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NOTE

Plant Growth-Promoting Genes can Switch to be Virulence Factors via Horizontal Gene Transfer

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Abstract

There are increasing evidences that horizontal gene transfer (HGT) is a critical mechanism of bacterial evolution, while its complete impact remains unclear. A main constraint of HGT effects on microbial evolution seems to be the conservation of the function of the horizontally transferred genes. From this perspective, inflexible nomenclature and functionality criteria have been established for some mobile genetic elements such as pathogenic and symbiotic islands. Adhesion is a universal prerequisite for both beneficial and pathogenic plant-microbe interactions, and thus, adhesion systems (e.g., the Lap cluster) are candidates to have a dual function depending on the genomic background. In this study, we showed that the virulent factor Lap of the phytopathogen *Erwinia carotovora* SCRI1043, which is located within a genomic island, was acquired by HGT and probably derived from *Pseudomonas*. The transformation of the phytopathogen *Erwinia pyrifoliae* Ep1/96 with the beneficial factor Lap from the plant growth-promoting bacterium *Pseudomonas fluorescens* Pf-5 significantly increased its natural virulence, experimentally recapitulating the beneficial-to-virulence functional switch of the Lap cluster via HGT. To our knowledge, this is the first report of a functional switch of an individual gene or a cluster of genes mediated by HGT.

Keywords HGT · Evolution · Erwinia · Pseudomonas · Lap cluster · Adhesion

Introduction

A main mechanism of bacterial evolution is horizontal gene transfer (HGT). In nature, HGT can be mediated by various types of mobile elements, including plasmids and integrative conjugative elements [1]. In turn, the availability of a growing number of bacterial genomes and bioinformatic tools has allowed the rapid identification of HGT events, and studies have shown that, in microbial communities, these events occur with high frequency [2]. Unlike the transfer of individual genes, the transfer of large DNA blocks (< 10 kb), also called genomic islands (GIs), is particularly interesting because they usually contain several genes and, as a result, encode complex functions that notably impact the host's lifestyle. Probably due to their negative effects on public health, the most well-known GIs are the pathogenic islands found within human microbial pathogens, which contain essential and accessory virulence factors [3]. However, GIs can be transferred in microbes with diverse lifestyles, including free-living and plant-associated microorganisms [4, 5]. For example, we have previously reported GIs related to the adaptation of free-living bacteria to extreme environments [6, 7], the promotion of plant growth via beneficial bacteria [8, 9], and the inhibition of plant growth through pathogenic microbes [10, 11]. In these cases and in other similar studies, both the transferred and original genes have been shown to have the same functions, limiting the contribution of HGT over microbial evolution. In this context, it is interesting to explore the putative duality of GIs, related to their ability to codify genes with dual roles in promoting and preventing plant growth depending on the host's lifestyle, which would further expand the role of HGT in bacterial evolution.

Since adhesion is a universal prerequisite for both beneficial and pathogenic plant-microbe interactions, bacterial genes involved in plant adhesion are natural candidates to have a dual function changed under different genomic backgrounds. Specifically, our model of study is the Lap cluster, a bacterial

