Plasmid – Chromosome interplay in natural and non-natural hosts: global transcription study of three Bacillus cereus group strains carrying pCER270 plasmid

Alicia Nevers, Markus Kranzler, Stéphane Perchat, M. Gohar, Alexei Sorokin, Didier Lereclus, Monika Ehling-Schulz, Vincent Sanchis-Borja

▶ To cite this version:

HAL Id: hal-04300453
https://hal.inrae.fr/hal-04300453

Submitted on 28 Nov 2023

Journal Pre-proof

Plasmid – chromosome interplay in natural and non-natural hosts: global transcription study of three *Bacillus cereus* group strains carrying pCER270 plasmid

Alicia Nevers, Markus Kranzler, Stéphane Perchat, Michel Gohar, Alexei Sorokin, Didier Lereclus, Monika Ehling-Schulz, Vincent Sanchis-Borja

Please cite this article as: A. Nevers, M. Kranzler, S. Perchat, M. Gohar, A. Sorokin, D. Lereclus, M. Ehling-Schulz, V. Sanchis-Borja, Plasmid – chromosome interplay in natural and non-natural hosts: global transcription study of three *Bacillus cereus* group strains carrying pCER270 plasmid, *Research in Microbiologoy*, https://doi.org/10.1016/j.resmic.2023.104074.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Plasmid – chromosome interplay in natural and non-natural hosts: global**

2 **transcription study of three *Bacillus cereus* group strains carrying pCER270**

3 **plasmid**

4

5 Alicia Nevers [a]*, Markus Kranzler [b], Stéphane Perchat [a], Michel Gohar [a], Alexei Sorokin [a],

6 Didier Lereclus [a], Monika Ehling-Schulz [b]*, Vincent Sanchis-Borja [a]*

7   [a] Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350 Jouy-en-Josas,

8   France

9   [b] Institute of Microbiology, Department of Pathobiology, University of Veterinary

10   Medicine Vienna; Vienna, Austria

11 alicia.nevers@inrae.fr

12 markus.kranzler@vetmeduni.ac.at

13 stephane.perchat@inrae.fr

14 michel.gohar@inrae.fr

15 alexei.sorokine@inrae.fr

16 didier.lereclus@inrae.fr

17 monika.ehling-schulz@vetmeduni.ac.at

18 *Correspondence and reprints: alicia.nevers@inrae.fr; vincent.sanchis-borja@inrae.fr &

19 monika.ehling-schulz@vetmeduni.ac.at

20

21 **Abstract:**

22 The *Bacillus cereus* group comprises genetically related Gram-positive spore-forming bacteria

23 that colonize a wide range of ecological niches and hosts. Despite their high degree of genome

24 conservation, extrachromosomal genetic material diverges between these species. The

25 discriminating properties of the *B. cereus* group strains are mainly due to plasmid-borne toxins,

26 reflecting the importance of horizontal gene transfers in bacterial evolution and species

27 definition. To investigate how a newly acquired megaplasmid can impact the transcriptome of

28 its host, we transferred the pCER270 from the emetic *B. cereus* strains to phylogenetically

29 distant *B. cereus* group strains. RNA-sequencing experiments allowed us to determine the

30 transcriptional influence of the plasmid on host gene expression and the impact of the host

31 genomic background on the pCER270 gene expression. Our results show a transcriptional

32 cross-regulation between the megaplasmid and the host genome. pCER270 impacted

33 carbohydrate metabolism and sporulation genes expression, with a higher effect in the natural

34 host of the plasmid, suggesting a role of the plasmid in the adaptation of the carrying strain to

35 its environment. In addition, the host genomes also modulated the expression of pCER270

36 genes. Altogether, these results provide an example of the involvement of megaplasmids in the

37 emergence of new pathogenic strains.

38 **Keywords**

41

42

1. Introduction

43

44   Plasmids are found throughout the bacterial kingdom and undergo a highly dynamic

45 evolution. Plasmid maintenance requires favorable encoded-traits that can compensate for the

46 associated fitness cost. On the other hand, their ability to be horizontally transferred and to

47 establish themselves in other hosts determines their spread (1).

48   The *Bacillus cereus* group is composed of Gram positive, sporulating bacteria that

49 includes several closely related species, such as *Bacillus anthracis*, *Bacillus cereus sensu stricto*

50 (ss), *Bacillus cytotoxicus*, *Bacillus thuringiensis*, and *Bacillus weihenstephanensis* (2). With the

51 exception of *B. cytotoxicus*, all defined species within this group exhibit a high conservation of

52 the core genome. However, these bacteria display a wide range of ecological lifestyles (3,4). *B.*

53 *thuringiensis* is an insect pathogen broadly used as biopesticide. *B. weihenstephanensis* is a

54 psychrotolerant species that can grow at temperatures ranging from 4°C to 37°C, whereas

55 human pathogens include *B. cereus* ss, *B. anthracis* and *B. cytotoxicus* strains (4).

56   Megaplasmids, defined as plasmids larger than 100 kb (5), have substantially

57 contributed to the diversity of the *B. cereus* group ecological niches. Plasmid-encoded genes,

58 particularly toxin-coding genes, are frequently used to distinguish species within the *B. cereus*

59 *sensu lato* (sl) group, as they often play a role in pathogenicity. *B. thuringiensis*, for example,

60 harbors plasmids carrying the insecticidal *cry* genes (6,7). Cry toxins do form parasporal

61 crystals which dissolve and take a major part in the infectious process, once ingested by their

62 target insects. *B. anthracis* is a highly pathogenic agent for mammals, including humans. This

63 bacterium causes anthrax disease due to the production of several toxins and a capsule, which

64 are all encoded on the pXO1 and pXO2 plasmids (8). *B. cereus* ss is the causative agent of

65 foodborne diseases, characterized by diarrheic or emetic syndromes(9). While the causes of

66 diarrheal syndromes are only partially understood, those responsible for emetic syndrome are

67 better characterized (2). Emetic strains of *B. cereus* harbor pCER270, a 270 kb megaplasmid,

68 sharing sequence similarities with *B. anthracis* pXO1, particularly at the region of the

69 replication origin (10). pCER270, like pXO1, is a non-conjugative plasmid, whose transfer

70 requires the assistance of a self-transmissible plasmid (11,12). Unique regions in pCER270

71 include the cereulide synthetase gene cluster (*ces*), encoding an enzymatic complex required

72 for the non-ribosomal synthesis of the emetic peptide toxin cereulide (13,14). Transcription of

73 *cesPTABCD* from one promoter generates a 23-kb polycistronic transcript, while *cesH*

74 transcription initiates from its own promoter (15). So far, *ces* expression has been assessed in

75 LB medium, and cereulide production has been monitored in LB and MOB medium

76 (sporulation medium) (16,17). The *ces* locus is expressed during late exponential phase,

77 allowing for cereulide accumulation which peaks during stationary phase. The timing of *ces*

78 expression in response to nutrients abundance has been shown to be controlled by CodY global

79 transcriptional regulator (18). AbrB, a transition-state regulator involved in repression of

80 stationary-phase genes during exponential phase, has also been involved in the negative control

81 of *ces* locus expression (19). More recently, a study showed that a pCER270-encoded

82 transcriptional regulator similar to PagR in *B. anthracis*, repressed *ces* expression (20).

