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Genome Analysis

Extracytoplasmic function sigma factors in *Pseudomonas syringae*

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Genome analyses of the plant pathogens *Pseudomonas syringae* pv. *tomato* DC3000, pv. *syringae* B728a and pv. *phaseolicola* 1448A reveal fewer extracytoplasmic function (ECF) sigma factors than in related Pseudomonads with different lifestyles. We highlight the presence of a *P. syringae*-specific ECF sigma factor that is an interesting target for future studies because of its potential role in the adaptation of *P. syringae* to its specialized phytopathogenic lifestyle.

In bacteria, the sigma factor protein binds to the core RNA polymerase and forms a holoenzyme able to recognize the promoter and initiate transcription. Bacterial sigma factors belong to two protein families: the σ^{54} and the σ^{70} families. The σ^{70} family can be further divided into four phylogenetic groups, with the extracytoplasmic function (ECF) group being the largest and most diverse [1–3]. Adaptation to a particular lifestyle clearly contributes to the final shape of bacterial genomes, and the number of transcription-associated proteins, namely sigma factors and two-component regulatory systems, might mirror the adaptability of the bacterium. Accordingly, it has been proposed that the number of sigma factors and transcription-associated proteins encoded in a genome correlates with the different lifestyles of bacteria [4–7]. ECF sigma factors offer a convenient mechanism for transcriptional regulation in response to specific environmental signals, and a correlation between bacterial lifestyles and the number of ECF sigma factors has become evident: bacteria with complex lifestyles involving different habitats have more ECF sigma factors than bacteria with simple lifestyles living in stable niches [7,8].

Genome sequences of *Pseudomonas syringae* pathovars

P. syringae is a common leaf-inhabiting Proteobacterium, existing either as a harmless commensal on the surface of leaves or as an agriculturally important plant pathogen [9]. *P. syringae* is a highly specialized plant parasite that interacts with a wide range of plants, and strains of *P. syringae* are assigned to a pathovar on the basis of their different host specificities. Disease development by *P. syringae* is often preceded by growth on leaf surfaces as an epiphyte that enters plant leaves through stomata, grows to large populations in intercellular spaces and injects virulence effector proteins into the cytoplasm of plant cells by means of the Hrp type III secretion system [9].

The genomes of *P. syringae* pv. *tomato* DC3000, pv. *syringae* B728a and pv. *phaseolicola* 1448A have been sequenced [10–12]. In *P. syringae* pathovars, between 10 and 12% of the genes have a regulatory role, which could reflect the need for rapid adaptation to the diverse environments encountered during epiphytic growth, plant colonization and pathogenesis [10–12]. The identification of genomic features that underlie the adaptation of *P. syringae* to its phytopathogenic lifestyle and the interactions with plant hosts is a major challenge. Hence, the ECF sigma factors of *P. syringae* are attractive goals for genomic studies that could help to elucidate their regulatory functions and molecular mechanisms, and to establish which gene sets contribute to the phytopathogenic lifestyle. The aim of this article is to analyze the ECF sigma factors in the complete genomic sequences of *P. syringae* pathovars and to compare them with the ECFs in the genomes of related Pseudomonads with different lifestyles. These are the completely sequenced genomes of the opportunistic animal and plant pathogen *Pseudomonas aeruginosa* PAO1 [13], the non-pathogenic saprophytic soil bacterium *Pseudomonas putida* KT2440 [14], the plant commensal *Pseudomonas fluorescens* Pf-5 [15], and the genomes in progress of the *P. aeruginosa* strain UCBPP-PA14, the nitrogen-fixing *Azotobacter vinelandii* AvOP and the *P. fluorescens* strains PfO-1 and SBW25 (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi).

A reduced number of ECF sigma factors in *P. syringae*

A limited number of ECF sigma factors have been characterized in plant pathogenic Proteobacteria, and two ECF sigma factors of *P. syringae* (HrpL and AlgT) have been studied in detail with regard to the transcriptional regulation of virulence genes [16–18]. The related species *P. aeruginosa* PAO1 and *P. putida* KT2440 contain 19 ECF sigma factors and many of the ECF sigma factors of *P. aeruginosa* PAO1 have a counterpart in *P. putida*

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Table 1. ECF sigma factors in the genomes of *Pseudomonas syringae* pathovars[a]

