Computational analysis suggests that virulence of *Chromobacterium violaceum* might be linked to biofilm formation and poly-NAG biosynthesis

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Abstract

Groups of genes that produce exopolysaccharide with a N-acetyl-d-glucosamine monomer are in the genome of several pathogenic bacteria. *Chromobacterium violaceum*, an opportunistic pathogen, has the operon hmsHFR-CV2940, whose proteins can synthesize such polysaccharide. In this work, multiple alignments among proteins from bacteria that synthesize such polysaccharide were used to verify the existence of amino acids that might be critical for pathogen activity. Three-dimensional models were generated for spatial visualization of these amino acid residues. The analysis carried out showed that the protein HmsR preserves the amino acids D135, D228, Q264 and R267, considered critical for the formation of biofilms and, furthermore, that these amino acids are close to each other. The protein HmsF of *C. violaceum* preserves the residues D86, D87, H156 and W115. It was also shown that these residues are also close to each other in their spatial arrangement. For the proteins HmsH and CV2940 there is evidence of conservation of the residues R104 and W94, respectively. Conservation and favorable spatial location of those critical amino acids that constitute the proteins of the operon indicates that they preserve the same enzymatic function in biofilm synthesis. This is an indicator that the operon hmsHFR-CV2940 is a possible target in *C. violaceum* pathogenicity.

Key words: biofilms, exopolysaccharide, *Chromobacterium violaceum* pathogenicity, comparative genomics.

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Introduction

Some microorganisms develop cooperative strategies in the formation of biofilms. Biofilm can be defined as interdependent communities of microorganisms, usually connected with a surface presenting high resistance to environmental stress. It consists of a complex symbiotic system of great importance to biotechnological and medical applications, since it is correlated with bacterial resistance to antibiotics and promotion of lethal infections.

The formation of biofilms in several microorganisms involves the presence of a complex matrix where the polymer poly[beta-1,6-N-acetyl-D-glucosamine], poly-NAG, can play an important role (Itoh et al., 2005), possibly related to resistance to antibiotics and promotion of bacterial infections. Poly-NAG has been implicated in the formation of biofilms in several pathogenic bacteria. The polymer, which is involved in cell adhesion in *Staphylococcus epidermidis* (Mack et al., 1996), is also involved in abiotic surface binding, and in intercellular adhesion formation of biofilm in *Escherichia coli* (Itoh et al., 2005).

The hmsHFRS operon has been involved in the formation of biofilms *in vitro* in *Yersinia pestis*. This operon was related to the blockage of the digestive system of fleas, and it is associated with the transmission of *Y. pestis* to mammals (Jarrett et al., 2004). The bacterium *Bordetella pertussis* has the operon bpsABCD related to a polymer that contributes to the formation of biofilms in the respiratory tract of rats (Sloan et al., 2007). An understanding of the relationship between the operons of those bacteria in the formation of biofilms could potentially lead to the development of a vaccine applicable to different bacterial species.

Recently, the sequencing of the complete genome of the *Chromobacterium violaceum*, strain ATCC 12472 (Brazilian National Genome Project Consortium, 2003; www.brgene.lncc.br; www.ncbi.nlm.nih.gov: access number NC_005085) was carried out to promote domestic genome projects. The investment was justified due to the great biotechnological and pharmaceutical potential of this microorganism. *Chromobacterium violaceum* is considered a possible pathogen for humans and animals, with several reported cases of infection in humans and animals in tropical and subtropical areas, where it is normally found.
Its potential pathogenicity in humans was first described in 1927 in Malaysia (Sneath et al., 1953). Although cases of infection with \textit{C. violaceum} are rare, it has a mortality rate of more than 57%, as reported in a case of chronic granulomatous disease in children (Macher \textit{et al.}, 1982). \textit{C. violaceum} reveals some potentially pathogenic genes (Brazilian National Genome Project Consortium, 2003); however, their extent is not yet known or how these genes are organized in their genome.

