habitats and show that for any two microbial isolates, the similarity of their annotated habitat (as measured by automated keyword comparisons) is strongly correlated to their evolutionary relatedness (Fig. 2, B and C). We observe such common habitat preferences surprisingly far back in time: Even strains related only at the level of taxonomic order are still significantly more frequently found in the same environment than a random pair of isolates (Fig. 2C). Thus, most microbial lineages remain associated with a certain environment for extended time periods, and successful competition in a new environment seems to be a rare event. The latter might require more than just the acquisition of a few essential functions; probably only a limited number of functionalities are self-sufficient enough, and provide sufficient advantage, to be pervasively transferred (*32*). For most other adaptations, fine-tuned regulation and/or subtle changes in the majority of proteins may be needed. Because this is difficult to achieve, well-adapted specialists might in fact rarely be challenged in their environment. This does not rule out the presence of a "long tail" of rare, atypical organisms in each environment (*33*), but most microbial clades do seem to have a preferred habitat.

Taken together, our alternative approach of taxonomic profiling of complex communities has sufficient resolution to uncover differences in evolutionary rates of entire communities, as well as long-lasting habitat preferences for bacterial clades. The latter raises the question of how many distinct environmental habitats there are on Earth—a factor that might ultimately determine the true extent of microbial biodiversity.

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Staphylococcus aureus Panton-Valentine Leukocidin Causes Necrotizing Pneumonia

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The *Staphylococcus aureus* Panton-Valentine leukocidin (PVL) is a pore-forming toxin secreted by strains epidemiologically associated with the current outbreak of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and with the often-lethal necrotizing pneumonia. To investigate the role of PVL in pulmonary disease, we tested the pathogenicity of clinical isolates, isogenic PVL-negative and PVL-positive *S. aureus* strains, as well as purified PVL, in a mouse acute pneumonia model. Here we show that PVL is sufficient to cause pneumonia and that the expression of this leukotoxin induces global changes in transcriptional levels of genes encoding secreted and cell wall–anchored staphylococcal proteins, including the lung inflammatory factor staphylococcal protein A (Spa).

The combined actions of many virulence factors enable *Staphylococcus aureus* to cause disease (*1, 2*). Depending on these factors and on the immune status of the host, staphylococci can cause diseases ranging from superficial skin infections to deep-seated and systemic conditions such as osteomyelitis, septic shock, and necrotizing pneumonia. Staphylococcal necrotizing pneumonia can affect young, immunocompetent patients. This disease, characterized by leukopenia, hemoptysis, and extensive necrosis of the lung tissue, is caused by *S. aureus* strains that produce Panton-Valentine leukocidin (PVL) (*3*). *S. aureus* PVL-positive strains are often methicillin-resistant (MRSA) and, in the USA, they are the predominant cause of community-associated infections (*4*).

PVL is a bi-component, pore-forming exotoxin (*5*) that targets cells of the immune system such as polymorphonuclear neutrophils (PMNs). The active form of PVL requires the assembly of two polypeptides, LukS-PV and LukF-PV, into a heterooligomeric pore. Although PVL has potent cytolytic and inflammatory activities in vitro (*6*, *7*), its role in necrotizing pneumonia has not been demonstrated. To analyze the molecular pathogenesis of PVL-expressing *S. aureus* strains, we have established a murine model of acute primary pneumonia.

We infected mice with strains isolated from necrotizing (PVL-positive) or nonnecrotizing

