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Conserved genes in a path from commensalism to pathogenicity

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Short Abstract: We focused on small variations of conserved genes from a conditional pathogen, Staphylococcus epidermidis. In addition to the previously investigated global changes, single-nucleotide polymorphisms in orthologs may reveal genes that contribute to adaptation of the bacteria to different environmental stimuli, allowing them to shift from commensalism to pathogenicity.

Long Abstract:
Staphylococcus epidermidis, long regarded as an innocuous commensal bacterium of the human skin, is recognized as the most frequent cause of nosocomial sepsis and infections associated with implanted medical devices. Such a conditional pathogen provides a model of choice to study some genome landmarks correlated with the transition between commensalism and pathogenicity. Traditional investigations stress differences in gene content. We focused here on conserved genes that have accumulated small mutation differences during the transition.
A comparison of S. epidermidis strain ATCC12228, a non-biofilm forming, non-infection associated strain and S. epidermidis strain RP62A, a methicillin-resistant biofilm clinical isolate, revealed consistent variation, mostly single-nucleotide polymorphisms (SNPs), in orthologous genes in addition to the previously investigated global changes in gene clusters. This polymorphism, scattered throughout the genome, may reveal genes that contribute to adaptation of the bacteria to different environmental stimuli, allowing them to shift from commensalism to pathogenicity. SNPs were detected in 931 pairs of orthologs with identical gene length, accounting for approximately 45% of the total 2053 pairs of orthologs. Assuming that non-synonymous mutations would be the hallmark of recent evolution, and hence be associated to the onset of the pathogenic process, analysis of ratios of non-synonymous SNPs vs synonymous SNPs suggested hypotheses about possible pathogenicity determinants.
In order to assess the effect of non-synonymous mutations upon the intraspecies differentiation of the two S. epidermidis strains, we calculated the ratios of total non-synonymous SNPs (N) vs total synonymous SNPs (S) of all genes, with some emphasis on virulence factors, surface proteins and translation, ribosomal structure and biogenesis-related proteins. This comparison would be a first proof of concept, while we were trying to uncover genes' illuminating or unexpected functions that would, in this way, suggest a participation in the evolution of pathogenicity. The N/S ratios for virulence factors and surface proteins differed significantly from that of all SNPs. In contrast, translation-related proteins also showed a significant bias when compared to total SNPs, but displayed mostly synonymous substitutions, indicative of a selective stabilization process leading to purifying

selection. This analysis shows that virulence factors and surface proteins evolved quickly, in parallel with the pathogenicity environment. That this is significant is emphasized by the observation that translation, ribosomal structure and biogenesis-related proteins, which are submitted to considerable structural and functional constraints and are highly expressed, evolved slowly (there is hardly any change in protein sequence, while the gene sequence has evolved).

Of those 931 SNPs gene pairs, 40 showed a disproportionate distribution of dN vs dS. As they may play important roles in pathogenic process, they were further analyzed. Two main groups were observed in the cluster of 40 orthologous pairs made of proteins with mostly non-synonymous substitutions: surface proteins, which are likely under pressure to escape the host immune system, and other genes that should be considered in priority as important for pathogenicity. Two conserved hypothetical proteins (SE0265 and SE0378) predicted to localize in the extracellular medium and several transmembrane proteins, such as transporter family proteins, belonged to the first group. YkyA(SE0790), which contains a lipoprotein signal and a hydrolase domain, might also be recognized by the host immune defense. In the second group, several proteins involved in lipid metabolism, likely to be important for S. epidermidis multiplying on skin, apparently evolved faster in the pathogen. Genes encoding fosfomycin resistance protein FofB and beta-lactamase detected in this group also may have evolved fast to benefit bacteria trying to survive in their host. Genes involved in the formation of biofilms and osmoprotection were also found in this group. PSMs belong to the class of surfactant peptides with putative biofilm-inhibitory properties. Repression of expression of PSMs in the biofilm stage enables bacterial cells to adhere together and to evade the host immune system. Several gene products were involved in DNA recombination and repair (such as SE1170, SE1302 and SE1828) suggesting adaptation to some chemical stress. Among those, the presence of the gene encoding methionine sulfoxide reductase suggested a possible involvement of reactive oxygen species. This prompted us to study the specific involvement of this process in the establishment of pathogenicity. Although the exact in vivo process of methionine oxygenation is not well established, it is supposed to be derived from ROS, in particular from reactions producing superoxide or H2O2. In an attempt to explore whether this prediction could be substantiated, both strains were challenged with increasing paraquat and H2O2 concentrations: interestingly, we observed that S. epidermidis ATCC12228 is indeed more sensitive to both paraquat and H2O2 than S. epidermidis RP62A, as predictedSome 16 genes of the list were of unknown function. We could suggest however that they were likely to belong to surface proteins or considered in priority as important for pathogenicity.

Our study proposed a new approach to identify genes involved in pathogenic processes and provided some insight about the molecular mechanisms leading a commensal inhabitant to become an invasive pathogen.