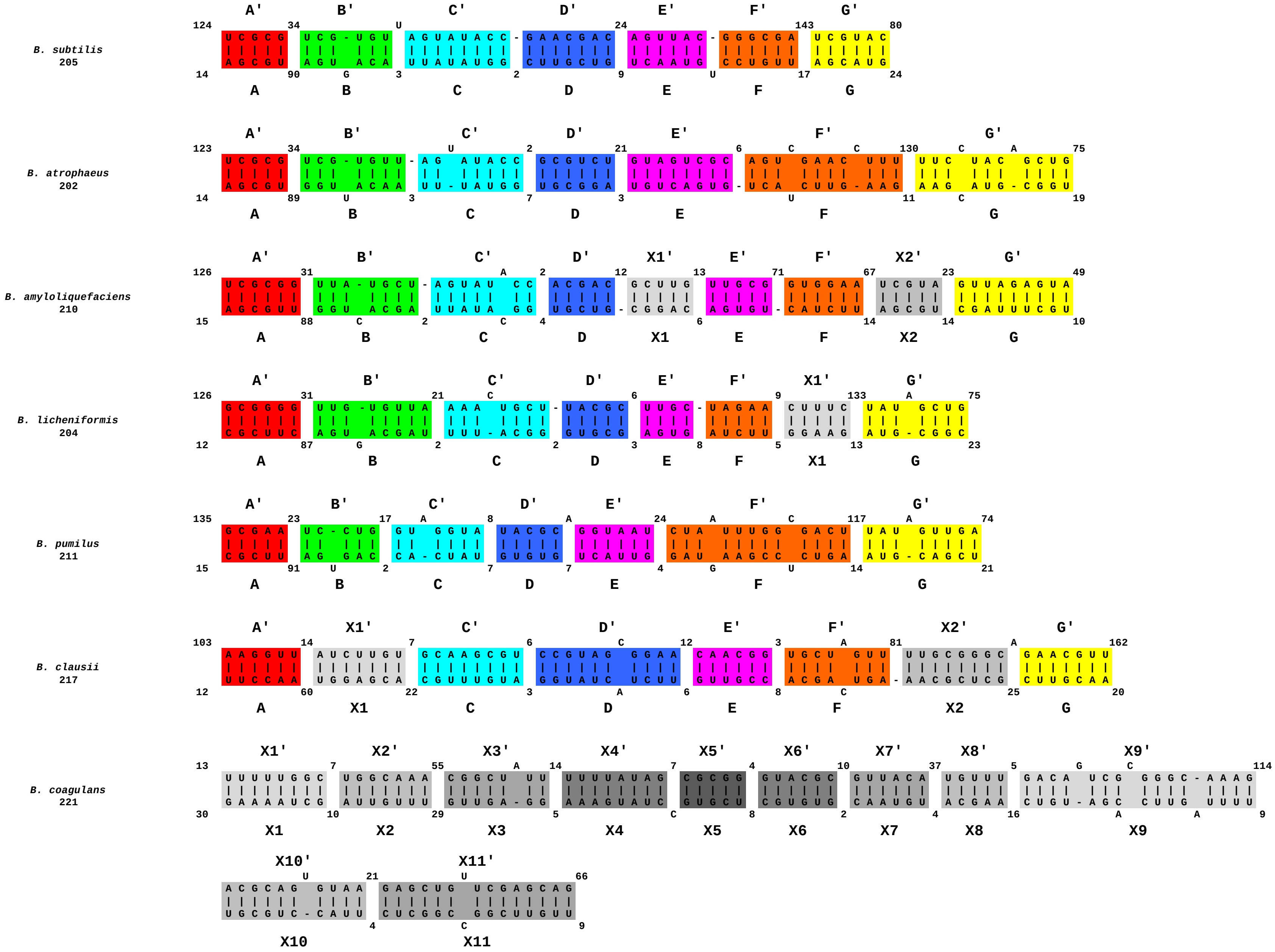
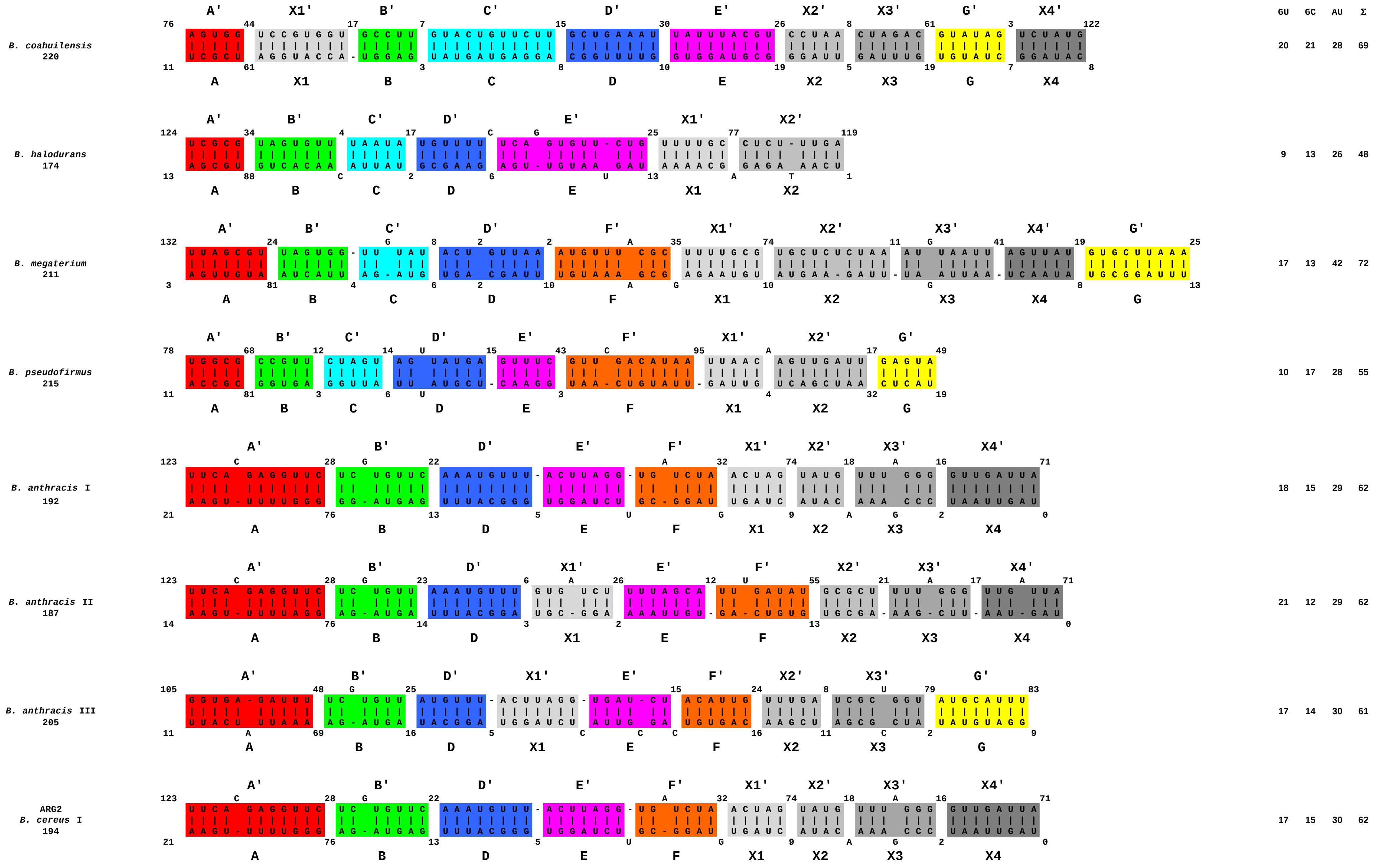


Figure S7. FASTA format text file of the ClustalW alignment of the *ahrC/arg* coding regions
Complementary regions are highlighted in colour as in Fig. S2.



