Supplementary files - Legend

Supplementary Figure 1: Size distribution of *S. ambofaciens* ATCC 23877 GIs

Using a minimal threshold of 15 CDS within the synteny break in at least one of the compared genome, 50 GIs (composed of 2 to 158 CDSs) and 22 insertion points (≤ 1 CDS within the synteny break) were identified in *S. ambofaciens* ATCC 23877 genome.

Supplementary Figure 2: Transcriptomes over growth of the four biosynthetic gene clusters encoding all known antibacterial activities of *Streptomyces ambofaciens* ATCC 23877

The genes expressed at the highest level is boxed in red for the congocidine, spiramycin and kinamycin BGCs. They correspond to *cgc1* (SAM23877_RS39345, encoding a transcriptional regulator), *srmB* (SAM23877_RS26680, encoding the ABC-F type ribosomal protection protein SrmB) and *alpZ* (SAM23877_RS00890, encoding a transcriptional regulator) genes, respectively. The gene encoding SrmS (SAM23877_RS26595), the transcriptional regulator of the spiramycin BGC, is boxed in black. The dots are joined by a line for clarity (but the lines do neither report a real observation nor a model).

Supplementary Figure 3: 3C-contact-maps for *S. ambofaciens* grown in the studied conditions

A. **Replicate of cells after 24 h in YEME growth medium, C6 condition**
B. **Replicate of after 24 h in MP5 growth medium, C1 condition**

C. **Replicate of after 48 h in MP5 growth medium, C4 condition.** The violet and pink arrows indicate the location of boundaries formed at desferrioxamin and congocidine BGCs, respectively.

D. **Results of the frontier index analysis including peaks that do not form boundaries**. This panel presents the frontier index analysis of the 3C-maps presented in **Fig.3** and **Fig.4**, showing single peaks that where not considered as indicative of a boundary. At each locus, two indices are computed, reflecting the intensity of the loss of contact frequencies when going downwards (green peaks) or upwards (orange peaks), respectively, to the locus. A boundary is defined as any bin in which there is a change in the right bias of contacts towards the left bias (± 2 bin, green and orange peaks, respectively). The detection of bins with only an upstream or a downstream significant peak reflects the intrinsically noisy nature of 3C-seq data.

Supplementary table 1: Collection of 125 *Streptomyces* genomes used in this study

Name: Species name

Genome Assembly ID: Unique identifier used in the NCBI Genome Assembly database

Chromosome length (bp): Chromosome length expressed in bp

Plasmid length (bp): Cumulative length of plasmids [Number of plasmids] expressed in bp

CDSs nb: Number of CDSs in chromosome [CDSs in plasmids]

TIR length (bp): Size of one copy of a TIR expressed in bp.

Supplementary table 2: List of the GIs identified in *S. ambofaciens* ATCC 23877 genome

Supplementary table 3: Gene distribution in the central region *versus* the extremities (defined by the first and last rDNA operons) of *Streptomyces ambofaciens* ATCC 23877 chromosome depending on features of interest

Supplementary table 4: List of the SMBGCs identified in *S. ambofaciens* ATCC 23877 genome

Supplementary table 5: Overall results from the comparative genomics, RNA-seq and 3C-seq analyses

ID: Gene identifier (NCBI annotation).

SUPPORT: genetic support, either chromosome (CHR) or pSAM1 plasmid.

TIR: 'TIR' if the gene is located within a TIR, 'NA' otherwise.

START: start position of the gene on the genetic support.

END: end position of the gene on the genetic support.

STRAND: orientation of the gene on the genetic support.

REPLICATION_ORIENTATION: orientation of the gene regarding the origin of replication, either on the "leading" or the "lagging" strand.

GENE_SIZE: size of the gene (in bp).

Protein_ID: Protein identifier (NCBI annotation).

GO_ID: Gene product identifier (Uniprot or RNA Central, suitable for GO analysis).