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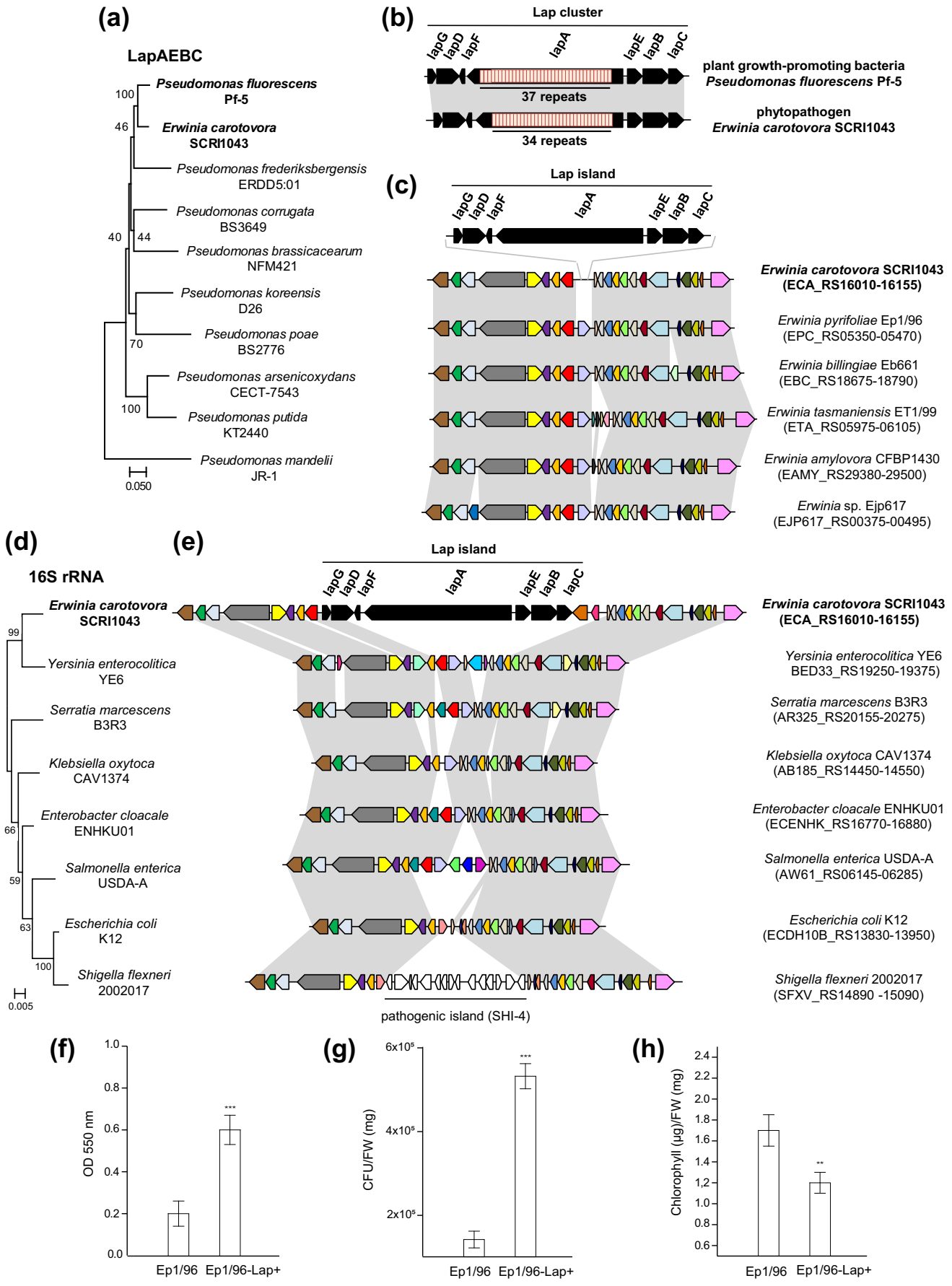
adhesion system, initially described as a critical component of biofilm formation by *Pseudomonas fluorescens* strains [12–14]. In addition to regulatory genes (*lapGDF*), the Lap cluster codifies for a large adhesion protein (LapA) and its specific ABC transporter (LapEBC). Previously, our group and Dr. Salmond's team at University of Cambridge (UK) have described important roles of the Lap cluster in both promoting and inhibiting plant growth in the beneficial bacterium *Pseudomonas fluorescens* Pf-5 (also called *Pseudomonas protegens* Pf-5) [9, 15] and in the phytopathogenic strain *Pectobacterium atrosepticum* SCRI1043 (formally known as *Erwinia carotovora* SCRI1043), respectively, by using mutants of Lap system [16, 17]. In this study, we showed that the Lap cluster of SCRI1043 is within a GI which is probably derived from an ancestral *Pseudomonas* strain. In addition, we experimentally recreated the beneficial-to-virulence transition of the Lap cluster via HGT.