	GU	GC	AU	Σ
B. subtilis 205	5	19	20	44
B. atrophaeus 202	12	20	21	53
B. amyloliquefaciens 210	14	20	21	55
B. licheniformis 204	12	17	18	47
B. pumilus 211	8	18	21	47
B. clausii 217	9	25	25	59
B. coagulans 221	21	33	37	91


GU
GC
AU
Σ
B. coahuilensis
220
A' X1' B' C' D' E' X2' X3' G' X4'
A X1 B C D E X2 X3 G X4
20 21 28 69
B. halodurans
174
A' B' C' D' E' X1' X2'
A B C D E X1 X2
9 13 26 48
B. megaterium
211
A' B' C' D' F' X1' X2' X3' X4' G'
A B C D F X1 X2 X3 X4 G
17 13 42 72
B. pseudofirmus
215
A' B' C' D' E' F' X1' X2' G'
A B C D E F X1 X2 G
10 17 28 55
B. anthracis I
192
A' B' D' E' F' X1' X2' X3' X4'
A B D E F X1 X2 X3 X4
18 15 29 62
B. anthracis II
187
A' B' D' X1' E' F' X2' X3' X4'
A B D X1 E F X2 X3 X4
21 12 29 62
B. anthracis III
205
A' B' D' X1' E' F' X2' X3' G'
A B D X1 E F X2 X3 G
17 14 30 61
ARG2
B. cereus I
194
A' B' D' E' F' X1' X2' X3' X4'
A B D E F X1 X2 X3 X4
17 15 30 62

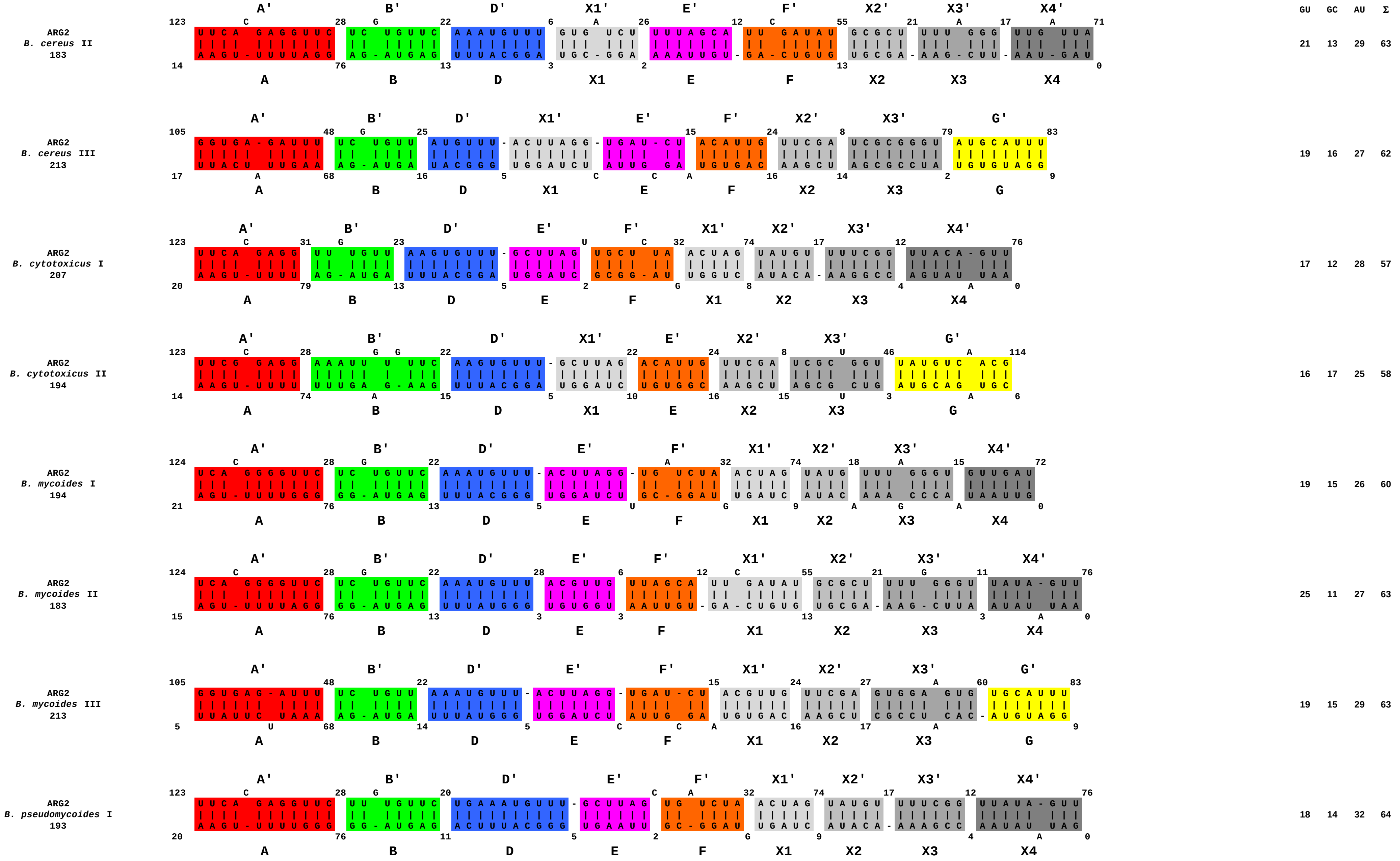
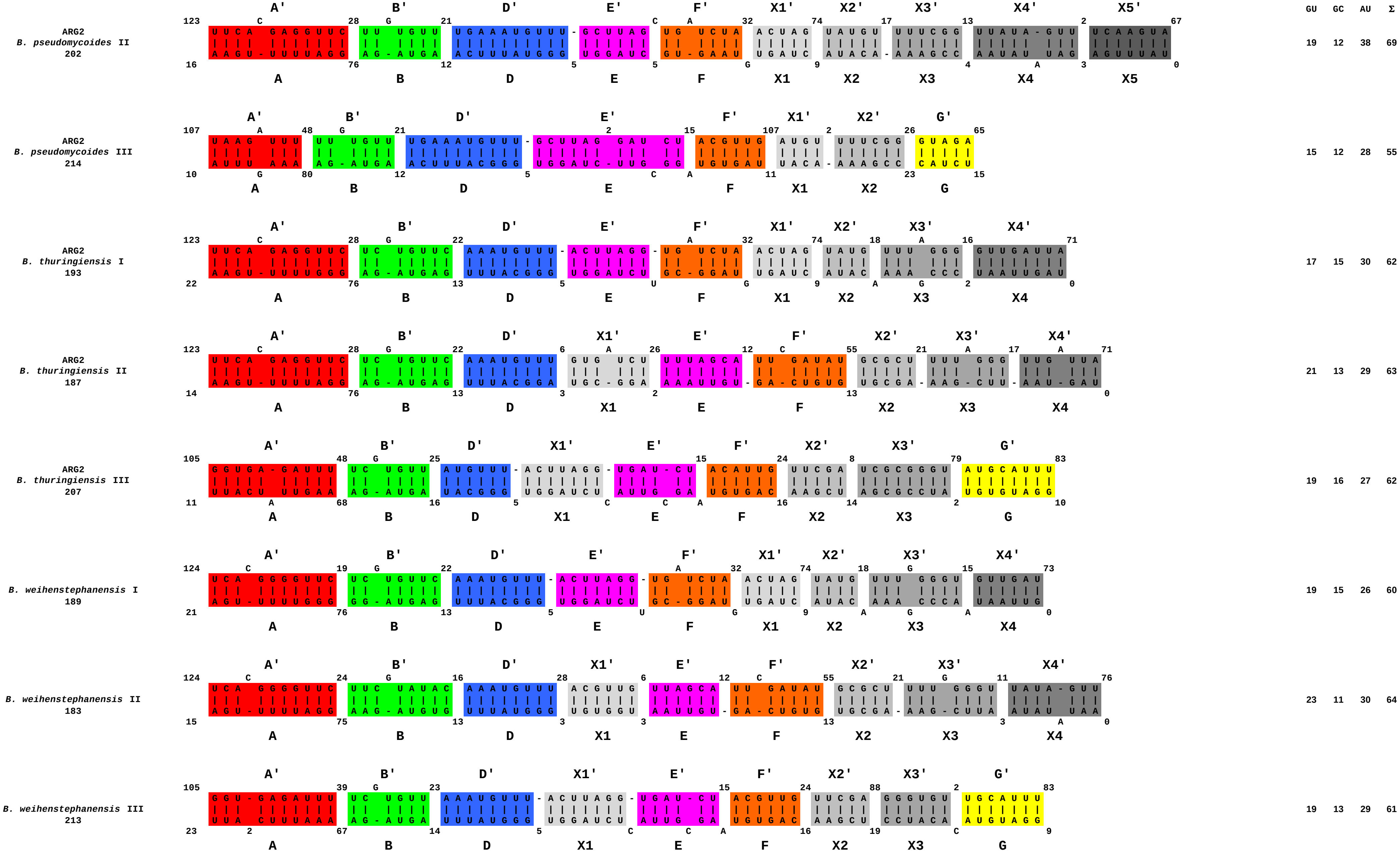
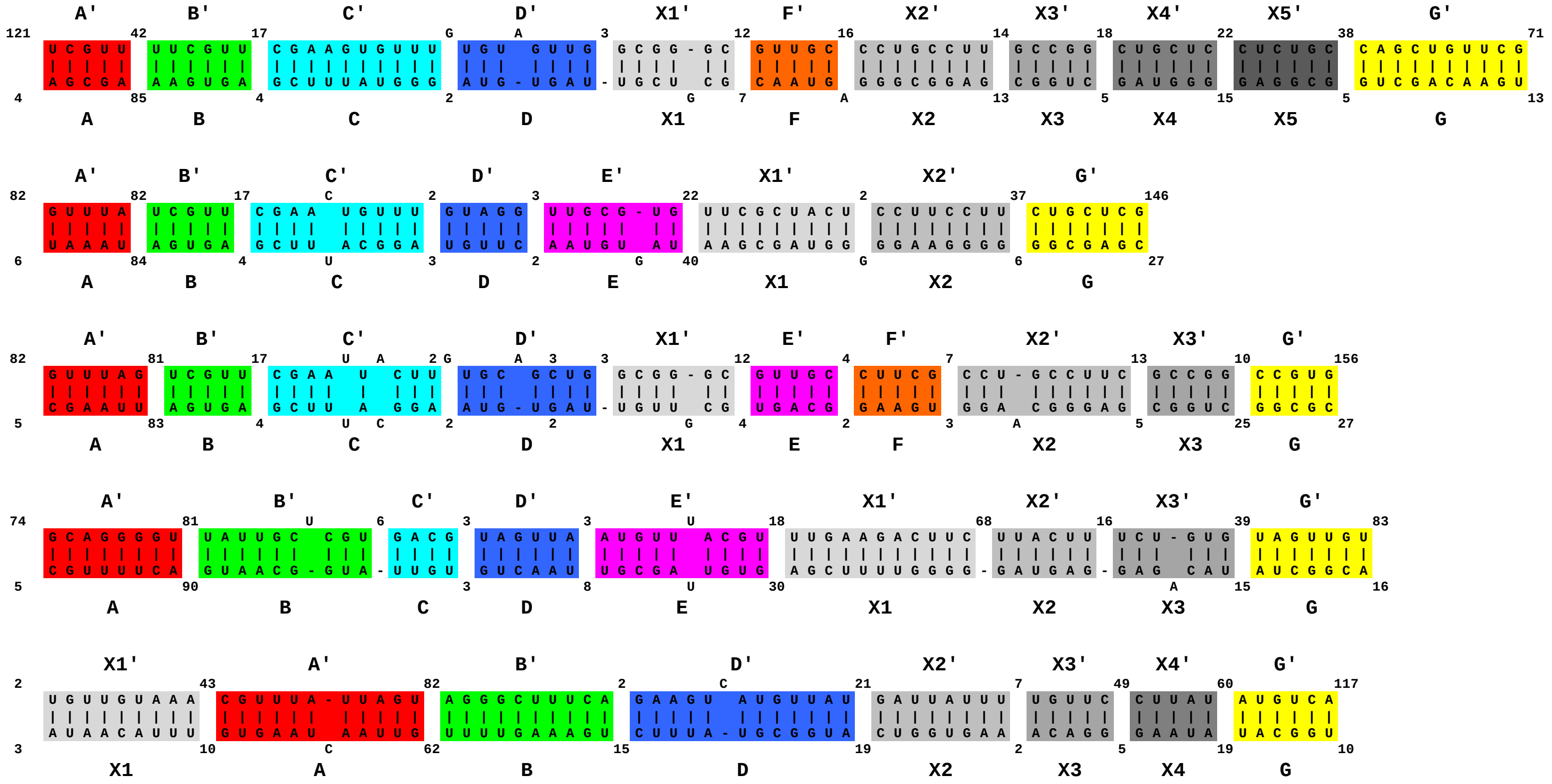

