Infection, Genetics and Evolution xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Genomics of *Streptococcus salivarius*, a major human commensal

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ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 30 September 2014
Accepted 2 October 2014
Available online xxxx

Keywords:
MLST
Comparative genomic
Streptococci
HGT
Diversity
Adaptation

ABSTRACT

The salivarius group of streptococci is of particular importance for humans. This group consists of three genetically similar species, *Streptococcus salivarius*, *Streptococcus vestibularis* and *Streptococcus thermophilus*. *S. salivarius* and *S. vestibularis* are commensal organisms that may occasionally cause opportunistic infections in humans, whereas *S. thermophilus* is a food bacterium widely used in dairy production. We developed Multilocus sequence typing (MLST) and comparative genomic analysis to confirm the clear separation of these three species. These analyses also identified a subgroup of four strains, with a core genome diverging by about 10%, in terms of its nucleotide sequence, from that of *S. salivarius sensu stricto*. *S. thermophilus* species displays a low level of nucleotide variability, due to its recent emergence with the development of agriculture. By contrast, nucleotide variability is high in the other two species of the salivarius group, reflecting their long-standing association with humans. The species of the salivarius group have genome sizes ranging from the smallest (~1.7 Mb for *S. thermophilus*) to the largest (~2.3 Mb for *S. salivarius*) among streptococci, reflecting genome reduction linked to a narrow, nutritionally rich environment for *S. thermophilus*, and natural, more competitive niches for the other two species. Analyses of genomic content have indicated that the core genes of *S. salivarius* account for about two thirds of the genome, indicating considerable variability of gene content and differences in potential adaptive features. Furthermore, we showed that the genome of this species is exceptionally rich in genes encoding surface factors, glycosyltransferases and response regulators. Evidence of widespread genetic exchanges was obtained, probably involving a natural competence system and the presence of diverse mobile elements. However, although the *S. salivarius* strains studied were isolated from several human body-related sites (all levels of the digestive tract, skin, breast milk, and body fluids) and included clinical strains, no genetic or genomic niche-specific features could be identified to discriminate specific group.

1. Introduction

The salivarius group consists of three genetically similar species (Kawamura et al., 1995; Poyart et al., 1998): *Streptococcus salivarius* and *Streptococcus vestibularis*, two commensal bacteria, and *Streptococcus thermophilus*, a nonpathogenic dairy species belonging to the viridans group of streptococci. The viridans group consists of 26 streptococcal species with similar phenotypic characteristics. These bacteria are mostly isolated from the human oral microbial ecosystem, and can be classified into five subgroups (anginosus, mitis, salivarius, sanguinis and mutans) (Facklam, 2002). The salivarius group of the genus *Streptococcus* is a cluster with significant support from sequence analysis using the 16S (Bentley et al., 1991; Kawamura et al., 1995), *sodA* (Poyart et al., 1998) and *rnpB* genes (Tapp et al., 2003) and, more recently, 136 genes from the core streptococcal genome (Richards et al., 2014). The most recent of these studies showed that *S. downei* and *S. criceti*, which are usually included in the mutans group, actually cluster tightly with the salivarius group on the phylogenetic tree (Richards et al., 2014).

S. salivarius, which can be isolated from human saliva and the tongue dorsum, is one of the first bacteria to colonize the mucosa in the first few days after birth (Pearce et al., 1995; Rotimi et al., 1985). This key streptococcal species is maintained as a dominant population in the human oral cavity and the upper airways, throughout the life of its human carrier (Nakajima et al., 2013; Tappuni and Challacombe, 1993). *S. salivarius* is also one of the primary inhabitants of the intestinal microbiota. A metagenomic analysis of the feces revealed its presence in more than 90% of the individuals analyzed to generate the first human gut microbial gene catalog (it was formerly described as *S. thermophilus* due to a lack of appropriate genomic references) (Qin et al., 2010). In the first few days after birth, *S. salivarius* has been recovered from fecal

