Characterization of the *Porphyromonas gingivalis* Type IX Secretion Trans-Envelope PorKLMNP Core Complex^{*}

Maxence S. Vincent[‡], Mickaël J. Canestrari[‡], Philippe Leone^{¶,§}, Julien Stathopulos^{¶,§}, Bérengère Ize[‡], Abdelrahim Zoued[‡], Christian Cambillau^{¶,§}, Christine Kellenberger^{¶,§}, Alain Roussel^{¶,§}, and Eric Cascales^{‡,1}

From [‡] Laboratoire d'Ingénierie des Systèmes Macromoléculaires, UMR7255, Institut de Microbiologie de la Méditerranée, Aix-Marseille Univ - CNRS, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France; [¶] Architecture et Fonction des Macromolécules Biologiques, Centre National de la Recherche Scientifique, UMR 7257, Campus de Luminy, Case 932, 13288 Marseille Cedex 09, France and [§] Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille Université, UMR 7257, Campus de Luminy, Case 932, 13288 Marseille Université, UMR 7257, Campus de Luminy, Case 932, 13288 Marseille Université, UMR 7257, Campus de Luminy, Case 932, 13288 Marseille Cedex 09, France.

Running title: T9SS membrane complex assembly

¹To whom correspondence should be addressed. Laboratoire d'Ingénierie des Systèmes Macromoléculaires, UMR7255, Institut de Microbiologie de la Méditerranée, Aix-Marseille Univ - CNRS, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France; Tel: +33 491-164-504; Fax: +33 491 712 124; E-mail: cascales@imm.cnrs.fr

* This work was supported by the Centre National de la Recherche Scientifique (CNRS), the Aix-Marseille Université and a grant from the Agence Nationale de la Recherche (ANR-15-CE11-0019-01). MSV is a recipient of a doctoral fellowship from the French Ministère de la Recherche. The work of AZ was funded by a end-of-thesis fellowship from the Fondation pour la Recherche Médicale (FDT20140931060).

Keywords: Type IX secretion, T9SS, *Porphyromonas, Flavobacterium*, protein transport, gingipains, membrane complex, membrane proteins, gliding motility, gingivitis, periodontitis; protein secretion, toxins, channel.

ABSTRACT

The transport of proteins at the cell surface of **Bacteriodetes** depends on a secretory apparatus known as Type IX secretion system (T9SS). This machine is responsible for the cell surface exposition of various proteins such as adhesins required for gliding motility in Flavobacteria, S-layer components in Tannerella forsythia and tooth tissue-degrading enzymes in the oral pathogen Porphyromonas gingivalis. While a number of subunits of the T9SS have been identified, we lack details on the architecture of this secretion apparatus. Here we provide evidence that five of the genes encoding the core complex of the T9SS are co-transcribed, and that the gene products are distributed in the cell envelope. Protein-protein interaction studies then revealed that these proteins oligomerize and interact through a dense network of contacts.

Porphyromonas gingivalis is the causative agent of gingivitis and periodontal diseases that are responsible for teeth loss (1, 2). It causes severe lesions in periodontal tissues such as the gingiva or the alveolar bone and yields to the disruption of the tooth-supporting structure (3). Periodontitis are considered a major public health concern as it affects ~ 35 % of the population. Tissue alterations and damages are mainly induced by a cocktail of toxin proteins secreted by the bacterium, the gingipains (4). Gingipains act as adhesins or proteases that help the bacterium to adhere to periodontal tissues and to promote gingival tissue invasion by the degradation of matrix proteins, fibrinogen and collagen (5, 6). The secretion of these proteins is a two-step mechanism: gingipains carry a N-terminal signal peptide and are first addressed to the periplasm by the Sec pathway before being transported to the cell surface or to the cell exterior (7). However, the machinery responsible for the translocation of gingipains through the outer membrane remained unknown as genes encoding a potential Type II secretion system (T2SS), the major two-step