This work aims to link, through comparative analysis, the genes \textit{hmsH}, \textit{hmsF}, \textit{hmsR} and the ORF CV2940 of \textit{C. violaceum} with the genes of organisms known and related to the formation of biofilm in an effort to shed light on the pathogenic potential of \textit{C. violaceum}. The analysis of the \textit{C. violaceum} genome reveals the presence of possible genes involved in the formation of biofilms, and the sequence of the group of genes \textit{hmsHFR} and CV2940 are potentially functional for it. Initially, the relationship among the proteins is carried out by the alignment of amino acid sequences and it is complemented by structural modeling in order to identify the importance of the amino acid through spatial viewing.

**Results**

In a multiple alignment comparison generated by the Clustal W server with other glycosyltransferase proteins, the critical amino acids related to formation of biofilms are preserved also in the HmsR protein of \textit{C. violaceum} (Figure 1). Conserved amino acids that are critical for function of HmsR of \textit{Y. pestis} (D176, D269, Q305 and R308) correspond to residues D135, D228, Q264 and R267 in \textit{C. violaceum}, respectively.

The structural model generated (Figure 2) comprises the critical amino acids for the function of this protein in the formation of biofilms arranged in a three-dimensional conformation with the possibility of fitting a substrate. The residue D228 is in exactly the same position as D191 (SpS\textit{A}) with other conserved residues (D135, Q264 and R267) also positioned on the active site.

The multiple alignment among organisms in this study shows that the polypeptide HmsF of \textit{C. violaceum} retains the amino acids that are crucial to the formation of extracellular matrix in biofilms (Figure 3). The D114 and D115 aspartate amino acids of the protein HmsF of \textit{Y. pestis}, which have HmsF deacetylase activity functionality, are conserved on all related organisms in this study, and in \textit{C. violaceum} correspond to aspartate D86 and D87. The important amino acids in the synthesis of extracellular matrix in \textit{Y. pestis} (W143 and H184), which correspond in \textit{C. violaceum} to W115 and H156, are also retained.

In the three-dimensional model of HmsF of \textit{C. violaceum} obtained (Figure 4), the 3D-JIGSAW server made use of the protein \textit{SpPgD} as a template (PDB code: 2c1g) (Blair \textit{et al.}, 2005), which is a \textit{N-acetyl-glucosaminate} deacetylase. The model generated for HmsF of \textit{C. violaceum} presenting the spatial location of critical residues in the biofilm formation to \textit{Y. pestis} shows that the residues D86, D87, W115 and H156 are closely located in space (Figure 4). These residues are situated around the active site.

In the multiple alignment of HmsH protein, it was observed that the residue arginine R113, which had a poor performance in the formation of biofilms in \textit{Y. pestis} (For-
man et al., 2006), and that in C. violaceum is the residue R104, was conserved in almost all organisms studied, but it was not observed in S. epidermidis, a Gram-positive bacterium (not shown). The multiple alignment performed among bacteria of this study for ORF CV2940 shows that the critical residue tryptophan W80 of Y. pestis is also conserved in Gram-negative bacteria E. coli and C. violaceum, corresponding to the residue W94 in C. violaceum.

The analysis of the three-dimensional model generated for HmsR of C. violaceum by the Ramachandran diagram shows that the model holds 99.1% of the residues in allowed regions, demonstrating a good quality. The analysis of the environment of each amino acid residue carried out by the Verify3D program presented two regions (residues 175 to 187 and 230 to 237) in the HmsR with a negative 3D-1D score, indicating a probable incorrect folding in this region. However, the critical residues for HmsR in the formation of biofilm do not appear in this region. The structure of HmsR in the formation of biofilm do not appear in this region. The structure of HmsR in the formation of biofilm do not appear in this region.

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HmsF preserves the residue arginine, which shows a moderate effect on the formation of biofilms in Y. pestis among all bacteria of this study, except for S. epidermidis which is a Gram-positive bacterium. In the three-dimensional model, the choice of the template of the OGT enzyme (PDB accession code 1w3b) was very useful, since this enzyme has an N-acetylg glucosamine (GlcNac) additive activity (Jinek et al., 2004) that is the basic unit of the polymer poly(beta-1,6-N-acetyl-D-glucosamine).