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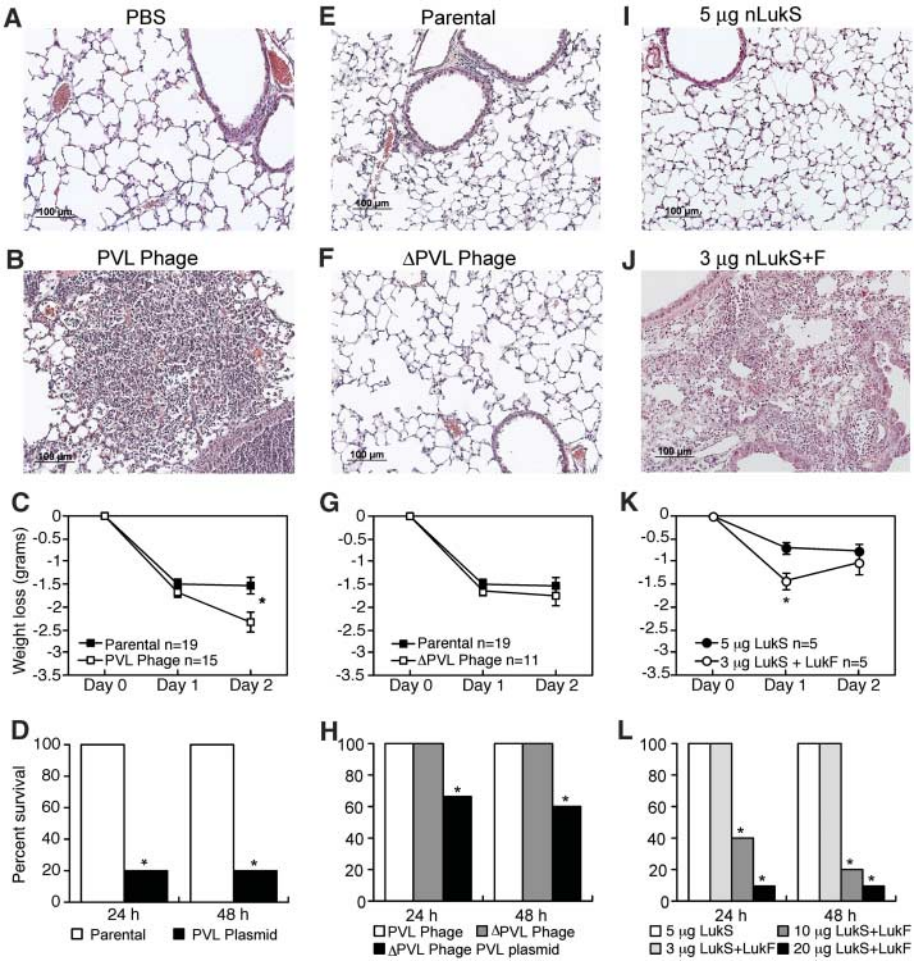

Fig. 1. Expression of PVL enhances the virulence of isogenic *S. aureus* strains. (**A**, **B**, **E**, **F**, **I**, and **J**) Lung histology of mice infected with PVL-positive and PVL-negative strains or inoculated with PVL toxin. The sections are representative of at least three separate experiments. Scale bar, 100 μm. (**C**, **G**, and **K**) Line graphs indicate weight loss in grams. (C) Parental versus PVL phage; $*P < 0.001$. (G) Parental versus ΔPVL phage; no statistical difference observed. (K) Animals inoculated with 3 μg of LukS+F-PV versus 5 μg LukS-PV; $*P < 0.01$ on day 1. (**D**, **H**, and **L**) Mouse survival. (D) Parental and PVL plasmid; (H) PVL phage, ΔPVL phage, and ΔPVL phage PVL plasmid; (L) 20 μg or 10 μg of LukS+LukF-PV versus 3 μg LukS+LukF-PV or 5 μg LukS-PV, $*P < 0.0001$.

(PVL-negative) staphylococcal pneumonia patients (table S1). PVL-positive strains caused murine necrotizing pneumonia with manifestations resembling human disease (fig. S1). In the PVL-positive strains, the *lukS-PV* and *lukF-PV* genes are organized as an operon within a phage (ϕSLT, or other similar phages) that could potentially contribute to the virulence of these strains. To define the role of PVL, we developed several isogenic strains (*8*). A PVL-negative, transformable *S. aureus* strain was lysogenized with ϕSLT or with a mutated ϕSLT in which the PVL operon (*luk-PV*) was deleted. We complemented the PVL-negative strains with a plasmid containing the *luk-PV* operon under the control of its own promoter (table S1).

