Conjugation Mediates Large-Scale Chromosomal Transfer in *Streptomyces* Driving Diversification of Antibiotic Biosynthetic Gene Clusters

Caroline Choufa [1], Pauline Gascht [1], Hugo Leblond [2], Anthony Gauthier [1], Michiel Vos [3], Cyril Bontemps [1,*], Pierre Leblond [1,*]

[1]Université de Lorraine, INRAe, DynAMic, Nancy, F-54000, France

[2]Université de Lorraine, CNRS, Inria, LORIA, Nancy, F-54000, France

[3]European Centre for Environment and Human Health, Penryn Campus, Penryn TR10 9FE, UK

***Corresponding authors:** Emails: cyril.bontemps@univ-lorraine.fr; pierre.leblond@univ-lorraine.fr.

Associate editor: Miriam Barlow

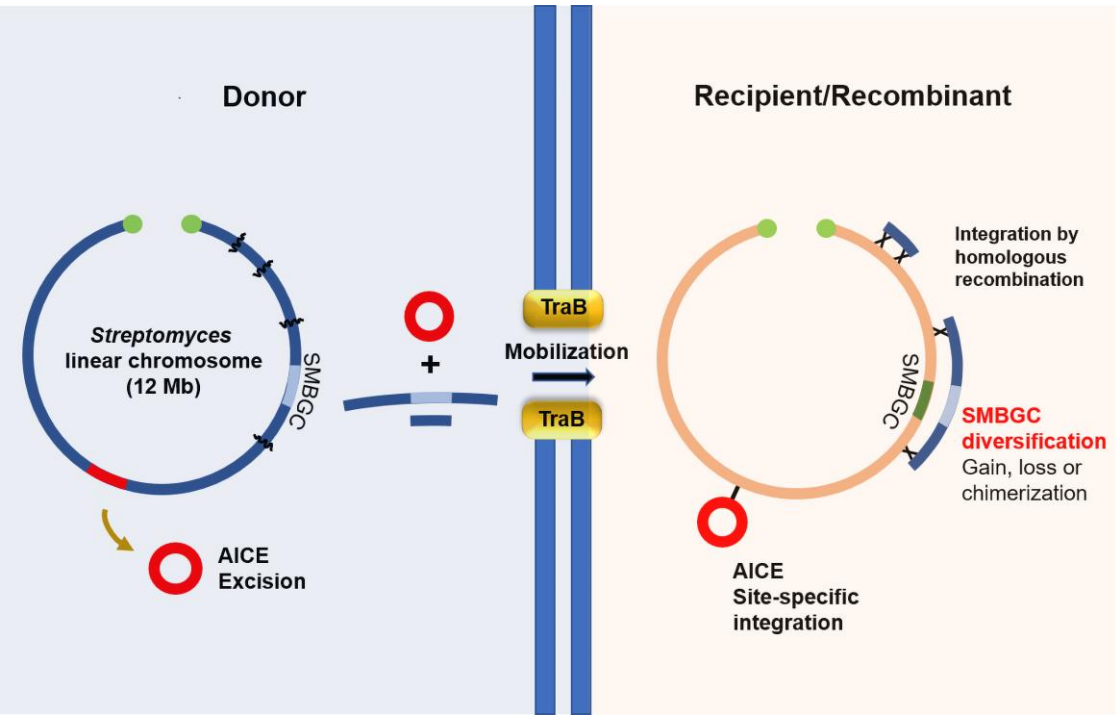
Abstract

***Streptomyces* are ubiquitous soil-dwelling bacteria with large, linear genomes that are of special importance as a source of metabolites used in human and veterinary medicine, agronomy, and industry. Conjugative elements (actinomycetes integrative and conjugative elements, AICEs) are the main drivers of *Streptomyces* Horizontal Gene Transfer. AICE transfer has long been known to be accompanied by mobilization of chromosomal DNA. However, the magnitude of DNA transfer, or the localization of acquired DNA across their linear chromosome, has remained undetermined. We here show that conjugative crossings in sympatric strains of *Streptomyces* result in the large-scale, genome-wide distributed replacement of up to one-third of the recipient chromosome, a phenomenon for which we propose the name "*Streptomyces* Chromosomal Transfer" (SCT). Such chromosome blending results in the acquisition, loss, and hybridization of Specialized Metabolite Biosynthetic Gene Clusters, leading to a novel metabolic arsenal in exconjugant offspring. Harnessing conjugation-mediated specialized metabolite biosynthesis gene cluster diversification holds great promise in the discovery of new bioactive compounds including antibiotics.**

Discoveries

Received: July 09, 2024. **Revised:** October 01, 2024. **Accepted:** October 21, 2024

Graphical Abstract



***Key words:* Streptomyces, conjugation, horizontal gene transfer, chromosome blending, specialized metabolite biosynthetic gene clusters, integrative and conjugative elements.**

Introduction

Horizontal gene transfer (HGT) is a major driver of the diversification and adaptation of bacteria (Gogarten et al. 2002). Conjugation, where gene transfer is dependent on cell–cell contact, is one of the main mechanisms of uptake of foreign DNA (Cabezón et al. 2015; Kohler et al. 2019; Virolle et al. 2020). Conjugation is mostly mediated by autonomous mobile genetic elements (MGEs) that ensure their own transfer from the donor to the recipient strain, either remaining extrachromosomal (plasmids) or integrating into the recipient's chromosome (integrative and conjugative elements, ICEs). Early work using the F-factor of *Escherichia coli* demonstrated that conjugation can also result in the transfer of chromosomal DNA flanking the integration site (Hfr) (Cavalli et al. 1953). A variety of other conjugation systems leading to large-scale chromosomal DNA transfer have since been described, including in *Mycobacterium smegmatis* (Gray and Derbyshire 2018) and *Mycoplasma agalactiae* (Dordet-Frisoni et al. 2014).

Streptomyces are filamentous Gram-positive bacteria ubiquitously distributed in soil, freshwater, and marine habitats, and can also be found in close association with plants and animals (van der Meij et al. 2017). *Streptomyces* exhibit a complex developmental cycle, which includes multi-cellular development, sporulation, and differentiation from primary to specialized metabolism (Bury-Moné et al. 2023). Members of the genus are a main source of natural products with applications in medicine, agriculture, and the food industry (Alam et al. 2022); e.g. streptomycin is critically important in human medicine used as part of the multi-drug treatment of pulmonary tuberculosis (Di Perri and Bonora 2004).

Pioneering work by Sir David A. Hopwood revealed that recombination was stimulated by so-called "Fertility Factors" in *Streptomyces coelicolor* A3(2) matings, a feature which was exploited to produce the first genetic maps in *Streptomyces* (Hopwood 2006). These fertility factors were further characterized as conjugative plasmids or AICEs (Actinomycete ICEs; the most prevalent MGEs in *Streptomyces* genomes) (te Poele et al. 2008; Bordeleau et al. 2012; Choufa et al. 2022). AICEs and conjugative plasmids (linear or circular) in *Streptomyces* utilize a fundamentally different system for self-transfer compared to canonical T4SS-dependent conjugative elements. During transfer, AICEs excise from the donor chromosome, replicate, and use the DNA translocase TraB, forming a conjugation pore, to self-transfer as dsDNA between hyphal compartments after which they reintegrate in both donor and recipient target insertion sites (Possoz et al. 2001, 2003).

Transfer of large chromosomal regions accompanying that of conjugative elements has long been suspected in *Streptomyces* based on the finding that recombinants often inherit distant markers from a parental donor strain (Hopwood 2006). We here quantify the extent of conjugal

transfer of chromosomal DNA in *Streptomyces* via mating assays coupled with the resequencing of transconjugant genomes. To do so, we employed pairs of strains belonging to the same environmental population with an overall genomic nucleotide similarity of 98.7% and 99% respectively within each pair; high enough to allow efficient homologous recombination, but divergent enough to enable *in silico* identification of donor DNA in recipient genomes. This approach allowed us, for the first time, to elucidate tract length and distribution of transferred chromosomal- and MGE DNAs in *Streptomyces*. Our experiments also provide an in-depth window into the acquisition and recombination of specialized metabolite biosynthesis gene clusters (SMBGCs) (Medema et al. 2015; Donadio et al. 2007), demonstrating that HGT greatly accelerates both the acquisition of novel SMBGCs (Fischbach et al. 2008; Ziemert et al. 2014; Nicault et al. 2020) and their rapid evolution by their recombination.