Materials and Methods

Molecular evolutionary analyses based on 16S rRNA, LapA, LapE, LapB, and LapC sequences were conducted using the MEGA 7 software [18]. The phylogenetic trees were generated using the neighbor-joining (NJ) method with the genetic distances computed using the Poisson correction model and root on midpoint. LapA protein sequences of *E. carotovora* SCRI1043 (WP_011094784) and *P. fluorescens* Pf-5 (AAY95545) were used as query to search against the genome database NCBI (www.ncbi.nlm.nih.gov) using BLASTP (amino acid identity cutoff ≤ 35%). With the exception of *Pseudomonas* genomes, no other microbial genomes containing the Lap cluster (*lapGDFAEBC*) were found. Genomic synteny comparisons among strains were performed by using the LASTZ plugin into Geneious v10.1 software [19]. The accession numbers of the genomes used in these molecular analyses were CP011020 for *P. chlororaphis* UFB2, CP017886 for *P. frederiksbergensis* ERDD5:01, LT629798 for *P. corrugata* BS3649, CP002585 for *P. brassicacearum* NFM421, LT629706 for *P. poae* BS2776, LT629687 for *P. koreensis* D26, LT629705 for *P. arsenicoxydans* CECT-7543, AE015451 for *P. putida* KT2440, CP005960 for *P. mandelii* JR-1, NC_004129 for *P. fluorescens* Pf-5, NC_004547 for *E. carotovora* SCRI1043, NC_014306 for *E. billingiae* Eb661, NC_010694 for *E. tasmaniensis* ET1/99, NC_013961 for *E. amylovora* CFBP1430, NC_017445 for *E.* sp. Ejp617, NC_012214 for *E. pyrifoliae* Ep1/96, NZ_CP016937 for *Y. enterolocolitica* YE6, NZ_CP013046 for *S. marcescens* B3R3, NZ_CP011636 for *K. oxytoca* CAV1374, NC_018405 for *E. cloacale* ENHKU01, NZ_CP014666 for *S. enterica* USDA-A, NC_010473 for *E. coli* K12 and NC_017328 for *S. flexneri* 2002017. The amino acid identity among proteins codified by genes within syntenic genomic regions was calculated by MastGAT v2.02 [20]. To avoid false-positive evolutionary relationships, orthologous genes were considered as genes that codified proteins with high amino acid identity (≤ 95%), a strict homology criterion [21]. Tandem repeats in LapA proteins were analyzed by the XSTREAM software [22].

For complementation analysis, the spontaneous mutant of *E. pyrifoliae* Ep1/96 with streptomycin resistance was conjugated with *E. coli* strain S17-1 carrying cosmid pVK102-Lap containing the Lap cluster from *P. fluorescens* Pf-5, as previously described [15]. Tetracycline-resistant transformants (Ep1/96-Lap+) were maintained on LB agar plus tetracycline. For the adhesion assays, strains Ep1/96 and Ep1/96-Lap+ were statically grown for 5 h in LB without antibiotics and biomass was stained with 0.1% crystal violet and quantified as previously described [23]. For the virulence assays, strains Ep1/96 and Ep1/96-Lap+ were grown overnight in LB and then centrifuged and resuspended in the same volume of saline solution (0.9% NaCl). After that, 5 mL of the bacterial suspension was used to inoculate alfalfa plants in 0.8-L pots during sowing. Two months post-inoculation, the number of streptomycin-tolerant endophytes