Mice infected with PVL-positive strains showed symptoms of severe illness: lethargy, hunched posture, ruffled fur, and significant weight loss. Lungs from infected mice were examined 48 hours postinoculation. Tissue sections from lungs infected with PVL-positive strains revealed a strong recruitment of neutrophils and significant inflammation in the lung parenchyma, bronchial epithelial damage, tissue necrosis, and hemorrhage (Fig. 1 and table S2). The lungs infected with PVL-negative strains showed normal lung structures, despite some leukocyte infiltration. By contrast, when the PVL-negative strains were complemented with a plasmid encoding PVL, we observed massive tissue damage and 35 to 80% mortality within 24 hours after inoculation (Fig. 1 and fig. S2). We stained lung sections using antibodies against LukS-PV (fig. S3) and showed that the toxin was detected in tissues infected with PVL-positive bacteria.

Administration of increasing equimolar amounts of native LukS-PV and LukF-PV resulted in concentration-dependent localized lesions, weight loss, and, at concentrations higher than 3 μg, high rates of mortality (Fig. 1). The protein-inoculated mice recovered the lost weight after 24 hours, whereas those infected with PVL-positive bacteria were still ill at 48 hours (Fig. 1C compared with Fig. 1K); these findings demonstrated that an active bacterial infection is required to cause severe morbidity.

Previous studies have demonstrated that PVL-positive strains had increased adherence to injured airway epithelium (*3*, 9). To examine the expression of surface proteins in these strains, we examined by SDS–polyacrylamide electrophoresis (SDS-PAGE) cell wall extracts and supernatants from cultures taken at both the exponential growth phase and the stationary growth phase (Fig. 2). Samples from a PVL-positive strain showed an enhanced expression of at least two cell wall–anchored polypeptides identified as SdrD and protein A (Spa) by N-terminal sequencing and Western analysis (Fig. 2, C to E, and fig. S4). The SdrD and Spa overexpression was not observed in a strain carrying the phage with the deleted *luk-PV* operon, which indicated that this effect was not mediated by products encoded by other phage genes or by its insertion in the chromosome of *S. aureus*. PVL-negative strains complemented with the *luk-PV* operon in a multicopy plasmid (table S1) also showed an increased expression of Spa during both logarithmic and stationary growth phases (Fig. 2F). A group of polypeptides with apparent molecular masses between 32 and 47.5 kD (Fig. 2B, dots) were present in the supernatants from the PVL-negative, but not the PVL-positive, strains. Some of the secreted polypeptides were identified as proteases by using zymograms (Fig. 2G). Thus, expression of the *luk-PV* operon resulted in an altered regulation of cell wall–anchored and secreted protein production.

Spa is a known virulence factor in mouse models of *S. aureus* infections, including pneumonia (*10*, *11*); therefore, we examined the role of Spa in necrotizing pneumonia. Animals infected with the *spa*-deleted isogenic strains had less severe symptoms of disease (Fig. 3A). However, the lungs from animals infected with *spa*-deleted, PVL-positive strains showed localized lesions with massive leukocyte infiltration (fig. S5), which demonstrated that PVL alone was sufficient to cause pneumonia. Complementation of Spa-positive strains with PVL rendered them lethal, whereas the Spa-negative, PVL-plasmid strains did not cause mortality (Fig. 3B). These data suggested that PVL and Spa may act together to cause the overwhelming inflammation and tissue damage that are seen in necrotizing pneumonia.

PVL-positive strains expressed Spa during both exponential and stationary growth phases (Fig. 2). To analyze the effect of PVL on *spa* transcription, PVL was introduced into mutants deficient in *spa* regulators. The Spa production was abolished in a *sarS*-deletion mutant (fig. S6), whether PVL was present or not, which indicated that PVL acts upstream of SarS. To evaluate the transcriptional profile of a PVL-positive strain