Materials and Methods

Bacterial Strains

This study employed six closely related *Streptomyces* strains that were isolated at a microscale from forest soil in a previous study (Tidjani et al. 2019). Pairs of strains were selected for their ability to form recombinants, as not all strain pairings are fertile (data not shown). To select for recombinants, each of the parental strains was labeled with a specific resistant gene (either *neo* for kanamycin or *aac(3)IV* for apramycin resistance) inserted at different chromosomal positions corresponding to intergenic regions present between two convergent genes (strains */**, supplementary table S1, Supplementary Material online). Briefly, resistance genes were amplified by PCR from the SuperCos1 (Agilent Technologies) and pIJ6902 (Huang et al. 2005) plasmids, respectively (Agilent, USA [supplementary table S2, Supplementary Material online]). Upstream and downstream regions of the targeted chromosomal site were amplified and then assembled by overlap extension-PCR (Hilgarth and Lanigan 2020) and cloned (site *Hin*dIII) into the derived pWED2 suicide vector (pWED2*) (Choufa et al. 2022). The *neo* or *aac(3)IV* genes were then inserted and cloned into an *Xba*I site designed for that purpose between the cloned upstream and downstream regions. These constructs were introduced into *Streptomyces* by intergeneric conjugation with *E. coli* ET12567/pUZ8002 (Kieser et al. 2000), and allelic replacement was selected on antibiotic resistance (*neo* or *aac(3)IV*) and susceptibility to vector-borne resistance. Alternatively, strains labeled by simple crossover integration of the construct in the chromosome (via recombination with the upstream or downstream region only) were also used (supplementary table S1, Supplementary Material online). For strain S1D4-23** (S1D4-23 NRPSaac(3)IV), a region close to the SMBGC nonribosomal peptide synthetase (NRPS)-nucleoside was amplified by PCR with primers NRPSBamHI_F and NRPSBamHI_R (supplementary table S2, Supplementary Material online), cloned into the suicide vector pIJ8668 (Sun et al. 1999). Integration into the chromosome was selected on the basis of the plasmid-borne resistance (apramycin). The labeled strains are listed in supplementary table S1, Supplementary Material online. In order to isolate recombinants, conjugations were performed with approximately 10^6 spores of each labeled parental strain cocultured on Hickey–Tresner (HT) agar medium (Hickey and Tresner 1952) for 7 d at 30 °C. The bacterial lawn was replicated on Mannitol Soy Flour medium (SFM) agar (Kieser et al. 2000) supplemented with 50 µg/ml kanamycin and 50 µg/ml apramycin, and grown for 5 d at 30 °C. The double-resistant colonies were then subcloned twice to ensure genetic homogeneity. The ratio of the number of double-resistant colonies to the total number of spores (both parental strains can be donors) was used to calculate recombination frequency.

Genome Sequencing

Genomic DNA extraction was performed according to Kieser et al. (2000). The genome of recombinants was sequenced by MiSeq sequencing (Illumina, CA, USA [mean coverage 100×]) at the Plateforme de Microbiologie Mutualisée (P2M) of the Pasteur Institute (Paris). The genome data are available on the NCBI database for the parental strains (supplementary table S1, Supplementary Material online), and raw sequencing data for the recombinant strains were deposited at Sequence Read Archive (SRA) (NCBI, Bioproject PRJNA912173).

Analysis of Recombinant Genomes

For each recombinant genome, sequencing reads were aligned with both parental strains to identify recipient and donor parent genome. The alignments were performed using the Geneious prime platform reference algorithm (Invitrogen Corp., version 2022.0.1) with the following parameters: 2 iterations, minimum quality mapping 30, minimum coverage 20× (if this threshold was not reached, Ns were added). The consensus of the recombinant sequence was aligned with the chromosomal sequences of the parents using the progressive MAUVE algorithm (Darling et al. 2004). The Single Nucleotide Polymorphism (SNP) call table was exported with MAUVE tool "export SNPs." Custom scripts (Python V.3.9) were used to call SNPs of both parental strains along the alignment (Github). In order to buffer against possible effects of point mutations which could lead to false identification of recombined fragments, we considered as a threshold the presence of at least two contiguous SNPs to assign a genomic region to one of the two parents. The size of a recombined fragment was calculated from the positions of the first and last SNPs delimiting the regions in the donor DNA, resulting in a conservative estimate of recombination fragment size. The parental origin of the TIRs of each recombinant could be unambiguously assigned thanks to the 116 to 220 kb to 246 to 261 kb which are specific to the parental chromosomes at the very ends of the TIRs in pairs A and B, respectively.

The output of analyses was manually checked to eliminate artifacts related to repeated sequences such as insertion sequences. Specialized Metabolite Biosynthetic Gene Clusters (SMBGCs) were predicted using antiSMASH (Blin et al. 2021). Whole chromosome structure of parental and recombinant strains was visualized in a circular layout produced by Circos (Krzywinski et al. 2009).

Results

Streptomyces Chromosome Transfer (SCT): Large-scale Conjugative Transfer of Chromosomal Regions in Streptomyces

To decipher how and to what extent conjugative elements promotes the transfer of the chromosomal DNA in *Streptomyces*, we performed mating experiments with closely related strains isolated from the same sympatric soil population (Tidjani et al. 2019). These strains that harbored a linear chromosome of ca. 12 Mb were previously shown to contain a great diversity of MGEs, including AICEs capable of effective transfer between strains (Choufa et al. 2022). Two strain pairs (pair A: S1A1-3* × S1D4-23*; pair B: RLB1-9* × SID4-23*; Fig. 1 and supplementary fig. S1, table S1, Supplementary Material online) able to reciprocally conjugate were selected for use in this study. Among these three strains, only RLB1-9* possessed plasmids (supplementary table S1, Supplementary Material online). Each strain was chromosomally labeled with different antibiotic resistance genes (labeled strain*/**, supplementary table S1, Supplementary Material online) and recombinants selected after mating on plates containing both antibiotics. Mating assays produced recombinants at a frequency of $\sim10^{-5}$ to 10^{-6}, which is in the same range as previously reported for different *Streptomyces* species and conjugative factors (Hopwood et al. 1984; Moretti et al. 1985; Smokvina et al. 1988; Hopwood and Kieser 1993). Twelve recombinants were picked for each strain pair, and their genomes were sequenced at high coverage (100×). The sequence for each recombinant strain was deduced from the alignment of sequencing reads on the parental chromosomes, and SNP analysis was used to identify the parental origin of each recombinant region.

For pair A, eight recombinants had an S1A1-3* genomic background (Fig. 1a), and four an S1D4-23* background (Fig. 1b). For pair B, ten recombinants had a RLB1-9* genomic background (Fig. 1c), and two an S1D4-23* background (Fig. 1d). None of the recombinants was identical to each other. The sizes of donor fragments in recombinant genomes varied from hundreds of kilobases down to extremely short stretches consisting of just a few nucleotides (Fig. 2, supplementary figs. S1 and S2, Supplementary Material online). As expected following double antibiotic selection, all recipient strains acquired a DNA fragment harboring the antibiotic resistance of the donor strain. For pair A, recombinants acquired 3 to 52 fragments of the donor DNA with an average total size of 1,825 kb (192 to 4,713 kb) representing 1.5% to 37.8% of total parental genome size (Fig. 2, supplementary table S3, Supplementary Material online). For pair B, 1 to 13 fragments were acquired with an average total size of 994 kb (255 to 2,197 kb), representing 2% to 17.5% of the total parental genome size. Overall, each recombinant acquired at least one large fragment (size, 152 to 2,020 kb), frequently corresponding to that containing the selected resistance from the donor. In one-third of the recombinants (5 out of 24), the second largest chromosomal fragment acquired (after the fragment containing the selection marker) corresponded to the end of the chromosome, including the long terminal inverted repeat (TIR, sizes from 303 to 408 kb) (Tidjani et al. 2020) capped by the telomere (supplementary table S3, Supplementary Material online). This phenomenon is particularly striking in pair A, where seven S1A1-3* background recombinants acquired both ends of the S1D4-23* chromosome (Fig. 1). None of the four recombinants analyzed in the other direction acquired the chromosome ends of the donor (S1A1-3*). For pair B, only one TIR replacement was observed for the recombinant B4 (Fig. 1c). Smaller fragments, defined as shorter than the median value of the recombined fragments (i.e. 4.9 kb), were predominantly located close to large acquired regions, resulting in "microheterogeneity" hotspots (Fig. 2 and supplementary fig. S2, Supplementary Material online). In addition to DNA acquisitions, deletions up to several hundreds of kilobases (Fig. 1) were observed in almost a third of the recombinants but these occurred independently of DNA acquisitions.