P. syringae pv. *syringae* B728a	*P. syringae* pv. *phaseolicola* 1448A	*P. syringae* pv. *tomato* DC3000	Homologue in *Pseudomonas aeruginosa* PAO1[b]	Homologue in *Pseudomonas putida* KT2440[b]	Homologue in *Pseudomonas fluorescens* Pf-5[b]
PSYR0362	PSPH0345 (94%)	PSPTO5176 (94%)	–	PP3006 (57%)	–
PSYR0892	PSPH0927 (97%)	PSPTO1043 (94%)	–	–	–
PSYR1040	PSPH1093 (94%)	PSPTO1209 (92%)	PA3899 (71%)	PP4611 (74%)	PFL0984 (76%)
PSYR1107	PSPH1175 (92%)	PSPTO1286 (88%)	–	PP1008 (70%)	PFL4625 (72%)
PSYR1217/HrpL	PSPH1294 (92%)	PSPTO1404 (88%)	–	–	–
PSYR1943	PSPH1909 (98%)	PSPTO2133 (97%)	PA2426/PvdS (85%)	PP4244/PfrI (79%)	PFL4190/PbrA (87%)
PSYR2096/SigX	PSPH2067 (98%)	PSPTO2298 (98%)	PA1776/SigX (94%)	PP2088/SigX (90%)	PFL1875 (95%)
PSYR2580	PSPH2747 (98%)	–	PA1912 (57%)	PP3577 (76%)	PFL2291 (55%)
PSYR3958/AlgT	PSPH3955 (100%)	PSPTO4224 (99%)	PA0762/AlgU (90%)	PP1427/AlgT (96%)	PFL1448 (97%)
PSYR4731	PSPH4765 (94%)	PSPTO0444 (92%)	PA0472 (78%)	PP0352 (82%)	PFL5704 (82%)
–	–	PSPTO1203	PA2468 (47%)	PP0162 (52%)	PFL0127 (52%)

[a]Percent amino acid identities with the corresponding homologue protein of *P. syringae* pv. *syringae* B728a are in parentheses (except for the last row, which is with the PSPTO1203 protein of *P. syringae* pv. *tomato* DC3000).
[b]Homologues were located in a similar genetic organization and were grouped together in the phylogenetic analysis.

KT2440, although these organisms have different lifestyles [19].

A profile Hidden Markov Model (HMM) capable of recognizing ECF sigma factors has been developed [5] and used to search for ECF sigma factors in the complete genomes of *P. syringae* pathovars and *P. fluorescens* Pf-5 (Table 1; for material related to this article, see http://www.cbs.dtu.dk/services/GenomeAtlas/suppl/TMsigmas/). In addition, BLAST searches revealed the presence of several homologues of *P. syringae* ECF sigma factors in the draft genomes of Pseudomonads. Despite the similar large genome sizes of *Pseudomonas* species (6–7 Mbp), only ten ECF sigma factors have been identified in the genome of each *P. syringae* pathovar (Table 1); this is significantly lower than the 19 ECF sigma factors in *P. aeruginosa* and *P. putida* [5] and the 28 ECF sigma factors predicted in *P. fluorescens* Pf-5. A consequence of lacking a given sigma factor could be the incapacity to express a set of genes in response to a particular stress or different environmental condition [5,20]. The reduced number of ECF sigma factor genes (e.g. nine less than *P. aeruginosa* and *P. putida*) in *P. syringae* therefore indicates an adaptation to its specialized lifestyle as a plant pathogen.

FecI-type ECF sigma factors

The genomes of *P. syringae* pathovars contain five FecI-type ECF sigma factors (Figure 1; Table 1), which is less than the FecI homologues identified in *P. putida* KT2440 (13 sigmas) [19], *P. aeruginosa* PAO1 (14 sigmas) [21] and *P. fluorescens* Pf-5 (20 sigmas). All FecI-type ECF sigma factor genes of *P. syringae* pathovars, except for homologues to PfrI/PvdS (PSYR1943, PSPTO2133 and PSPPH1909), are located within gene clusters containing FecR-like transmembrane sensors and distinct outer membrane receptors, organized in a similar gene order to the ferric citrate regulatory system *fecIRA* of *Escherichia coli* (Figure 1) [21], which suggests that they are mainly involved in the regulation of different iron transport systems.

An ECF sigma factor specific for *P. syringae*

Strikingly, the unique PSYR0892 homologues are present in *P. syringae* pv. *tomato* DC3000 (PSPTO1043) and pv. *phaseolicola* 1448A (PSPPH0927) (Table 1), and homologues of PSYR0892 are absent in the other Pseudomonad genomes and in BLAST searches with current databases. Several ECF sigma factors are co-transcribed with their negative regulators, known as anti-sigma factors [2], and the transcriptional activator PSYR0891 (PSPTO1042 in *P. syringae* pv *tomato* DC3000 and PSPPH0926 in *P. syringae* pv. *phaseolicola* 1448A) can function as an anti-sigma factor of PSYR0892 (Figure 1). PSYR0892 can therefore be described as a *P. syringae*-specific ECF sigma factor and the PSYR0892/PSYR0891 regulatory system is an obvious target for future experimental studies because of the potential role of the system in the adaptation of *P. syringae* to its specialized pathogenic lifestyle on plants.

Future perspectives

Genome comparisons indicate that *P. syringae* is significantly different from other *Pseudomonas* species [5], suggesting that in the adaptation to the phytopathogenic lifestyle its genome must have undergone fundamental changes without a reduction in size. Moreover, the substantial differentiation of the genomes of *P. syringae* pathovars has involved a reduction in the complement of ECF sigma factors [5], and consequently it seems likely that adaptation of *Pseudomonas* species to different lifestyles might, in part, rely on the possession of different arrays of ECF sigma factors. A key area of future research in *P. syringae* will therefore be the elucidation of the ECF sigma factors implicated in the adaptation to a phytopathogenic lifestyle and in the association with plant hosts. In particular, the identification of PSYR0892 as a *P. syringae*-specific ECF sigma factor is a significant advance and raises many additional questions to be addressed in the laboratory about the function, regulatory network and signal-transduction mechanism of this unique ECF sigma factor.