Gene_Name: Gene name (from NCBI and manual annotation from SMBGCs encoding known antibiotics).

UNIPROT_Gene_Name: Uniprot gene name, old locus tag.

Product (NCBI annotation): Product annotation from NCBI.

Product (Uniprot annotation): Product annotation from Uniprot.

ACTINO_SIGN: 'ACTINO_SIGN' if the gene encodes a protein of the actinobacterial signature, 'NA' otherwise.

CORE: 'CORE' if the gene belongs to the core CDSs identified by comparing 125 *Streptomyces* genomes (see Methods section for details, as well as Supplementary Table 1).

PERSIST95: 'PERSIST95' if the gene persistence is superior to 95 %, 'NOT_PERSIST95' otherwise.

GI: annotation as to whether or not the gene belongs to a GI (see details about GI names on Supplementary Table 2).

Strain-specific_CDSs: 'UNIQ' if the gene is only present in *S. ambofaciens* ATCC23877 (in a panel of 125 *Streptomyces* genomes described in Supplementary Table 1), 'NOT_UNIQ' otherwise.

Func_RNA: annotation as to whether or not the gene encodes a functional RNA (tRNA, tmRNA, SRP RNA, RNAse P RNA) excluding rRNA.

Transl._CDS: 'translation' if the CDS encodes a function related to translation and/or RNA stability, 'NA' otherwise

NAPSF: 'NAPSF' if the gene encodes a nucleoid-associated protein or a chromosome structural protein and/or a protein experimentally found associated to *Streptomyces* chromatin (see Methods for details), 'NA' otherwise.

SMBGC: annotation as to whether or not the gene belongs to a SMBGC (see details about SMBGC names on Supplementary Table 4).

pSAM2: 'pSAM2' if the gene belongs to pSAM2 integrative plasmid, 'NA' otherwise.

PROPHAGE: 'PROPHAGE' if the gene belongs to the prophage integrated in *S. ambofaciens* ATCC23877 genome, 'NA' otherwise

PERSIST_INDEX: Persistence index (N_{orth}/N, where N_{orth} is the number of genomes carrying a given orthologue and N the number of genomes searched, 124 in our case). This persistence index was calculated only for CDSs (functional RNAs were not considered).

'SENSE' columns: Number of normalized counts (DESeq2 normalization and normalized on gene size) per kb for each gene in the sense orientation, as the mean of the number of counts in all tested conditions ('SENSE_Mean') or of the replicates in each condition considered individually ('SENSE_C1' to 'SENSE_C7')

'ANTISENSE_INDEX' columns: Number of reads in the antisense orientation over the total (sense plus antisense) number of reads for each gene in all tested conditions ('ANTISENSE_INDEX_Mean') or in each condition considered individually ('ANTISENSE_INDEX _C1' to 'ANTISENSE_INDEX _C7')

'DIFF' columns: annotation as to whether or not the gene is statistically differentially expressed compared to C1 condition, with an adjusted p value ≤ 0.05 ('DIFF0.05'), 0.01 ('DIFF0.01') or 0.001 ('DIFF0.001').

'CAT' columns: Gene expression level category in all tested conditions ('CAT _Mean'), in each condition considered individually ('CAT _C1' to 'CAT _C7'), maximal category in all tested conditions ('CAT_MAX') and minimal category in all tested conditions ('CAT_MIN').

SWITCH: annotation as to whether or not the gene expression level switches from CAT_0 to a maximal CAT_3 ('SWITCH0->3') or CAT_4 ('SWITCH0->4') category, when considering all studied conditions.

‘BORDER’ columns: annotation as to whether or not the gene belongs to a boundary observed on the map contact obtained in C1 (‘C1_BORDER’), C4 (‘C4_BORDER’) and/or C6 condition (‘C4_BORDER’). The boundaries are numbered according to their order in the genome. ‘rRNA’ indicates that the boundary also contains an rDNA operon.