83 Cereulide is resistant to proteolysis, high temperatures and acidic pH, and therefore it

84 accumulates in cooked food and causes vomiting when ingested (9). Many studies noticed that

85 the low prevalence of emetic *B. cereus* in the environment is largely increased in food samples,

86 questioning the role of pCER270 in this prevalence (21,22). Interestingly, some *B.*

87 *weihenstephanensis* strains have been found as being able to produce cereulide. These

88 psychrotolerant emetic strains harbor their *ces* genes either encoded on large plasmids or on the

89 chromosome, most likely as a result of an horizontal gene transfer (HGT) event (22–24).

90   The importance of megaplasmids in defining *B. cereus* sl species raises questions about

91 the emergence of new pathogens, especially when HGT and plasmid sharing are frequent in the

92 environment. Indeed, previous studies on *B. thuringiensis* showed that conjugation can occur

93 in soil with frequencies that are not neglectable, and that plasmid transfer is common in dead

94 insect larvae (25,26). Furthermore, conjugative plasmid transfer appears to be more efficient in

95 food matrices, especially milk, than in the widely used LB medium (Lysogeny Broth) (27).

96   In this context, it seems particularly relevant to study the consequences of megaplasmids

97 transfer within new hosts. Indeed, the initial establishment of a plasmid in its new genomic

98 environment is the first step that is required for its maintenance and co-evolution with the core

99 genome. Here, we focused on the transcriptional regulations associated with the transfer of

100 pCER270 to phylogenetically distant *B. cereus* sl hosts. The *B. cereus* group forms about five

101 major phylogenetic clades, in which different *B. cereus* group species are intermingled (2).

102 Thus, we aimed to (i) choose host-strains that are only distantly related to the emetic *B. cereus*

103 lineage and (ii) represent non-*B. cereus* sensu stricto species of economic importance, such as

104 *B. thuringiensis* (commercially used biopesticide) and *B. weihenstephanensis* (known for its

105 food spoilage potential). According to the *panC* gene sequence

106 (https://toolcereusid.shinyapps.io/Bcereus, (3)), the *B. weihenstephanensis* KBAB6 strain

107 belongs to the same phylogenetic group as the previously described *B. weihenstephanensis*

108 emetic strains (group VI). In particular, we questioned the interaction of pCER270 with the

109 chromosome, by identifying differentially expressed genes in presence of the plasmid, and

110 analyzing pCER270 gene regulation in the different genetic backgrounds.

111

2. Materials and methods

2.1. Strains construction and culture conditions

Spontaneous streptomycin resistant mutants were selected from spores preparation plated on 300 µg/ml of streptomycin for *B. cereus* F4810-72 emetic strain (synonymous to *B. cereus* AH187) cured for pCER270 plasmid (B.ce F4810-72), *B. thuringiensis* serovar *kurstaki* HD73 (B.th HD73) and *B. weihenstephanensis* KBAB6 strains (B.we KBAB6). The B.we KBAB6 strain is closely related to the more generally used B.we KBAB4 strain, but competent for high efficiency conjugation with pXO16 containing donors. These streptomycin resistant strains were used as the reference "pCER270-" plasmidless strains for the transcriptomic studies, and as recipient strains for conjugation. A spectinomycin resistance gene was inserted into pCER270 using CRISPR methodology based on the vector pJOE9282 (28) with modifications (unpublished), thus obtaining pCER270-spec. This non-conjugative plasmid was transferred into recipient strains, by a pXO16-assisted mobilization (unpublished). The details of conjugation experiments will be described elsewhere. The pCER270+ transconjugants were selected as resistant to spectinomycin (300 µg/ml), sensitive to tetracycline (pXO16 marker) and further checked by PCR to ensure the presence of an essential part of pCER270, intrinsic host plasmids and the loss of pXO16.

For the three strains tested, the pCER270- and pCER270+ strains were grown in parallel in either LB medium, or HCT medium, a sporulation medium (29). The onset of the stationary phase is determined as the end of the exponential growth and corresponds to an inflection of the growth curve. For RNA preparation, LB cultures were collected two hours (early stationary phase) after the beginning of stationary phase, whereas in HCT medium the cultures were collected four hours after the onset of the stationary phase. At this bacterial growth phase, direct-targets of Spo0A, such as *spoIIE*, are induced (30). This time point corresponds to the onset of the sporulation, when the asymmetric septum is not yet formed. Three biological

137   replicates of each strain were processed for RNA-seq experiments and the following statistical

138   analysis.

139   *2.2. RNA extraction*

140   RNA extraction was performed using Trizol and 1-bromo-3-chloropropane as

141   previously described (31). Total RNAs were treated with DNAse using the Invitrogen™ Kit

142   TURBO DNA-free (AM1907). RNA quality was checked on agarose gel and on Bioanalyzer.

143   Ribosomal RNAs were depleted from total RNA samples using the RiboZero kit (Illumina)

144   according to the manufacturer's instructions.

145   *2.3. Libraries preparation and sequencing*

146   RNA-seq libraries were generated using the NextSeq 500/550 High Output Kit v2 (75

147   cycles). A single read -75 sequencing run was performed on a NextSeq instrument at the I2BC

148   High-throughput sequencing facility (CNRS, Gif-sur-Yvette).

149   *2.4. Data processing*

150   Reads were demultiplexed using bcl2fastq2-2.18.12 and adapters trimmed using

151   Cutadapt 3.2. The sequencing data were then uploaded to the Galaxy web platform, and data

152   analysis was performed on the usegalaxy.org public server (32). The reads were aligned to each

153   of three species genomes concatenated with the pCER270 sequence (accession number

154   CP001179) using Bowtie2. MulticovBed was used to generate the read counts. Genomic

155   sequences with accession numbers CP001177 (chromosome) and CP001178-CP001181

156   (plasmids) were used for B.ce F4810-72. CP004069 (chromosome) and CP004069-CP004076

157   (plasmids), excluding CP004072 (pAW63, not found in this strain) for B.th HD73. For B.we

158   KBAB6, an in-house unpublished genome was used. The B.we KBAB6 wild type strain were

159   deposited at NCBI as biosample SAMN14639501. Under the same biosample, there will soon

160   be the assembly and annotation versions of this genome. Processed data were analyzed using R

161   and Deseq2 differential analysis of total RNA-sequencing using SARTools R Pipeline (33).

