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Mini-Review

The conjugative DNA-transfer apparatus of *Streptomyces*

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ABSTRACT

Conjugation is a major route of horizontal gene transfer, an important driving force in the evolution of bacterial genomes. Since antibiotic producing streptomycetes represent a natural reservoir of antibiotic resistance genes, the *Streptomyces* conjugation system might have a particular role in the dissemination of the resistance genes.

Streptomycetes transfer DNA in a unique process, clearly distinguished from the well-known DNA-transfer by type IV secretion systems. A single plasmid-encoded DNA-translocase, TraB, transfers a double-stranded DNA-molecule to the recipient. Elucidation of the structure, pore forming ability and DNA binding characteristics of TraB indicated that the TraB conjugation system is derived from an FtsK-like ancestor protein suggesting that *Streptomyces* adapted the FtsK/SpoIIIE chromosome segregation system to transfer DNA between two distinct *Streptomyces* cells. Following the primary transfer, a multi-protein DNA-translocation apparatus consisting of TraB and several Spd-proteins spreads the newly transferred DNA to the neighbouring mycelial compartments resulting in the rapid colonization of the recipient mycelium by the donor DNA.

Introduction

Conjugation in *Streptomyces* was discovered more than 50 years ago, when Sermonti and Spada-Sermonti observed "synthrophic tufts of exuberant growth" at the areas of contact between two auxotrophic *Streptomyces* colonies and demonstrated genetic recombination occurring in these strains (Sermonti and Spada-Sermonti, 1955, 1956). Shortly later, genetic linkage of the markers was described (Hopwood, 1959, 1965). Genetic evidence suggested involvement of a plasmid (SCP1) in *Streptomyces* conjugation (Vivian and Hopwood, 1970), which later was identified as a linear plasmid of 350 kb by pulsed-field gel electrophoresis (Kinashi and Shimaji-Murayama, 1991). Beside such huge linear plasmids, *Streptomyces* strains contain a wide variety of conjugative plasmids, including small multi-copy plasmids of 8–15 kb in size, larger low-copy number plasmids and an extensive group of integrative plasmids, the actinomycete integrating chromosomal elements (AICE; te Poele et al., 2007). These elements are conjugative, able to replicate autonomously as a circular molecule and integrate into the host chromosome by site specific recombination via an attachment site that overlaps a tRNA gene.

Mycelial growth of *Streptomyces*

The process of conjugal DNA transfer in *Streptomyces* is certainly influenced by its growth characteristics. Unlike most other bacteria that divide by binary fission, *Streptomyces* grow by apical tip extension at a rate of ~16 μm h^{-1} (Wolanski et al., 2011). The incorporation of new peptidoglycan (PG) precursors at the tips depends on the coiled coil proteins DivIVA, Scy and FilP, which form the polarisome (Hempel et al., 2008; Fuchino et al., 2013). Occasionally, septal cross walls are formed and branching points are initiated. *Streptomyces* mycelium is multinuclear and contains several copies of a linear chromosome with terminally attached telomerase proteins. *Streptomyces* genomes with a size of 5.3–11.8 Mb (Chandra and Chater, 2013) belong to the largest bacterial genomes. During replication the replisomes follow tips at a speed equivalent to the rate of hyphal extension (Wolanski et al., 2011).

A process resembling cell division only occurs during morphological differentiation. When nutrients become limited, long unbranched aerial hyphae are erected from the partially lysing substrate mycelium. Simultaneously more than 50 septal cross walls are formed within the aerial hyphae, the multiple chromosome copies are segregated into the prespores and after thickening of the spore wall a chain of uninucleoic spores, each one containing a single chromosome, is formed (Flardh and Buttner, 2009). Surprisingly, synthesis of the thickened spore wall involves a multiprotein

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complex very similar to that directing rod-shape morphology in other bacteria by positioning lateral wall synthesis (White et al., 2010; Heichlinger et al., 2011; Kleinschnitz et al., 2011).

Conjugative DNA transfer takes place only on solid agar and in the early stage of the life cycle, when *Streptomyces* grows as substrate mycelium (Pettis and Cohen, 1996). Growth as a multiply branching mycelium might make a "mating pair formation" system superfluous, since on solid agar there is a good chance that branching hyphae of two colonies meet.

Unique translocation system transferring a double-stranded DNA molecule

For quite a long time it was thought that bacterial sex always proceeds like the F-factor. This DNA-transfer system is derived from a specialized type IV (T4SS) protein secretion system (Alvarez-Martinez and Christie, 2009), a complex translocation apparatus consisting of up to 15 proteins. The plasmid-encoded relaxase nicks a specific sequence within the *oriT* and becomes covalently linked to the 5′-end of the DNA strand to be transferred (Lanka and Wilkins, 1995). The relaxase is then secreted as the pilot protein with the bound single-stranded plasmid molecule through the channel of the T4SS (Christie et al., 2014; Low et al., 2014). Mobilization of chromosomal markers requires the previous integration of the plasmid into the chromosome and starting at the plasmid *oriT* the adjacent chromosomal markers are transferred in a linear progression.

Single components of T4SS, including conjugative relaxases, have been also detected on few *Streptomyces* plasmids (Zhang et al., 2008), but their ability to direct DNA-transfer has not been addressed so far. If these genes are functional, they would depend on other T4SS components provided by helper plasmids or the host chromosome.