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http://dx.doi.org/10.1016/j.meegid.2014.10.001
1567-1348/

samples collected from preterm infants and newborns (Favier et al., 2002; Millar et al., 1996; Park et al., 2005). The proportions of streptococci in the bacterial community differ along the length of the human intestinal tract, with these species predominating in the jejunum, present in the distal ileum and absent from the ascending colon and rectum (Booijink et al., 2010; Wang et al., 2005). *S. salivarius* was isolated from jejunum biopsy specimens and from samples collected from subjects undergoing ileostomy (van den Bogert et al., 2013b; Wang et al., 2005). *S. salivarius* was also detected in microbiota from the human stomach, cecum and rectosigmoidal colon (Hakalehto et al., 2011; Hayashi et al., 2005). The abundance of streptococci among which *S. salivarius* represents a significant part in intestinal metagenomic samples indicates that these bacteria achieve a true development rather than being washed down with saliva flow (Qin et al., 2010; Qin et al., 2014; van den Bogert et al., 2013b; Zoetendal et al., 2012). No *S. salivarius* strain has yet been unambiguously characterized from non-human sources, suggesting that this bacterium may be considered specific to humans.

Several reports have indicated that *S. salivarius* plays a positive role in oral and digestive tract ecology. *S. salivarius* may exert its impact on human health through effects on the stability of microbiota composition, bacterial interference and interaction with the host. *Streptococcus* is the dominant genus in the tonsillar crypts of children aged two to four years with tonsillar hyperplasia, and a decrease in the abundance of *S. salivarius*/*S. vestibularis* has been reported in children with recurrent tonsillitis (Jensen et al., 2013). The predominance of *S. salivarius* in microbial profiles is associated with stabilization, as opposed to acute cystic fibrosis (Filkins et al., 2012). A low abundance of *S. salivarius* in patients with halitosis has been reported, but not in all studies (Kazor et al., 2003; Riggio et al., 2008). Negative bacterial interference plays a fundamental role in surface colonization and in the limitation of pathogen emergence. The antagonistic effects of *S. salivarius* have been described in competition with *S. mutans* and *Streptococcus sobrinus* for tooth sites during initial oral colonization (Tanzer et al., 1985), and during the adhesion of *Streptococcus pyogenes* to the human pharyngeal cell layer (Guglielmetti et al., 2010). *S. salivarius* has also been shown to inhibit epithelial colonization by the periodontal pathogen *Aggregatibacter actinomycetemcomitans* (Sliepen et al., 2009b; Teughels et al., 2007). This predominant colonizer of the oral cavity is also the main producer of bacteriocins, such as lantibiotics in particular (Hyink et al., 2007; Ross et al., 1993; Wescombe et al., 2012a; Wescombe et al., 2011). These compounds inhibit the growth of *Streptococcus pneumoniae* (Santagati et al., 2012), *S. pyogenes* (Dempster and Tagg, 1982) and other species (Wescombe et al., 2011). Antimicrobial peptides with broad-spectrum activity, including those from the streptococci cited above, have also been characterized from strains recovered from fecal samples (Birri et al., 2012; O'Shea et al., 2009). *S. salivarius* K12, a bacteriocin-producing strain, is used as an oral probiotic, initially in New Zealand and now widely in the world, for the prevention of streptococcal pharyngitis (Burton et al., 2011; Tagg and Dierksen, 2003). Diverse colonization outcomes and potential applications of this strain in the control of respiratory infections have been reported (Dierksen et al., 2007; Power et al., 2008; Wescombe et al., 2012b). A lower incidence of bacterial throat and ear infections was recently reported in a preliminary trial of *S. salivarius* K12 administration in children (Di Pierro et al., 2012). This strain, which inhibits the growth of the salivary bacteria responsible for halitosis, can modify the factors underlying bad breath and the composition of the oral microbiota in subjects with halitosis (Burton et al., 2006). In addition, *S. salivarius* K12 has been shown to protect against *Candida albicans* invasion, by inhibiting adhesion through mechanisms independent of its antimicrobial activity (Ishijima et al., 2012). *S. salivarius* has also been shown to affect the immune response, by inhibiting the inflammatory pathways activated by different pathogens. *S. salivarius* inhibited the IL-8 production and NF-κB activation induced by the enteric pathogens *Pseudomonas aeruginosa* and *Salmonella* enterica serovar Typhimurium and a periodontal pathogen (Cosseau et al., 2008; Frick et al., 2007; Sliepen et al., 2009a). An immunomodulatory response to *S. salivarius* was established in several oral cell lines but also in intestinal epithelial cells (Frick et al., 2007; Kaci et al., 2011). The downregulation of other molecular functions was demonstrated in global analyses of human bronchial epithelial cells (Cosseau et al., 2008). The anti-inflammatory potential of *S. salivarius* was recently confirmed, in studies demonstrating an inhibition of inflammation in mouse models of severe and moderate colitis. The *in vitro* and *in vivo* anti-inflammatory responses were observed with live bacteria but not with heat-killed bacteria. These responses are, therefore, linked to the metabolic activity of *S. salivarius* (Kaci et al., 2014; Kaci et al., 2011).