162 Significantly differentially expressed genes are characterized by a p-value that is lower than

163 0.05 and a log2(fold change) higher than 0.7 or lower than -0.7 for up-regulated and down-

164 regulated genes, respectively. Integrative Genomic Viewer (IGV) software allowed to visualize

165 sequencing reads distribution from Wig files, generated with Galaxy BAM-to-Wiggle tool and

166 normalized with bigwigCompare tool.

167 Raw files and processed data are available at NCBI Gene Expression Omnibus

168 Database, with the accession number GSE220688.

169 *2.4. Functional groups identification in the different hosts of pCER270*

170 In order to define functional groups in the different strains SyntTax

171 (https://archaea.i2bc.paris-saclay.fr/synttax/) was used to define orthologs in B.ce F4810-72,

172 B.th HD73 and the published B.we KBAB4, highly similar to B.we KBAB6 (34).

173   **3.** Results

174   *3.1. B.ce F4810-72 is more sensitive to condition-specific gene regulations induced by the*

175   *pCER270*

176   To better understand how the pCER270 megaplasmid can establish itself in a new host

177   and to estimate the consequences of its transfer on the transcriptional landscape of the recipient

178   strain, we analyzed the transcription of B.th HD73 and B.we KBAB6 strains, in comparison to

179   the natural pCER270- harboring strain (B.ce F4810-72). The spectinomycin-tagged pCER270

180   plasmid was transferred to its recipient strains via a pXO16-assisted conjugation. For reference,

181   transfer of the plasmid was performed in the B.ce F4810-72 emetic strain previously cured of

182   its pCER270. Recipient strains, referred to as "pCER270-" and validated transconjugants

183   referred to as "pCER270+" strains, were grown in parallel in LB and HCT media. RNA-seq

184   samples were collected two hours and four hours after the onset of stationary phase, in LB and

185   HCT medium respectively. Indeed, since a number of adaptative processes (production of

186   virulence factors, biofilm formation, sporulation) occurs at this time, the transition phase

187   between the exponential phase and the stationary phase was chosen for this transcriptional

188   study. *ces* expression, for example, starts at the end of exponential phase (16).

189   Deseq2 RNA-sequencing analysis enabled the identification of differentially expressed

190   genes in pCER270+ *versus* pCER270- strains. Volcanoplots were generated for the different

191   hosts of the megaplasmid as well as for the two conditions tested (Fig.1A). These graphical

192   representations of transcriptomic data demonstrate that pCER270 genes (in red) are expressed

193   and have a similar profile across all genetic backgrounds. Moreover, Volcanoplots shapes

194   suggest that the presence of the megaplasmid has a much greater impact on host gene expression

195   when the strains were grown in sporulation (HCT) medium than in standard (LB) medium.

196   Indeed, in LB medium, only a few host genes are located above the statistical strength and fold

197   change thresholds (dotted gray lines), while the number of differentially expressed genes in the

198   pCER270+ is distinctly increased in HCT. The Venn diagram representations, corresponding

199   to LB medium (Fig.1B), show that 17, 2 and 2 genes are significantly up-regulated in B.ce

200   F4810-72, B.th HD73 and B.we KBAB6, respectively, while 52, 8 and 3 genes are down-

201   regulated, respectively (Table.S1). Under sporulation inducing conditions, the transcriptional

202   impact of pCER270 was more pronounced, with 1507, 83 and 123 up-regulated genes and 1569,

203   64 and 495 down-regulated genes in B.ce F4810-72, B.th HD73 and B.we KBAB6 strains,

204   respectively (Fig.1B, Table.S2). The identified genes are not markedly shared between the two

205   conditions, with calculated overlaps ranging from 0.2% to 7.5%.

206   All these findings suggest that the host transcriptome is less impacted by the pCER270

207   presence during LB-growth compared to HCT-growth. Furthermore, the B.ce F4810-72, the

208   natural host of pCER270, showed a greatly increased number of pCER270-impacted genes

209   compared with its two artificial hosts. B.th HD73 genome transcription seems to be less

210   sensitive to pCER270 transfer.

211   *3.2. pCER270 increases expression of TCA cycle genes in HCT medium but only in the B.ce*

212   *F4810-72 strain*

213   The tricarboxylic acid cycle (TCA cycle) is a metabolic pathway which plays a crucial

214   role in bacterial energetic metabolism. From the 13 genes described for this metabolic pathway

215   in *B. subtilis* (http://subtiwiki.uni-goettingen.de (35)), we have defined the sets of TCA cycle

216   genes in each of the different strains. TCA cycle genes are up-regulated in the B.ce F4810-72

217   strain harboring pCER270. The boxplots for this functional group of genes show an overall

218   increase of their expression in the natural host strain of the plasmid (B.ce F4810-72 pCER270+

219   strain), grown in the HCT medium (Fig.2A). Indeed, the Deseq2 analysis tool identified 12

220   genes out of 13 of this functional group that were significantly up-regulated (Fig.2B). However,

221   pCER270 had no effect on the expression of these genes in B.th HD73 and B.we KBAB6

222   strains, suggesting they are less sensitive to pCER270-induced regulation than the B.ce F4810-

223   72 strain. In LB medium, pCER270 did not impact TCA cycle genes expression, even in its

224   B.ce F4810-72 natural host (Fig.S1). In summary, pCER270 specifically increases TCA cycle

225   genes expression in the HCT sporulation-inducing medium and in the emetic context, as shown

226   by IGV profiles corresponding to some representative genes within this functional group

227   (Fig.2C).

228   *3.3. pCER270 up-regulates the acetoin catabolism genes in B.ce F4810 and B.th HD73 strains*

229   *growing in HCT medium, but down-regulates them in B.we KBAB6.*

230   Acetoin is an extracellular metabolite that can be metabolized as a carbon source in the

231   absence of glucose (36). The expression of acetoin breakdown genes *acoL*, *acoA*, *acoB* and

232   *acoC* was increased in the pCER270+ B.ce F4810-72 natural host strain. In HCT conditions,

233   all these genes were up-regulated compared to the pCER270- strain (Fig.3A and 3B). The

234   presence of pCER270 in the B.th HD73 strain also resulted in a higher expression of acetoin

235   metabolism genes in HCT medium. Interestingly, pCER270 seems to induce the reverse

236   regulation of acetoin consumption genes in the B.we KBAB6 pCER270+ strain since the global

237   expression level of this group of genes is significantly lowered in this strain (Fig.3C). According

238   to Deseq2 results for the *B.we* KBAB6 strains, all individual genes for this group are down-

239   regulated. However, according to Deseq2 analysis, none of the *aco* genes seems to be

240   differentially expressed in LB medium (Fig.S2).