GU GC AU Σ
ARG2 B. cereus II 183
21 13 29 63
ARG2 B. cereus III 213
19 16 27 62
ARG2 B. cytotoxicus I 207
17 12 28 57
ARG2 B. cytotoxicus II 194
16 17 25 58
ARG2 B. mycoides I 194
19 15 26 60
ARG2 B. mycoides II 183
25 11 27 63
ARG2 B. mycoides III 213
19 15 29 63
ARG2 B. pseudomycoides I 193
18 14 32 64
A' B' D' X1' E' F' X2' X3' X4' G'
A B D X1 E F X2 X3 X4 G

Figure S8 - page 4 of 7





	GU	GC	AU	Σ
Geob.kaustophilus 229	23	32	19	74
Geob. thermoglucosidasius 229	15	19	21	55
Geob. thermodenitrificans 225	17	28	16	61
Anoxyb. flavithermus 235	23	17	25	65
Brevib. brevis 212	17	12	37	66

ARG1
B. cereus I
194

```
               Y1'                     Y2'                 Y3'               Y4'               Y5'             Y6'           Y7'             Y8'           Y9'
158             A             10                  62                  26                15                3           C         G       13          10              81
    A A A A U U   U U G G U A   U G U G U U A G U   A C U C U U U U G   U U - C C A U G   G A G U G C U U   G C G C U   U U U G   C A U   U A U G U   G A U U G U U
    | | | | | |   | | | | | |   | | | | | | | | |   | | | | | | | | |   | |   | | | | |   | | | | | | | |   | | | | |   | | | |   | | |   | | | | |   | | | | | | |
    U U U U G G - G A U U A U   G U A C A A U U G   U G A G A A A G U - A A   G G U A C - U U U A C G G G   U G U G G   G G A U - G U G   A U A C A   U U G A U A G
22                            52                  13                        C                             3           7                 12          12              0
               Y1                      Y2                  Y3                Y4                Y5              Y6            Y7              Y8            Y9
```

ARG1
B. cereus II
183

```
               Y1'                    Y2'                Y3'                Y4'               Y5'            Y6'            Y7'               Y8'            Y9'           Y10'
158             A             12                61                  25    U           15                3           C                 5       A                     10                80
    A A A A U U   U U G G U A   U G U U A G U U   A C U C U U U U G   C U   C C A U G   G A G U G C U U   G C G C U   U U U G G C A U   A C A   U G A U - U A U G U   G A U U G U U A
    | | | | | |   | | | | | |   | | | | | | | |   | | | | | | | | |   | |   | | | | |   | | | | | | | |   | | | | |   | | | | | | | |   | | |   | | | |   | | | | |   | | | | | | | |
    U U U U A G - G A C C A U   A C A A U C G G   U G A G A A A A C - G A   G G U A C - U U U A C G G A   U G C G G   A A A U U G U G   U G U - G C U A   A U G C G   U U A A U G A U
17                            54                12                        C                             3           3                 2                 9           5                 0
               Y1                     Y2                 Y3                 Y4                Y5             Y6             Y7                Y8             Y9             Y10
```

