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Modeling the onset of virulence in a pectinolytic bacterium

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Abstract

Building up from experimental knowledge of the regulatory network of the *pel* genes in the bacteria *E. chrysanthemi*, we propose for the first time a qualitative modeling of the infectious transition of this bacteria when it is hosted in a plant. We show that this infectious transition can be understood as the *excitable* dynamics of a metabolico-genetic network. Our mathematical model can account for the main phases which are observed in the onset of the pathogenecity by *Erwinia chrysanthemi*, namely the silent, latent and virulent stages. Like in many infectious agents, the silent state corresponds to the growth phase of the bacteria, where they multiply without significantly producing molecules which could trigger a counter attack of the invaded host. The latent stage is characterized by a moderate but unequivocal expression of the virulence gene, waiting for a number of conditions which have to fulfill in order to trigger a fully developed infection. In the virulent state the bacteria synthesize a massive production of virulence factors including pectate lyases (Pel) which favor the invasion of the host plant tissues. Our model is able to show cases of transitions from the silent to the virulent stages of the infection, using the method of the piecewise-affine (PA) differential equations and its implementation in the *genetic network analyser* software (GNA). The obtained qualitative dynamics of the models are consistent with the current experimental data about this system. Moreover it can be interpreted with respect to the relatively complex structure of the binding sites of *pel*. From the biological point of view, our simulations validate the picture that the promoter of *pel* has evolved to form a security device preventing a hastened expression of these virulent genes. This first modeling of the regulation of *pel* genes opens the way to new confrontations between theoretical ideas with experiments and possible strategies to fight the soft-rot disease of plants.

Keywords: *Erwinia chrysanthemi*; Pectinases; Gene regulatory network; Piecewise-affine differential equations

1. Introduction

Coordination of virulence factor synthesis is central to pathogenicity. Thus, understanding of the basic mechanisms allowing the coordination of the bacterial virulence gene expression is of fundamental interest and a prerequisite for the development of new methods to fight against pathogenic bacteria. In this study, we present a comprehensive and integrative model of the regulatory network directing the synthesis of the *Erwinia chrysanthemi* essential virulence factors, allowing to identify important events which happen during the infectious transition.

The pectinolytic *Erwiniae* spp. are soft-rotting Gram-negative bacteria that cause severe diseases in a wide range of plant species, including many crops of economical importance such as vegetables and ornamentals. Soft rot results from the general disorganization of plant tissues following the degradation of pectin, the major component of primary cell walls. Bacterial soft rots are diseases difficult to control because of the ubiquity of the soft-rot *Erwiniae*. They are widespread in surface water (Cother et al., 1992); they are competitive saprophytes in the rhizosphere (Stanghellini, 1982); and they can aggressively utilize pectate as a carbon source (Burr and Schroth, 1977). The virulence of the pectinolytic *Erwiniae* is mainly correlated with their ability to produce and secrete cell-wall degrading enzymes mainly pectate lyases (Pel) (Collmer and Keen, 1986; Hugouvieux-Cotte-Pattat et al., 1996). Nevertheless, as illustrated in Fig. 1 plant

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colonization by pectinolytic *Erwiniae* is a multifactorial process requiring numerous additional factors, including iron assimilation (Enard et al., 1988), the Hrp system (Bauer et al., 1994), lipopolysaccharide (Schoonejans et al., 1987), exopolysaccharides (EPS) (Condemine et al., 1999) and proteins involved in resistance against plant defense mechanisms (Lopez-Solanilla et al., 1998; El Hassouni et al., 1999; Santos et al., 2001; Reverchon et al., 2002).