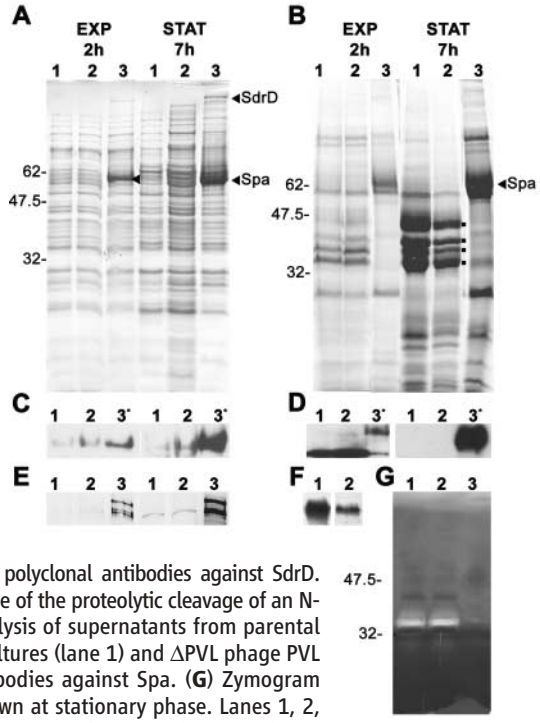

Fig. 2. PVL alters the expression pattern of cell wall–anchored and secreted proteins. (**A** to **E**) Lanes 1, 2, and 3 represent extracts from parental, ΔPVL phage, and PVL phage strains respectively; samples were isolated from bacterial cultures grown at exponential (EXP) and stationary (STAT) phases of growth. (A) Lysostaphin extracts analyzed by SDS-PAGE. The arrows point to overexpressed proteins. (B) Exoproteins from culture supernatants harvested from bacterial cultures analyzed by SDS-PAGE. Arrows point to overexpressed proteins; dots indicate exoproteins reduced or absent in PVL-positive strains. (C and D) Western blot analysis of lysostaphin extracts and supernatants using a monoclonal antibody against Spa. Complete gels shown in fig. S4 (*8*). *Samples were diluted 1:100. (E) Western blot analysis of lysostaphin extracts using polyclonal antibodies against SdrD. SdrD is detected as two polypeptides because of the proteolytic cleavage of an N-terminal subdomain. (**F**) Western blot analysis of supernatants from parental PVL plasmid stationary phase (7-hour) cultures (lane 1) and ΔPVL phage PVL plasmid (lane 2) using monoclonal antibodies against Spa. (**G**) Zymogram analysis of exoproteins from cultures grown at stationary phase. Lanes 1, 2, and 3 represent proteins extracted from parental, ΔPVL phage, and PVL phage, respectively.

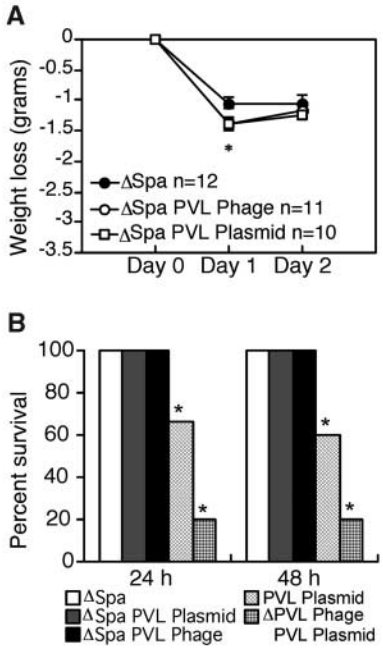

Fig. 3. Spa enhances the virulence of PVL-positive strains. (**A**) Line graph indicates weight loss in grams, *P < 0.01 ΔSpa versus ΔSpa PVL phage or ΔSpa PVL plasmid. (**B**) Percent survival of animals infected with ΔSpa, ΔSpa PVL plasmid, ΔSpa PVL phage, PVL plasmid, and ΔPVL phage PVL plasmid, *P < 0.001 ΔSpa, ΔSpa PVL phage and ΔSpa PVL plasmid versus PVL plasmid or ΔPVL phage PVL plasmid.

compared with the PVL-negative strain, we used microarray analysis: 28 genes showed a distinct expression pattern during exponential growth (table S3), whereas, during the stationary phase, 133 genes showed differential expression (table S4). The *agr* transcripts and several exoproteins were repressed, whereas genes encoding for cell wall–anchored proteins and the *spa* activator *sarS* (*12*) were up-regulated (Fig. 4A). Elevated expression of the *spa* and *sdrD* transcripts correlated with the enhanced production of Spa and SdrD observed in Western blot analysis (Fig. 2 and fig. S4). The repression of exoprotein transcripts paralleled the absence of the 32- to 47.5-kD exoproteins in the supernatants of PVL-positive strains (Fig. 2). This pattern of transcription implicated PVL in an interaction with a factor (or factors) that controls gene expression during the transition from the logarithmic growth to stationary phase.