Fig. 1 Evidence for horizontal gene transfer (HGT) of Lap cluster from *Pseudomonas* to *Erwinia* and empirical recapitulation of the beneficial-to-virulence transition of the Lap cluster via HGT. a Phylogenetic relationships of Lap cluster based on NJ analysis of the LapAEBC proteins. Bootstrap percentages of 40% or more are indicated at the branch points. This evolutionary analysis showed a clear affinity between LapAEBC proteins of *Erwinia carotovora* SCRI1043 and *Pseudomonas fluorescens* Pf-5. **b** Schematic representation of the structure of the Lap cluster in the plant-promoting strain Pf-5 and in the phytopathogen strain SCRI1043. The Lap clusters of the strains Pf-5 and SCRI1043 exhibit a conserved structure where the only difference is within the central repetitive region (gray rectangles) of the large adhesion protein LapA. Specifically, LapA proteins of Pf-5 and SCRI1043 have 37 and 34 repeats of 100 amino acids, respectively. **c** The Lap cluster of *E. carotovora* SCRI1043 is located within a genomic island, which is absence in other *Erwinia* species including phytopathogen strain *Erwinia pyrifoliae* Ep1/96. **d** Phylogenetic relationships between representative species of the order *Enterobacteriales* based on NJ analysis of the 16S rRNA gene. Bootstrap percentages of 40% or more are indicated at the branch points. **e** The Lap cluster of *E. carotovora* SCRI1043 is situated within a 26-kb DNA block, named here Lap island, which is inserted into a conserved syntenic region of *Enterobacteriales* genomes. In this putative chromosomal integration hot spot, *Shigella flexneri* 2002017 exhibits a virulence island. In synthetic analyses (Fig. 1c and e), orthologous genes are shown in the same color, the orthologous block of genes is represented with a gray shadow, and arrows represent gene orientation. To study the effects of the transformation of the phytopathogen *Erwinia pyrifoliae* Ep1/96 (Ep1/96) with the Lap cluster of Pf-5, wild-type strain Ep1/96 and its derived recombinant strain (Ep1/96-Lap+) were compared in their ability to (**f**) form biofilm, (**g**) colonize plants, and (**h**) damage plants. Values are means ± SE of five (**f**, **g**) or four (h) biological replicates. The significance of the difference between strains is indicated (** $P < 0.01$, *** $P < 0.001$, ANOVA)


(a)
LapAEBC
Pseudomonas fluorescens Pf-5
Erwinia carotovora SCRI1043
Pseudomonas frederiksbergensis ERDD5:01
Pseudomonas corrugata BS3649
Pseudomonas brassicacearum NFM421
Pseudomonas koreensis D26
Pseudomonas poae BS2776
Pseudomonas arsenicoxydans CECT-7543
Pseudomonas putida KT2440
Pseudomonas mandelii JR-1
100
46
40
44
70
100
0.050
(b)
Lap cluster
lapG lapD lapF lapA lapE lapB lapC
37 repeats
34 repeats
plant growth-promoting bacteria Pseudomonas fluorescens Pf-5
phytopathogen Erwinia carotovora SCRI1043
(c)
Lap island
lapG lapD lapF lapA lapE lapB lapC
Erwinia carotovora SCRI1043 (ECA_RS16010-16155)
Erwinia pyrifoliae Ep1/96 (EPC_RS05350-05470)
Erwinia billingiae Eb661 (EBC_RS18675-18790)
Erwinia tasmaniensis ET1/99 (ETA_RS05975-06105)
Erwinia amylovora CFBP1430 (EAMY_RS29380-29500)
Erwinia sp. Ejp617 (EJP617_RS00375-00495)
(d)
16S rRNA
Erwinia carotovora SCRI1043
Yersinia enterocolitica YE6
Serratia marcescens B3R3
Klebsiella oxytoca CAV1374
Enterobacter cloacale ENHKU01
Salmonella enterica USDA-A
Escherichia coli K12
Shigella flexneri 2002017
99
66
59
63
100
0.005
(e)
Lap island
lapG lapD lapF lapA lapE lapB lapC
Erwinia carotovora SCRI1043 (ECA_RS16010-16155)
Yersinia enterocolitica YE6 BED33_RS19250-19375)
Serratia marcescens B3R3 (AR325_RS20155-20275)
Klebsiella oxytoca CAV1374 (AB185_RS14450-14550)
Enterobacter cloacale ENHKU01 (ECENHK_RS16770-16880)
Salmonella enterica USDA-A (AW61_RS06145-06285)
Escherichia coli K12 (ECDH10B_RS13830-13950)
Shigella flexneri 2002017 (SFXV_RS14890 -15090)
pathogenic island (SHI-4)
(f)
OD 550 nm
1.0
0.8
0.6
0.4
0.2
0.0
***
Ep1/96
Ep1/96-Lap+
(g)
CFU/FW (mg)
6x10⁵
4x10⁵
2x10⁵
***
Ep1/96
Ep1/96-Lap+
(h)
Chlorophyll (µg)/FW (mg)
2.4
2.2
2.0
1.8
1.6
1.4
1,2
1.0
0.8
0.6
**
Ep1/96
Ep1/96-Lap+

and the levels of chlorophyll in alfalfa leaves were analyzed as previously [9, 11].

