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Diversity and evolution of oligopeptide permease systems in staphylococcal species

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ABSTRACT

Several oligopeptide permease (Opp) systems have been found in staphylococcal species, including Opp1-4, Opp3′ and the arginine catabolic mobile element (ACME)-encoded Opp system (ACME-Opp). They confer upon bacteria the increasing fitness, but their evolutionary histories remain unclear. In this work, we performed a genome-wide identification of Opp systems in staphylococcal species. Novel Opp systems were identified, including the duplicate of Opp4 in *Staphylococcus pseudintermedius* and the ACME-Opp-like systems in coagulase-negative staphylococci (CoNS). Phylogenetic analysis revealed that all of the identified Opp systems were derived from Opp3 system by operon duplication during species divergence, while lateral gene transfer might also confer to the dissemination of Opp in staphylococci. In addition, we proposed an improved theory on evolution of ACME: the Opp and arginine-deiminase systems were firstly transferred from *Staphylococcus haemolyticus* to *Staphylococcus epidermidis* independently; in *S. epidermidis* they were assembled together and then transferred to *Staphylococcus aureus*.

1. Introduction

Oligopeptide permease (Opp) systems are widely distributed in both gram-positive and negative bacteria. Genes encoding the Opp components are commonly organized as a cluster, including one encoding the substrate binding protein (OppA), two transmembrane proteins (OppB and OppC) which form the translocate channel, and two membrane-bound cytoplasmic ATP-binding proteins (OppD and OppF) which provide energy for peptide importing [1]. Opp systems are mainly involved in nutritional uptaking and environmental sensing [2,3], but they may play other roles such as drug resistance and virulence [4–6].

In *Staphylococcus aureus*, several Opp systems have been identified, namely Opp1-3 and Opp4/Opp3′. Of them, both *opp4* and *opp3′* operons are tandemly arranged at the downstream of *opp3* operon in chromosome. However, they have varied a lot at the level of sequence, indicating that they arose through a remote duplication event [7,8]. Biological roles of Opp systems in *S. aureus* have been investigated: Opp2 and Opp3 participate in nickel uptaking and nitrogen utilization, respectively [7,9], while in some models Opp2 is also involved in virulence [10,11]. These Opp systems are conserved across different *S. aureus* isolates in terms of both sequence and gene organization. However, Opp systems in other staphylococcal species have not been well characterized.

In addition to core chromosome-encoded Opp systems, a mobile element-encoded Opp system was identified in a community-associated methicillin resistant *S. aureus* (CA-MRSA) clone USA300 (ST8-MRSA-Iva). It was originally reported as Opp3 and hereafter referred to as ACME-Opp since it is located within an arginine catabolic mobile element (ACME) [12]. Different ACMEs that have been detected in *S. aureus* and *Staphylococcus epidermidis* can be divided into three types according to the presence of arginine-deiminase system (Arc) and Opp system, *i.e.* type I containing both Arc and Opp systems, while types II and III containing only one of them [12–17].

ACMEs were firstly assumed to contribute to the successful spread of *S. aureus*, but later were proved to be unnecessary virulence factors for *S. aureus* [18,19]. Meanwhile, ACMEs in *S. epidermidis* have been found to be associated with low antibiotic resistance and low pathogenicity [20]. What is worth noting is that the function of Arc and Opp systems is independent from each other. While the ACME-Arc system allows *S. aureus* to thrive in acidic environments [21], the function of ACME-Opp system remains unclear. In addition, due to the high similarity of ACMEs from *S. aureus* and *S. epidermidis*, *S. epidermidis* is considered as the most probable donor of the ACME from *S. aureus*. However, it is not well described how gene transfer took place or in which manner Arc and Opp systems are assembled together [13,22]. In this work we performed genome-wide identification of Opp systems in a total of 51 isolates of seven staphylococcal species, which revealed the diversity

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of Opp systems in staphylococci systematically. Moreover, based on comparative and phylogenetic analysis, we made a more solid hypothesis about the evolution of ACME-Opp system.

2. Results

2.1. Identification of Opp1-4 systems in staphylococci

41 selected *S. aureus* isolates were classified into two classes according to the Opp systems they carried, *i.e.* class I (36 isolates) carrying Opp1-4 and class II (5 isolates) carrying Opp1-3 and Opp3′ (Fig. 1, Table S1). Multi-locus sequence typing analysis showed that the *S. aureus* isolates from class I had diverse genetic backgrounds, while class II contained ST398, ST93 and ST151 isolates only. Interestingly, the two *S. aureus* classes cannot separate their clones from each other, so that duplication of Opp3 system may have occurred several times in *S. aureus* independently and the duplicates, *i.e.* Opp3′ or Opp4, are not the major cause leading to the wide spread of ST5 and ST8 clones (Fig. 2).