AICEs Drive Chromosomal Mobilization

All 24 recombinants but one acquired at least one AICEs. Eleven AICEs, all possessing the three key functions (i.e. integration, replication, and transfer) enabling their autonomous mobilization, were present in the parental strains. Eight out of 11 were transferred in the recombinants. Several types of AICE acquisition were observed. Self-transfers of three AICEs (AICE05, AICE11, AICE13; Fig. 3b), i.e. the result of AICE-mediated excision and integration (evidenced by AICE flanking sequences from the recipient strain), was observed in 19 out of 24 recombinants (Fig. 3b). In one case, the self-transfer of two AICEs was observed (recombinant B2, Fig. 3b). In 14 recombinants, AICEs were acquired not by self-transfer but likely through homologous recombination events with the chromosomal regions flanking the elements. In nine of these recombinants, this non-autonomous acquisition occurred along with self-transferred AICEs; in four cases (A2, A8, B9, B12), there is no evidence of AICE self-transfer and/or integration. In recombinant A2, the acquired AICE (AICE05) was flanked by chromosomal DNA of the donor on only one side revealing the mobilization of DNA on only one side of the element (monodirectional mobilization) (Hopwood 2006). Interestingly, two recombinants (A3 and B11) were found to have acquired a chromosomal fragment which harbored an AICE in the donor but not in the recipient, possibly because the AICE was

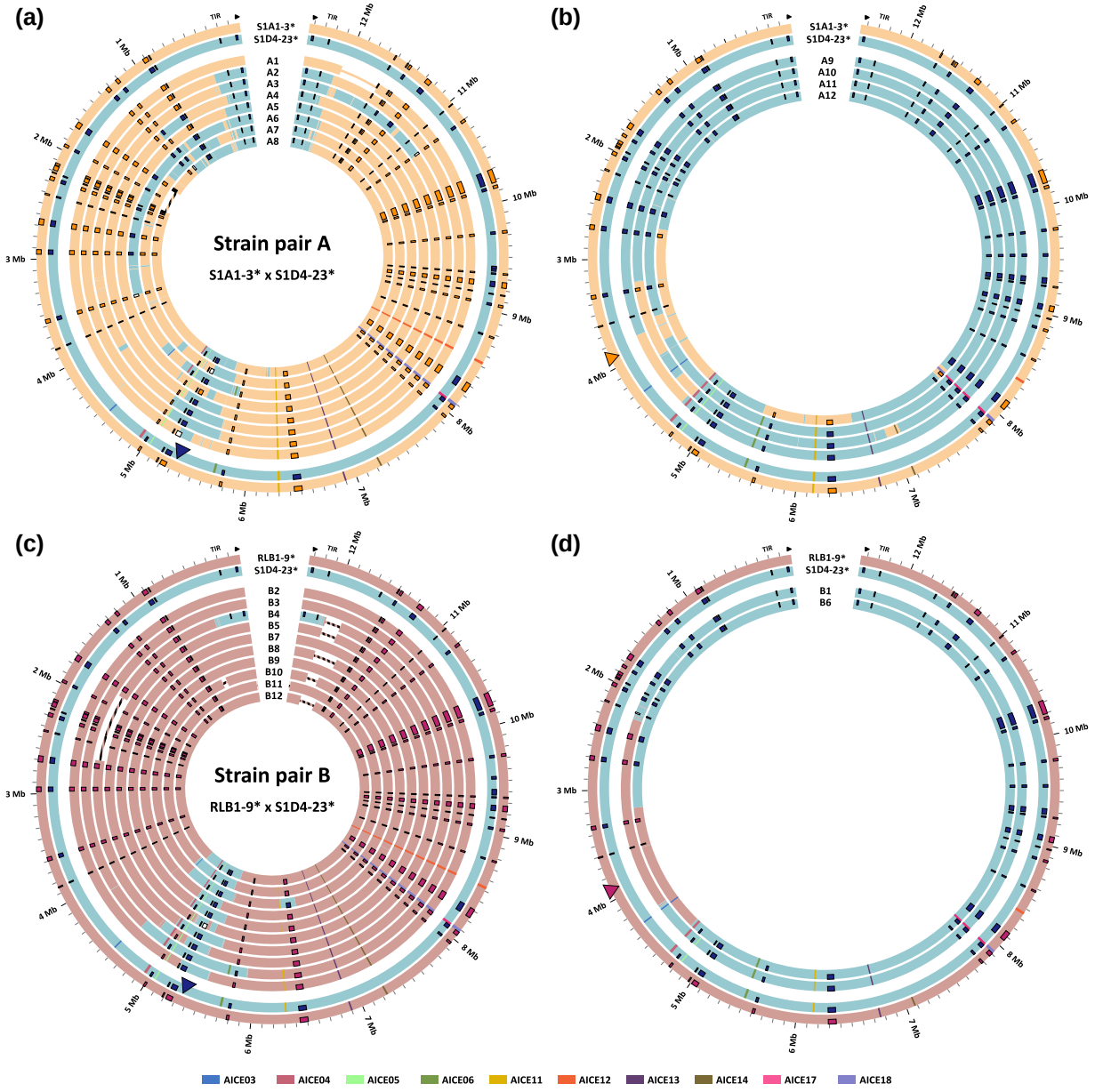


Fig. 1. Chromosomal DNA acquisition in recombinant strains. The linear chromosomes of the parental and recombinant strains for mating pairs A (S1A1-3* × S2D4-23*) and B (RLB1-9* × S1D4-23*) are represented as open concentric circles (Circos). a) Recombinants A1 to A8 with an S1A1-3* genomic background. b) Recombinants A9 to A12 with an S1D4-23* background. c) B2 to B5 and B7 to B12 recombinants with an RLB1-9* genomic background. d) Recombinants B1 and B6 with an S1D4-23* genomic background. For each strain pair, the parental chromosomes correspond to the two outer circles (S1A1-3*, S1D4-23*, and RLB1-9*), and the recombinants to the inner circles. Terminal inverted repeats (TIRs) symbolized by arrows are shown on the external ring for the parents only. AICEs are represented by rectangles in the concentric circles. SMBGCs are represented by rectangles on the upper edge of the concentric circles. Chimeric and hybrid (rearranged) SMBGCs are colored white and black, respectively. Large deletions are represented by a hatched line. All recombinants have inherited the donor chromosomal selection marker (colored triangle). Each locus (including the selected antibiotic resistance gene) was positioned relative to the chromosomal positions of the recipient genome. The AICE code corresponds to the different AICE families as defined in Choufa et al. (2022).

subsequently lost during the mobilization process by the defect of the integration step in the recipient chromosome.

During the conjugation process, gain or loss of plasmids in the recombinants was also observed. In pair B (the only pair involving plasmids), the acquisition concerned the linear plasmid pRLB1-9.2 in recombinants B1 and B6. Loss of the circular plasmid pRLB1-9.1 was observed in two-thirds of the RLB1-9* recombinants (Fig. 3).