In *E. chrysanthemi*, production of these virulence factors, particularly the pectate lyases, is tightly regulated and responds to several environmental and metabolic stimuli, including growth phase-dependent induction, the presence of pectin or plant extract and catabolic repression (Hugouvieux-Cotte-Pattat et al., 1996). Several regulators (KdgR, PecS, PecT, CRP, H-NS and ExpR) modulating the expression of virulence genes in *E. chrysanthemi* have been characterized previously (Reverchon et al., 1991, 1994, 1997; Praillet et al., 1996; Surgey et al., 1996; Nasser et al., 2001). Most of these systems are not limited to the control of Pel production; they also affect other factors that are involved in virulence, such as motility, production of a cellulase, synthesis of compounds involved in the resistance to the products of oxidative burst and EPS production (Reverchon et al., 1994, 1997, 2002; Castillo et al., 1998; Condemine et al., 1999). KdgR, PecT and PecS mainly act as repressors whereas H-NS, ExpR and CRP are activators of *pel* gene expression. The induction of *pel* gene expression by 2-keto-3-deoxygluconate (KDG), an inducer derived from pectin catabolism, is mediated by KdgR (Reverchon et al., 1991; Nasser et al., 1994). ExpR is a *quorum sensing* (QS) regulator that moderately participates in the modulation of *pel* gene expression in response to *N*-acyl-homoserine lactones (acyl-HSLs) generated by the synthase ExpI (Nasser et al., 1998; Reverchon et al., 1998). Catabolic repression is mediated by the cAMP–CRP complex, which acts as the main activator of the *pel* gene expression. The signals to which PecS, PecT and H-NS respond remain elusive. However H-NS was supposed to respond to change in environmental conditions particularly to nutritional stress and variation in temperature whereas PecS was supposed to respond to phenolic compounds or reactive oxygen species (ROS) produced by the plant defense reactions (Nasser and Reverchon, 2002; Reverchon et al., 2002). Moreover, these regulators are organized in a complex network (Reverchon et al., 1998; Nasser and Reverchon, 2002; Rodionov et al., 2004). Undermining this regulatory network may be a promising therapeutic strategy against the pectinolytic *Erwiniae*.

Like many infectious agents, *E. chrysanthemi* has two main behaviors: silent and virulent. In the silent state, the bacteria multiply without producing molecules which could trigger the defense mechanisms of the invaded host. In a second phase, when the bacteria are subjected to some signals (bacterial density, pectin or other plant cell compounds, temperature, nutritional starvation) they are able to induce a massive production of virulence factors including Pel which favor the invasion of the host plant tissues. Although this transition is well observed and reproducible in *in vitro* and *in vivo* experiments, a full understanding of the conditions which lead to an inexorable infection is unclear, due to the number of factors which are involved in the regulation of pectinases. Therefore a theoretical model of this transition, as well as its mathematical analysis, could help to clarify this question. Moreover, computational studies of the model can open the way to possible strategies to struggle against the soft-rot disease and propose new experiments to test.

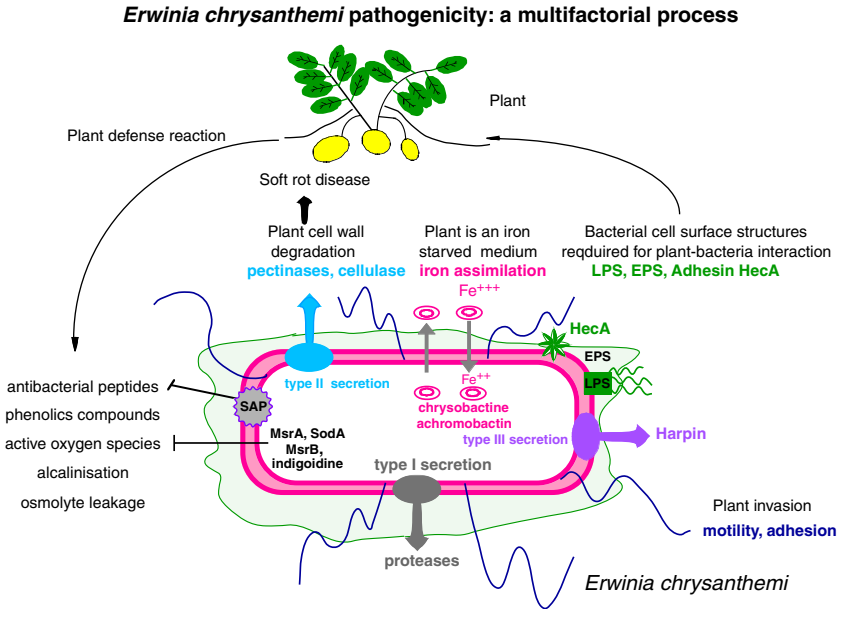

Fig. 1. Schematic description of the interactions between *Erwinia chrysanthemi* and the host plant. In this paper we concentrate on modeling the production of pectinases (pectate lyases) leading to the soft-rot disease.