The bacteria of the viridans group of streptococci are commensal organisms, but they may cause both local and systemic infections (Doern and Burnham, 2010; Maeda et al., 2011). *S. salivarius* displays only weakly virulent but, in opportunistic infections, it can cause invasive disease, such as bacteremia in immunocompromised patients (Corredoira et al., 2005; Han et al., 2006), endocarditis (Kitten et al., 2012) and meningitis after brain abscess, cranial trauma, and cerebrospinal fluid (CSF) fistula (Wilson et al., 2012). *S. salivarius* is one species of the viridans group most frequently recovered from patients with meningitis following spinal procedures because of contamination of the procedure site (Rubin et al., 2007; Trautmann et al., 2002). In several recent cases of iatrogenic meningitis, the strains recovered from the CSF of patients and the saliva of the physician concerned were identical (Chitnis et al., 2012; Shewmaker et al., 2010; Srinivasan et al., 2012), showing a direct contamination of the patient via a respiratory droplet from the physician who was not wearing a face mask. In this review, we summarize the available and new data for the phylogeny, ecology, genomics and metabolism of the *S. salivarius* species.

2. Materials and methods

2.1. Phylogenetic analyses

The nucleotide sequences of internal fragments from the five selected genes of the strains identified in Fig. 1 were concatenated (2.359-bp): *ddlA* (encoding D-alanine ligase), *pyrE* (encoding orotate phosphoribosyltransferase), *thrS* (encoding threonyl-tRNA synthetase), *dnaE* (encoding DNA polymerase III) and *sodA* (superoxide-dismutase). Phylogenetic trees of the nucleotide sequences for each housekeeping locus were constructed by the neighbor-joining (NJ) method using MEGA version 4 software (http://www.megasoftware.net) (Tamura et al., 2007). A model of the Kimura 2-parameter distance was used to estimate distances for nucleotide sequences. The significance of the observed clusters in the trees constructed by the NJ method was assessed by bootstrap analysis with 1000 replicates.

2.2. Sequence data and genome mining

Details of the genome sequences used in our analyses are presented in Tables S1 and S2. All genome sequences were obtained directly from National Center for Biotechnology Information (NCBI). RAST facilities were used for whole-genome comparisons (Overbeek et al., 2014). Genomes were screened for urease genes by dedicated Blast searches using urease genes from *S. salivarius* JIM8777. Carbohydrate and amino-acid biosynthesis pathways

Please cite this article in press as: Delorme, C., et al. Genomics of *Streptococcus salivarius*, a major human commensal. Infect. Genet. Evol. (2014), http://dx.doi.org/10.1016/j.meegid.2014.10.001