241   *3.4. The expression of genes implicated in the initiation of the sporulation is affected by*

242   *pCER270*

243   In *Bacilli*, Spo0A is a master regulator controlling various transition phase functions

244   and sporulation initiation (37). Its transcription is tightly regulated and Spo0A activity depends

245   on a phosphorelay regulated by the quorum sensing system Rap-Phr (38). Deseq2 analysis of

246   B.ce F4810-72 and B.th HD73 strains indicate that *spo0A* expression is significantly up-

247   regulated in presence of pCER270 (padj<0.05 in both cases). However, this is not the case for

248   the B.we KBAB6 strain, despite the slight increase visible on IGV profile. These IGV profiles

249   illustrate the distribution of reads at the genomic loci but do not provide information on the

250   statistical strength of these observations, as they are only average profiles of the biological

251   replicates. For the B.we KBAB6 strain, the heterogeneity between biological replicates had an

252   impact on the p-value (padj>0.05) for the *spo0A* gene, therefore the fold increase cannot be

253   considered as significant (Fig.4A).

254   Interestingly, the expression of SigF forespore specific factor expression is also

255   statistically up-regulated in B.ce F4810-72 and B.th HD73 pCER270+ strains but not in B.we

256   KBAB6 pCER270+ strain. The expression of the SigE mother-cell specific sporulation factor

257   is also increased in B.th HD73 pCER270+ strain but remains stable in the two other hosts, with

258   or without the plasmid. On the other hand, the expression of Yttp transcriptional regulator

259   ortholog, involved in sporulation septum formation, is up-regulated in the B.ce F4810-72 and

260   B.th HD73 strains but not in the B.we KBAB6 strain.

261   When generalizing to all direct Spo0A targets involved in the initiation of sporulation

262   (39), we can see that pCER270 has a variable impact on the expression of these genes depending

263   on the host. The B.ce F4810-72 natural host strain is again the most impacted by the

264   megaplasmid (Fig.4B), with 9 genes out of 13 being significantly up-regulated in the

265   pCER270+ strain. This proportion is reduced to 5 out of 13 and 3 out of 13 for B.th HD73 and

266   B.we KBAB6 strains, respectively (Fig.4C).

267   The only sporulation-related regulation induced by pCER270 that is conserved across

268   all pCER270 hosts, is the increased expression of *sinR* and *sinI* (Fig.4D), which likely relates

269   to the transcriptional up-regulation of Spo0A.

270   *3.5. The expression of pCER270 genes is impacted by the host genomic background*

271   According to Genbank (CP001179.1), the pCER270 plasmid contains 277 defined

272   transcriptional features, of which only 112 have annotated functions, including the virulence

273   *ces* operon. The pCER270 plasmid also contains 17 transcriptional regulator genes.

274   To determine whether the host genomic context could influence pCER270 expression

275   and describe the locus-specific regulation of the pCER270 genes in relation to its hosts, global

276   expression levels of the megaplasmid were compared in all pCER270+ strains. Then, a Deseq2

277   normalization and analysis restricted to pCER270 transcriptional features was performed.

278   The results show that the overall expression level of pCER270 genes was equivalent in

279   all media, albeit slightly lower in the B.we KBAB6 strain and LB medium. Thus, global

280   pCER270 expression does not appear to be regulated by the host (Fig. 5A). However, some

281   specific genes were differentially regulated depending on the genomic context. An analysis was

282   done with the pCER270-restricted Deseq2 normalization, to compare the hosts for a same

283   global expression of pCER270. This pCER270-centered analysis allowed us to identify the

284   number of genes that are down-regulated or up-regulated in B.th HD73 or B.we KBAB6 strains

285   when compared to the B.ce F4810-72 natural host strain (Fig.5B, Table.S3). The number of

286   genes showing a clear up- or down-regulated transcription in the B.th HD73 compared to the

287   B.ce F4810-72 was higher in HCT than in LB but this was not the case in the B.we KBAB6

288   strain. 12% and 8% of pCER270 genes shared the same regulation in both B.th HD73 and B.we

289   KBAB6 strains as compared to the B.ce F4810-72 strain in LB and HCT media, respectively.

290   We evaluated the impact of the genomic background on *ces* transcription, and found

291   that the expression of the pCER270 cereulide synthetase locus was sustained in all the genomic

292   backgrounds (Fig. 5C). The expression of *ces* locus was similar in the three strains grown in

293   HCT medium. Surprisingly, in LB medium, compared to the two other strains, this expression

294   was increased during the early stationary phase in the B.th HD73 strain. This suggests that the

295   impact of the host on the expression of pCER270 virulence genes is dependent on the growth

296   conditions. Cereulide was synthesized in the HD73 and the KBAB6 genetic backgrounds. In

297   line with the RNA-seq data corresponding to LB medium, higher levels of cereulide were found

298   in HD73 than in KBAB6 (unpublished).

299   *3.6. pCER270 transcriptional regulator expression varies in remote hosts*

300   To gain insights on the regulation of pCER270 gene expression, depending on the

301   genomic context, we focused on the expression of putative transcriptional factors in the

302   different contexts. Indeed, genes encoding DNA-binding protein are most likely involved in the

303   local regulation of their adjacent genes present on the megaplasmid. Among the 17 genes

304   encoding transcriptional factors, only four are showing an important variation in their host-

305   specific expression when the pCER270 hosts are growing in LB medium (Fig. 6 & Table 1).

306   Nevertheless, 13 transcriptional regulators out of 17 are differentially expressed in remote hosts

307   compared to B.ce F4810-72 natural host strain in HCT medium (Table 1). The genes

308   BCAH187_C0191 and BCAH187_C0192, coding for a helix-turn-helix (HTH) domain protein,

309   and for a protein belonging to the LacI regulatory family, respectively, are strongly down-

310   regulated in both B.th HD73 and B.we KBAB6 strains, compared to the B.ce F4810-72 strain

311   in LB, but not in HCT medium. Gene BCAH187_C0240, which also encodes an HTH-domain

312   protein, is down-regulated in pCER270 remote hosts in both LB and HCT (Fig.6, Table 1).