Large-scale Recombination of Specialized Metabolite Biosynthetic Gene Clusters (SMBGCs)

Specialized metabolite biosynthesis gene clusters (SMBGCs) are large genomic regions that encompass both biosynthesis genes together with regulatory and resistance genes (in case of toxic activity such as antibiotics). Conjugative HGT was found to result in profound reassortment of parental SMBGCs in recombinants. Twenty-two out of 24 recombinants differed in SMBGC content

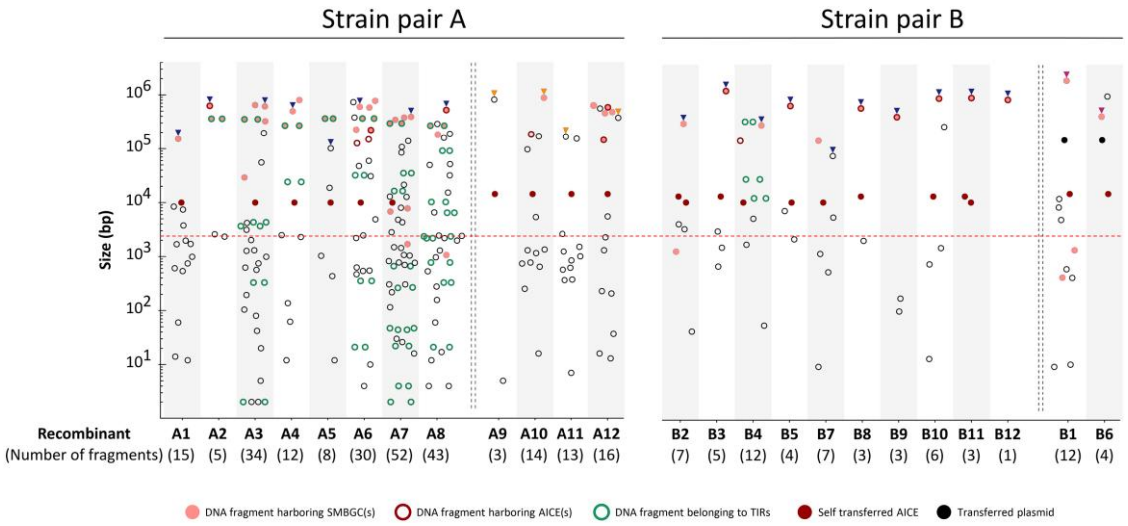


Fig. 2. Number and size of incorporated DNA fragments in recombinant progeny. Size length distribution of incorporated donor DNA in all recombinants obtained for strain pairs A and B (A1 to B12). The number of acquired fragments for each recombinant is indicated in brackets. The different recipient genomic backgrounds are separated by a vertical double dotted line. The dotted red horizontal line at 4.9 kb represents the median length of the recombinant DNA fragments.

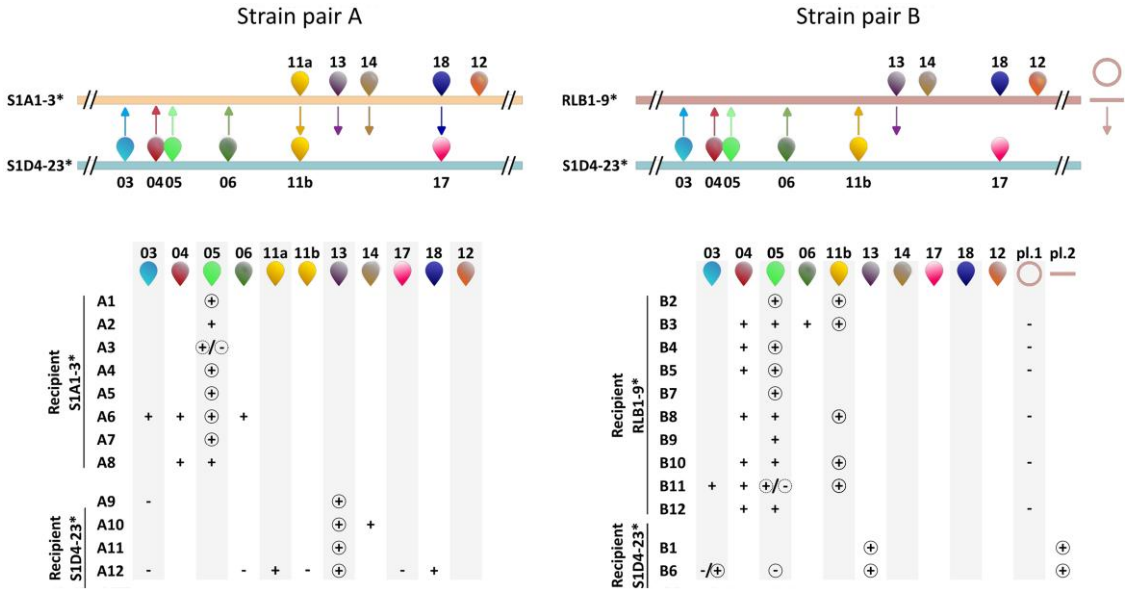


Fig. 3. MGE transfer in recombinants. a) AICE content of the parental strains. AICE mobility is indicated by colored arrows. The mobility of AICE11a and AICE18 (pair a) results in the replacement of AICE11b and AICE17, respectively, at the same insertion site; AICE11a and AICE 11b are different elements but belong to the same family (same color code). b) Describes the acquired (+)/lost (−) AICEs for each recombinant of the two pairs A and B. When the AICE is self-transferred, the + symbol is circled. In recombinants A3 and B11, the +/− circled by a dotted line symbolizes the probable loss (by self-excision) of AICE05 during transfer in either the donor or the recipient. Note that the loss of AICE05 in recombinant B6 probably results from its excision not followed by reintegration in its original chromosome. AICE03 in B6 was probably lost by replacement of the homologous region of the donor devoid of AICE03, but reintegrated at its target site after DNA acquisition. The plasmids pRLB1-9.1 (circular) and pRLB1-9.2 (linear) are represented by a circle and a line, respectively.

relative to the two parental strains which contain between 36 and 40 SMBGCs each (Table 1, supplementary fig. S3, Supplementary Material online). As most SMBGCs were common to both parental strains (supplementary fig. S3, Supplementary Material online), whole-sale replacement of recipient SMBGCs by the homologous SMBGCs of the donor was commonly observed. Twenty out of 24 recombinants showed the replacement of 1 to 14 SMBGCs. These replacements resulted from recombination events occurring in the regions flanking the SMBGCs. Since homologous SMBGCs can differ in gene content (supplementary fig. S4, Supplementary Material online), such substitutions potentially result in a significant change in biosynthesis capacity. In a third of the recombinants, a recombination breakpoint occurred within the SMBGC itself resulting in the formation of chimeric SMBGCs (Fig. 4). In five out of

Table 1 SMBGC reassortment in recombinants

Recombinant	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	B2	B3	B4	B5	B7	B8	B9	B10	B11	B12	B1	B6
Gain	-	2	2	2	2	2	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss	2	-	1	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-
Substitution[a]	-	2	6	5	-	14	4	5	-	2	-	6	1	4	2	3	1	3	3	4	3	3	4	2
Chimerization[a]	1	-	1	-	-	1	2	1	-	-	-	-	1	-	-	-	1	-	-	-	-	-	1	-
Recombined DNA (kbp)	192	1,350	2,607	1,898	858	4,713	2,242	2,272	833	1,332	345	3,259	356	1,354	1,256	709	255	635	426	1,239	993	891	2,197	1,613

Gain: corresponds to the acquisition of a BGC for which no homolog was present in the recipient; loss: corresponds to the acquisition of a BGC native to the recipient.
[a]Replacement of a complete SMBGC in the recipient by its homologue from the donor leads to the formation of a substitution. When the recombination event occurs within the SMBGC, a chimeric version of the cluster is produced.

nine chimeric SMBGCs, the recombination breakpoint(s) occurred in one of the SMBGC biosynthetic genes. This could reflect the recombinogenic nature of SMBGCs *via* sequence repeats associated with redundant protein motifs within multidomain biosynthetic genes (Fischbach et al. 2008). Figure 4 illustrates such cases with recombination in A1 in the NRPS-like/T1PKS (Fig. 4a), in recombinant A7 in the aminopolycarboxylic acid biosynthetic gene (Fig. 4b) and in recombinant B2 in the siderophore synthase of NI-siderophore (Fig. 4c). In the last case (B2), the siderophore SMBGC constitutes the border of a large acquired region, and as depicted in Fig. 4c, several recombination breakpoints were identified very close near to each other forming a microheterogeneity hotspot within the siderophore synthase gene.

Acquisition of donor-specific SMBGCs by recipients was observed mainly in terminal regions. Two SMBGCs (*terpene* and *Ripp-like*) were acquired in the same DNA fragment in eight recombinants (A2 to A8 and B4, Table 1). As the terminal regions are duplicated, all the SMBGCs present in these regions are *de facto* in two copies. Loss of SMBGCs (from one to six) was observed in five recombinants (A1, A3, A6, A8, and B4; Table 1). The simplest event corresponded to the replacement of a region harboring an SMBGC in the recipient by its donor homolog devoid of the SMBGC (A3 and A6). In three recombinants (A1, A8, and B4), the partial loss of an SMBGC was not directly linked to recombination with incoming DNA, but to rearrangement of the host genome. In A8, a deletion of about 416 kb resulted in the partial loss of two SMBGCs, and also in the creation of a NRPS and T3PKS hybrid SMBGC at the junction point (supplementary fig. S5, Supplementary Material online).

