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Sensor domain of histidine kinase ComD confers competence pherotype specificity in *Streptoccoccus pneumoniae*

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Abstract

Competence for genetic transformation in *Streptococcus pneumoniae* is regulated by a quorum-sensing mechanism involving the pheromone competence stimulating peptide (CSP) encoded by *comC* and a two-component signal transduction system, ComD–ComE (TCS12). In the presence of CSP, the transmembrane histidine kinase ComD receptor activates the response regulator ComE. The *comC*, *comD* and *comE* genes are part of an operon denoted as *comCDE*. In this work, the *comCDE* locus of 17 *S. pneumoniae* strains was characterized by DNA sequencing. Two major allelic combinations, *comC1–comD1* and *comC2–comD2* were present. Two further allelic combinations, *comC1–comD3* and *comC1–comD4*, were also present. Comparison of the deduced amino acid sequences of the four ComD allelic variants showed that all variations are localized in the N-terminal sensor domain. In order to have the four *comD* alleles in the same genetic background, we constructed four different isogenic strains in which *comC* was deleted and the DNA encoding the sensor domain of ComD was exchanged. To formally demonstrate that the sensor domain of ComD is responsible for competence pherotype specificity, CSP-1 and CSP-2 peptides were used to induce competence in the isogenic strains: (i) strains expressing the ComD1, ComD3 and ComD4 variants were induced to competence by CSP1; (ii) the strain expressing ComD2 was induced by CSP2. Moreover, cross-induction of competence by both CSPs was observed in the ComD2 and ComD3-carrying strains in the presence of high CSP doses. This is the first formal confirmation that the ComD sensor domain is responsible for competence pherotype specificity in *S. pneumoniae*.

Keywords: *Streptococcus pneumoniae*; Competence; Transformation; Two-component system; Pheromone; Quorum-sensing; Histidine kinase

1. Introduction

Genetic transformation in *Streptococcus pneumoniae* (the pneumococcus) is responsible for the genetic variability of this important human pathogen. Recombinational exchanges cause serotype switching, assembly of mosaic antibiotic resistance genes, pherotype changes and polymorphisms in surface protein loci [1]. Furthermore, transformation is a powerful tool for genetic manipulation of this organism, commonly used as a model pathogen to study new drugs and vaccines.

Competence for genetic transformation is a transient physiological state that in *S. pneumoniae* develops in the exponential growth phase. Development of competence is regulated by a quorum-sensing mechanism, based on a two-component signal transduction system, and a peptide pheromone [2]. In *S. pneumoniae* the competence quorum-sensing mechanism involves the pheromone

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competence stimulating peptide (CSP) encoded by *comC* [3] and a two-component signal transduction system, ComD–ComE (TCS12) [4,5]. In streptococci, *comC*, *comD* and *comE* are part of an operon denoted as *comCDE* [4,6]. The unmodified heptadecapeptide pheromone CSP, purified from pneumococcus strain Rx1 [3], expressed as a 41-residue precursor, is processed and secreted by an ABC-transporter, known as ComAB [7]. It is likely, by analogy with other two-component regulatory systems, that binding of CSP to the transmembrane histidine kinase ComD receptor provokes autophosphorylation and then the phosphoryl group is transferred to the response regulator ComE [8]. ComE is a DNA-binding protein that interacts with a sequence located near the promoter of the *comCDE* and *comAB* operons and regulates their expression [9]. Late competence genes involved in DNA uptake and recombination contain an unusual consensus sequence (cin-box) in the promoter region [10], and are activated by ComE through ComX, which is proposed to be a competence specific sigma factor [11].

Histidine kinases have been grouped into two classes on the basis of their domain organization. In class I, histidine kinases the N-terminal transmembrane region represents the sensor domain and the C-terminal transmitter domain contains the conserved histidine residue [5,12]. ComD has the general organization typical of the histidine kinases of class I, characterized by an N-terminal sensor domain spanning amino acids positions 1–210. Overall topology prediction of the pneumococcal ComD sensor domain indicates the presence of 5–7 putative transmembrane segments as in the *Streptococcus gordonii* ComD receptor [4,6,13].

Genetic polymorphism of the competence quorum-sensing system has already been reported in streptococci [6,13–15]. In a previous study, we characterized the nucleotide sequence of *comC* in 42 pneumococcal clinical isolates belonging to different serotypes. Two allelic variants *comC1* and *comC2* were identified. Their predicted gene products were shown to differ by eight amino acid residues. CSP-1 and CSP-2 synthetic peptides were used to induce competence in the 42 strains. About half of the strains became competent after CSP addition and pherotype specificity was found: strains carrying *comC1* were induced by CSP-1 while CSP-2 induced strains carrying *comC2*. Cross-induction by both CSP1 and CSP2 was also found [15]. In a following study the presence of two major alleles *comD1* and *comD2* (associated to *comC1* and *comC2*, respectively) were described, although rare *comC* and *comD* alleles were discovered [13]. Also in *S. gordonii*, the presence of two allelic combinations *comC1–comD1* and *comC2–comD2*, associated to different pherotypes, was reported [6]. In this work, it was demonstrated that a *S. gordonii* chimeric strain expressing both ComD1 and ComD2 receptors is induced to competence by the CSP1 and CSP2 peptides, linking the pheromone specificity to the ComD receptor.

Allelic variation of peptide-pheromone quorum-sensing systems is a common feature and has also been reported for the second pneumococcal quorum-sensing bacteriocin-like peptide system, the *agr* quorum-sensing system in *Staphylococcus* spp. and the competence quorum-sensing system in *Bacillus subtilis* [16–19].

In this study, we characterized the competence *comCDE* locus in *S. pneumoniae* isolates whose data of competence induction by synthetic CSPs are available. We found four *comD* allelic variants. Four isogenic *comC*-negative strains each carrying a different *comD* allele were used to formally demonstrate that the sensor domain of ComD is responsible for the competence pherotype specificity in *S. pneumoniae*.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Pneumococcal strains used in this work are the Avery's strain A66 (type 3) in which *comC2* was originally described, the rough strain Rx1 (type 2) and its novobiocin resistant DP1002 derivative strain [20]; one strain from the American Type Culture Collection and 11 isolates from an Italian clinical collection [15]. Bacteria were grown at 37 °C in tryptic soy broth (TSB) or in tryptic soy agar (Difco) supplemented with 3% horse blood. Novobiocin (10 μg/ml) or chloramphenicol (3 μg/ml) was added to the media where appropriate.

2.2. Pneumococcal transformation

Competent cell preparation and transformation were carried out essentially according to previously described protocols [20,21]. Pneumococcal competent cells were incubated for 30 min at 37 °C with the transforming DNA and CSP. The DNA purified from the strain DP1002 was added at 1 μg per ml of competent cells.

2.3. Amplification, sequencing and sequence analysis

A 2788-bp PCR fragment, containing the *comCDE* locus, was amplified from the pneumococcal chromosome using the primer pair IF46-IF79. Primer IF46 (5′-GAA GTT TAG GAT TGT CAT CAT CTG-3′) corresponds to nucleotides 243–266, and IF79 (5′-GGA TAA AAT AGT CCG TAC GGG-3′) is complementary to nucleotides 3030–3010 of the Rx1 *comCDE* locus (GenBank Accession No. U33315). PCR and sequence experiments were performed as previously reported [21,22]. BLAST search was carried out at GenBank (http://www.ncbi.nlm.nih.gov/) and at The Institute for Genomic Research (TIGR) (http://www.tigr.org/). Protein analysis was carried out using the tools available at the Expasy website (http://www.expasy.org/).