ARG1
B. cereus III
213

```
         Y1'              Y2'              Y3'              Y4'              Y5'                Y6'              Y7'            Y8'             Y9'
144               32                6               24        A       2               65                    27          10                38            36
    U A A U G G U   U G U U A G U U   G U U G U U G   A U A U   G U C   A C A U U U A   A G U G C U U A U A   U A U G U   G A U U G U U A   A U G U U U
    | | | | | | |   | | | | | | | |   | | | | | | |   | | | |   | | |   | | | | | | |   | | | | | | | | | |   | | | | |   | | | | | | | |   | | | | | |
    A U U A C U A   A C A A U U G G   C A A C A G U   U A U A - C G G   U G U G G A U   U U G C G A A U G U   A U A C A   U U A A U A A U   U G U A G G
16                56                20              2                 4               4                     13          5                 19            9
         Y1               Y2               Y3               Y4               Y5                 Y6               Y7             Y8              Y9
```

ARG1
B. cytotoxicus I
198

```
             Y1'                   Y2'                  Y3'              Y4'          Y5'            Y6'              Y7'               Y8'
158             A         12      A           62      C             2           5             33              25                10                80
    A A A A U U   U U G G   U G U   U U A G U   G C U   U U U U U A   U A U A U   U U A G G A   G C U U A U A   U C U - G U G U   G G U U A U U A
    | | | | | |   | | | |   | | |   | | | | |   | | |   | | | | | |   | | | | |   | | | | | |   | | | | | | |   | | |   | | | |   | | | | | | | |
    U U U U G G - G A U U   G U A   A A U C G   U G A - A A A G G U   A U G U G - G A U C U U   C G G A U G U   G G A   C A U A   C C A G U A G U
23                        54      C           13                    18                        G               8       A         7                 11
             Y1                    Y2                   Y3               Y4           Y5             Y6               Y7                Y8
```

ARG1
B. cytotoxicus II
214

```
           Y1'                 Y2'                   Y3'                   Y4'                 Y5'              Y6'           Y7'             Y8'                Y9'
144       U           28      A           6           A           19        A         2                   6             89            9                 36          A       35
    U A A   G G U C C   U G U   U U A G U   U G U U G   U G A A G   A C G U   U A G G   A A C A U U U A C   G C U C U U   U G U G U G   G G U U A U U A   A G A U G   U U A
    | | |   | | | | |   | | |   | | | | |   | | | | |   | | | | |   | | | |   | | | |   | | | | | | | | |   | | | | | |   | | | | | |   | | | | | | | |   | | | | |   | | |
    G U U   U U A G G   G U A   A A U C G   A C A G U - A C U U U   U G U G   A U C U   U U G U G A A U G   U G A G A A - A C A U G U   U U A A U A G U   U C U G C   A A U
11        U           57      C           23                      8         G         2                   6                           6                 12          G       14
           Y1                  Y2                    Y3                    Y4                  Y5               Y6            Y7              Y8                 Y9
```

ARG1
B. pseudomycoides I
193

```
             Y1'                   Y2'                 Y3'              Y4'             Y5'             Y6'           Y7'           Y8'
158             A         12                  62                  27    A       15                5             11            19            89
    A A A A U U   U U A A   U G U G U U A G U   A C U U U U U U A   C C   A U G   A A G U G C U U   G C A C U U   A C A U U G   U U G U U A
    | | | | | |   | | | |   | | | | | | | | |   | | | | | | | | |   | |   | | |   | | | | | | | |   | | | | | |   | | | | | |   | | | | | |
    U U U U G G - G A U U   G U A C A A U U G   U G A G A A A G U   G G - U A C - U U U A C G G G   U G U G A A   U G U G A U   A A U A A U
24                        54                  13                  3                               3             9             22            6
             Y1                    Y2                  Y3               Y4              Y5              Y6            Y7            Y8
```

ARG1
B. pseudomycoides II
202

```
           Y1'               Y2'            Y3'             Y4'               Y5'                Y6'                 Y7'             Y8'          Y9'
158             A     16              29            27                49                5         U         5           A       6           7             89
    A A A A U U   U U   U G U U A G U   G A A A U U   A C U U U U U U   A A G U G C U U   G C A C   U U A G   A A C A C   U U G   U G G U A   U U G U U A
    | | | | | |   | |   | | | | | | |   | | | | | |   | | | | | | | |   | | | | | | | |   | | | |   | | | |   | | | | |   | | |   | | | | |   | | | | | |
    U U U U A G - G A   A C A A U U G   C U U U G A   U G A G A A A A   U U U A U G G G   U G U G - G A U C   U U G U G - A A U   A U C A U   A G U A A U
21                    58              4             2                 8                 3                   3                   3           19            15
           Y1                Y2             Y3              Y4                Y5                 Y6                  Y7              Y8           Y9
```

ARG1
B. pseudomycoides III
214

```
          Y1'              Y2'              Y3'            Y4'           Y5'                Y6'                Y7'             Y8'           Y9'
107                 69              6                 15          14              65                    26              12              27          56
    U U A G A U U U   U G U U A G U   U G U U G U U G   G A A A U   A C A U U U G   A G U G C U U A U A   U G - A U G U   U U G U U A A   G U G U G
    | | | | | | | |   | | | | | | |   | | | | | | | |   | | | | |   | | | | | | |   | | | | | | | | | |   | |   | | | |   | | | | | | |   | | | | |
    G A U U U G A A   A C A A U C G   A C A A C A G U   C U U U A   U G U G G A U   U U G C G G A U G U   G C   U A C A   A A U A A U U   C A C A U
9                   65              20                A           7               C                     11    A         7               12          17
          Y1               Y2               Y3             Y4            Y5                 Y6                 Y7              Y8            Y9
```

	GU	GC	AU	Σ
ARG1 *B. cereus* I 194	26	12	31	69
ARG1 *B. cereus* II 183	18	20	39	77
ARG1 *B. cereus* III 213	18	10	37	65
ARG1 *B. cytotoxicus* I 198	21	9	30	60
ARG1 *B. cytotoxicus* II 214	21	14	36	71
ARG1 *B. pseudomycoides* I 193	16	10	33	59
ARG1 *B. pseudomycoides* II 202	16	10	38	64
ARG1 *B. pseudomycoides* III 214	16	12	35	63

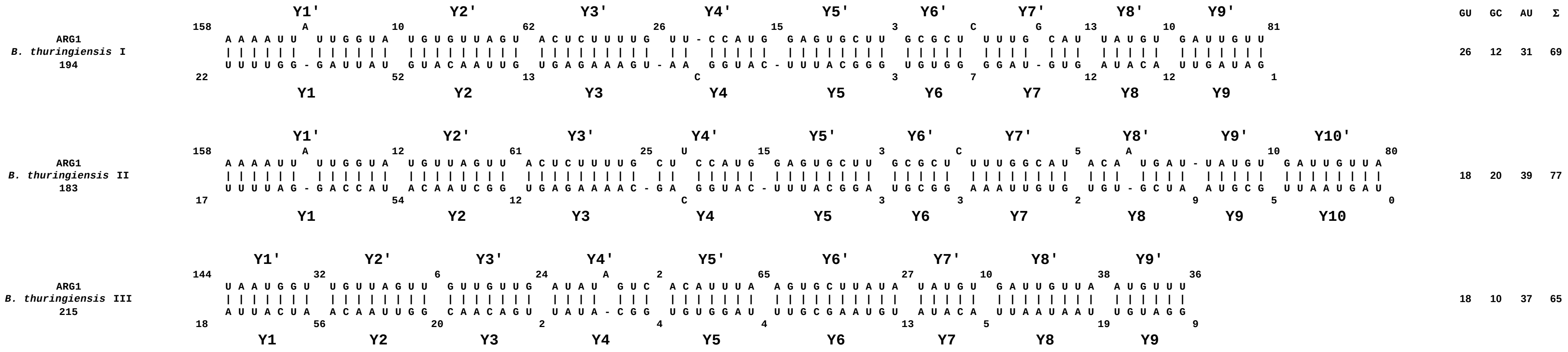


Figure S8. Overview of the predicted SR1-*ahrC/arg* interactions

The potential base pairing between different regions of SR1 homologues and the corresponding *ahrC/arg* coding regions were predicted using IRNA (E. Barth, unpublished) as well as the experimentally verified interactions of SR1 and *ahrC* in *B. subtilis*. All species possess at least seven regions of complementarity with a minimum of 44 bp in total.