Seven staphylococcal species other than *S. aureus* were also investigated in this work, which showed a pronounced diversity of Opp systems (Table 1). For those characterized Opp systems, only Opp3 was conserved in all of the staphylococcal species. Notably, Opp3 and Opp4 were tandemly arranged in *S. aureus* (class I), but they were not adjacent to each other in *Staphylococcus carnosus* TM300 and *Staphylococcus pseudintermedius* ED99. Moreover, tandem duplication of Opp4 (designated Opp4′) was identified in *S. pseudintermedius* ED99, and operon *opp4′* was directly located at the downstream of *opp4* operon in chromosome. The protein identity between Opp4 components to their counterparts in Opp4′ system was 76–87%. Remarkably, both Opp4 and Opp4′ were absent from *S. pseudintermedius* strain HKU10-03, indicating that the acquisition of Opp4 and its subsequent duplication in ED99 both occurred recently. The Opp2 system was identified in the non-*S. aureus* species except *Staphylococcus haemolyticus* and *Staphylococcus lugdunensis*. In *S. carnosus*, *S. pseudintermedius* and *Staphylococcus saprophyticus*, Opp2 system had an extra component, Opp2A, a substrate binding protein that showed 60 to 65% protein identity to Opp5A in *S. aureus*.

2.2. ACME-Opp-like (ACME-OPPL) systems in staphylococci

In *S. aureus*, the entire structure of ACME-Opp system has been exclusively characterized in USA300 strains, *i.e.* FPR3757 and TCH1516, which are nearly identical [12,23]. In this work, genome-wide identification of their homologues in other staphylococcal species was performed, which found several ACME-OPPL systems in *S. haemolyticus*, *S. lugdunensis* and *Staphylococcus warneri* (Table 1). Notably, components of the two ACME-OPPL systems in *S. haemolyticus* showed varied protein identity to their *S. aureus* counterparts, *i.e.* 84–91% for ACME-OppLH1 (SH0147-151) and 49–59% for ACME-OppLH2 (SH0288-292). In contrast, ACME-OPPL systems in *S. lugdunensis* showed lower sequence identity to *S. aureus* homologues, *e.g.* 54–72% for ACME-OppLL1 (SLGD_00199-203) and 47–59% for ACME-OppLL2 (SLGD_00621-626).

Moreover, in contrary to *S. aureus* and *S. epidermidis*, in which the ACMEs are adjacent to staphylococcal cassette chromosome (SCC) element regions, the ACME-OPPL systems in *S. lugdunensis* and *S. haemolyticus* are not contained in ACME elements and are distant from SCC*mec*. For example, ACME-OppLH1 and ACME-OppLH2 in *S. haemolyticus* are 25 kb and 165 kb away from the SCC composite island, respectively [24].

2.3. Pseudogenes in opp gene clusters

In this work, we identified over one hundred Opp systems in staphylococcal species. Most of them are intact and their coding genes are clustered. However, *opp* pseudogenes can also be identified in six *S. aureus* isolates and in three other non-*S. aureus* species. Frame shift and interruption by insert sequence (IS) were two main reasons of gene inactivation, which were responsible for eight and four pseudogenes, respectively (Table 2). Interestingly, the four IS interrupted genes were exclusively *opp4* genes, indicating that *opp4* was a hotspot for insertion events.

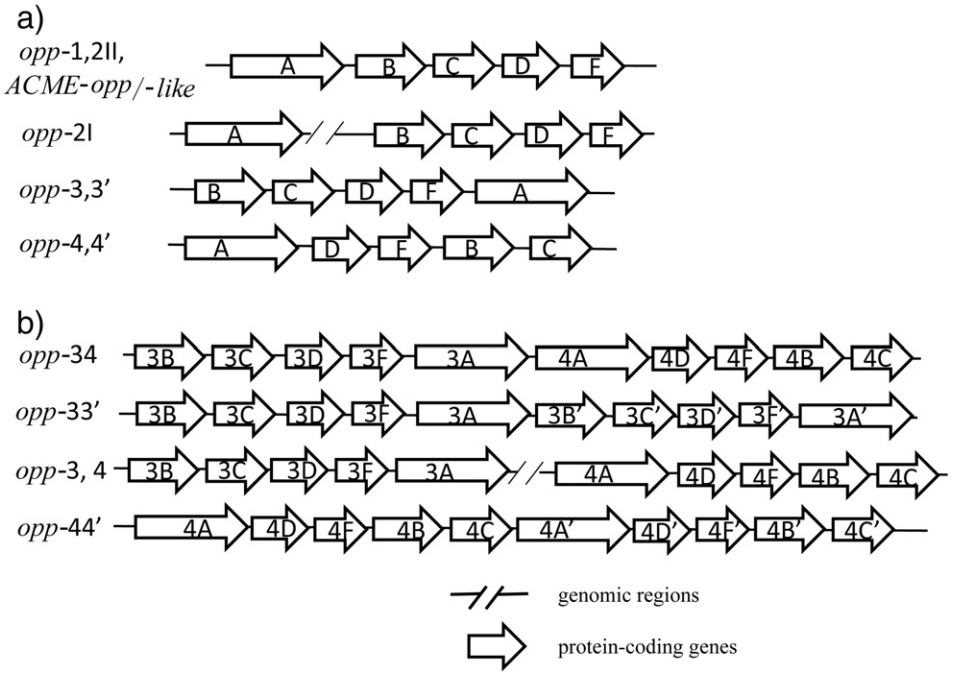

Fig. 1. Opp operons in staphylococci. a) Gene organization of *opp* operons identified in staphylococcal species. Coding genes for Opp2II are organized in a cluster, but for Opp2I, the *oppA* gene is separated from others. b) Arrangement of *opp3* and *opp4* operons (or their duplicates) in staphylococcal chromosomes. Operons opp3 and opp4 are tandemly arranged in *Staphylococcus aureus*, but not in *S. carnosus* and *S. pseudintermedius*.