**Fig. S1**: ANI-based species relationship within *B. cereus* sensu lato.

Neighbor network was calculated using ANI distances of 142 *B. cereus* sensu lato genomes. Entire genomes (completed or draft) including all available plasmids were used. Phylogenetic groups are designated according to [1]. *B. cereus* F528/94 (#242) is intermediate between clusters II and III.
**Ancient duplication of hbl**

The second hbl operon hbl\(_a\) is common in clusters II, V and VI and probably was acquired by HGT in cluster IV (Fig. 1, grey crosses; Fig. S2B, grey circles). Several strains seem to have lost their second hbl operon and it is not found at all in cluster III. Intra-operon recombination analysis (Table S4) revealed three significant recombination events that all include *B. cereus* MHI 226 (#140, II) as a parental sequence and took place within hbl\(_D\). The duplication of hblCDA seems to be an ancient and unique event, because it occurs in all phylogenetic groups but III (loss of hbl\(_a\) at furcation of II and III) and VII (Fig. 1). The topologies of hbl and hbl\(_a\) phylogenies are similar but not identical, which could be explained by HGT (Fig. S2 and Fig. 4). Possibly due to directional selection hblCDA\(_a\) is as conserved as hblCDA (Fig. 4B). Hbl\(_a\) shows overall nucleotide sequence identity of 75 – 82 % towards the hbl genes, which are 89 – 100 % identical among themselves. Hbl\(_a\) are 93 – 100 % identical among each other. Six strains (#85, #97, #137, #140, #152, and #211) possess only hblCDA. Their version of hbl is homologous to hblCDA\(_a\) and they may have lost hblCDAB. *B. mycoides* Rock3-17 (#152, I) and *B. mycoides* Rock1-4 possess an hblCDA that differs from both hbl variants described above. *B. mycoides* Rock3-17 hblCDA shows 80 – 82 % identity to hblCDA\(_a\), but 86 – 89 % identity to hblCDA. We suggest that these two strains have no hbl\(_a\), but their hblCDAB developed differently and lost hblB.
**Fig. S2:** *Hbl* in *B. cereus* sensu lato.

**A:** Phylogenetic tree (Maximum Likelihood Method) of the concatenated sequences of 94 *hblCDAB* genes. Seven strains within the set 142 contain only *hblCDA* (empty circle and star) or an incomplete *hbl* operon (#6, IV) and were excluded. **B:** Phylogenetic tree based on the seven housekeeping genes of 101 *hbl*-containing *B. cereus* sensu lato strains.
Recombinational mechanism and boundaries of hbl

Since hbl occurs chromosomally as well as plasmid-bound, we analyzed the immediate vicinity of hbl and hbl\textsubscript{a} with regard to potential indications of transposon activity in all 16 hbl-containing strains that were sequenced in this study. It has been speculated that hbl is part of a large 18 kb transposon [2-4]. A comparison of putative transposon regions including hbl or hbl\textsubscript{a} is shown in Figure S3. Sequence analysis and annotation with RAST [5] revealed that half of the hblCDAB operons are inserted within the uvrC gene as described earlier [3], but in the rest neither insertion sites nor length of the inserting region or adjacent genes are conserved. The lowest common denominator of inserting regions from 16 B. cereus sensu lato strains consists only of a transcriptional regulator gene of the araC family and hblCDAB itself. Inverted repeats (IR and bcr1) [3, 6], which mark the insertion site interrupting uvrC as telltale signs of transposons, could not be found in half of the investigated strains. A transposase gene could only be detected in the vicinity of hbl\textsubscript{a} of B. cereus 6/27/S (#245, IV), but not adjacent to hbl (Fig. S3). Studied hbl\textsubscript{a} are located close to antibiotic resistance genes and do not contain known inverted repeats. Thus, one may speculate that the hbl operon is part of a highly degraded transposon which is in most cases not functional anymore.

Furthermore, the gene pagA encoding a protective antigen similar to a gene located on the B. anthracis pXO1 virulence plasmid, has been inserted into the chromosome of B. cereus, B. mycoides and B. weihenstephanensis (Fig. S3), proving that recombination between virulence plasmids and the bacterial chromosome occurs frequently. Nhe and hbl duplications occur chromosomally as well as plasmid-bound and, hence, are mobile within B. cereus sensu lato.

These results show that recombination within B. cereus sensu lato is limited only by preservation of gene/protein functionality. Consequently, the pathogenic potential of (psychrotolerant) environmental strains or probiotic strains can change rapidly with a single and simple exchange of genetic material. This observation may render the current risk assessment strategies questionable.
**Fig. S3:** Genomic organization of *hbl* operons and adjacent regions of 17 *B. cereus* sensu lato strains.

**A:** 15 of the strains sequenced in this study contain *hblCDAB*, they are shown in comparison to type strain *B. cereus* ATCC 14579.

**B:** Six of the strains sequenced in this study contain *hblCDA*.

Fig. S4: CytK in B. cereus sensu lato.

A: Phylogenetic tree (Maximum Likelihood Method) based on 68 cytK gene sequences. B: Phylogenetic tree based on the concatenated sequence of seven housekeeping genes from 68 cytK-containing B. cereus sensu lato strains.

References