The regulatory model proposed here was inferred by using strains derived from RN6390, a strain harboring a deletion in *rsbU*, which encodes a regulator necessary for the activity of the stress sigma factor σ^B. Strains with a reduced σ^B (*13*) activity display, among other traits, a decreased production of cell wall–anchored proteins and an increased production of exoproteins. We subsequently generated isogenic PVL-positive and PVL-negative strains in the SH1000 (*14*) *rsbU*+ background and observed overexpression of Spa during the stationary phase of growth (fig. S7A). The SH1000-derived PVL-positive strain was more virulent than its PVL-negative isogenic pair (fig. S7, C and D). When compared with RN6390, the SH1000 lineage showed an increased expression of Spa, but produced ≥20% PVL, although some variability was seen (fig. S7A) (*15*). This suggested that RsbU/σ^B partially regulates PVL.

We show here that PVL is a significant *S. aureus* virulence factor and that PVL-positive strains can cause murine necrotizing pneumonia with manifestations that resemble those observed in human patients. Our results demonstrate that the expression of the genes that encode PVL (*lukS-PV* and *lukF-PV*) or direct inoculation with native toxin is sufficient to induce pneumonia in mice. The expression of the *luk-PV* operon also resulted in an altered expression of multiple proteins, including the tightly regulated (*16*, *17*) proinflammatory factor Spa.

In PVL-positive strains, many secreted proteins are down-regulated (Fig. 4B), similarly to data reported by Vojtov *et. al.* (*18*), who demonstrated that two staphylococcal superantigens, toxic shock syndrome toxin-1 (TSST-1) and enterotoxin B (SEB), strongly repressed production of secreted proteins. It is possible that these toxins act similarly to PVL, interacting with unknown factors that interfere with regulatory networks.

Several genes encoding putative and known microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (including SdrD) are up-regulated in the PVL-positive strains (Fig. 4A). The up-regulation of MSCRAMMs may lead to enhanced tissue adherence and colonization of PVL-expressing strains, thereby contributing to the virulence potential of these strains (*19*, *20*).

Spa is highly expressed in PVL-positive strains. Our in vivo data underscore the documented role of Spa as a proinflammatory factor in pneumonia (*11*). Increased production of Spa, coupled with the ability of PVL to lyse PMNs and macrophages (*6*), could lead to a vicious cycle of cell recruitment, lysis, and release of inflammatory mediators (*7*), resulting in overwhelming tissue inflammation and necrosis.

Here, we show not only that PVL is a key virulence factor in pulmonary infections but also that expression of the *luk-PV* genes interferes with global regulatory networks, which may also enhance virulence. A detailed analysis of such dysregulation will be useful to identify targets for the potential development of novel therapies to treat *S. aureus* infections.

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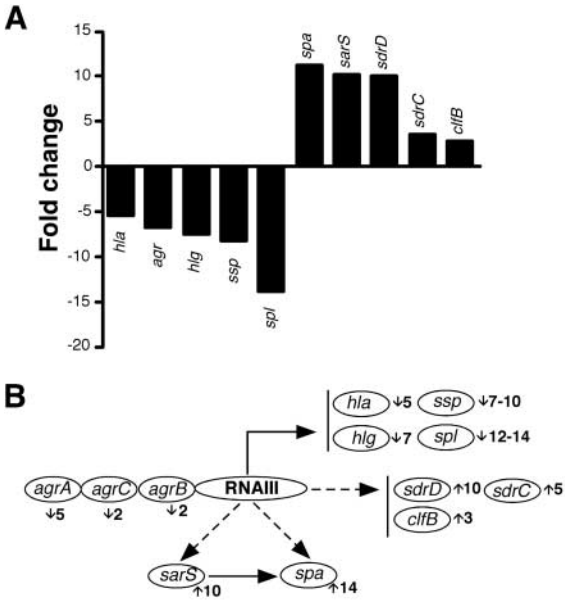

