



Key Role of Transconjugants for Dissemination of the Integrative Conjugative Element ICEBs1 in Biofilms

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ABSTRACT In this issue of the *Journal of Bacteriology*, J.-S. Bourassa, G. Jeannotte, S. Lebel-Beaucage, and P. B. Beauregard (*J Bacteriol* 204:e00181-22, 2022, <https://doi.org/10.1128/jb.00181-22>) showed that ICEBs1 propagation in *Bacillus subtilis* biofilm relies almost exclusively on transconjugants. It appears restricted to clusters of bacteria in a close neighborhood of initial donor cells, which are heterogeneously distributed in the biofilm and expand vertically toward the air-liquid interface.

KEYWORDS integrative conjugative elements, biofilms, conjugation, single cell

Mobile genetic elements (MGEs) play a major role in bacterial genome evolution and largely contribute to the adaptation of bacteria to their changing environment. Integrative conjugative elements (ICEs), although less studied than plasmids, appear more frequently in many bacterial species (1). ICEs encode their own excision from the chromosome of the donor cell, transfer by conjugation through a type IV secretion system and integration in the chromosome of the recipient cell, giving rise to a new cell, called a transconjugant (or exconjugant), that hosts the ICE (2). Conjugation is a DNA-transferring process that requires tight cell-to-cell contact between donor and recipient cells. In natural environments, bacteria frequently grow in biofilm, i.e. as surface-associated communities embedded in a matrix of extracellular polymeric substances (3). These conditions of growth protect bacteria from a wide array of environmental and chemical stressors, including antibiotics (4). Biofilms offer close and stable contacts between neighboring bacterial cells and thus appear propitious for conjugation. ICEBs1 found in *Bacillus subtilis* has been thoroughly characterized (5). Like other ICEs, it remains silent as an integrated form in the majority of the cells of a bacterial population and is induced only in particular conditions. Conjugation of ICEBs1 has been reported to increase dramatically (up to 10,000-fold) in biofilm conditions (6). In this issue of the *Journal of Bacteriology*, Bourassa et al. (7) use a single-cell approach to study the spatiotemporal dynamic of ICEBs1 propagation in biofilm. They used the undomesticated wild strain *B. subtilis* NCIB3610 in order to ensure proper structuration of the bacterial communities in the biofilm (8). They showed that conjugation appears restricted to clusters of bacteria in a close neighborhood of initial donor cells. These clusters appear heterogeneously distributed in the biofilm, forming close to the air-biofilm interface and expanding vertically. They demonstrated that ICE propagation to neighboring cells relies almost exclusively on transconjugants (99% of the transfer events) rather than on multiple transfer events from a donor cell or vertical transmission by cell division. An efficient gene transfer has been reported in cell chains for ICEBs1 (9); however, this does not seem to be a major factor in ICE propagation in biofilms (as shown by construction of autolysin mutants).

TIME COURSE OF REGULATION OF ICE TRANSFER

Whatever the ICE considered, activation of the ICE appears in a very small percentage of the host population (Table 1). This requires a tight regulation circuit to control the expression of the excision and conjugation genes of the ICE. Each ICE displays its own regulation cascade,

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TABLE 1 Comparison of the activation rates and regulation actors of different ICEs

ICE	Preferential host	ICE regulons	Excision rate (%)	Frequency of transfer (per donor cell except with MMC)	Reference(s)
ICEBs1	<i>Bacillus subtilis</i>	Rapl-Phrl (quorum-sensing), CI-like ImmR repressor and protease ImmA	0.005 (uninduced), 0.2 in biofilms, >90 with RapI overproduction	10^{-7} to 3×10^{-5} if uninduced, 10^{-5} with MMC, ^a 10^{-2} (in biofilm or with RapI overproduction)	5, 6
Tn916	<i>Enterococcus faecalis</i>	Attenuation, repressor Orf9, antisense RNA <i>orf9</i> , Orf7 and Orf8 activators		10^{-9} to 10^{-4} ($\times 12$ with tetracycline)	11, 21, 22
ICEst3	<i>Streptococcus thermophilus</i>	ImmR and ImmA homologs and other putative regulators	5 (uninduced), 90 with MMC	3×10^{-6} to 10^{-4} if uninduced, $\times 25$ with MMC	15, 23, 24
ICEclc	<i>Pseudomonas knackmussii</i>	MfsR (repressor), TciR, BisR, BIsDC, InrR (activators)	3 in stationary phase	10^{-3} to 10^{-2} (with <i>P. putida</i> as donor)	12
ICESXT	<i>Vibrio cholerae</i>	CI-like SetR, SetCD, CroS	1	2×10^{-7} to 2×10^{-2} , $\times 100$ with MMC	11

^aMMC, mitomycin C (DNA damage).

but the common feature is the complexity of the regulation with interlaced pathways and amplification/feedback loops (10, 11) (Table 1).

Two pathways of ICE regulation have been described for ICEBs1, regulation through quorum sensing that relies on the RapI-PhrI components and CI-like repressor ImmR (5). Due to the kinetics of peptide diffusion in biofilm and the delay in protein production, the principal actors of the repression of ICE genes, PhrI and ImmR, are absent in cells that have just acquired an ICE. Regulation through quorum sensing appears quite original for ICEs, but ImmR homologs exist for many ICEs (10, 11). The absence of this regulator in the recipient cell upon ICE entry would have the same consequence for other ICEs. This window of “full activity” of the ICE explains transconjugants’ proficiency in ICE transfer.