The upper strand equates to *ahrC* mRNA shown in 3’ to 5’ direction, the bottom strand to SR1 in 5’ to 3’ direction. The numbers above or below the sequences denote the number of nt between two complementary regions or the predicted ends of SR1 or *ahrC* coding region, respectively. In the case of the triplicate/duplicate SR1 species from the *B. cereus* group members, interactions between every single unit and the corresponding *ahrC/arg* homologue were predicted and the last nt predicted for SR1/*arg2* RNA interaction was denoted as the end of the SR1 unit. The number below the organisms indicates the length of the corresponding SR1 homologue. On the right margin, the total numbers of all base-pairing nt (G-C, A-U, G-U) are shown.

Designations of regions complementary to SR1 with letters and colours are as in Figs. S2 and S7.

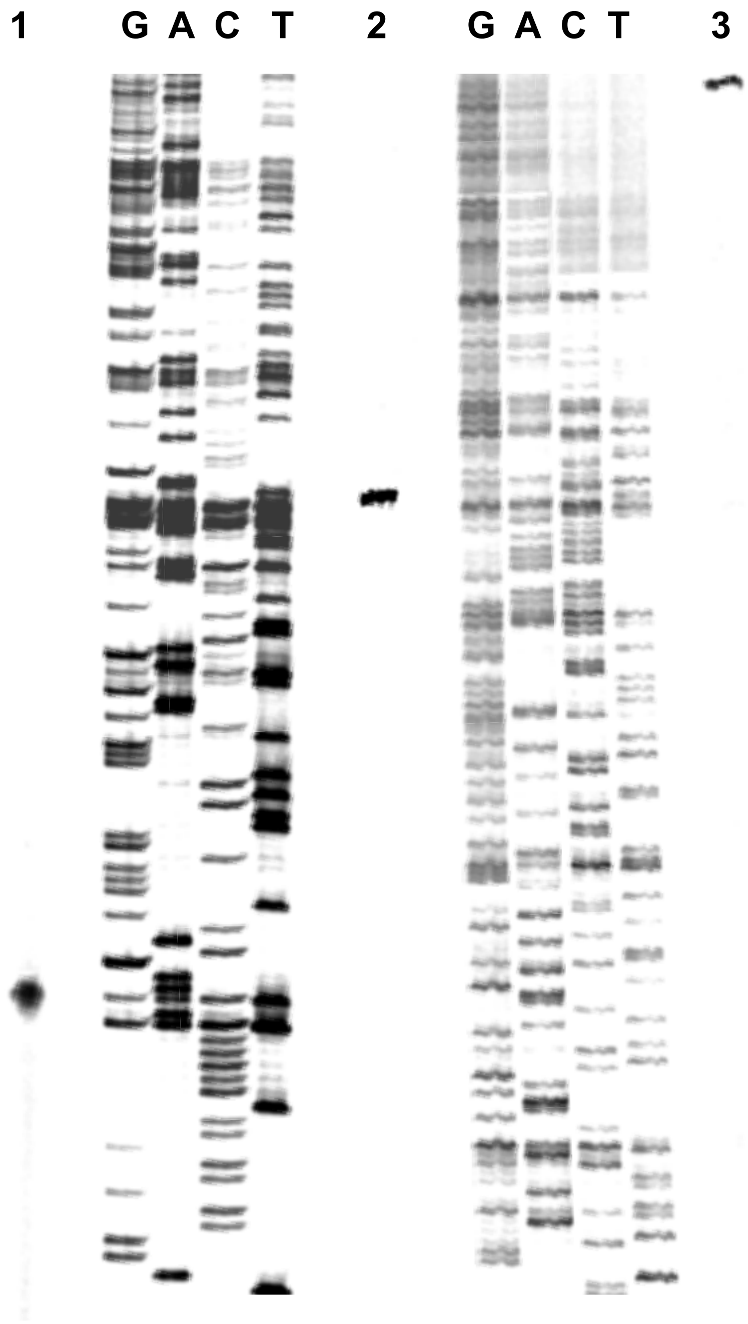


Figure S9. Determination of 5' end of SR1 from *B. thuringiensis*
Lanes 1, 2 and 3 show primer extension reactions with total RNA prepared from *B. thuringiensis* grown in complex TY medium at 37 °C until onset of stationary phase (OD_{560} = 15). For comparison, sequencing reactions were loaded. The following primers were used: lane 1, primer 1941 complementary to SR1-I; lane 2, primer SB1963 complementary to SR1-II and lane 3, primer SB1962 complementary to SR1-III. From all three reactions, the same 5' end of *B. thuringiensis* SR1 could be determined, which was used in the alignment in Figure S2. No indication for a processing event was found.

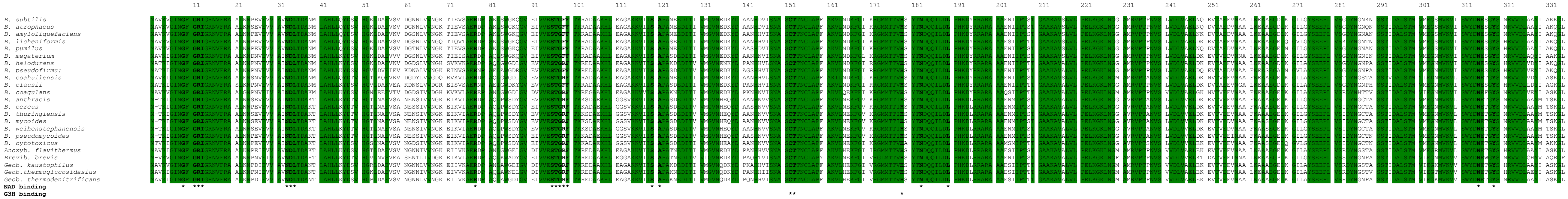


Figure S10. Alignment of the amino acid sequences of the corresponding GapA species.
ClustalW was used to align the amino acid sequences of the GapA species. Identical amino acids are shown in green. Residues involved in binding of NAD and glyceraldehyde-3-phosphate are in bold and labelled with an asterisk.