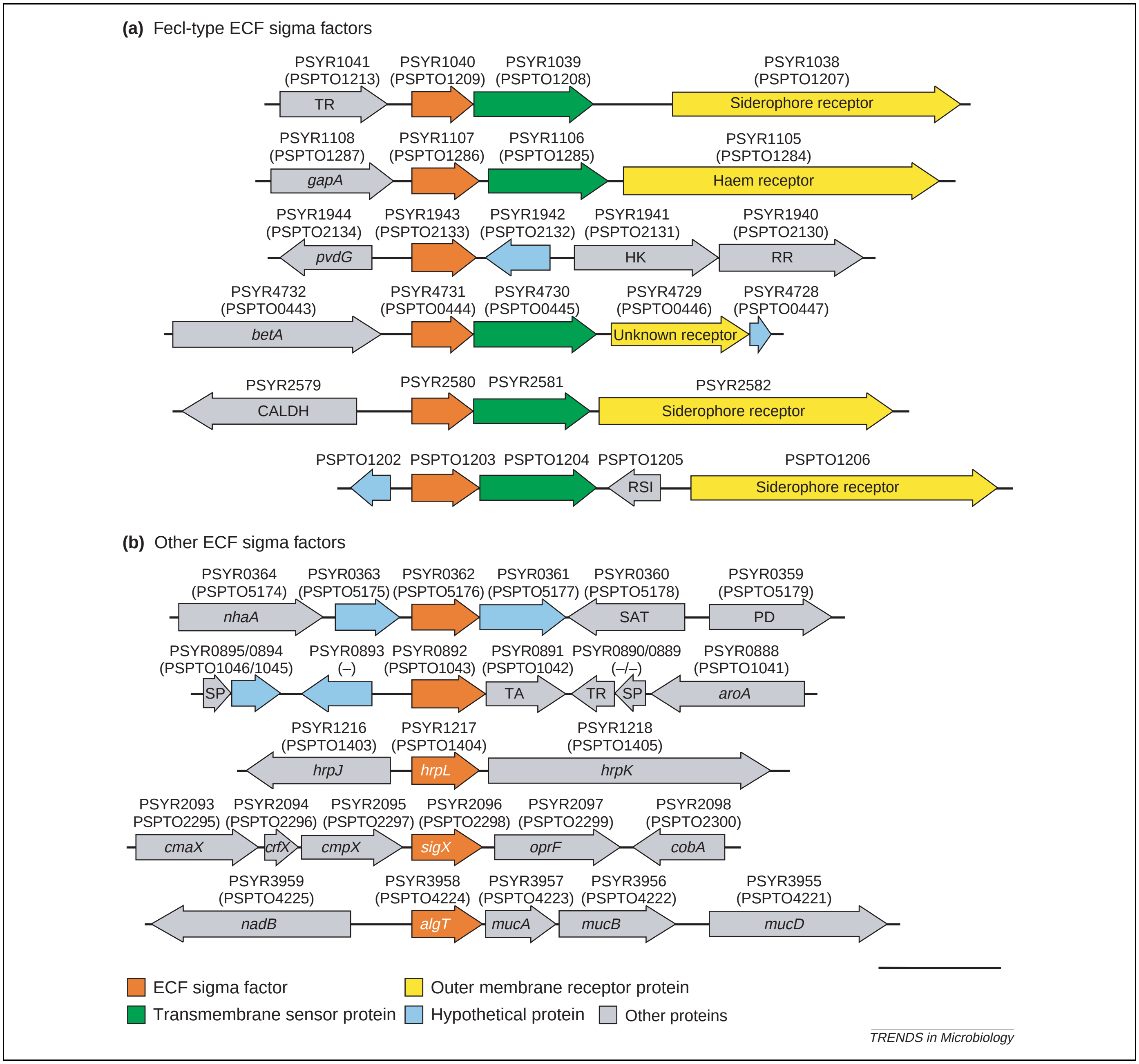

Figure 1. ECF sigma factor genes in *Pseudomonas syringae* pv. *syringae* B728a (PSYR) and pv. *tomato* DC3000 (PSPTO). **(a)** Genomic context of the FecI-type ECF sigma factor genes. **(b)** Genomic context of ECF sigma factor genes involved in the regulation of other systems. Genes are coloured according to the functional category of their encoded proteins. Scale bar = 1 kb. Abbreviations: CALDH, coniferyl aldehyde dehydrogenase; HK, histidine kinase; PD, pyridoxal phosphate-dependent deaminase; RR, response regulator; RSI, ribosomal subunit interface protein; SAT, serine *O*-acetyltransferase; SP, SpoVT/AbrB-like protein; TA, transcriptional activator; TR, transcriptional regulator.

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