313

314 **4.** Discussion

315   In order to determine the role of pCER270 in strain adaptation, virulence and ecological

316 niche specificity, we artificially transferred the pCER270 into different strains of the *B. cereus*

317 sl group. These included the pCER270 natural host-strain *B. cereus* F4810-72, the *B.*

318 *thuringiensis* serovar *kurstaki* HD73 and the *B. weihenstephanensis* KBAB6 strains, that are

319 known for their emetic, entomopathogenic and psychrotrophic properties, respectively. This

320 study provides the first analysis of the consequences, at the transcriptional level, of the transfer

321 of the pCER270 megaplasmid into new hosts.

322   The pCER270 plasmid has an effect on *B. cereus* sl gene expression, especially in

323 sporulating conditions (HCT medium). Indeed, pCER270 has an impact on carbohydrate

324 metabolism and on sporulation in all its hosts (Fig.7). As sporulation is an energy-costly

325 process, a strict control of the energetic metabolism and choice of carbon sources is crucial for

326 the bacterial self-maintenance and development in the environment (40). TCA cycle and acetoin

327 metabolism genes induction has been described previously in *B. subtilis* starved cells (41).

328 During starvation, when cells activate the sporulation genetic program, the pCER270 host-

329 strain B.ce F4810-72 was the most responsive to gene regulation induced by the megaplasmid.

330 Indeed, in the B.ce F4810-72 strain, pCER270 appears to accentuate the transcriptional

331 response to a nutritional starvation by increasing the induction magnitude of the TCA cycle and

332 acetoin catabolism genes. Transcriptional regulations are less impacted by pCER270 in its

333 artificial hosts suggesting that gene regulation in response to the environmental conditions is

334 partly host-specific.

335   We show here that after sporulation induction, pCER270 influences the expression of

336 sporulation initiation genes by affecting the transcription rate of *spo0A*. The *spo0A* transcription

337 has been described as an essential step in the control of sporulation during developmental

338   transition (42). The up-regulation of *spo0A* mediated by pCER270 is more pronounced in its

339   natural B.ce F4810-72 host-strain. This partly explains the higher number of differentially

340   regulated genes identified in this strain and highlights the physiological implications of hosting

341   the pCER270 megaplasmid at the regulatory level.

342   On the other hand, our transcriptome study provides evidence that the host genomic

343   background also has a regulatory role on the expression of the pCER270 genes (Fig.7). This

344   regulation of pCER270 genes is locus-specific rather than global, highly dependent on growth

345   conditions, and may have an impact on the expression of the virulence *ces* locus. Indeed, the

346   expression of the *ces* locus genes and of putative transcriptional regulators is specifically and

347   differentially regulated in response to growth conditions and in different genetic host

348   backgrounds. This suggests that pCER270 plays a role in virulence and adaptive properties,

349   which may differ significantly according to its hosts.

350   The condition-specific impact of the megaplasmid on chromosomal gene expression

351   may represent compensatory regulations to alleviate the burden of plasmid carriage in

352   pCER270+ strains (43). Nevertheless, the pCER270-induced regulation may also help the host

353   to cope with new environmental conditions, providing an adaptive advantage to the host

354   carrying the plasmid. Artificial hosts of pCER270, which have very different ecological niches,

355   may be less sensitive at the molecular level to the plasmid's regulatory potential. However,

356   plasmid-chromosome incompatibility is expected to decrease after a longer co-evolution of the

357   megaplasmid with the host genome. The domestication of the plasmid (43) could then

358   subsequently increase the potential prevalence of the artificial hosts in new environments, such

359   as food, and would be a concern in terms of new pathogen emergence. Furthermore, the new

360   genetic background could in turn affect the expression of virulence and regulatory factors

361   encoded on pCER270 depending on the conditions, and possibly lead to increased pathogenicity

362   of the new strains harboring the pCER270.

363   In conclusion, our study shows that the pCER270 megaplasmid can be transferred to

364 new hosts, with the help of pXO16 conjugative plasmid, and expressed at the transcriptional

365 level. Depending on the environmental conditions, the megaplasmid impacts its host

366 transcriptome, and the genetic background of pCER270 can in turn specifically impact plasmid

367 gene expression (Fig.7). However, the pCER270 interaction with its host genomes is greater

368 with its natural host, the F4810-72 strain, than with remote hosts, suggesting a co-evolution

369 between the plasmid and its genetic background.

370 **Acknowledgements**

378

379

References

380   **References**

381   1.   San Millan A, MacLean RC. Fitness Costs of Plasmids: a Limit to Plasmid Transmission.

382   Microbiol Spectr. 2017;5(5):5.5.02.

383   2.   Ehling-Schulz M, Lereclus D, Koehler TM. The *Bacillus cereus* Group: Bacillus Species

384   with Pathogenic Potential. 2020;35.

385   3.   Guinebretière MH, Thompson FL, Sorokin A, Normand P, Dawyndt P, Ehling-Schulz M,

386   et al. Ecological diversification in the *Bacillus cereus* Group. Environ Microbiol. avr

387   2008;10(4):851-65.

388   4.   Patiño-Navarrete R, Sanchis V. Evolutionary processes and environmental factors

389   underlying the genetic diversity and lifestyles of *Bacillus cereus* group bacteria. Res

390   Microbiol. 2017;168(4):309-18.

391   5.   Hall JPJ, Botelho J, Cazares A, Baltrus DA. What makes a megaplasmid? Philos Trans R

392   Soc B Biol Sci. 2022;377(1842):20200472.

393   6.   Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, et al. *Bacillus*

394   *thuringiensis* and Its Pesticidal Crystal Proteins. Microbiol Mol Biol Rev.1998;62(3):775-806.

395   7.   González JoséM, Carlton BC. A large transmissible plasmid is required for crystal toxin

396   production in *Bacillus thuringiensis* variety israelensis. Plasmid. 1984;11(1):28-38.

397   8.   Kolstø AB, Tourasse NJ, Økstad OA. What Sets *Bacillus anthracis* Apart from Other

398   Bacillus Species? Annu Rev Microbiol. 1 oct 2009;63(1):451-76.

399   9.   Rouzeau-Szynalski K, Stollewerk K, Messelhäusser U, Ehling-Schulz M. Why be serious

400   about emetic *Bacillus cereus*: Cereulide production and industrial challenges. Food Microbiol.

401   2020;85:103279.

402   10. Rasko DA, Rosovitz MJ, Økstad OA, Fouts DE, Jiang L, Cer RZ, et al. Complete

403   Sequence Analysis of Novel Plasmids from Emetic and Periodontal *Bacillus cereus* Isolates

404   Reveals a Common Evolutionary History among the *B. cereus* -Group Plasmids, Including

405   *Bacillus anthracis* pXO1. J Bacteriol. 2007;189(1):52-64.

