Our case is unusual in that the genes originate from a fungus and have a known ecological role in the recipient. In view of the widespread dependence of animals on carotenoids, it is perhaps curious that acquisition of genes underlying carotenoid biosynthesis has not been more frequent. Whereas the phylogenies for these genes suggest several events of horizontal gene transfer among divergent bacterial lineages (Fig. 2), the trees support only a single acquisition by plants (from their plastid symbionts) and a single origin within Fungi (Fig. 2). Likewise, the transfer documented here, from a fungus to an aphid ancestor, is, so far, the only acquisition of carotenoid biosynthetic machinery known in animals.

References and Notes
2. P. D. Fraser, P. M. Bramley, Prog. Lipid Res. 43, 228 (2004).
12. Materials and methods are available as supporting material on Science Online.
35. We are indebted to K. Vogel for samples of aphids, including crosses he performed for other experiments. We thank N. Craft and staff at Craft Technologies for high-performance liquid chromatography analyses of aphid samples, K. Hammond for care of pea aphid colonies, K. Hammond and H. Dunbar for noticing the mutant 5AY female, J. Russell and S. Via for aphid clones, B. Nankiwew for preparation of figures and of the manuscript, H. Ochman for comments on the paper, members of the Ochman-Moran laboratory for comments on the project, and A. Badyaev for discussions of carotenoids in animals. This project was funded by NSF 0626716 to N.G. Merchant to access the DNA sequences in GenBank.

Supporting Online Material
www.sciencemag.org/cgi/content/full/328/5978/624/DC1
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15 January 2010; accepted 1 March 2010
10.1126/science.1187113

D-Amino Acids Trigger Biofilm Disassembly
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Bacteria form communities known as biofilms, which disassemble over time. In our studies outlined here, we found that, before biofilm disassembly, Bacillus subtilis produced a factor that prevented biofilm formation and could break down existing biofilms. The factor was shown to be a mixture of α-leucine, α-methionine, α-tyrosine, and α-tryptophan that could act at nanomolar concentrations. α-Amino acid treatment caused the release of amyloid fibers that linked cells in the biofilm together. Mutants able to form biofilms in the presence of α-amino acids contained alterations in a protein (YqzM) required for the formation and anchoring of the fibers to the cell. α-Amino acids also prevented biofilm formation by Staphylococcus aureus and Pseudomonas aeruginosa. α-amino acids are produced by many bacteria and, thus, may be a widespread signal for biofilm disassembly.

Most bacteria form multicellular communities known as biofilms in which cells are protected from environmental insults (1, 2). However, as biofilms age, nutrients become limiting, waste products accumulate, and it is advantageous for the biofilm-associated bacteria to return to a planktonic existence (2). Thus, biofilms have a finite lifetime, characterized by eventual disassembly. Bacillus subtilis forms communities on semi-solid surfaces and thick pellicles at the air/liquid interface of standing cultures (1, 3–5). Cells in the biofilm are held together by an extracellular matrix consisting of exopolysaccharide and amyloid fibers composed of the protein TasA (5–7). The exopolysaccharide is produced by the epsA-O operon, and the TasA protein is encoded by the yqzM-sipW-tasA operon (8). After 3 days of incubation in a biofilm-inducing medium, B. subtilis formed thick pellicles at the air/liquid interface of standing cultures (Fig. 1A). Upon incubation for an additional 3 to 5 days, however, the pellicles lost their integrity (Fig. 1B). To investigate whether mature biofilms produce a factor that triggers biofilm disassembly, we asked whether a conditioned medium would prevent pellicle formation when added to a fresh medium (9). Medium from an 8-day-old culture was applied to a C18 column (Sep Pak, Waters, Milford, MA), and concentrated eluate from the column was added to a freshly inoculated culture. The eluate was sufficient to prevent pellicle formation (Fig. 1C). Concentrated eluate from a 3-day-old culture had little effect on pellicle formation (Fig. 1D). Further purification of the factor was achieved by eluting the cartridge stepwise with methanol. Elution with 40% methanol resulted in a fraction that was active in inhibiting pellicle formation (Fig. 1E), but had little effect on cell growth (fig. S1). The activity was resistant to heating at 100°C for 2 hours and proteinase K treatment (Fig. 1F).