Work done on ICEclc regulation nicely illustrates how ICE regulation can be complex, with three regulatory nodes and at least five gene clusters constituting the full ICE regulon (11, 12). A “bistability” generator orchestrates the choice between the two routes of ICE life cycle (remaining silent or being activated). Expression of all gene clusters is restricted to the small cell subpopulation that will transfer ICE. This reduces the fitness impact of ICE transfer on the population.

ENVELOPE STRESS DURING CONJUGATION

Assembly of the large secretion apparatus of the ICE requires a local degradation of the bacterial cell wall. For this purpose, ICEs encode a dedicated enzyme (CwIT for ICEBs1) with two catalytic activities (muramidase and endopeptidase) (13). To be efficient without killing the cell, peptidoglycan digestion should be mild and synchronized with cell wall synthesis occurring during cell division. Experiments done with *Bacillus anthracis* as recipient cells of ICEBs1 showed that mature cells appear resistant to digestion by CwIT (13). This indicates that digestion should occur before modification of peptidoglycan. Mutants of *Bacillus subtilis* affected in cell wall composition (in particular in wall teichoic acids [WTAs]) showed a drastic defect in ICEBs1 transfer (14). The same observation was made for donor cells of *Streptococcus thermophilus* (15). Providing an osmoprotective mating surface enables the donor cells to bypass the need for WTAs in ICEBs1 conjugation (14). This suggests that the ICEBs1 conjugation machinery interferes with cell wall biosynthesis in a way that is incompatible with WTA depletion. Interestingly, Tn916 transfer is less affected by WTA depletion, suggesting some specificity of ICE-host interactions. WTA could also control CwIT activity (as described for autolysins), with WTA depletion leading to an excess of enzyme activity and cell death. WTA-depleted cells (of *Bacillus subtilis* and also *S. thermophilus*) exhibit severe cell shape defects and irregularities in cell wall thickness (15, 16). These cells are likely too fragile to endure assembly and/or activation of the ICE secretion apparatus. It seems likely that a cell cannot handle multiple transfer events, explaining the limited propagation of ICE and the appearance of isolated clusters. Membrane damage and increased levels of reactive oxygen species have been described for cells that activate another ICE, ICEclc, in *Pseudomonas putida* (17). Donors with activated ICEclc are characterized by reduced cell division, growth arrest, and lysis (18). Sacrifice of donor cells could be a

general phenomenon explaining why activation is confined to a small proportion of cells, to keep fitness loss at the population level as low as possible.

MANIPULATION OF HOST PATHWAYS

Activation of *ICEBs1* leads to an inhibition of biofilm-associated gene expression by *DevI*, likely through inhibition of *SpoOA* (19). Hence, *ICEBs1* can manipulate host pathways (including the sporulation one). Cells activating *ICEBs1* “cheat” by decreasing the costly expression of biofilm matrix genes compared to cells without *ICEBs1*. Activation of *ICEBs1* thus confers a selective advantage to the cells. Since this use of the “common good” is restricted to the small fraction of the population that has activated *ICEBs1*, this does not have too much impact on the whole bacterial population. More biofilm matrix components could be synthesized at the air-liquid interface (as shown by Vlamakis and colleagues [20] at the air-biofilm interface with experiments done in agar medium), thus affording better cell-cell contacts for conjugation to occur. This likely explains the preferential localization and vertical expansion of clusters where conjugation events occur. In addition, the energy required for matrix production creates microenvironments in which nutrients are severely depleted. The selective advantage offered by *ICEBs1* activation described above could help transconjugants to outcompete other cells in these areas of biofilm where nutrients are limited.

PERSPECTIVES

Bacterial populations are heterogeneous, and studying physiological processes at the population level gives an average view and masks the differences that can exist between individual components of the population. The development of single-cell studies enables to go further in the understanding of the specific behavior of some cells and the spatiotemporal progress of the processes. This is particularly relevant for studies done in biofilms since these environments are known to be heterogeneous. Despite not being crucial for *ICEBs1* transfer in biofilm, intrachain spreading could be a general and important feature of conjugative DNA transfer, particularly in ovo/coccoid species that form long chains (*S. thermophilus* in particular), but offer fewer lateral surfaces of exchange than rod-shaped bacteria. Determination of the number and distribution at the cell surface of ICE secretion machineries would also constitute a major step in our understanding of ICE transfer.

Transconjugants appear to play a crucial role in *ICEBs1* propagation in biofilm. This is likely the case in other growth conditions and for the other ICEs that involve repressors in their regulation circuit. This would ensure that, even with a small proportion of initial activated donor cells, ICE propagates efficiently in the bacterial population.

Despite being embedded in a host, MGEs form separate entities that have undergone selection in order to optimize their fitness in a particular host. The two routes of ICE lifestyle (remaining silent or being activated) can be detrimental to the host cell and inflict serious cell damage. This explains why ICE activation is so tightly controlled and restricted to a small proportion of the host population. Complex regulation cascades have been described for the few ICEs that have been studied intensively. Characterization of the regulation circuits of other ICEs would help in defining common features (or not) between elements. Manipulation of host pathways is likely a common function of many of the as-yet-uncharacterized cargo genes of ICEs. Further studies are needed to elucidate these ICE-host interactions and their specificity.

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