406   11. Makart L, Commans F, Gillis A, Mahillon J. Horizontal transfer of chromosomal markers

407   mediated by the large conjugative plasmid pXO16 from *Bacillus thuringiensis* serovar

408   israelensis. Plasmid. 2017;91:76-81.

409   12. Green BD, Battisti L, Thorne CB. Involvement of Tn4430 in transfer of *Bacillus*

410   *anthracis* plasmids mediated by *Bacillus thuringiensis* plasmid pXO12. J Bacteriol.

411   1989;171(1):104-13.

412   13. Ehling-Schulz M, Fricker M, Grallert H, Rieck P, Wagner M, Scherer S. Cereulide

413   synthetase gene cluster from emetic *Bacillus cereus*: Structure and location on a mega

414   virulence plasmid related to *Bacillus anthracis* toxin plasmid pXO1. BMC Microbiol.

415   2006;6(1):20.

416   14. Ehling-Schulz M, Vukov N, Schulz A, Shaheen R, Andersson M, Märtlbauer E, et al.

417   Identification and Partial Characterization of the Nonribosomal Peptide Synthetase Gene

418   Responsible for Cereulide Production in Emetic *Bacillus cereus*. Appl Environ Microbiol.

419   2005;71(1):105-13.

420   15. Dommel MK, Frenzel E, Strasser B, Blöchinger C, Scherer S, Ehling-Schulz M.

421   Identification of the Main Promoter Directing Cereulide Biosynthesis in Emetic *Bacillus*

422   *cereus* and Its Application for Real-Time Monitoring of *ces* Gene Expression in Foods. Appl

423   Environ Microbiol. 2010;76(4):1232-40.

424   16. Dommel MK, Lücking G, Scherer S, Ehling-Schulz M. Transcriptional kinetic analyses

425   of cereulide synthetase genes with respect to growth, sporulation and emetic toxin production

426   in *Bacillus cereus*. Food Microbiol. avr 2011;28(2):284-90.

427   17. Lücking G, Frenzel E, Rütschle A, Marxen S, Stark TD, Hofmann T, et al. *Ces* locus

428   embedded proteins control the non-ribosomal synthesis of the cereulide toxin in emetic

429   Bacillus cereus on multiple levels. Front Microbiol. 2015 ;6.

430   18. Frenzel E, Doll V, Pauthner M, Lücking G, Scherer S, Ehling-Schulz M. CodY

431   orchestrates the expression of virulence determinants in emetic *Bacillus cereus* by impacting

432   key regulatory circuits: CodY modulates virulence of *Bacillus cereus*. Mol Microbiol.

433   2012;85(1):67-88.

434   19. Lücking G, Dommel MK, Scherer S, Fouet A, Ehling-Schulz M. Cereulide synthesis in

435   emetic *Bacillus cereus* is controlled by the transition state regulator AbrB, but not by the

436   virulence regulator PlcR. Microbiology. 2009;155(3):922-31.

437   20. Kalbhenn EM, Kranzler M, Gacek-Matthews A, Grass G, Stark TD, Frenzel E, et al.

438   Impact of a Novel PagR-like Transcriptional Regulator on Cereulide Toxin Synthesis in

439   Emetic *Bacillus cereus*. Int J Mol Sci. 2022;23(19):11479.

440   21. Ehling-Schulz M, Frenzel E, Gohar M. Food–bacteria interplay: pathometabolism of

441   emetic *Bacillus cereus*. Front Microbiol. 2015 ;6.

442   22. Hoton FM, Fornelos N, N'Guessan E, Hu X, Swiecicka I, Dierick K, et al. Family

443   portrait of *Bacillus cereus* and *Bacillus weihenstephanensis* cereulide-producing strains.

444   Environ Microbiol Rep. 2009;1(3):177-83.

445 23. Mei X, Xu K, Yang L, Yuan Z, Mahillon J, Hu X. The genetic diversity of cereulide

446 biosynthesis gene cluster indicates a composite transposon Tnces in emetic *Bacillus*

447 *weihenstephanensis*. BMC Microbiol. 2014 ;14(1) :149.

448 24. Guérin A, Rønning HT, Dargaignaratz C, Clavel T, Broussolle V, Mahillon J, et al.

449 Cereulide production by *Bacillus weihenstephanensis* strains during growth at different pH

450 values and temperatures. Food Microbiol. 2017; 65:130-5.

451 25. Vilas-Bôas GFLT, Vilas-BÃ´as LA, Lereclus D, Arantes OMN. *Bacillus thuringiensis*

452 conjugation under environmental conditions. FEMS Microbiol Ecol. 1998;25(4):369-74.

453 26. Jarrett P, Stephenson M. Plasmid transfer between strains of *Bacillus thuringiensis*

454 infecting *Galleria mellonella* and *Spodoptera littoralis*. Appl Environ Microbiol.

455 1990;56(6):1608-14.

456 27. Van der Auwera GA, Timmery S, Hoton F, Mahillon J. Plasmid exchanges among

457 members of the *Bacillus cereus* group in foodstuffs. Int J Food Microbiol.

458 2007;113(2):164-72.

459 28. Altenbuchner J. Editing of the *Bacillus subtilis* Genome by the CRISPR-Cas9 System.

460 Appl Environ Microbiol. 2016;82(17):5421-7.

461 29. Lecadet MM, Blondel MO, Ribier J. Generalized Transduction in *Bacillus thuringiensis*

462 var. berliner 1715 using Bacteriophage CP-54Ber. J Gen Microbiol. 1980;(121(1)) :203-12.

463 30. Cardoso P, Fazion F, Perchat S, Buisson C, Vilas-Bôas G, Lereclus D, Rap-Phr Systems

464 from Plasmids pAW63 and pHT8-1 Act Together To Regulate Sporulation in the *Bacillus*

465 *thuringiensis* Serovar kurstaki HD73 Strain. Applied and Environmental

466 Microbiology;86(18).

467

468   31. Gélis-Jeanvoine S, Canette A, Gohar M, Caradec T, Lemy C, Gominet M, et al. Genetic

469   and functional analyses of krs, a locus encoding kurstakin, a lipopeptide produced by *Bacillus*

470   *thuringiensis*. Res Microbiol. 2017;168(4):356-68.

471   32. Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Čech M, et al. The Galaxy

472   platform for accessible, reproducible and collaborative biomedical analyses: 2018 update.

473   Nucleic Acids Res. 2018;46(W1):W537-44.

474   33. Varet H, Brillet-Guéguen L, Coppée JY, Dillies MA. SARTools: A DESeq2- and EdgeR-

475   Based R Pipeline for Comprehensive Differential Analysis of RNA-Seq Data. PLOS ONE. 9

476   juin 2016;11(6):e0157022.

