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Uncovering the commensalism vs pathogenicity in Staphylococcus epidermidis – a combined genomic/proteomic approach

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Introduction: Staphylococcus epidermidis (SE) include the skin microbiota and contribute to homeostasis and protection against pathogens. However, they are the most frequent cause of medical device-associated infections. Skin isolates belonging to clonal complex 2 (CC2) lineage are the major colonizers sharing their ecological niche with other minor genetic backgrounds (non-CC2). CC2 strains are the more frequent in infection. However, the reasons lying behind the enhanced pathogenicity of this lineage are unclear.
Objective: Comprehensive determination of the molecular factors and processes associated to SE pathogenicity and commensalism. The genomes and proteomes of representatives from both CC2 and non-CC2 strains were compared. Their ability to resist to antibiotics and heavy metals was validated by phenotypic assays.
Results: The comparison between the genomes of both SE strains showed the presence of 73 and 55 specific genes, respectively for the CC2 and non-CC2 strains. A total of 739 proteins were quantified, of which 149 and 119 were accumulated in CC2 and non-CC2 strains, respectively. Central carbon metabolism follows different pathways in each strain allowing the support of diverse biological processes. As expected for SE in aerobic conditions and in the presence of an enriched medium, CC2-strain oxidizes pyruvate to acetate since TCA cycle activity is largely repressed. In contrast, the non-CC2 favors the citric cycle allowing a sustained increase of NADH. Also for the peptidoglycan biosynthesis relevant differences between the two strains were noticed. The level of glycyl-tRNA that adds glycine residues to the cross-linking peptides, is highly incremented in CC2 relative to non-CC2 strain. This suggests differences in the structure of both peptidoglycans. CC2 strain holds a heavier artillery regarding virulence either in its encoded specific genes or as for the relatively accumulated proteins. As corroborated by phenotypic assays, CC2 showed a higher antibiotic resistance and non-CC2 a higher resistance do arsenic and an increased extracellular proteolytic activity.
Conclusions: Although both CC2 and non-CC2 strains are colonizers of human skin, they display totally different metabolic and phenotypic profiles in the same environmental conditions, suggesting that each type of strain plays a specific role in skin ecology. Overall, having the opportunity, CC2 strain appears to be better equipped to cause infection than non-CC2 strain.
Impact: Understanding the differences in the metabolic and biological processes between the two types of commensal SE strains constitutes a major starting point for the design of specific antimicrobials toward the pathogenic strains.
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Metabolic modelling reveals pH-dependent differences in pathogenic and commensal ***Staphylococcus epidermidis*** **strains**

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Background: *Staphylococcus epidermidis*, a common human skin colonizer, causes infections associated with the use of medical devices. Strains belonging to clonal complex 2 (CC2) and non-CC2 coexist in human skin as commensals, but have different pathogenic potential[1]. This dual behavior is poorly understood and improved knowledge on the factors that determine the pathogenicity of *S. epidermidis* is needed to design more effective prevention and treatment strategies.

Methods: To investigate the differences in metabolism of CC2 and non-CC2 *S. epidermidis* strains at different pH that mimic skin and blood (5.5 and 7.4), we collected time-course exometabolomic data during bacterial growth and integrated it with genome-scale metabolic models (GEM). The models specific to CC2 and non-CC2 strains were built based on the genome annotation of representative strains, using a manually curated model of a reference strain (RP62 strain of CC2 lineage) as a template[2]. To simulate the two strain metabolisms at different pH, we performed flux balance analysis using biomass production as optimization function and exometabolomic data as constraints. Previously acquired intracellular metabolomics and proteomics data under these pH conditions[3] were further used to constrain and validate the GEMs.

Results: Unique genes were associated with each of the lineages: 73 genes associated with antibiotic resistance, virulence, and their regulation were specific to the CC2 strain; while 55 genes associated with sulfur metabolism, metal ion resistance, detoxification, membrane transport, and osmoregulation were specific to the non-CC2 strain. Exometabolomics analysis revealed that the two strains had different uptake patterns of several amino acids and saccharides. For several metabolites (2,3-butanediol, acetoin, and formate) the effluxes were discriminative between each of the strains at different pH. Differences in the genomic

background and the influx/efflux rates were used to constrain the GEMs, leading to more reliable metabolic models under relevant biological pH states.

Conclusions The generated experimental datasets and strain-specific GEMs provide an important tool to investigate *S. epidermidis* metabolism and to predict the responses of CC2 and non-CC2 strains to compounds used to prevent or treat *S. epidermidis* infections.

References:

1. Espadinha, D. *et al. Front. Microbiol.* **10**, 1 (2019).
2. Calvo, T. D. *et al. Metabolites* **12**, 136 (2022).
3. Gonçalves, L.G. et al Front. Microbiol. 13, 1000737 (2022).

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