To test whether conjugative transfer can mobilize specialized metabolite biosynthesis pathways, a selection marker (*aac(3)IV* for apramycin resistance) was inserted close to specific SMBGCs in two strains. One marker was inserted close to an SMBGC encoding a lanthipeptide in RLB3-17* (at 80.5 kb), and another close to an SMBGC encoding an NRPS-nucleoside in S1D4-23** (at 27.5 kb). In pair C (RLB3-17* × RLB1-8*, ANI 99.0%), seven recombinants were whole-genome sequenced (supplementary fig. S6, Supplementary Material online). Five of them were found to have acquired the lanthipeptide SMBGC. The size of the fragment carrying both the SMBGC and the selection marker varied between 341.9 kb and 609.5 kb. In pair D (S1D4-23** × S1D4-14*, ANI 98.7%), the three recombinants analyzed acquired NRPS-containing fragments ranging in size from 221 to 509 kb. The size of recombined chromosomal DNA fragments, several hundred kilobases around the selection marker, is in the same range as that of the SMBGCs, enabling their whole-sale transfer.

Discussion

Here, we show that AICE transfer in *Streptomyces* is accompanied by the uptake and homologous recombination of vast tracts of unlinked chromosomal DNA. A single

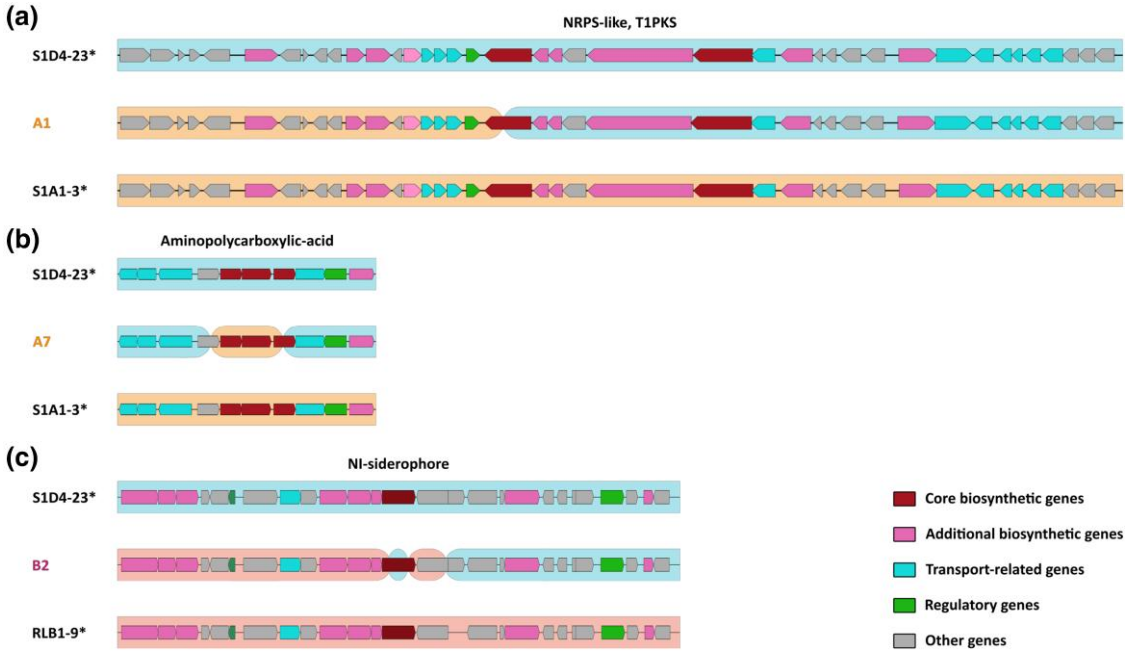


Fig. 4. Representation of three different chimeric SMBGCs obtained in the recombinant progeny. Homologous parental SMBGCs predicted by antiSMASH are represented according to this software nomenclature. The donor strain SMBGC is represented at the top of each scheme and the recipient strain SMBGC at the bottom; both SMBGCs are highlighted with a strain specific color according to Fig. 1. Chimeric SMBGCs identified in recombinants (A1, A7, and B2) are represented in-between parental SMBGCs. The DNA parental origin in a chimeric SMBGC is identified according to its background color that correspond to those specifically highlighting parental SMBGCs.

conjugation event can result in the replacement of 1.5% to 37.8% of the recipient genome, distributed in fragments across the chromosome: a phenomenon for which we propose the term *Streptomyces* chromosomal transfer (SCT). The size and distribution of the fragments indicates that one or more large DNA fragments are transferred followed by recombination with the host chromosome. Microheterogeneity at the edge of large transferred DNA stretches probably result from gene conversion at the recombination site, which is responsible for alternating parental sequences in recombinants (Cole et al. 2012). Microheterogeneity hotspots have also been observed in *Mycobacterium* (Gray and Derbyshire 2018) and *Mycoplasma* (Dordet-Frisoni et al. 2019), two genera in which large-scale HGT events and chromosomal mosaicism have been previously demonstrated (Gray and Derbyshire 2018; Dordet-Frisoni et al. 2019).

SCT can be hypothesized to occur *via* three distinct TraB-dependent mechanisms (Fig. 5). In a first scenario originally put forward by Sir D.A. Hopwood, AICEs transfer without being excised from the donor chromosome (Hopwood et al. 1969, 1973) (Fig. 5a). This hypothesis was proposed based on analysis of chromosome marker mobilization by the integrated form of the conjugative plasmid SCP1 in *Streptomyces coelicolor* A3(2) (Hopwood 2006). Mobilization of the element is here assumed to be based on the recognition of a *cis*-acting pattern, the *clt* site for *c*is-acting *l*ocus of *t*ransfer (Franco et al. 2003; Vogelmann et al. 2011), by the TraB complex (hexamer) forming a conjugation pore from the donor to the recipient cells. The mobilization of the conjugative element from its chromosomal locus, without excision, would here mediate the transfer of chromosomal DNA to one or both sides of the AICE in a mono- or bidirectional pattern, in a similar fashion to the polarized mobilization induced by the F-factor integrated into the *E. coli* chromosome (Hfr strain). Such a mechanism of SCT would explain the observation of recombinants formed without concomitant acquisition of autonomous AICE. In a second scenario initially suggested by Pettis and Cohen (Pettis and Cohen 1994) and further supported by the work of G. Muth's group (Vogelmann et al. 2011), double-stranded chromosomal DNA is transferred through the TraB pore into the recipient mycelium alongside an independently moving free AICE (Fig. 5b). Transfer here is hypothesized to be mediated by *clt*-like sequences distributed through the chromosome (*clc* for *clt*-like chromosomal sequences) (Vogelmann et al. 2011).

Recombination was frequently accompanied by acquisition of the terminal parts of the chromosome of the donor (7 and 1 out of 12 in crossings A and B, respectively). The concomitant acquisition of the two chromosomal extremities can be explained by the simultaneous acquisition of chromosome ends. It can also result from the acquisition of a copy of a chromosome arm, the transient production of a hybrid chromosome with divergent TIRs, followed by homogenization of the ends *via* homologous recombination. This homogenization phenomenon is possible thanks to the strong DNA identity between the parental TIRs in their internal parts (between 147 and 188 kb from the start of the TIRs of the parental strains are