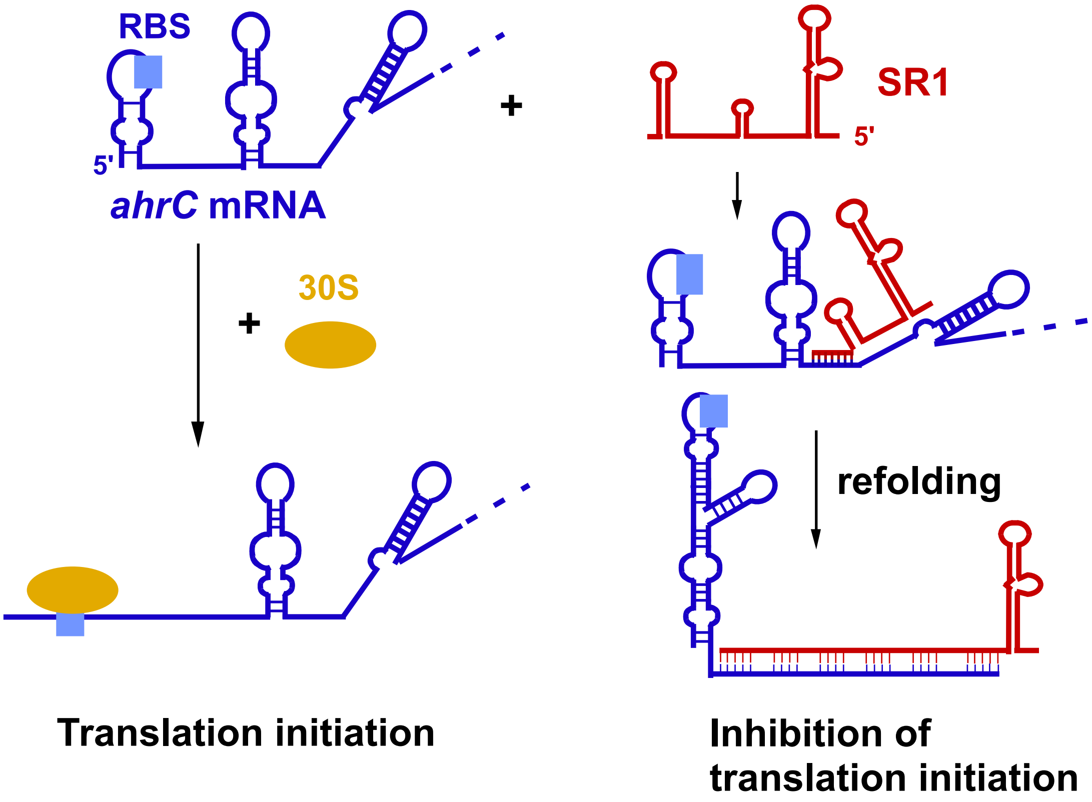


Figure S11. Mechanism of action of *B. subtilis* SR1 on *ahrC* mRNA
Red, SR1; blue, *ahrC* mRNA; light blue box, ribosome binding site (RBS); yellow, 30S ribosomal subunit. In the absence of SR1, the 30S subunit can bind to the *ahrC* SD sequence and initiate translation. Binding of SR1 ≈90 nt downstream of the *ahrC* SD sequence induces structural changes in *ahrC* mRNA immediately downstream of the RBS, which prevent 30S binding and, consequently, translation initiation. Based on (2).

Table 1S: Oligodeoxyribonucleotides used in this study

Name	Sequence	purpose
SB1434	5' TTT CAG TTG ACG GTA ACA	NB, gapA
SB1083	AGG AGC AGA GAT GAT TAC	NB, gapA
SB303	5' GTA ATC CTT GCA TTC CTT CG	NB, SR1 *B. sub., B. pum., B. thur.*
SB309	5' GCG TAT TGA GGC GAT GCA CC	NB, SR1 *B. subtilis*
SB317	5′ GAA TTC AAG CTT AAA AAA TAA AAA GCA TGC GGC TT	all pWSR1 mutants
SB348	5′ TCG AGT TCA TGA AAA ACT	all pWSR1 mutants
SB1623	5' CAT CAC AGC AAT GAC AGT	NB, SR1 *B. pumilis*
SB1625	5' TGT GAT CAC ATC CGC AAG	NB, SR1 *B. thuringensis*
SB1750	5' GGG GAT GAG TAG ATG GGA	NB, SR1 *B. halodurans*
SB1751	5' TAT CAT TAC AAC TAG GGC	NB, SR1 *B. halodurans*
SB1752	5' GGG GAG TTT ACA AAA TGG	NB, SR1 *B. megaterium*
SB1753	5' TAA ATC TTC ATC TGC TTT	NB, SR1 *B. megaterium*
SB1754	5' GGG GTT TTG GGG ATG GGC	NB, SR1 *G. kaustophilus*
SB1755	5' TTC TCC GCC CTC ATT GTC	NB, SR1 *G. kaustophilus*
C767	5′ GGG TGT GAC CTC TTC GCT ATC GCC ACC	5S rRNA probe *B. subtilis*
SB746	5′ ACC CGA CAA GGA ATT TCG C	23 S rRNA probe *B. subtilis*
SB1319	5′ TGA TCT ACC CAT GTC CAG	23 S rRNA probe *B. amyloliquef.*
SB1740	5' ACG GAA TTC AAG GAT TGC GTC ATA AAG	*B. amyloliquefaciens* pACS54
SB1741	5' GCG GGA TCC TTG TTG TTA TTA TAT TAT	*B. amyloliquefaciens* pACS54
SB1742	5' ACG GAA TTC ACC TTA AGC AGG AAT ACA	*B. licheniformis* pACS55
SB1743	5' GCG GGA TCC TTG TTA CCC CTA TAA TAT	*B. licheniformis* pACS55
SB1744	5' ACG GAA TTC TCG TAC TTA TAA ATA TGG	*B. thuringiensis* pACS56
SB1745	5' GCG GGA TCC TTT CTG TCT TTA TTT TAA	*B. thuringiensis* pACS56
SB 1726	5' ACG GAA TTC TAG ATA TGA TGC TAA GCG TTC A	*B. megaterium* pACS59
SB1727	5' GCG GGA TCC TTA CAA CTC TTG ATA TTA	*B. megaterium* pACS59/66/67
SB1728	5' ACG GAA TTC ATG TTT TGA ACC AAC CAG CA	*B. halodurans* pACS58
SB1729	5' GCG GGA TCC ACA TTT GAT TTA TTT ACA	*B. halodurans* pACS58/64/65
SB1730	5' ACG GAA TTC CAA AAT ACA ATT GAA ACG T	*B. pumilus* pACS57
SB1731	5' GCG GGA TCC ATC TAT TTC ACA CTT CTA	*B. pumilus* pACS57
SB1748	5' ACG GAA TTC CGT ACG TAT AAA TAC GGC	*B. anthracis* pACS60
SB1749	5' GCG GGA TCC TTT CTG TCT TTA TTT TAA	*B. anthracis* pACS60
SB1746	5' ACG GAA TTC ATT GTA CCA TAT GTT GGC	*G. kaustophilus* pACS61
SB1747	5' GCG GGA TCC CAC ATT TCA ATT GTC AAT	*G. kaustophilus* pACS61
SB1450	5' ATA GAA TTC ATA AAA TGT GTT ATA CAG	*B. subtilis*, pACS62
SB831	5' GCG GGA TCC TTT CTT TTG TTG TTA TTA	*B. subtilis*, pACS62, pACS63
SB1448	5' GTA GAA TTC TTG ACA TTT TAA ATG TGA	*B. subtilis*, pACS63
SB1782	5' ACG GAA TTC TAT GAC ATA CAA TTT AGC	*B. halodurans*, pACS64
SB1783	5' ACG GAA TTC TTG ACG GGA TAA ATG ATA	*B. halodurans*, pACS65