477   34. Oberto J. SyntTax: a web server linking synteny to prokaryotic taxonomy. BMC

478   Bioinformatics. 2013;14(1):4.

479   35. Pedreira T, Elfmann C, Stülke J. The current state of Subti Wiki, the database for the

480   model organism *Bacillus subtilis*. Nucleic Acids Res. 2022;50(D1):D875-82.

481   36. Peng Q, Zhao X, Wen J, Huang M, Zhang J, Song F. Transcription in the acetoin

482   catabolic pathway is regulated by AcoR and CcpA in *Bacillus thuringiensis*. Microbiol Res.

483   2020;235:126438.

484   37. Sonenshein AL. Control of sporulation initiation in *Bacillus subtilis*. Curr Opin

485   Microbiol. 2000;3(6):561-6.

486   38. Perego M, Hoch JA. Cell-cell communication regulates the effects of protein aspartate

487   phosphatases on the phosphorelay controlling development in *Bacillus subtilis*. Proc Natl

488   Acad Sci. 1996;93(4):1549-53.

489   39. Molle V, Fujita M, Jensen ST, Eichenberger P, González-Pastor JE, Liu JS, et al. The

490   Spo0A regulon of *Bacillus subtilis*: The Spo0A regulon. Mol Microbiol.

491   2003;50(5):1683-701.

492   40. Pisithkul T, Schroeder JW, Trujillo EA, Yeesin P, Stevenson DM, Chaiamarit T, et al.

493   Metabolic Remodeling during Biofilm Development of *Bacillus subtilis*. Ghigo JM, mBio.

494   2019;10(3):e00623-19.

495   41. Koburger T, Weibezahn J, Bernhardt J, Homuth G, Hecker M. Genome-wide mRNA

496   profiling in glucose starved *Bacillus subtilis cells*. Mol Genet Genomics. 2005;274(1):1-12.

497   42. Mirouze N, Prepiak P, Dubnau D. Fluctuations in *spo0A* Transcription Control Rare

498   Developmental Transitions in *Bacillus subtilis*. PLoS Genet. 2011;7(4):e1002048.

499   43. Vial L, Hommais F. Plasmid-chromosome cross-talks. Environ Microbiol.

500   2020;22(2):540-56.

501

502

503

504 **Legends to figures**

505 <u>Fig.1</u>. **B.ce F4810-72 is more sensitive to condition-specific gene regulations induced by**

506 **the pCER270**

507 (A) Volcano plots showing differentially expressed genes in pCER270+ versus pCER270-

508 context in B.ce F4810-72, B.th HD73, and B.we KBAB6 strains. Statistical significance is

509 represented as a function of variation in expression level and calculated using the Deseq2 tool.

510 All transcriptional features are represented using black dots, while red dots correspond to

511 pCER270 genes. (B) Venn diagrams showing differentially regulated gene repartition, in LB or

512 HCT, of B.ce F4810-72, B.th HD73, and B.we KBAB6 strains after the transfer of the pCER270

513 plasmid.

514 <u>Fig.2</u>. **pCER270 increases expression of TCA cycle genes in HCT medium in the B.ce**

515 **F4810-72 strain**

516 (A) Boxplots representation of the distribution of tricarboxylic acid (TCA) cycle gene reads, in

517 HCT samples, for the B.ce F4810-72, B.th HD73, and B.we KBAB6 strains. (B) Pie charts

518 showing differentially expressed genes within TCA genes, as defined by Deseq2 analysis, in

519 HCT samples. (C) IGV profiles corresponding to *mdh*, *citC* and *citZ* ortholog genes

520 representative of this functional group. For each strain, the blue color represents the pCER270-

521 context, while the red color represents the pCER270+ context.

522 <u>Fig.3</u>. **pCER270 increases acetoin catabolism genes expression in B.ce F4810 and B.th**

523 **HD73 strains**

524 (A) Boxplots depicting the distribution of acetoin synthesis gene reads, in B.ce F4810-72, B.th

525 HD73, and B.we KBAB6 strains. (B) Pie charts showing differentially expressed genes within

526 *aco* genes as defined by Deseq2 analysis. (C) IGV profiles corresponding to genes of this

527   functional group. For each strain, the blue color represents the pCER270- context, while the red

528   color represents the pCER270+ context.

529   Fig.4. **Expression of early sporulation genes is affected in the presence of pCER270 in**

530   **HCT medium** (A) *spo0A* IGV profiles in the different hosts of the pCER270 and table

531   representation of Deseq2 output values. (B) Boxplots representation of the distribution of reads

532   for direct Spo0A-targets genes involved in sporulation, in B.ce F4810-72, B.th HD73, and B.we

533   KBAB6 strains. (C) Pie charts showing differentially expressed sporulation genes directly

534   controlled by Spo0A as defined by Deseq2 analysis. (D) IGV profile of the *sinI-sinR* locus in

535   the different hosts of the pCER270. For each strain, the blue color represents the pCER270-

536   context, while the red color represents the pCER270+ context.

537   Fig.5. **pCER270 gene expression is impacted by the host genomic background** (A) Boxplots

538   representation of distribution of reads corresponding to pCER270 genes, in B.ce F4810-72, B.th

539   HD73, and B.we KBAB6 strains. (B) Volcano plots showing differentially expressed genes in

540   artificial *versus* natural hosts of the megaplasmid. Statistical significance is represented as a

541   function of variation in expression level and calculated using Deseq2 tool. (C) IGV profiles of

542   *ces* gene expression in LB or HCT samples. For each pCER270+ strain, grey, yellow and blue

543   read distributions correspond to B.ce F4810-72, B.th HD73, and B.we KBAB6 genomic

544   backgrounds, respectively.

545   Fig.6. **The expression of pCER270 transcriptional regulator genes varies in remote hosts.**

546   Volcano plots showing differentially expressed genes in artificial versus natural hosts of the

547   megaplasmid. Statistical significance is represented as a function of variation in expression

548   level and calculated using the Deseq2 tool. The putative transcriptional regulators are presented

549   using purple dots.

550

551 <u>Fig.7</u>. **Transcriptional interactions between pCER270 and the chromosome.** Summary of

552 the interactions between the pCER270 plasmid and the host genome. pCER270 has a regulatory

553 potential for chromosomal genes in B.ce F4810-72, B.th HD73, and B.we KBAB6 strains. The

554 genomic background has in turn an impact on pCER270 gene expression.

555 <u>Table 1</u>. Summary of the expression variations of pCER270-encoded transcriptional regulators

556 in remote hosts, compared with the natural host of the megaplasmid B.ce F4810-72 strain.

