The role of bacteriocins as selfish genetic elements

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Bacteria produce a wide arsenal of toxic compounds in order to kill competing species. Bacteriocins, protein-based toxins produced by nearly all bacteria, have generally been considered a ubiquitous anti-competitor strategy, used to kill competing bacterial strains. Some of these bacteriocins are encoded on plasmids, which also code for closely linked immunity compounds (thereby rendering toxin producing cells immune to their own toxin). However, the production of bacteriocins can also be interpreted as a means to promote plasmid stability by preferentially selecting for cells carrying the plasmid. If, for example, a cell were to lose the plasmid, it would no longer produce the immunity compound and would be killed by its bacteriocin-producing clone mates. In this respect, bacteriocins can be regarded as similar to previously described toxin–antitoxin systems that are able to promote the stable transmission of plasmids to daughter cells. In order to test this prediction, we carried out an experimental evolution study using the bacterium Escherichia coli, finding that bacteriocins can indeed select for the stable maintenance of plasmids. This suggests that bacteriocins can act primarily as selfish genetic elements promoting their own transmission in the population, which may help explain their unique ecology and evolution.

1. Introduction

Bacteria inhabit highly competitive environments where the production of toxins to kill other microbial species is common [1]. Bacteriocins (toxins that generally target closely related bacterial strains) represent an important class of antimicrobials used in these types of competitive interactions [2,3]. Some are encoded on plasmids and can be horizontally transferred via mobile genetic elements [4]. Bacteriocin genetic systems are composed of a bacteriocin gene, which encodes the toxin, and an immunity gene, which encodes a protein that binds to the bacteriocin and renders it non-toxic [2]. This genetic operon often includes a lysis gene, as cell lysis is sometimes required for the release of the bacteriocins into the surrounding environment (as is the case with our experimental system). Although much is known about the evolution and ecology of bacteriocins, i.e. the conditions that favour the production of anti-competitor toxins [2,4–7], one aspect has been largely overlooked: their role in promoting their own stable genetic transmission, similar to the stabilizing effects that are often attributed to toxin–antitoxin (TA) systems.

Bacterial TA systems are widespread amongst bacteria, and, similarly to bacteriocins, are composed of closely linked genes encoding a toxin and an antitoxin, which provides immunity from the toxin [8,9]. However, unlike bacteriocins, the toxins are not secreted and, importantly, the antitoxin is less stable than the toxin. Although TA systems have been implicated in numerous functions from the regulation of bacterial persistence [10] to mediating interplasmid competition [9,11], studies have shown that they can act simply as
selfish elements, promoting their own genetic transmission to future generations [12–14]. Bacterial cells that lose the TA gene complex eventually die because of the differential half-lives of the toxin and antitoxin ensuring elimination of TA-free cells and the stable maintenance of the TA genes in the population as a whole.

Our goal here is to test whether bacteriocins can potentially act in a similar manner to TA systems by promoting their own stable inheritance. In this case, if a bacterial cell were to lose the bacteriocin gene complex (and hence its immunity compound), it would not be killed by its own toxin, but rather by the bacteriocins produced by its clone mates. In order to test this prediction experimentally, we evolved independent, replicate lines of Escherichia coli and analysed the effects of colicins (bacteriocins produced by E. coli) on the maintenance and loss of plasmids. We worked with three different plasmids differing only in their colicin production: a colicin-free ancestor plasmid (pBR322) and its two derivatives encoding two different colicins (colicins A or E2). Two strains of E. coli were used as hosts for the plasmids: one was sensitive (BZB1011) and another resistant (BZB1030) to both colicins (table 1). We analysed the plasmid stability of all six combinations of the two strains and three plasmids.

2. Material and methods

(a) Bacterial strains and plasmids

The strains and plasmids used in this study are summarized in table 1. Colicins A and E2 were chosen as they represent two functionally different types of bacteriocins (i.e. pore-former versus DNase activity), but bind to the same cell-surface receptor (BtuB). The constructed plasmids (see below) were purified using Accuprep plasmid extraction kit (BioNeer, Seoul, South Korea) and transformed into the E. coli strains specified in table 1. Transformants were selected on the basis of antibiotic resistance and their identities were confirmed by PCR.