Conservation and favorable spatial arrangement of those critical amino acids that constitute the proteins encoded by the elucidated operon indicate that they may express the necessary enzymatic function for resistant biofilm formation. Therefore the conclusion is that the operon hmsHFR-CV2940 might be linked to C. violaceum pathogenicity.

References

Bates PA, Kelley LA, Macallum RM and Sternberg MJ (2001) Enhancement of protein modeling by human intervention in applying the automatic programs 3D-JIGSAW and 3D-PSSM. Proteins 5:39-46.

Blair DE, Schuttelkopf AW, Macrae JI and Van Aalten DM (2005) Structure and metal-dependent mechanism of peptidoglycan deacetylase, a streptococcal virulence factor. Proc Natl Acad Sci USA 102:15429-15434.

Brazilian National Genome Project Consortium (2003) The complete genome sequence of Chromobacterium violaceum reveals remarkable and exploitable bacterial adaptability. Proc Natl Acad Sci USA 100:11660-11665.

Eisenberg D, Luthy R and Bowie JU (1997) VERIFY3D: Assessment of protein models with three-dimensional profiles. Meth Enzymol 277:396-404.

Forman S, Bobrov AG, Kirillina O, Craig SK, Abney J, Fetherston JD and Perry RD (2006) Identification of critical amino acid residues in the plague biofilm Hms proteins. Microbiology 152:3399-3410.

Itoh Y, Wang X, Hinnebusch BJ, Preston JF and Romeo T (2005) Depolymerization of beta-1,6-N-acetyl-D-glucosamine disrupts the integrity of diverse bacterial biofilms. J Bacteriol 187:382-387.

Jarrett CO, Deak E, Isherwood KE, Oyston PC, Fischer ER, Whitney AR, Kobayashi SD, Deleo FR and Hinnebusch BJ (2004) Transmission of Yersinia pestis from an infectious biofilm in the flea vector. J Infect Dis 190:783-792.

Jeannotte F, Thompson JD, Gouy M, Higgins DG and Gibson TJ (1998) Multiple sequence alignment with Clustal X. Trends Biochem Sci 23:403-405.

Jinek M, Rehwinkel J, Lazarus BD, Izaurralde E, Hanover JA and Conti E (2004) The superhelical TPR-repeat domain of O-linked GlcNAc transferase exhibits structural similarities to importin alpha. Nat Struct Mol Biol 11:1001-1007.

Kelley LA, Macallum RM and Sternberg MJ (2000) Enhanced genome annotation using structural profiles in the program 3D-PSSM. J Mol Biol 299:499-520.

Laskowski RA, Rullmann JA, Macarthur MW, Kaptein R and Thornton JM (1996) AQUA and PROCHECK-NMR: Programs for checking the quality of protein structures solved by NMR. J Biomol NMR 8:477-486.
Macher AM, Casale TB and Fauci AS (1982) Chronic granulomatous disease of childhood and Chromobacterium violaceum infections in the southeastern United States. Ann Intern Med 97:51-55.

Mack D, Fischer W, Krokotsch A, Leopold K, Hartmann R, Egge H and Laufs R (1996) The intercellular adhesin involved in biofilm accumulation of Staphylococcus epidermidis is a linear beta-1,6-linked glucosaminoglycan: Purification and structural analysis. J Bacteriol 178:175-183.

Ramachandran GN, Ramakrishnan C and Sasisekharan V (1963) Stereochemistry of polypeptide chain configurations. J Mol Biol 7:95-99.

Sloan GP, Love CF, Sukumar N, Mishra M and Deora R (2007) The Bordetella Bps polysaccharide is critical for biofilm development in the mouse respiratory tract. J Bacteriol 189:8270-8276.

Sneath PH, Whelan JP, Bhagwan SR and Edwards D (1953) Fatal infection by Chromobacterium violaceum. Lancet 265:276-277.

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