Bacteria produce α-amino acids in stationary phase (10). We asked whether the biofilm-inhibiting factor was composed of one or more α-amino acids. Indeed, α-tyrosine, α-leucine, α-tryptophan, and α-methionine were active in inhibiting biofilm formation in a liquid medium, as well as on a solid medium (Fig. 1, G and H, and figs. S2 and S3). In contrast, the corresponding L-isomers and D-isomers of other amino acids (such as D-tyrosine and D-phenylalanine) were inert in our biofilm-inhibition assay. Next, we determined the minimum concentration needed to prevent biofilm formation. Individual α-amino acids varied in their activity, with α-tyrosine being more effective (3 μM) than α-methionine.

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or similarity to known racemases. Strains mutant for whose predicted products exhibit sequence sim-
ification, in contrast to conditioned me-
ited with TasA-mCherry (Fig. 3A). In contrast,
were blocked in biofilm forma-
tion by day 6, but only at concentrations of <10 nM at day 3. In contrast, the ylmE racX
double mutant was blocked in D-tyrosine pro-
duction and impaired in D-leucine production at day 6 (table S1).

How do D-amino acids disassemble biofilms? D-amino acids did not inhibit growth (Fig. S6), nor did they inhibit the expression of the matrix operons epsA-O and yqxA-M-sipW-tasA (Fig. S7). D-amino acids are incorporated into the peptide side chains of peptidoglycan in place of the terminal L-alanine (10). Using 14C-D-tyrosine, we confirmed that tyrosine (but not 14C-L-proline) was incorporated into the cell wall (Fig. S8), with incorporation beginning at day 3 (Fig. S9). Finally, in keeping with the idea that D-amino acids act via their incorporation into the wall, the effects of D-tyrosine and other D-amino acids were prevented by D-alanine (Fig. 1, K and L, and Fig. S2).

We hypothesized that TasA fibers are anchored to the cell wall and that the incorpora-
tion of biofilm-disassembling D-amino acids into the cell wall might disengage the fibers from their anchor. To investigate this possibility, we exam-
inized the localization of a functional fusion of TasA with the fluorescent reporter mCherry. Treatment with D-tyrosine had little effect on the accumulation of TasA-mCherry (Fig. S10). In contrast, when the cells were washed by cen-
trifugation, re-suspended, and then examined by fluorescence microscopy, untreated cells, which were often in clumps, were intensely deco-
drated with TasA-mCherry (Fig. 3A). In contrast, D-tyrosine–treated cells, which were largely unclumped, showed only low levels of fluorescence (17-fold lower; table S2). Similar results were obtained with D-leucine and with the D-amino acid mixture. We also carried out electron micro-
scopy (EM) with gold-labeled antibodies to TasA (anti-TasA) to visualize unmodified TasA. TasA fibers were anchored to the cells of untreated pellets (Fig. 3B, images 1 and 2). In contrast, cells treated with D-tyrosine consisted of

![Image](https://www.sciencemag.org)

**Fig. 1.** Conditioned medium blocks pellicle formation. *B. subtilis* strain NCIB3610 was grown at 22°C in 12-well plates in a liquid biofilm-inducing medium for 3 days (A) or 8 days (B). (C and D) Cells grown for 3 days in a medium with added dried and resuspended methanol eluate (1:100 v/v) from a C18 column (Sep Pak) that had been loaded with conditioned (cond.) medium from a 6- to 8-day-old culture (C) or a 3-day-old culture (D). The final concentration of concentrated factor added to the wells represented a 1:4 dilution on a volume basis of the original conditioned medium. (E) The factor was further purified on the C18 column by stepwise elution with methanol. The result of adding 3 μl of the 40% methanol eluate is shown. (F) Before addition to fresh medium, the 40% methanol eluate was incubated with proteinase K beads. (G to L) Wells containing M5ag medium supplemented with D-tyrosine (3 μM), D-leucine (8.5 mM), L-tyrosine (7 mM), L-leucine (8.5 mM), D-tyrosine (3 μM), and D-methionine (5 μM) were present at concentrations at or above those needed to inhibit biofilm formation by day 6, but only at concentrations of <10 nM at day 3. In contrast, the ylmE racX double mutant was blocked in D-tyrosine production and impaired in D-leucine production at day 6 (table S1).