SB1780	5' ACG GAA TTC TGT GAC ATA CTA ATT AGT	*B. megaterium*, pACS59/66
SB1781	5' ACG GAA TTC TTG ACA TAT TTA ATG TGA	*B. megaterium*, pACS67
SB1620	5' AGT AAG CTT AGA AAG TTA AGC GGT TAC	*B. thuringensis*, pWSR1/M51
SB1619	5' GCC GCA TGC TTT TTT TTA TTT TTC GAA CTG CGG GTG GCT CCA TTG GGC TTT TGT ATG CTC	*B. thuringensis*, pWSR1/M51
SB1622	5' AGT AAG CTT AAC AAG TAA AAA TTA AGC	*B. amyloliquefaciens*, pWSR1M52
SB1621	5' GCC GCA TGC TTT TTT TTA TTT TTC GAA CTG CGG GTG GCT CCA CTC TTC TTC AAG ATG ACA	*B. amyloliquefaciens*, pWSR1M52
SB1651	5' AGT AAG CTT TGA AAT AGA TTA AAC GCT	*B. pumilis*, pWSR1/M55
SB1652	5' GCC GCA TGC TTT TTT TTA TTT TTC GAA CTG CGG GTG GCT CCA TTC AGA GGC TTC ATC ACA	*B. pumilis*, pWSR1/M55
SB1662	5' AGT AAG CTT GGT AAC AAA TTT AAA CGC TTC CAT	*B. licheniformis*, pWSR1/M57
SB1661	5' GCC GCA TGC TTT TTT TTA TTT TTC GAA CTG CGG GTG GCT CCA TTC TTC CTC TTT AAG ATG GCA	*B. licheniformis*, pWSR1/M57
SB1706	5' GCC TCA TGC TTT TTT TTA TTT TTC GAA CTG CGG GTG GCT CCA TCT CTC GTT TTT TGT TTC TT	*B. halodurans*, pWSR1/M58
SB1705	5' AGT AAG CTT AAA TCA AAT GTT AAG CGT	*B. halodurans*, pWSR1/M58
SB1707	5' AGT AAG CTT AAG AGT TGT AAA ACG CTT	*B. megaterium*, pWSR1/M59
SB1708	5' GCC GCA TGC TTT TTT TTA TTT TTC GAA CTG CGG GTG GCT CCA ATC TAA ATC TTC ATC TGC TT	*B. megaterium*, pWSR1/M59
SB1702	5' AGT CTG CAG AAG CTT AGA AAG TTA AGC GGT TACA	*B. anthracis*, pWSR1/M64/63 *B. thuringensis*, pWSR1/M60
SB1704	5' GCC GCA TGC TTT TTT TTA TTT TTC GAA CTG CGG GTG GCT CCA TTG GGC TTT TGT ATG CAC TGT	*B. anthracis*, pWSR1/M64
SB1703	5' AGT CTG CAG GGT ACC GAT GCA ATC GTT GCA ACA	*B. anthracis*, pWSR1/M63 *B. thuringensis*, pWSR1/M60
SB1633	5' GCC GCA TGC TTT TTT TTA TTT TTC GAA CTG CGG GTG GCT CCA TTC TTT TTC TCC GCC CTCA	*G. kaustophilus*, pWSR1/M55
SB1671	ATG GGC ACG ATC GTA TGT CAA	*G. kaustophilus*, pWSR1/M55
SB1555	5′ GGT ACG ATT GTT TCC CAA GAT TCC AAC GAA GCC ATT	*B. subtilis*, pWSR1/M32
SB1556	5′ AAT GGC TTC GTT GGA ATC TTG GGA AAC AAT CGT ACC	*B. subtilis*, pWSR1/M32
SB1628	5′ GGA ACT TCC TCC GGA TAA TGT CAA TGT CCT GTT	*B. subtilis*, pWSR1/M54
SB1629	5′ AAC AGG ACA TTG ACA TTA TCC GGA GGA AGT TCC	*B. subtilis*, pWSR1/M54
SB1402	5' GTC AAG CTT AAA AAA TAA AAAGCA TGC GGC TTT AAG CCG CAT GCT TTT TTT TA	pWSR1/MX, addition of Strep-tag
SB625	5′ GAA ATT AAT ACG ACT CAC TAT AGG ACA AAA GAA ATT AAG CGT	T7-SR1-up *B. subtilis*
SB829	5′ AGC CGC ATG CTT TTT TTT ACT	SR1-down *B. subtilis*
SB643	5′GAA ATT AAT ACG ACT CAC TAT AGG TCG ACA TGC TA AGC AGG A	T7-*ahrC*-up *B. subtilis*

SB644	5′ GTT ACA GCA GTT CAA GGA GC	*ahrC*-down *B. subtilis*
SB1874	5′GAA ATT AAT ACG ACT CAC TAT AGG AAG TAA 5′ AAA TTA AGC GTT TTC	T7-SR1-up *B. amyloliqu.*
SB1950	5′ CGA AAT CGG TTT CGG GCC T	SR1-down *B. amyloliq.*
SB1878	5′ GAA ATT AAT ACG ACT CAC TAT AGG TTG ACA TGC TGA GAG AAG AAG	T7-*ahrC*-up *B. amyloliq.*
SB1877	5′ GCT ACA GCA GCT CCA AAA GAC	*ahrC*-down *B. amyloliq.*
SB1881	5′ GAA ATT AAT ACG ACT CAC TAT AGG TAA ATA AAT AGA TTA CTA TTG	T7-SR1-III-up *B. thuri.*
SB1882	5′ TTT CCT ACA CAT GTA GGC GCT	SR1-III- down *B. thuri.*
SB1883	5′ GAA ATT AAT ACG ACT CAC TAT AGG ATG AAT TAG TTG ATA TTT TAC	T7-*arg2*-up *B. thuri.*
SB1884	5′ GTT ACA ACA TAT TTA AGA AAC	*arg2*-down *B. thuri.*
SB1885	5′ GAA ATT AAT ACG ACT CAC TAT AGG AAC AAG CGA ATT TAT CAT	T7-SR1-up *G. kaustoph.*
SB1886	5′ ACA GAC TTG TCG ACA TGC GCG	SR1-down *G. kaustoph.*
SB1887	5′ GAA ATT AAT ACG ACT CAC TAT AGG ACG AGC TTG TCG ACC GGC TGA	T7-*ahrC*-up *G. kaustoph.*
SB1888	5′ CCT AGA GCA TGG ACA ACA GCT	*ahrC*-down *G. kaustoph.*
SB1915	5′ GAA ATT AAT ACG ACT CAC TAT AGG AAC AAA TTT AAA CGC TTC TAT	T7-SR1-up *B. licheniformis*
SB1916	CTG TAA ATG GCC GCA TGC TTT	SR1-down *B. licheniformis*
SB1938	5′ GAA ATT AAT ACG ACT CAC TAT AGG AGC TTG TCG ATA TTT TAA AGA CGG	T7-*ahrC*-up *B. licheniformis*
SB1939	5′ TTA TAA AAG CTC CAA TAT TTT CTT CTG	*ahrC*-down *B. licheniformis*
SB1919	5′ GAA ATT AAT ACG ACT CAC TAT AGG AAA ACG CTT ACA AA AAA GAC	T7-SR1-up *B. megaterium*
SB1955	5′ GAA AAT CCG CAC CTT TTT TTA TTG	SR1-down *B. megaterium*
SB1921	5′ GAA ATT AAT ACG ACT CAC TAT AGG CAA GGT TTT AAT GTA ACA CAG	T7-*ahrC*-up *B. megaterium*
SB1922	5′ TTA TAA CAT TTC TAA GAA TCG	*ahrC*-down *B. megaterium*
SB1900	5′ GAA GCC GCC TTT CAA TTT CGA	R-out 16S rRNA *B. thuri*
SB1901	5′ GAT GGA TRCC GTC CGC CGC TAA CTT CAT AAG A	R-in 16S rRNA *B. thuri*
SB1894	5′ GGA CAT TTT GAA GAT GAA AAA TCA	R-out *B. thuri* SR1
SB1897	5′ AGC GAA TTC TCT CAT TGC GAC TGT GAC CAT	R-in *B. thuri* SR1
SB1941	5′ GTG TGC AAT TGT ACC TTC ACA ATC	PT *B. thuri* SR1-I
SB1962	5′ TTT CCC GTA TAG TAC TGT TGA TTT TTC	PT *B. thuri* SR1-III
SB1963	5′ CCA CAT TTT CCG TAA AGT ACT GTC GTT	PT *B. thuri* SR1-II

NB, oligos for Northern blots. Oligos designated with -up and -down were used in PCR for the generation of templates for *in vitro* transcription with T7 RNA polymerase. All other oligos were used for cloning, and the resulting plasmids are shown at the right margin. R, primer for 3′ RACE. PT, primer for primer extension.