(b) Plasmid construction

Colicin A and colicin E2 operons were PCR-amplified, including cAa cAI cAl (GenBank accession no. M26369), and ce2a ce2i ce2l (GenBank accession no. M29885) encoding the activity, immunity and lysis genes, respectively. The following forward and reverse primers were, respectively, used: 5′-TTTATGAATCTT-GAATAGTTG-3′ and 5′-TAATAGCCTTCATAAAG-3′ for amplifying the colicin A operon using pColA-CA31 (table 1) as template. For amplifying the colicin E2 operon the reverse and forward primers 5′-AATTCTGAATCTTACGGATTG-3′ and 5′-GCCTACGGAAGCTTGTGAC-3′ were used with pColE2-P9 (table 1) as template. Cycle parameters were set to an initial 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 2 min then the reaction was incubated at 72°C for 10 min. The resulting amplicon and a pBR322 vector (Fermentas, Burlington, Canada) were digested with EcoRI and HindIII restriction enzymes (Fermentas) and fused to form the pBR-ColA and pBR-ColE2 plasmids. The plasmids pBR322, pBR-ColA and pBR-ColE2 were transformed into E. coli strains BZB1011 (a susceptible strain) and BZB1030 (colicins A and E2 resistant strainowing to mutation in the colicin binding protein BtuB). Transformants were selected and confirmed by antibiotic and colicin resistance. The verified strains were used to evaluate the persistence of the plasmids in each host.

(c) Growth conditions

M9 minimal salt medium was prepared according to the manufacturer’s instructions (Sigma, St Louis, MO, USA) and supplemented with 4 g l⁻¹ Casein Digest (Difco, Lawrence, KS, USA). The medium was supplemented with ampicillin at 100 mg l⁻¹ as required. Suspended cultures were grown in an incubator (New Brunswick, Edison, NJ, USA) at 37 °C with shaking at 200 r.p.m.

(d) Growth kinetics

Growth kinetics were measured in supplemented M9 for all strains used in this study and generation time was determined. Overnight cultures grown in supplemented M9 media were diluted and grown to early exponential phase (A600 = 0.2) and culture aliquots (25 µl) were inoculated into the wells of sterile, transparent, 96-well microtiter plates. The plates were incubated in an Infinite M200 (Tecan, Grödig, Austria) microplate reader at 37°C with orbital shaking. The optical density was monitored every 20 min at 600 nm wavelength and the generation time of each colony was calculated. Growth kinetics for each strain was measured in triplicate. No significant differences in growth rate were observed between all strain and plasmid combinations (table 1).

(e) Evolution experiment

Bacterial populations were grown in 24-well plates containing 1.5 ml of M9 minimal glucose liquid medium, shaking at 220 rpm and incubated at 37°C. Six replicate, evolving lines were initiated for each strain and plasmid combination, with 1.5 µl of each well (approximately 10⁶ cells) being transferred to fresh media every 24 h, for 18 transfer (approximately 180 bacterial generations).

The presence or absence of plasmids in the different replicate populations was assayed every 3 days by (i) serially diluting and plating each bacterial population on Luria-Bertani (LB) agar, to assay the absolute density of bacterial cells by counting colony forming units, and (ii) replica plating these colonies using sterile velvetine squares onto LB agar containing 100 µg ml⁻¹ ampicillin, to assay the proportion of cells still harbouring the plasmids [15].

(f) Analysis

Individual regression lines were fitted for each replicate sample over the course of the experiment, thereby calculating the slope. This slope can be considered the rate at which plasmids were lost in each sample. Subsequently Student’s t-tests were carried out on all treatments to determine if these rates of loss differed significantly from zero (after a sequential Bonferroni correction was applied to correct for multiple tests [16]). A Tukey–Kramer honestly significant difference (HSD) comparison of means was also carried out in order to determine any difference between all experimental treatments. All statistics were performed in R v. 2.15.0.

All data have been deposited in dryad: doi:10.5061/dryad.s622m.

Table 1. Growth rate of E. coli strains.