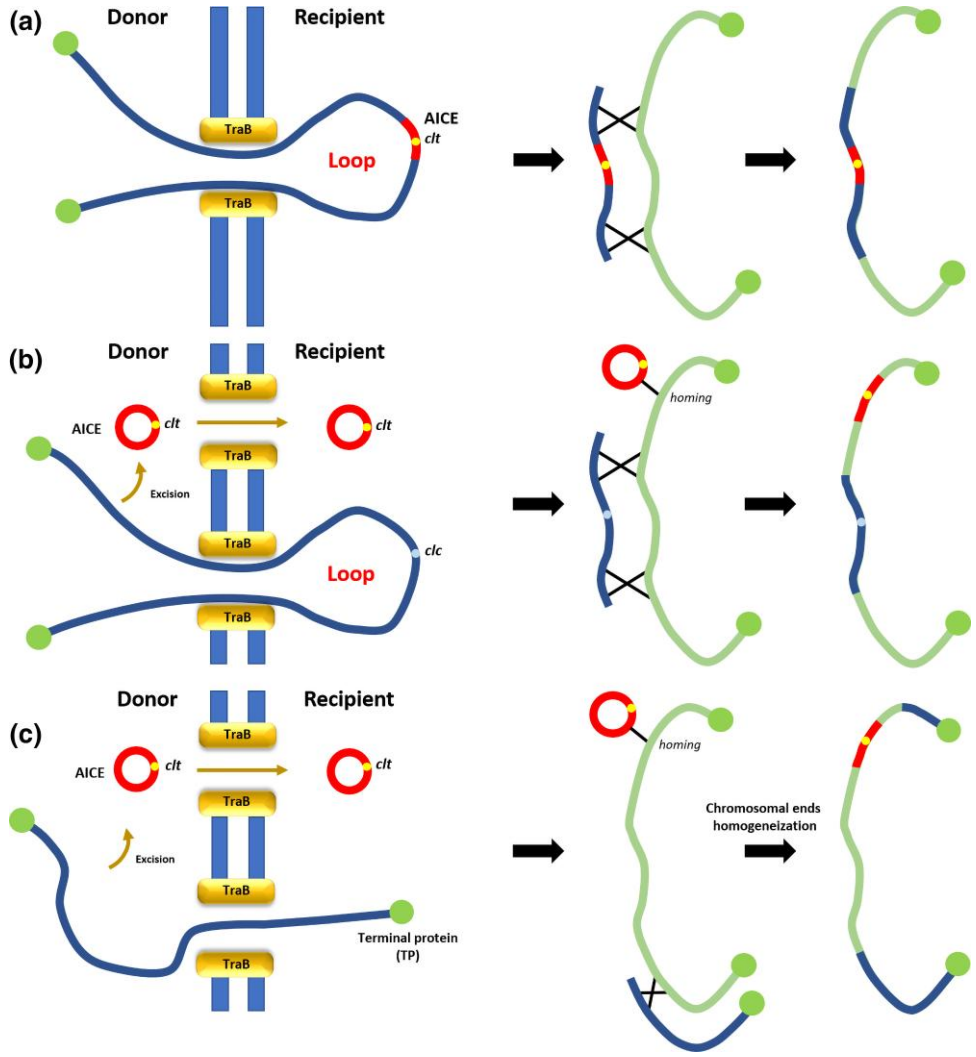


Fig. 5. Scenarios for TraB-dependent DNA mobilization. a) *cis*-mobilization: mobilization of the conjugative element from its chromosomal locus thanks to its *clt* site for *c*is-acting *l*ocus of *t*ransfer, by the TraB complex (hexamer) forming a conjugation pore from the donor to the recipient cells. A large DNA loop is extruded in the recipient cell. After transfer, replacement of a large region is achieved by homologous recombination. b) *trans*-mobilization: chromosomal DNA is transferred through the TraB pore into the recipient mycelium independently from an excised AICE. Transfer of chromosomal DNA is hypothesized to be mediated by *clc* (for *clt*-like chromosomal sequences) distributed through the chromosome. After transfer, self-integration of the AICE occurs at its integration site while chromosomal DNA is integrated by homologous recombination. c) "End-first" hypothesis: each chromosomal end could serve as transfer origin and mobilize chromosomal information by the ends. An AICE could self-transfer autonomously. After transfer, the chromosomal end would replace one end and produce a chromosome with two distinct ends; such hybrid structure could be homogenized by terminal recombination. If both ends are transferred and recombined, the concomitant acquisition of the two chromosomal extremities can be obtained. For b and c, mobilization of chromosomal and AICE DNA could occur through a unique TraB pore in the form of double-stranded DNA.

homologous for pairs A and B, respectively) (Tidjani et al. 2020); the more distal region till the chromosome extremity is specific of each strain do not support homologous recombination. A close look at the origin of the SNPs of the terminal regions of the recombinant revealed that frequent exchanges occurred between the homologous parts of the parental TIRs supporting a homogenization mechanism powered by homologous recombination. A third scenario is supported by our recombinant analysis, the so-called "end-first" model proposed by C.W. Chen (Wang et al. 1999; Lee et al. 2011) where chromosomal ends could serve as transfer origins (Fig. 5c). In these experiments mobilization of the chromosomal ends was associated with the presence of conjugative plasmids. In our experiments, parental strains of pair A were devoid of conjugative plasmids showing that, in this scenario, AICEs would also be able to promote this end-first mobilization. As all three types of recombination were observed within the population, or even within a single recombinant, the three mechanisms are not mutually exclusive and could operate simultaneously.

Streptomyces are well-known for their production of antibiotics and other specialized metabolites (van der Meij et al. 2017). The biotechnological exploitation of this biosynthetic arsenal is a key objective in the arms race against the emergence of antibiotic resistance in pathogenic

bacteria (Allahverdiyev et al. 2013; Dávila Costa et al. 2022; Gavriilidou et al. 2022). Cloning and transfer of an entire biosynthetic pathway (often approaching 100 kb in size) is often a hindrance to heterologous expression approaches to identify the metabolite encoded by the SMBGC (Nah et al. 2017; Zhao et al. 2017; Nepal and Wang 2019; Liu et al. 2021). Our mating assays demonstrated that conjugative transfer resulted in the transfer of complete SMBGCs as well as the shuffling of parental SMBGCs within recombinants. Gain, loss, duplication and (wholesale) SMBGC replacement was observed, especially in terminal regions which are known to be enriched in SMBGCs (Bury-Moné et al. 2023). To our knowledge, this is the first experimental demonstration of chromosomal SMBGC transfer and recombination in *Streptomyces*.

Recombination within SMBGCs (i.e. formation of chimeric and hybrid SMBGCs) was also frequently observed, which could be aided by their repetitive structure (Fischbach et al. 2008; Nivina et al. 2021). Polyketide synthase (PKS) and NRPS synthases are large multidomain enzymes encoded by long highly repetitive DNA sequences that can also exhibit short repeated DNA motifs (Nivina et al. 2021), favoring recombination and gene conversion (Fischbach et al. 2008). SCT can thus result in inter- and intra-domain recombination, deletions, or insertions of modular enzymes opening the possibility for diversifying function of BGCs and their products (Chevrette et al. 2020). It is known that genome shuffling can induce drastic alterations of the specialized metabolism leading to new antibiotic variants and/or increased antibiotic yields in *Streptomyces* (Zhang et al. 2002; Xu et al. 2008; Tong et al. 2018). The capacity for large-scale chromosomal recombination could be hypothesized to aid adaptation in (small-scale) *Streptomyces* populations (Tidjani et al. 2019; Tidjani et al. 2020). The rearrangement of biosynthetic gene cluster (BGC) could lead to rapid niche differentiation or play roles in intra- and interspecific "bacterial warfare" (Czárán et al. 2002). On longer timescales, recombination is expected to greatly influence speciation processes (Vos 2011; Shapiro and Polz 2014). The natural products and enzymes produced by actinomycetes have also been shown to impact other members of the ecological niche by protecting them against pathogens, promoting their growth or providing nutriments by the degradation of complex natural polymers such as lignocellulose (van der Meij et al. 2017; Grundmann et al. 2024). Apart from its relevance to the ecology and evolution of *Streptomyces*, the rapid generation of SMBGC diversification in simple mating type assays also offers a new avenue into natural product discovery by providing new combinations of biosynthesis genes, reassociation of functional domains within SMBGCs, and identification of new molecules of interest.

Supplementary Material

Supplementary material is available at *Molecular Biology and Evolution* online.

Acknowledgments

We would like to thank Jean-Luc Pernodet (I2BC, CNRS) for his valuable advice on improving the manuscript.

Funding

This work was supported by a grant overseen by the French National Research Agency (ANR) (ANR-11-LABX-0002 to 0001, Lab of Excellence ARBRE). C. Choufa received co-funding support from the Région Grand Est.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Data Availability

Raw sequencing data for the recombinant strains were deposited at SRA (NCBI, Bioproject PRJNA912173).