**Fig. 2.** D-amino acids break down pellets, and inhibition of pellicle formation by conditioned medium depends on racemase genes. (A) Three-day-old cultures with added (as a 10 μl drop to the surface of the pellets to achieve the indicated final concentration) L-tyrosine (7 mM), a mixture of L-amino acids (5 mM each), D-tyrosine (3 μM), or a mixture of D-amino acids (2.5 mM each). (B) Effect of concentrated C18 column (Sep Pak) eluate from a conditioned medium from an 8-day-old culture from the wild type or from a strain (IKG55) doubly mutant for ylmE and racX. Scale bars, 7 mm.
a mixture of cells that were largely undecorated with TasA fibers and free TasA fibers or aggregates of fibers that were not anchored to cells (Fig. 3B, images 3 to 6).

Next, we isolated \(\alpha\)-amino acid–resistant mutants (Fig. 4A). Wrinkled papillae appeared spontaneously on the flat colonies formed during growth on a solid medium containing D-tyrosine (Fig. 4A) or D-leucine (fig. S2). When purified, these spontaneous mutants gave rise to wrinkled colonies and pellicles in the presence of individual \(\alpha\)-amino acids. We isolated several such mutants and found that they contained mutations in the 3’ region of yqxM (table S3). Two mutations that conferred resistance to D-tyrosine were examined in detail: (i) yqxM2 was an insertion of G:C at base pair 728 and (ii) yqxM6 was a deletion of A:T at base pair 569 (Fig. 4B). The presence of yqxM2 and yqxM6 restored clumping and cell decoration by TasA-mCherry to cells treated with D-tyrosine (Fig. 3A and fig. S12; see above text). Because YqXM is required for the association of TasA with cells (6), the discovery that the biofilm-inhibiting effect of \(\alpha\)-amino acids could be overcome by mutants of YqXM reinforces the view that the effect of \(\alpha\)-amino acid incorporation into the cell wall is to impair the anchoring of the TasA fibers to the cell. A domain near the C terminus of YqXM could trigger the release of TasA in response to the presence of \(\alpha\)-amino acids in the cell wall.

Finally, we wondered whether \(\alpha\)-amino acids would inhibit biofilm formation by other bacteria. The pathogens Staphylococcus aureus and Pseudomonas aeruginosa form biofilms on plastic surfaces (12), which can be detected by washing away unbound cells and staining the bound cells with crystal violet. D-tyrosine and L-tyrosine had no effect. Furthermore, the effect of \(\alpha\)-amino acids was prevented by the presence of L-alanine (fig. S13), suggesting that \(\alpha\)-amino acids act to block biofilm formation by replacement of L-alanine in the peptide side chain. Given that many bacteria produce \(\alpha\)-amino acids, these amino acids may provide a general strategy for biofilm disassembly. If so, then \(\alpha\)-amino acids might prove widely useful in medical and industrial applications for the prevention or eradication of biofilms.

References and Notes
9. Materials and methods are available as supporting material on Science Online.
13. We thank T. Norman for the analysis of table S2. I.K.-G. and D.R. are postdoctoral fellows of the Human Frontier Science Program and Fulbright/Ministerio de Educación y Ciencia (Spain), respectively. This work was funded by NIH grants to R.K. (GM58213), J.C. (GM086258 and CA24487), and R.L. (GM18546), as well as grants from BASF to R.K. and R.L. Additionally, we have filed a patent application on the use of \(\alpha\)-amino acids for biofilm disassembly.

Supporting Online Material
www.sciencemag.org/cgi/content/full/328/5978/627/DC1
Materials and Methods
Figs. S1 to S13
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References
22 February 2010; accepted 18 March 2010
10.1126/science.1188628