Fig. 4. *S. aureus* PVL-positive strains show an altered transcriptional profile. (**A**) Fold increase or decrease levels of transcript from selected genes. Total RNA extracted from cultures grown to stationary phase. Genes were considered to be induced or repressed in the PVL phage if they were transcribed at least twice or half as much as those of ΔPVL phage. The shown transcripts encode *agrA-C*, accessory gene regulator system; *sar*S, staphylococcal accessory regulator S; *spa*, staphylococcal protein A; *sdr*D, serine-aspartate repeat protein D; *sdr*C, serine-aspartate repeat protein C; *clf*B, clumping factor B; *hla*, alpha toxin; *ssp*, a representative of serine proteases *ssp*B and *ssp*C; *spl*, a representative of *splA-F* proteases. (**B**) A schematic overview of the interactions between regulators involved in cell wall–anchored and secreted protein genes (full and broken lines indicate positive and negative regulation, respectively) based on previously published data. Numbers next to the gene name indicate fold change based on microarray analysis (upward arrow indicates up-regulation, downward arrow indicates down-regulation). The down-regulation of RNAIII (the effector of the *agr* system) results in the down-regulation of secreted protein genes (*hla*, *hlg*C, *hlg*B, and proteases) and the up-regulation of *sar*S and cell wall–anchored proteins (*spa*, *sdr*D, *sdr*C, *clf*B). In addition, the up-regulation of *sar*S results in the up-regulation of *spa.*

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Regulation of *Drosophila* Life Span by Olfaction and Food-Derived Odors

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Smell is an ancient sensory system present in organisms from bacteria to humans. In the nematode *Caenorhabditis elegans*, gustatory and olfactory neurons regulate aging and longevity. Using the fruit fly, *Drosophila melanogaster*, we showed that exposure to nutrient-derived odorants can modulate life span and partially reverse the longevity-extending effects of dietary restriction. Furthermore, mutation of odorant receptor *Or83b* resulted in severe olfactory defects, altered adult metabolism, enhanced stress resistance, and extended life span. Our findings indicate that olfaction affects adult physiology and aging in *Drosophila*, possibly through the perceived availability of nutritional resources, and that olfactory regulation of life span is evolutionarily conserved.

As in many species, reduced nutrient availability (dietary restriction) increases life span in the fruit fly, *Drosophila melanogaster*, and leads to alterations in age-dependent patterns of gene expression, physiology, and behavior (*1*–*4*). Acute nutrient manipulation causes sudden and rapid changes in age-specific mortality (*5*, *6*). Whole-genome expression data, containing age-dependent patterns of gene expression in diet-restricted long-lived flies and fully fed control flies (*1*), revealed that expression of genes encoding odorant-binding proteins was strongly affected by both age and nutrient availability (fig. S1).

To determine whether detection of food-related odors is sufficient to affect fly life span, we measured the life spans of flies in the presence and absence of odorants from live yeast. Yeast odorants were used because demographic and gene-expression data suggested that yeast availability is a major component of the longevity response to diet in *Drosophila* (*7*–*9*). Exposure to yeast odorants reduced life span in long-lived flies from two laboratory fly strains (Canton-S and yw) that had been subjected to dietary restriction (Fig. 1, A and C). Life span was further reduced when flies were allowed to consume yeast paste. The magnitude of the odorant effect was variable and usually small, relative to that caused by the consumption of yeast paste; odorant-mediated life-span reductions ranged from 6 to 18% in Canton-S flies and from 7 to 8% in yw flies (Fig. 1C). Such variability is reminiscent of the dietary-restriction response in flies, which depends on genetic background (*8*). Odorants are therefore sufficient to modulate life span, and currently unidentified odors may alter longevity with greater potency.

We tested whether diet-restricted flies might exhibit altered feeding behavior or altered in-

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