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- ### Balancing the loss and acquisition of pathogenic traits in food-associated bacteria
- The recent description and comparative genome analysis of the lactic acid bacterium *Streptococcus thermophilus* (Bolotin *et al.*, 2004) provided insights into adaptive evolutionary mechanisms that led to the assembly of a ‘generally recognized as safe’ (GRAS) species, starting from closely related deadly human pathogens such as *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus agalactiae*.
- Bolotin *et al.* (2004) argued that the species *S. thermophilus* evolved from close phylogenetically related pathogenic streptococci through loss-of-function events. The regressive evolution of *S. thermophilus* was supported by the finding of an inactive vestige of pathogenesis-related genes (10% of the total sequence), counterbalanced by the acquisition of relevant traits, like lactose utilization, that have allowed the assembly of a new genome organization suitable for the colonization of the dairy niche.
- Surprisingly, in the *S. thermophilus* genome analysis, Bolotin *et al.* (2004) made no reference to the urease gene cluster that accounts for 0.4% of the total coding sequence and 0.6% of the total coding sequence belonging to known functional categories. This urease gene cluster, composed of eight genes organized in an operon (Mora *et al.*, 2004), has been found in all *S. thermophilus* strains characterized until now, including natural urease-negative mutants that, however, have only been isolated with relatively low frequency (Monnet *et al.*, 2004). Since the close pathogenic streptococci considered by Bolotin *et al.* (2004) do not carry ureolytic determinants, we could postulate that the urease gene cluster in *S. thermophilus* could be a function complementation, probably derived from other bacterial pathogens and fixed on a genomic backbone ancestrally regressed from pathogenic streptococci.
- The urease operon, which plays an important technological role in dairy fermentation processes (Mora *et al.*, 2004; Monnet *et al.*, 2004), is often associated with pathogenic bacteria (Mobley *et al.*, 1995; Burne & Chen, 2000). In fact ureolytic activity has a key role in pathogenesis elicited by bacteria like *Clostridium perfringens*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella* sp., *Staphylococcus saprophyticus*, *Ureoplasma urealyticum* and *Yersinia enterocolitica* (Mobley *et al.*, 1995; Burne & Chen, 2000), and such activity has been reported in diseases like urolithiasis, pyelonephritis, ammonia encephalopathy, hepatic encephalopathy, hepatic coma and gastro-duodenal infections (Mobley *et al.*, 1995).
- The role of urease is primarily linked to nitrogen assimilation. Ammonium,

released from the urea present in the secretions of major and minor exocrine glands (1–10 mM in saliva), provides a nitrogen source for bacteria that colonize the human body, as has been demonstrated for the oral bacteria *Streptococcus salivarius* (Chen *et al.*, 2000) and *Actinomyces naeslundii* (Morou-Bermudez & Burne, 2000). Moreover, the release of alkaline equivalents, mediated by urease, is a major cause of damage to host tissues, a key factor in pathogen persistence (Burne & Chen, 2000), and can even increase pathogen fitness by counteracting the environmental acidity as observed for *Helicobacter pylori* in the human stomach.

In the light of these considerations, the maintenance of urease determinants within the *S. thermophilus* genome is certainly dependent on how they contribute to the fitness of the organism in its environment. From an ecological perspective, the availability of an enzyme such as urease could, apart from offering its host the previously mentioned advantages, also positively affect the association between *S. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, or that with other lactic acid bacteria in milk, providing ammonium and carbon dioxide from the hydrolysis of milk urea (3–6 mM) and buffering the generated acidity. In *S. thermophilus* it has been recently shown that one of the functions of urease is to supply ammonia for the synthesis of glutamine (Monnet *et al.*, 2005).

Among the 59 recognized species of the genus *Streptococcus*, the urease operon is restricted to *S. alactolyticus*, *S. hyointestinalis*, *S. salivarius*, *S. thermophilus* and *S. vestibularis*. Structural *S. thermophilus* urease genes show a greater than 96 % identity with the close oral bacterium *S. salivarius* (Mora *et al.*, 2004), an opportunistic pathogen rather than a GRAS bacterium. *S. salivarius* has been associated with dental caries, infective endocarditis and meningitis and is classified in the Risk Group 2 according to the EU directive 90/679/EEC (German definition). Like *S. thermophilus*, *S. salivarius* is exposed to milk in the mouth during nursing and shares with *S. thermophilus* not only urease but also another relevant metabolic trait, the specific symporter of lactose. This lactose uptake system, which has 95 % DNA/DNA

identity between the two species, is structurally different from the lactose uptake genes of all the other lactic acid bacteria (Vaillancourt *et al.*, 2002).

Other virulence-related genes clusters are still present and functional in the *S. thermophilus* genome. The genes involved in the synthesis of the polysaccharide capsule that surrounds pathogenic streptococci are maintained in the *S. thermophilus* genome but, unlikely to be considered pathogenic traits, they contribute to the production of external polysaccharides that confer the desirable texture of yogurt (Tettelin, 2004). Also, the genes encoding the oligopeptide transport system (AmiA oligopeptide-binding proteins) resemble those of *S. pneumoniae* on the basis of both structural organization and overlapping specificities and are differently organized compared with those of *Lactococcus lactis* (Opp system), another GRAS dairy lactic acid bacterium. Nevertheless, on the basis of substrate specificity the oligopeptide system of *S. thermophilus* resembles that of *L. lactis*, showing a broad specificity of peptide transport that renders these lactic acid bacteria species well adapted to their natural habitat, milk (Juille *et al.*, 2005).

A conclusion we would like to emphasize is that a gene or a cluster of genes, and the related products, which have been shown to be involved in virulence mechanisms may change their role whilst still retaining their catalytic function (i.e. for urease, the hydrolysis of urea), according to the environmental 'choice' made by the host organism during speciation.

We could therefore postulate that beneficial organisms, even though regressed from pathogens, could still take advantage of characteristics that play an important role in controlling pathogenesis. Hence, the presence of traits related to pathogens, like urease in the GRAS *S. thermophilus*, indicates that the positive, or negative, value of a bacterium for human beings derives from the proper assembly of suitable genetic loci (linked or not to pathogenesis) driven by environmental constraints.

In the light of such a consideration, the GRAS status of a micro-organism cannot be definitely predicted by a standard comparative genome analysis looking for

the presence or absence of genes whose virulence-related function has been demonstrated for bacteria with a different environmental background. Likewise, strains belonging to bacterial taxa considered to be potentially pathogenic for humans may actually be not, if they are members of a defined subpopulation adapted to an environmental niche other than the human body, like animal milk and milk-derived products.

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