Results and Discussion

To analyze the origin of the Lap cluster in *Erwinia carotovora* SCRI1043, we examined the phylogenetic relationships of the four structural proteins of this adhesion system (LapAEBC). An unexpected clustering was observed for strain SCRI1043 within the *Pseudomonas* genus in the LapAEBC consensus tree, where this phytopathogenic strain showed a high affinity to *Pseudomonas fluorescens* Pf-5 (Fig. 1a). In concordance with this evolutionary analysis, we observed an extreme similarity of the complete Lap clusters (*lapGDFAEBC*) of strains Pf-5 and SCRI1043 in terms of nucleotide identity and gene organization (Fig. 1b), and the absence of the Lap cluster in other *Erwinia* species (Fig. 1c), including the pathogenic *Erwinia pyrifoliae* Ep1/96 [24]. Therefore, the most parsimonious explanation for the occurrence of the Lap cluster in strain SCRI1043 is its horizontal acquisition from an ancestral *Pseudomonas* strain. In addition, comparison of the nucleotide sequence of the region around the Lap cluster of strain SCRI1043 with other genomes of phylogenetically related *Enterobacteriales* strains (Fig. 1d), including the model bacterium *Escherichia coli* K12, showed a conserved synteny without large-scale DNA rearrangements (Fig. 1e). A 26-kb DNA block containing the Lap cluster was found to be unique to strain SCRI1043 (Fig. 1e). This result further supports that the Lap cluster is not an ancestral feature of *Enterobacteriales* and thus, that this cluster was not inherited by vertical transfer in strain SCRI1043. Since this 26-kb DNA block occupies a block absent from other *Enterobacteriales*, this chromosomal region can be classified as a genomic island (GI), which we named the Lap island (Fig. 1e). In addition, the Lap island is in the same position as the known virulence island of *Shigella flexneri* 2002017 (Fig. 1e) [25], suggesting that these GIs are located at a chromosomal integration hot spot.

After defining that the Lap cluster in strain SCRI1043 was acquired via HGT and that this virulent cluster has probably a *Pseudomonas* origin related to plant-beneficial functions, we here experimentally recreated the hypothetical ancestral switch of the Lap cluster function via a horizontal transfer event. In concordance with the expected heterologous functionality of the Lap cluster in bacterial adhesion processes, strain Ep1/96 transformed with the Lap cluster from strain Pf-5 (Ep1/96-Lap+) showed higher levels of biofilm production (Fig. 1f) and plant colonization (Fig. 1g) than its parental wild-type strain (Ep1/96). In comparison with that observed in Ep1/96-treated plants, inoculation of plants with Ep1/96-Lap+ showed stronger plant damage (Fig. 1h), confirming that, in a pathogenic background, a beneficial factor can act as a virulence factor.

Conclusions

Adhesion is a universal prerequisite for both plant growth-promoting bacteria and phypathogens to efficiently deliver their repertoire of beneficial and virulence factors and exert effects on the plant cells. Here we experimentally recapitulate the beneficial-to-virulence functional switch of the Lap cluster via HGT. Specifically, our results show that the transformation of the phytopathogen strain Ep1/96 with the beneficial factor Lap from the plant growth-promoting strain Pf-5 increased its natural virulence. Further studies involving other plant-microbe interaction factors and microbial strains are necessary to determine whether the alteration of functional properties of genes via HGT found in this work is a general mechanism of gene evolution and microbial diversification. To our knowledge, this is the first report of a functional switch of an individual gene or a cluster of genes mediated by HGT.

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