The discovery that even small *Streptomyces* plasmids of less than 10 kb in size are fully conjugative already suggested that *Streptomyces* conjugation follows a different principle, not involving such a complex plasmid-encoded DNA transfer machinery. In a very elegant genetic experiment Possoz et al. demonstrated that a double-stranded DNA molecule is transferred (Possoz et al., 2001). This was shown by the sensitivity of conjugal DNA transfer to the presence of the type II restriction enzyme SalI in the recipient, which only recognizes ds-DNA as a substrate but not ss-DNA (Possoz et al., 2001).

Pock formation and the concept of plasmid spreading

A further discriminating feature in *Streptomyces* conjugation is the formation of inhibition zones (pocks) that mark areas of conjugal transfer and make them visible to the mere eye (Fig. 1). This phenomenon was interpreted as the result of plasmid spreading within the recipient mycelium and the toxicity of plasmid-encoded proteins, becoming expressed in the new host cell (Bibb and Hopwood, 1981; Hopwood and Kieser, 1993). Plasmid spreading might be a specific feature of filamentous actinomycetes relating to the mycelial growth characteristics. If a plasmid enters a hypha of the recipient mycelium by conjugation, the subsequent plasmid spreading within the recipient mycelium ensures the rapid colonization of the recipient colony with the incoming plasmid.

Moreover, plasmid spreading can be also seen as a stability function. Mycelial fragments that have accidentally lost a plasmid, might recover it from the neighbouring mycelial compartment. The concept of plasmid spreading acting also as a stability function is supported by experimental data reported for the *S. lividans* linear plasmid SLP2. Inactivation of *spdB* resulted in 90% loss of SLP2 after a single round of sporulation (Hsu and Chen, 2010).

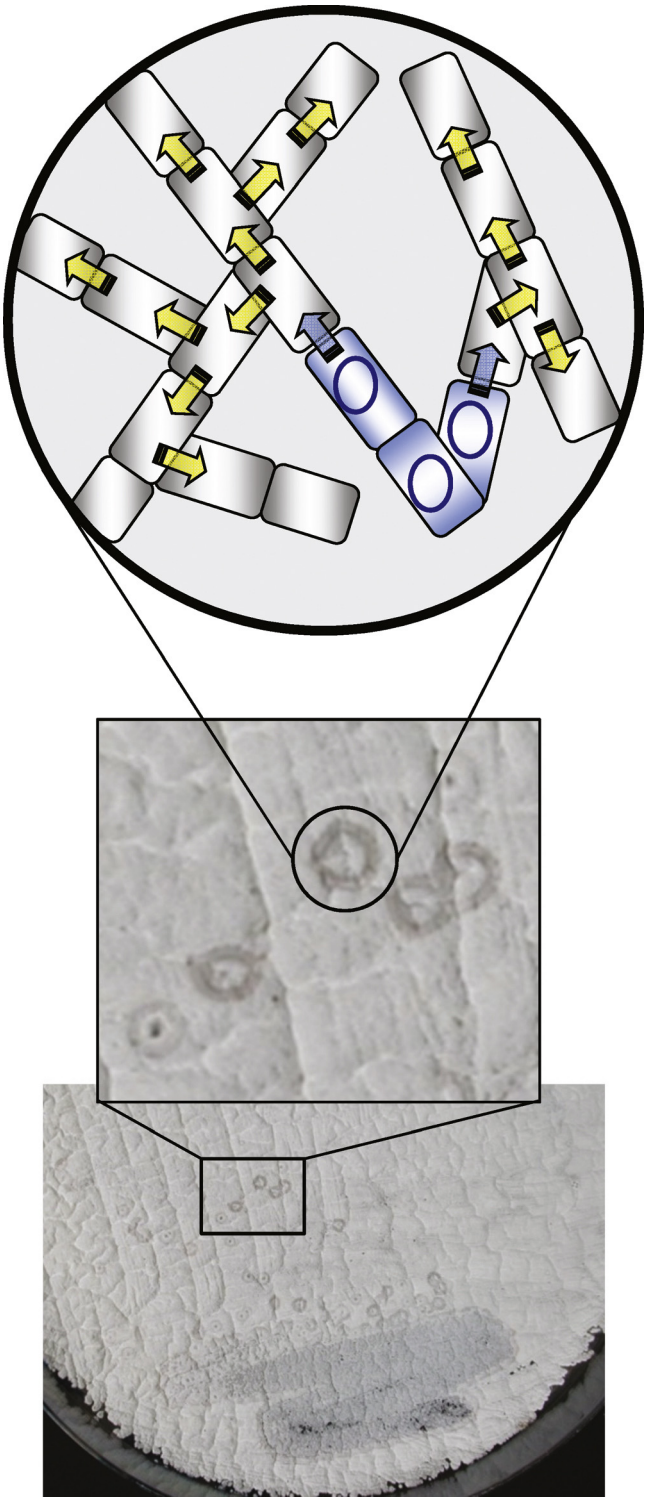

Fig. 1. Pock formation and the concept of intramycelial plasmid spreading. Dilutions of *S. lividans* containing plasmid pEB211 were streaked on a lawn (~10^5 spores) of plasmid-free *S. lividans* on R5 plates. After incubation, pock structures (enlarged image) develop in the sporulating recipient lawn, indicating conjugative plasmid transfer. The schematic drawing illustrates the concept of primary plasmid transfer (blue arrows) from the donor (blue segments) into the recipient (grey segments) and the subsequent intramycelial plasmid spreading (yellow arrows) across the septal cross walls of the recipient mycelium.