Table S2: Plasmids used in this study

Plasmid	Description	Reference
pWSR1	tet-inducible overexpression vector for SR1, Km^R	17
pWSR1/M25	overexpression vector for Strep-tagged SR1P, Km^R	22
pWSR1/M2	as pWSR1, but with C6T	22
pWSR1/M3	as pWSR1, but with D8L, C9T	22
pWSR1/M7	as pWSR1, but with C28S, C29S	22
pACC1	vector for integration of a *cggR-lacZ* fusion, Cm^R	22
pACT87	vector for integration of a transcriptional *B. subtilis sr1-lacZ* fusion, Cm^R, 87 nt upstream of -35 box of p_{sr1}	17
pACS54	as pACT87, but for *B. amyloliquefaciens*	this study
pACS55	as pACT87, but for *B. licheniformis*	this study
pACS56	as pACT87, but for *B. thuringiensis*	this study
pACS57	as pACT87, but for *B. pumilus*	this study
pACS58	as pACT87, but for *B. halodurans*	this study
pACS59	as pACT87, but for *B. megaterium*	this study
pACS60	as pACT87, but for *B. anthracis*	this study
pACS61	as pACT87, but for *Geobacillus kaustophilus*	this study
pACS62	as pATC87, but with only 19 nt upstream of the -35 box of p_{sr1}	this study
pACS63	as pATC87, but with 0 nt upstream of the -35 box of p_{sr1}	this study
pACS64	as pACS58, but with only 19 nt upstream of the -35 box of p_{sr1}	this study
pACS65	as pACS58, but with only 0 nt upstream of the -35 box of p_{sr1}	this study
pACS66	as pACS59, but with only 19 nt upstream of the -35 box of p_{sr1}	this study
pACS67	as pACS59, but with only 0 nt upstream of the -35 box of p_{sr1}	this study
pWSR1/M51	as pWSR1/M25, but for *B. thuringiensis* first SR1P1	this study
pWSR1/M52	as pWSR1/M25, but for *B. amyloliquefaciens* SR1P	this study
pWSR1/M55	as pWSR1/M25, but for *Geobacillus kaustophilus* SR1P	this study
pWSR1/M56	as pWSR1/M25, but for *B. pumilus* SR1P	this study
pWSR1/M57	as pWSR1/M25, but for *B. licheniformis* SR1P	this study
pWSR1/M58	as pWSR1/M25, but for *B. halodurans* SR1P	this study
pWSR1/M59	as pWSR1/M25, but for *B. megaterium* SR1P	this study
pWSR1/M64	as pWSR1/M25, but for *B. anthracis* SR1P	this study
pWSR1/M60	as pWSR, but for entire *B. thuringiensis sr1* (encoding SR1P 1 to 3)	this study
pWSR1/M65	as pWSR1/M25, but for *B. thuringiensis* SR1P2	this study
pWSR1/M66	as pWSR1/M25, but for *B. thuringiensis* SR1P3	this study
pWSR1/M32	as pWSR1/M25, but with C6S, C9S	this study
pWSR1/M54	as pWSR1/M25, but with C28S, C29S and Δ31-39	this study

Table S3: Characteristics of SR1 homologues

Species	RNA length [nt] / identity [%]	SR1P length [nt] / identity [%]	Base-pairing interactions complementary regions / total number of pairings		Transcriptional terminator
			Arg2	Arg1	
B. subtilis	205 / 100	39 / 100	7 / 44		AAAAAAAAGCATGCGGCTTAAAGCCGCATGCTTTTTA*TTTTTT*
B. atrophaeus	202 / 92	39 / 90	7 / 53		AAAAGCATGCGGTGTAAAAGCCGCATGC*TTTTTT*
B. amyloliquefaciens	210 / 88	39 / 93	9 / 55		AAAAAGGCCCGAAACGATTTCGTTTCGGGC*TTCTTT*
B. licheniformis	204 / 87	39 / 79	8 / 47		AAAAAAGCATGCGGCCATTTACAGTGCCGCATGC*TTTT*
B. pumilus	211 / 83	39 / 79	7 / 47		AAAAAAGCATGCAGCTCCATTTGGGGCTGCATGTTTC*TTTTTT*
B. megaterium	213 / 82	41 / 63	10 / 73		AAAAAAAAGGTGCGGATTTCCGCACC*TTTTTT*
B. halodurans	174 * / 82	41 / 57	7 / 48		AAAAAAGACTCATCTATGGGGGAGATGAGTC*TTTTTTT*
B. coahuilensis	220 / 79	38 / 45	10 / 69		AAAAAGATGTATCCGAGGACGGATACATC*TTTTTT*
B. coagulans	220 / 81	36 / 57	11 / 91		GGCCGGCTTGTTGCCCGGCT*TTTTTT*
B. clausii	220 / 81	45 / 55	8 / 59		GAACACCTTGCAAGGATGACTGCAAGGTG*TTTTTTTT*
B. pseudofirmus	215 / 84	41 / 53	9 / 55		AAAAGAAGCATGCCCCTCATAACGGGGGCATGCTTC*TTTGTT*
B. anthracis	584				AAAAAAGCGCCTACATATGTAGGTGC*TTTTTT*
B. anthracis I	195 / 83	42 / 64	9 / 62		
B. anthracis II	192 / 83	42 / 57	9 / 62		
B. anthracis III	197 / 82	42 / 57	9 / 61		
B. thuringiensis	587				GAAAAAGCGCCTACATGTGTAGGCGC*TTTTTT*
B. thuringiensis I	196 / 85	42 / 64	9 / 62	9 / 69	
B. thuringiensis II	192 / 82	42 / 57	9 / 63	10 / 77	
B. thuringiensis III	199 / 80	42 / 57	9 / 62	9 / 65	
B. cereus	586				GAAAAAGCGCCTACATGTGTAGGCGC*TTTTTT*
B. cereus I	195 / 85	42 / 64	9 / 62	9 / 69	
B. cereus II	192 / 82	42 / 57	9 / 63	10 / 77	
B. cereus III	199 / 80	42 / 57	9 / 62	9 / 65	
B. weihenstephanensis	586				GAAAAAACGCCTACACATGTAGGCG*TTTTTTT*
B. weihenstephanensis I	194 / 82	42 / 64	9 / 60		
B. weihenstephanensis II	192 / 82	42 / 55	9 / 64		
B. weihenstephanensis III	200 / 78	42 / 55	9 / 63		
B. mycoides	586				GAAAAAACGCCTACACATGTAGGCG*TTTTTTT*
B. mycoides I	194 / 82	42 / 64	9 / 60		
B. mycoides II	192 / 82	42 / 57	9 / 63		
B. mycoides III	200 / 77	42 / 57	9 / 63		
B. pseudomycoides	609				AAAAAGGCGCCTGCACATCTGCAGGCGCC*TTTTTT*
B. pseudomycoides I	194 / 82	42 / 64	9 / 64	8 / 59	
B. pseudomycoides II	201 / 82	43 / 59	10 / 70	9 / 64	
B. pseudomycoides III	214 / 79	42 / 57	7 / 55	9 / 63	
B. cytotoxicus	413				AAAAAAGCGTCTGCGAATGCAGATGC*TTTTTT*
B. cytotoxicus I	194 / 82	42 / 67	9 / 58	8 / 61	
B. cytotoxicus II	215 / 78	42 / 57	8 / 58	9 / 69	
Anoxyb. flavithermus	237 / 85	37 / 64	9 / 67		GGGAAGGGATCGGCATAGCCGGTCTC*TTTTT*
Brev. brevis	212 / 80	41 / 58	8 / 66		AACAGGCCTACGGTAGGCCTG*TTTTTTTT*
Geob. kaustophilus	229 / 83	41 / 71	11 / 74		GGCGCGCATGTCGACAAGTCACATGCGCGCCG*TTTATTTTT*
Geob. thermoglucosidasius	229 / 80	41 / 67	8 / 57		GGCGAGCATGTGAAAGGGGCGCATGCTCGCCCA*TTTTTTT*
Geob. thermodenitrificans	225 / 81	41 / 74	10 / 61		GGCGCACATGTGAAAGGGGCACATGTGCGCCA*TTTATTTT*

23 SR1 homologues were identified. The three peptide-encoding sequences in the *B. cereus* group members are numbered with I, II and III. RNA and SR1P identities are relative to *B. subtilis* SR1 and SR1P, respectively. The base-pairing interactions with *arg2* RNA, which is highly homologous to *B. subtilis ahrC* RNA, and, in species that contain two *arg* genes, to *arg1* RNA, which contains the two arginine binding motifs, but is otherwise divergent from *arg2* RNA, are shown. The number of total pairings indicates the added number of base pairs of the 7 to 11 complementary regions. The exact location of all complementary regions for the single SR1 species is shown in Fig. S1.

*exact length could not be determined due to insertion of IS652