Code Availability

The codes to reproduce the main results have been deposited on GitHub: https://github.com/leblond14u/Large-scale-chromosomal-DNA-transfer.

References

Alam K, Mazumder A, Sikdar S, Zhao Y-M, Hao J, Song C, Wang Y, Sarkar R, Islam S, Zhang Y, et al. Streptomyces: the biofactory of secondary metabolites. Front Microbiol. 2022:**13**:968053. https://doi.org/10.3389/fmicb.2022.968053.

Allahverdiyev AM, Bagirova M, Abamor ES, Ates SC, Koc RC, Miraloglu M, Elcicek S, Yaman S, Unal G. The use of platensimycin and platencin to fight antibiotic resistance. Infect Drug Resist. 2013:**6**:99–114. https://doi.org/10.2147/IDR.S25076.

Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, Medema MH, Weber T. antiSMASH 6.0: improving cluster detection and comparison capabilities. Nucleic Acids Res. 2021:**49**(W1):W29–W35. https://doi.org/10.1093/nar/gkab335.

Bordeleau E, Ghinet MG, Burrus V. Diversity of integrating conjugative elements in actinobacteria: coexistence of two mechanistically different DNA-translocation systems. Mob Genet Elements. 2012:**2**(2):119–124. https://doi.org/10.4161/mge.20498.

Bury-Moné S, Thibessard A, Lioy VS, Leblond P. Dynamics of the Streptomyces chromosome: chance and necessity. Trends Genet. 2023:**39**(11):873–887. https://doi.org/10.1016/j.tig.2023.07.008.

Cabezón E, Ripoll-Rozada J, Peña A, de la Cruz F, Arechaga I. Towards an integrated model of bacterial conjugation. FEMS Microbiol Rev. 2015:**39**(1):81–95. https://doi.org/10.1111/1574-6976.12085.

Cavalli LL, Lederberg J, Lederberg EM. An infective factor controlling sex compatibility in Bacterium coli. J Gen Microbiol. 1953:**8**(1):89–103. https://doi.org/10.1099/00221287-8-1-89.

Chevrette MG, Gutiérrez-García K, Selem-Mojica N, Aguilar-Martínez C, Yañez-Olvera A, Ramos-Aboites HE, Hoskisson PA, Barona-Gómez F. Evolutionary dynamics of natural product biosynthesis in bacteria. Nat Prod Rep. 2020:**37**(4):566–599. https://doi.org/10.1039/C9NP00048H.

Choufa C, Tidjani A-R, Gauthier A, Harb M, Lao J, Leblond-Bourget N, Vos M, Leblond P, Bontemps C. Prevalence and mobility of integrative and conjugative elements within a Streptomyces natural

population. Front Microbiol. 2022:**13**:970179. https://doi.org/10.3389/fmicb.2022.970179.
Cole F, Keeney S, Jasin M. Preaching about the converted: how meiotic gene conversion influences genomic diversity. Ann N Y Acad Sci. 2012:**1267**(1):95–102. https://doi.org/10.1111/j.1749-6632.2012.06595.x.
Czárán TL, Hoekstra RF, Pagie L. Chemical warfare between microbes promotes biodiversity. Proc Natl Acad Sci U S A. 2002:**99**(2):786–790. https://doi.org/10.1073/pnas.012399899.
Darling ACE, Mau B, Blattner FR, Perna NT. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res. 2004:**14**(7):1394–1403. https://doi.org/10.1101/gr.2289704.
Dávila Costa JS, Guerrero DS, Romero CM. Streptomyces: connecting red-nano and grey biotechnology fields. Crit Rev Microbiol. 2022:**48**(5):565–576. https://doi.org/10.1080/1040841X.2021.1991272.
Di Perri G, Bonora S. Which agents should we use for the treatment of multidrug-resistant Mycobacterium tuberculosis? J Antimicrob Chemother. 2004:**54**(3):593–602. https://doi.org/10.1093/jac/dkh377.
Donadio S, Monciardini P, Sosio M. Polyketide synthases and nonribosomal peptide synthetases: the emerging view from bacterial genomics. Nat Prod Rep. 2007:**24**(5):1073–1109. https://doi.org/10.1039/b514050c.
Dordet-Frisoni E, Faucher M, Sagné E, Baranowski E, Tardy F, Nouvel LX, Citti C. Mycoplasma chromosomal transfer: a distributive, conjugative process creating an infinite variety of mosaic genomes. Front Microbiol. 2019:**10**:2441. https://doi.org/10.3389/fmicb.2019.02441.
Dordet-Frisoni E, Sagné E, Baranowski E, Breton M, Nouvel LX, Blanchard A, Marenda MS, Tardy F, Sirand-Pugnet P, Citti C. Chromosomal transfers in mycoplasmas: when minimal genomes go mobile. mBio. 2014:**5**(6):e01958. https://doi.org/10.1128/mBio.01958-14.
Fischbach MA, Walsh CT, Clardy J. The evolution of gene collectives: how natural selection drives chemical innovation. Proc Natl Acad Sci U S A. 2008:**105**(12):4601–4608. https://doi.org/10.1073/pnas.0709132105.
Franco B, González-Cerón G, Servín-González L. Direct repeat sequences are essential for function of the cis-acting locus of transfer (clt) of Streptomyces phaeochromogenes plasmid pJV1. Plasmid. 2003:**50**(3):242–247. https://doi.org/10.1016/S0147-619X(03)00063-5.
Gavriilidou A, Kautsar SA, Zaburannyi N, Krug D, Müller R, Medema MH, Ziemert N. Compendium of specialized metabolite biosynthetic diversity encoded in bacterial genomes. Nat Microbiol. 2022:**7**(5):726–735. https://doi.org/10.1038/s41564-022-01110-2.
Gogarten JP, Doolittle WF, Lawrence JG. Prokaryotic evolution in light of gene transfer. Mol Biol Evol. 2002:**19**(12):2226–2238. https://doi.org/10.1093/oxfordjournals.molbev.a004046.
Gray TA, Derbyshire KM. Blending genomes: distributive conjugal transfer in mycobacteria, a sexier form of HGT. Mol Microbiol. 2018:**108**(6):601–613. https://doi.org/10.1111/mmi.13971.
Grundmann CO, Guzman J, Vilcinskas A, Pupo MT. The insect microbiome is a vast source of bioactive small molecules. Nat Prod Rep. 2024:**41**(6):935–967. https://doi.org/10.1039/D3NP00054K.
Hickey RJ, Tresner HD. A cobalt-containing medium for sporulation of Streptomyces species. J Bacteriol. 1952:**64**(6):891–892. https://doi.org/10.1128/jb.64.6.891-892.1952.
Hilgarth RS, Lanigan TM. Optimization of overlap extension PCR for efficient transgene construction. MethodsX. 2020:**7**:100759. https://doi.org/10.1016/j.mex.2019.12.001.
Hopwood DA. Soil to genomics: the Streptomyces chromosome. Annu Rev Genet. 2006:**40**(1):1–23. https://doi.org/10.1146/annurev.genet.40.110405.090639.
Hopwood DA, Chater KF, Dowding JE, Vivian A. Advances in Streptomyces coelicolor genetics. Bacteriol Rev. 1973:**37**(3):371–405. https://doi.org/10.1128/br.37.3.371-405.1973.
Hopwood DA, Harold RJ, Vivian A, Ferguson HM. A new kind of fertility variant in Streptomyces coelicolor. Genetics. 1969:**62**(3):461–477. https://doi.org/10.1093/genetics/62.3.461.
Hopwood DA, Hintermann G, Kieser T, Wright HM. Integrated DNA sequences in three streptomycetes form related autonomous plasmids after transfer to Streptomyces lividans. Plasmid. 1984:**11**(1):1–16. https://doi.org/10.1016/0147-619X(84)90002-7.
Hopwood DA, Kieser T. Conjugative plasmids of Streptomyces. In: Clewell DB, editors. Bacterial conjugation. Boston (MA): Springer US; 1993. p. 293–311.
Huang J, Shi J, Molle V, Sohlberg B, Weaver D, Bibb MJ, Karoonuthaisiri N, Lih C-J, Kao CM, Buttner MJ, et al. Cross-regulation among disparate antibiotic biosynthetic pathways of Streptomyces coelicolor. Mol Microbiol. 2005:**58**(5):1276–1287. https://doi.org/10.1111/j.1365-2958.2005.04879.x.
Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA. Practical Streptomyces genetics. Norwich: The John Innes Foundation; 2000.
Kohler V, Keller W, Grohmann E. Regulation of gram-positive conjugation. Front Microbiol. 2019:**10**:1134. https://doi.org/10.3389/fmicb.2019.01134.
Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. Circos: an information aesthetic for comparative genomics. Genome Res. 2009:**19**(9):1639–1645. https://doi.org/10.1101/gr.092759.109.
Lee H-H, Hsu C-C, Lin Y-L, Chen CW. Linear plasmids mobilize linear but not circular chromosomes in Streptomyces: support for the "end first" model of conjugal transfer. Microbiology (Reading). 2011:**157**(9):2556–2568. https://doi.org/10.1099/mic.0.051441-0.
Liu Z, Zhao Y, Huang C, Luo Y. Recent advances in silent gene cluster activation in Streptomyces. Front Bioeng Biotechnol. 2021:**9**:632230. https://doi.org/10.3389/fbioe.2021.632230.
Medema MH, Kottmann R, Yilmaz P, Cummings M, Biggins JB, Blin K, de Bruijn I, Chooi YH, Claesen J, Coates RC, et al. Minimum information about a biosynthetic gene cluster. Nat Chem Biol. 2015:**11**(9):625–631. https://doi.org/10.1038/nchembio.1890.
Moretti P, Hintermann G, Hütter R. Isolation and characterization of an extrachromosomal element from Nocardia mediterranei. Plasmid. 1985:**14**(2):126–133. https://doi.org/10.1016/0147-619X(85)90072-1.
Nah H-J, Pyeon H-R, Kang S-H, Choi S-S, Kim E-S. Cloning and heterologous expression of a large-sized natural product biosynthetic gene cluster in Streptomyces species. Front Microbiol. 2017:**8**:394. https://doi.org/10.3389/fmicb.2017.00394.
Nepal KK, Wang G. Streptomycetes: surrogate hosts for the genetic manipulation of biosynthetic gene clusters and production of natural products. Biotechnol Adv. 2019:**37**(1):1–20. https://doi.org/10.1016/j.biotechadv.2018.10.003.
Nicault M, Tidjani A-R, Gauthier A, Dumarcay S, Gelhaye E, Bontemps C, Leblond P. Mining the biosynthetic potential for specialized metabolism of a Streptomyces soil community. Antibiotics (Basel). 2020:**9**(5):271. https://doi.org/10.3390/antibiotics9050271.
Nivina A, Herrera Paredes S, Fraser HB, Khosla C. GRINS: genetic elements that recode assembly-line polyketide synthases and accelerate their diversification. Proc Natl Acad Sci U S A. 2021:**118**(26):e2100751118. https://doi.org/10.1073/pnas.2100751118.
Pettis GS, Cohen SN. Transfer of the pIJ101 plasmid in Streptomyces lividans requires a cis-acting function dispensable for chromosomal gene transfer. Mol Microbiol. 1994:**13**(6):955–964. https://doi.org/10.1111/j.1365-2958.1994.tb00487.x.
Possoz C, Gagnat J, Sezonov G, Guérineau M, Pernodet J-L. Conjugal immunity of Streptomyces strains carrying the integrative element pSAM2 is due to the pif gene (pSAM2 immunity factor). Mol Microbiol. 2003:**47**(5):1385–1393. https://doi.org/10.1046/j.1365-2958.2003.03380.x.
Possoz C, Ribard C, Gagnat J, Pernodet J-L, Guérineau M. The integrative element pSAM2 from Streptomyces: kinetics and mode of