<table>
<thead>
<tr>
<th>E. coli strains</th>
<th>plasmids</th>
<th>growth rate (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZB1011</td>
<td>pBR-ColA</td>
<td>0.51 ± 0.11</td>
</tr>
<tr>
<td>BZB1030</td>
<td>pBR-ColA</td>
<td>0.45 ± 0.07</td>
</tr>
<tr>
<td>BZB1011</td>
<td>pBR-ColE2</td>
<td>0.48 ± 0.16</td>
</tr>
<tr>
<td>BZB1030</td>
<td>pBR-ColE2</td>
<td>0.53 ± 0.09</td>
</tr>
<tr>
<td>BZB1011</td>
<td>pBR322</td>
<td>0.55 ± 0.10</td>
</tr>
<tr>
<td>BZB1030</td>
<td>pBR322</td>
<td>0.45 ± 0.09</td>
</tr>
</tbody>
</table>

*Growth rate is expressed in generations per h.*
In this respect, bacteriocins could be more efficient than ‘traditional’ TA systems in selecting for plasmid containing cells. One possible criticism of the notion that TA systems play an important role in preventing plasmid loss is their mutational instability: a single mutation that inactivates the toxin should lead to selection for cells no longer containing the TA complex (assuming carriage of the TA complex is costly and there is no horizontal gene transfer between cells). However, in the case of bacteriocin production, where selection for the plasmid is mediated externally via clone mates, individual cells still require the immunity compound encoded on the plasmid, regardless of whether they are able to produce the bacteriocin.

Interestingly, our results show that even under conditions where plasmids are lost, there is no difference in the rate of loss or prevalence between empty plasmid backbones and plasmids encoding bacteriocins (figure 1). This suggests that the major fitness cost associated with carrying these bacteriocin-encoding plasmids is not in fact the production of the bacteriocins, but rather the production and carriage of the plasmid. Furthermore, this may also indicate that bacteriocin production is not as costly as initially assumed [4], presumably owing to the tight regulation of bacteriocin expression that is generally highly repressed via dual SOS regulatory systems [2]. We also find no difference in loss rate between the two different classes of bacteriocins (i.e. pore-former versus DNA degrader). This suggests that although mechanistically different, both these classes of bacteriocins act in an analogous manner in promoting plasmid stability.

Although it has been consistently shown that bacteriocin production can provide bacteria with an advantage against competing bacteria, our results illustrate that bacteriocin production can also play an important role in plasmid maintenance. In the latter case, the bacteriocin acts as a selfish genetic element promoting the stability of the plasmid in the population and may help explain why bacteriocins target cell-surface receptors found on the host cell (which has been shown to decrease the overall effectiveness of the bacteriocin through clones mates neutralizing the bacteriocin [18]). Furthermore, this result may explain why bacteriocins are able to persist in environments where potential competitors are often resistant [19,20]. Hence, to better understand the ecology and evolution of plasmid borne bacteriocins and bacteriocins in general, we should not only take into account their effect on the host cell and interacting bacterial community but also their role as selfish genetic elements promoting their own stable inheritance.

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3. Results and discussion

Evolution experiments carried out over approximately 180 bacterial generations supported our hypothesis. We found that plasmids were frequently and continuously lost in both E. coli strains (BZB1011 and 1030) when no bacteriocin was encoded (figure 1, p < 0.001). We attribute this loss to the competitive growth advantage of E. coli cells that no longer carry the plasmid and to the lack of any mechanism to ensure the equal segregation of plasmid into daughter cells during cell division [17]. Plasmids loss was also observed in the bacteriocin resistant strain (BZB1030) carrying the plasmids encoding either colicin A or colicin E2 (figure 1, p < 0.001). As cells are already resistant to the colicins, and, therefore, do not require the immunity compound encoded on the plasmid, plasmid loss is entirely expected.

However, when E. coli strains were sensitive to the bacteriocin (BZB1011), no plasmid loss was observed when encoding either bacteriocin (figure 1, p > 0.05). Under these conditions, cells that lose the plasmid become sensitive to the bacteriocin present in the environment and so are immediately removed from the population, thereby allowing the stable maintenance of bacteriocins at the population level.

References