557

558   **Supplementary material**

559   Fig.S1. **pCER70 does not impact TCA cycle genes expression in LB medium** (A) Boxplots

560   representation of read distribution for tricarboxylic acid (TCA) cycle genes, in B.ce F4810-72,

561   B.th HD73, and B.we KBAB6 strains, in LB medium. (B) Pie charts showing differentially

562   expressed genes within TCA genes, defined from Deseq2 analysis, in LB medium

563   Fig.S2. **pCER70 does not affect acetoin catabolism genes expression in LB medium** (A)

564   Boxplots representation of reads distribution for acetoin utilization genes, in B.ce F4810-72,

565   B.th HD73, and B.we KBAB6 strains. (B) Pie charts showing differentially expressed genes

566   within aco genes as defined from Deseq2 analysis.

567   Table S1. **Differential expressed genes in pCER270+ versus pCER270- for all strains**

568   **grown in LB medium.**

569   Table S2. **Differential expressed genes in pCER270+ versus pCER270- for all strains**

570   **grown in HCT medium.**

571   Table S3. **Differential expressed genes in remote versus natural hosts of pCER270,**

572   **pCER270-centered Deseq2 analysis in LB and HCT media.**

573

Table 1: Summary of the expression variations of pCER270-encoded transcriptional

regulators in remote hosts, compared with the natural host of the megaplasmid B.ce F4810-72

strain.

ID	Product	LB	HCT
BCAH187_C0028	HTH transcriptional regulator	H_up	H_down
BCAH187_C0033	transcriptional regulator ArsR family		H_down
BCAH187_C0048	transcriptional regulator ArsR family protein		
BCAH187_C0050	transcription factor RsfA family		H_up
BCAH187_C0051	HTH domain-containing protein		K_up
BCAH187_C0098	transcriptional regulator MerR family		H_down
BCAH187_C0183	DNA-binding protein HU 1		
BCAH187_C0187	transcriptional regulator AbrB/MazE/SpoVT family		
BCAH187_C0191	HTH domain-containing protein	H&K_down	H_down
BCAH187_C0192	transcriptional regulator LacI family protein	H&K_down	H_down
BCAH187_C0200	transcriptional regulator ArsR family		K_up
BCAH187_C0205	transcriptional activator AraC family		H_down
BCAH187_C0213	transcriptional regulator AraC family protein		
BCAH187_C0218	transcription regulator Cro/CI family		H_up
BCAH187_C0240	helix-turn-helix HxlR type	H&K_down	H&K_down
BCAH187_C0255	DNA-binding response regulator		K_down
BCAH187_C0268	transcriptional repressor PagR		H&K_down

H-up: up-regulated in B.th HD73 strain, K_up: up-regulated in B.we KBAB6 strain,

H-down: down-regulated in B.th HD73 strain, K_down: down-regulated in B.we KBAB6

strain, H&K_down : down-regulated in both B.th HD73 and B.we KBAB6 strains.

Strain specific gene regulation by pCER270 plasmid

Impact on metabolism and sporulation genes :
handle energetic resources and control sporulation kinetics

pCER270

Condition-specific
cross-regulation

Host genome
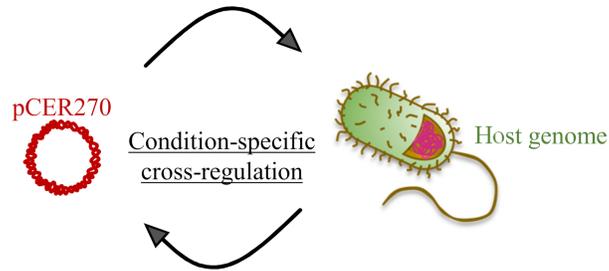
pCER270 genes regulation by genomic background

Differential expression of transcriptional regulators and *ces* cluster genes :
control of adaptative properties and virulence depending on the host

A

	LB medium Early stationary phase	HCT medium Commitment to sporulation

● pCER270 genes

B.ce
F4810-72

B.th
HD73

B.we
KBAB6

B

B.ce F4810-72	B.th HD73	B.we KBAB6

LB **HCT**

9   8   1499

Up-regulated

LB **HCT**

1   1   82

Up-regulated

LB **HCT**

0   2   121

Up-regulated

LB **HCT**

33   19   1550

Down-regulated

LB **HCT**

3   5   59

Down-regulated

LB **HCT**

2   1   494

Down-regulated

TCA cycle genes (n=13)

A

| B.ce F4810-72 | B.th HD73 | B.we KBAB6 |

pCER270 -   pCER270 +

B

| B.ce F4810-72 | B.th HD73 | B.we KBAB6 |

pCER270+ vs pCER270-

Up-regulated
Down-regulated
Unchanged

C

B.ce F4810-72

pCER270 -
pCER270 +

BCAH187_A4717   mdh   citC   citZ

B.th HD73

pCER270 -
pCER270 +

HD73_4886   HD73_4887   HD73_4888   HD73_4889

B.we KBAB6

pCER270 -
pCER270 +

peg.4469   peg.4470   peg.4471   peg.4472

Acetoin catabolism genes (n=4)

spo0A gene:

A

Strain	Gene ID for s*po0A*	log2FoldChange	padj
B.ce F48-10-72	BCAH187_A4301	3.638	2.83e-13
B.th HD73	HD73_4474	0.926	8.45e-4
B.we KBAB6	peg.4068	0.344	2.11e-1

Sporulation genes and direct targets of Spo0A (n=13)

B

C

pCER270+ vs pCER270-

- 🟧 Up-regulated
- 🟩 Down-regulated
- ⬛ Unchanged

sinI – sinR genes

D

A

LB medium
Early stationary phase

HCT medium
Commitment to sporulation

B.ce
F4810-72

B.th
HD73

B.we
KBAB6

pCER270 genes expression level

ns — ns

*

ns — ns

ns

B

LB medium
Early stationary phase

HCT medium
Commitment to sporulation

B.th HD73

N=29   N=26

-log(p-value)

log2(Fold Change artificial/natural host)

B.th HD73

N=73

N=80

-log(p-value)

log2(Fold Change artificial/natural host)

B.we KBAB6

N=37   N=19

-log(p-value)

log2(Fold Change artificial/natural host)

B.we KBAB6

N=26   N=12

-log(p-value)

log2(Fold Change artificial/natural host)

Up-regulated in HD73   Down-regulated in HD73
Up-regulated in KBAB6   Down-regulated in KBAB6

C

LB medium
Early stationary phase

B.ce F4810-72
B.th HD73
B.we KBAB6

HCT medium
Commitment to sporulation

B.ce F4810-72
B.th HD73
B.we KBAB6

cesD   *cesB*   *cesA*   *cesP*
   cesC