conjugal transfer. Mol Microbiol. 2001:**42**(1):159–166. https://doi.org/10.1046/j.1365-2958.2001.02618.x.
Shapiro BJ, Polz MF. Ordering microbial diversity into ecologically and genetically cohesive units. Trends Microbiol. 2014:**22**(5):235–247. https://doi.org/10.1016/j.tim.2014.02.006.
Smokvina T, Francou F, Luzzati M. Genetic analysis in Streptomyces ambofaciens. J Gen Microbiol. 1988:**134**(2):395–402. https://doi.org/10.1099/00221287-134-2-395.
Sun J, Kelemen GH, Fernández-Abalos JM, Bibb MJ. Green fluorescent protein as a reporter for spatial and temporal gene expression in Streptomyces coelicolor A3(2). Microbiology (Reading). 1999:**145**(Pt 9):2221–2227. https://doi.org/10.1099/00221287-145-9-2221.
te Poele EM, Bolhuis H, Dijkhuizen L. Actinomycete integrative and conjugative elements. Antonie Van Leeuwenhoek. 2008:**94**(1):127–143. https://doi.org/10.1007/s10482-008-9255-x.
Tidjani A-R, Bontemps C, Leblond P. Telomeric and sub-telomeric regions undergo rapid turnover within a Streptomyces population. Sci Rep. 2020:**10**(1):7720. https://doi.org/10.1038/s41598-020-63912-w.
Tidjani A-R, Lorenzi J-N, Toussaint M, van Dijk E, Naquin D, Lespinet O, Bontemps C, Leblond P. Massive gene flux drives genome diversity between sympatric Streptomyces conspecifics. mBio. 2019:**10**(5):e01533-19. https://doi.org/10.1128/mBio.01533-19.
Tong Q-Q, Zhou Y-H, Chen X-S, Wu J-Y, Wei P, Yuan L-X, Yao J-M. Genome shuffling and ribosome engineering of Streptomyces virginiae for improved virginiamycin production. Bioprocess Biosyst Eng. 2018:**41**(5):729–738. https://doi.org/10.1007/s00449-018-1906-3.
van der Meij A, Worsley SF, Hutchings MI, van Wezel GP. Chemical ecology of antibiotic production by actinomycetes. FEMS Microbiol Rev. 2017:**41**(3):392–416. https://doi.org/10.1093/femsre/fux005.
Virolle C, Goldlust K, Djermoun S, Bigot S, Lesterlin C. Plasmid transfer by conjugation in gram-negative bacteria: from the cellular to the community level. Genes (Basel). 2020:**11**(11):1239. https://doi.org/10.3390/genes11111239.
Vogelmann J, Ammelburg M, Finger C, Guezguez J, Linke D, Flötenmeyer M, Stierhof Y-D, Wohlleben W, Muth G. Conjugal plasmid transfer in Streptomyces resembles bacterial chromosome segregation by FtsK/SpoIIIE. EMBO J. 2011:**30**(11):2246–2254. https://doi.org/10.1038/emboj.2011.121.
Vos M. A species concept for bacteria based on adaptive divergence. Trends Microbiol. 2011:**19**(1):1–7. https://doi.org/10.1016/j.tim.2010.10.003.
Wang S-J, Chang H-M, Lin Y-S, Huang C-H, Chen CW. Streptomyces genomes: circular genetic maps from the linear chromosomes. Microbiology (Reading). 1999:**145**(Pt 9):2209–2220. https://doi.org/10.1099/00221287-145-9-2209.
Xu B, Jin Z, Wang H, Jin Q, Jin X, Cen P. Evolution of Streptomyces pristinaespiralis for resistance and production of pristinamycin by genome shuffling. Appl Microbiol Biotechnol. 2008:**80**(2):261–267. https://doi.org/10.1007/s00253-008-1540-0.
Zhang Y-X, Perry K, Vinci VA, Powell K, Stemmer WPC, del Cardayré SB. Genome shuffling leads to rapid phenotypic improvement in bacteria. Nature. 2002:**415**(6872):644–646. https://doi.org/10.1038/415644a.
Zhao C, Huang Y, Guo C, Yang B, Zhang Y, Lan Z, Guan X, Song Y, Zhang X. Heterologous expression of spinosyn biosynthetic gene cluster in Streptomyces species is dependent on the expression of rhamnose biosynthesis genes. J Mol Microbiol Biotechnol. 2017:**27**(3):190–198. https://doi.org/10.1159/000477543.
Ziemert N, Lechner A, Wietz M, Millán-Aguiñaga N, Chavarria KL, Jensen PR. Diversity and evolution of secondary metabolism in the marine Actinomycete genus Salinispora. Proc Natl Acad Sci U S A. 2014:**111**(12):E1130–E1139. https://doi.org/10.1073/pnas.1324161111.