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Phenylalanine induces *Burkholderia cenocepacia* phenylacetic acid catabolism through degradation to phenylacetyl-CoA in synthetic cystic fibrosis sputum medium

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ABSTRACT

Synthetic cystic fibrosis sputum medium (SCFM) is rich in amino acids and supports robust growth of *Burkholderia cenocepacia,* a member of the *Burkholderia cepacia* complex (Bcc). Previous work demonstrated that *B. cenocepacia* phenylacetic acid (PA) catabolic genes are up-regulated during growth in SCFM and are required for full virulence in a *Caenorhabditis elegans* host model. In this work, we investigated the role of phenylalanine, one of the aromatic amino acids present in SCFM, as an inducer of the PA catabolic pathway. Phenylalanine degradation intermediates were used as sole carbon sources for growth and gene reporter experiments. In addition to phenylalanine and PA, phenylethylamine, phenylpyruvate, and 2-phenylacetamide were usable as sole carbon sources by wild type *B. cenocepacia* K56-2, but not by a PA catabolism-defective mutant. EMSA analysis showed that the binding of PaaR, the negative regulator protein of *B. cenocepacia* PA catabolism, to PA regulatory DNA could only be relieved by phenylacetyl-Coenzyme A (PA-CoA), but not by any of the putative phenylalanine degradation intermediates. Taken together, our results show that in *B. cenocepacia*, phenylalanine is catabolized to PA and induces PA catabolism through PA activation to PA-CoA. Thus, PaaR shares the same inducer with PaaX, the regulator of PA catabolism in *Escherichia coli*, despite belonging to a different protein family.

1. Introduction

The *Burkholderia cepacia* complex (Bcc) comprises a group of at least fifteen taxonomically related species of extremely versatile Gram-negative bacteria [1,2]. Initially exploited for biocontrol and bioremediation, Bcc strains are now well known for their capacity to cause infections in patients with the genetic disease cystic fibrosis (CF) [3–5]. Bcc has evolved large genomes that allow them to deal with a variety of nutrient sources, predation, and competition. The three chromosomes of *B. cenocepacia*, one of the most common Bcc species found in CF patients [6], encode a broad array of catabolic functions, many of them seemingly redundant. Yet, the contribution of these metabolic capacities to *B. cenocepacia*'s ability to colonize and grow in its host is unclear.

The particular environment of the CF lung may influence the first events leading to bacterial colonization and growth. The CF airways are characterized by impaired ion transport across the lung epithelia, poor hydration [7], and a significant increase in sputum amino acid levels [8]. Recent progress in mimicking the nutritional components of CF sputum in the laboratory, through the use of a synthetic CF sputum medium (SCFM) [9], has shown that the nutrient composition of CF sputum induces a transcriptional response in *Pseudomonas aeruginosa* [9] and *B. cenocepacia* [10]. In particular, the global gene expression profile of *B. cenocepacia* during growth in SCFM showed that several genes encoding phenylacetic acid (PA) degradation enzymes [11–13] were highly induced [10]. Although the physiological significance of such activation remains elusive, a link between PA degradation and pathogenicity is emerging. Our laboratory recently demonstrated that interruption of different steps of the *B. cenocepacia* PA catabolic pathway produces diverse pathogenic phenotypes [14], and that the promoter of *B. cenocepacia paaA* gene is activated during growth in SCFM and in the presence of phenylalanine, one of the aromatic amino acids of SCFM [15]. In this work, we investigated the role of phenylalanine, as an inducer of the PA catabolic pathway. We show that phenylalanine, and putative intermediates of

Abbreviations: SCFM, synthetic cystic fibrosis sputum medium; PA, phenylacetic acid; PA-CoA, phenylacetyl-Coenzyme A; Bcc, *Burkholderia cepacia* complex; CF, cystic fibrosis; EMSA, electrophoretic mobility shift assay.

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phenylalanine catabolism, mediate activation of PA catabolism through degradation to PA-CoA, which, in turn, binds to the TetR-like negative regulator, PaaR [15], releasing the interaction between the regulatory protein and promoters.

2. Results

2.1. Phenylalanine activates PA catabolism reporter systems during growth in SCFM

The PA catabolic pathway is a central route through which the catabolism of many aromatic compounds, such as styrene, phenylethylamine and poly-hydroxyphenyl alkanoates converge, and are directed to the TCA cycle [12,16]. In *Pseudomonas* sp. strain Y2, styrene degradation and PA degradation are co-regulated [17,18] through the action of PaaX, a negative regulator of the GntR family [19,20]. Because the only aromatic compounds of the SCFM are the amino acids phenylalanine, tyrosine, and tryptophan, we sought to investigate the possible co-regulation of aromatic amino acid degradation and PA catabolism. To find out if the aromatic amino acids present in SCFM activated the expression of the PA catabolic pathway, we conducted gene reporter analyses with the translational reporter plasmids pJH6, pJH7 and pJH8 [15], that contain the enhanced green fluorescent protein gene (*eGFP*) [21] under control of P_{paaA}, P_{paaH} and P_{paaZ}, the promoters of *paaA*, *paaH* and *paaZ*, respectively. When bacteria were grown in MOPS minimal medium containing glycerol plus each of the aromatic amino acids at the same concentration found in SCFM (Fig. 1) phenylalanine was capable of activating the *paaA*, *paaH* and *paaZ* reporter systems. As previously noted [15], the *paaA* reporter strain showed the strongest levels of relative fluorescence (Fig. 1 top right panel), while eGFP expression driven by P_{paaH} and P_{paaZ} was lower (Fig. 1 bottom left and right panels, respectively). Relative fluorescence of the reporter strains grown in the presence of tyrosine or tryptophan was comparable to the one of strains grown with glycerol only, and to background fluorescence of *B. cenocepacia* K56-2 cells (Fig. 3 top left panel). Only the *paaA* reporter strain showed an increase in relative fluorescence in the presence of tyrosine (Fig. 1 top right panel) although this activation was negligible in comparison with the strongest signal due to phenylalanine. Relative fluorescence of the reporter strains grown in the presence of tryptophan was comparable to that of the strains grown with glycerol only, and to background fluorescence of *B. cenocepacia* K56-2 cells (Fig. 3 top left panel).

To further confirm that phenylalanine was the major inducer of PA catabolism during growth in SCFM, the reporter strains were grown in phenylalanine-depleted-SCFM and their fluorescence compared with

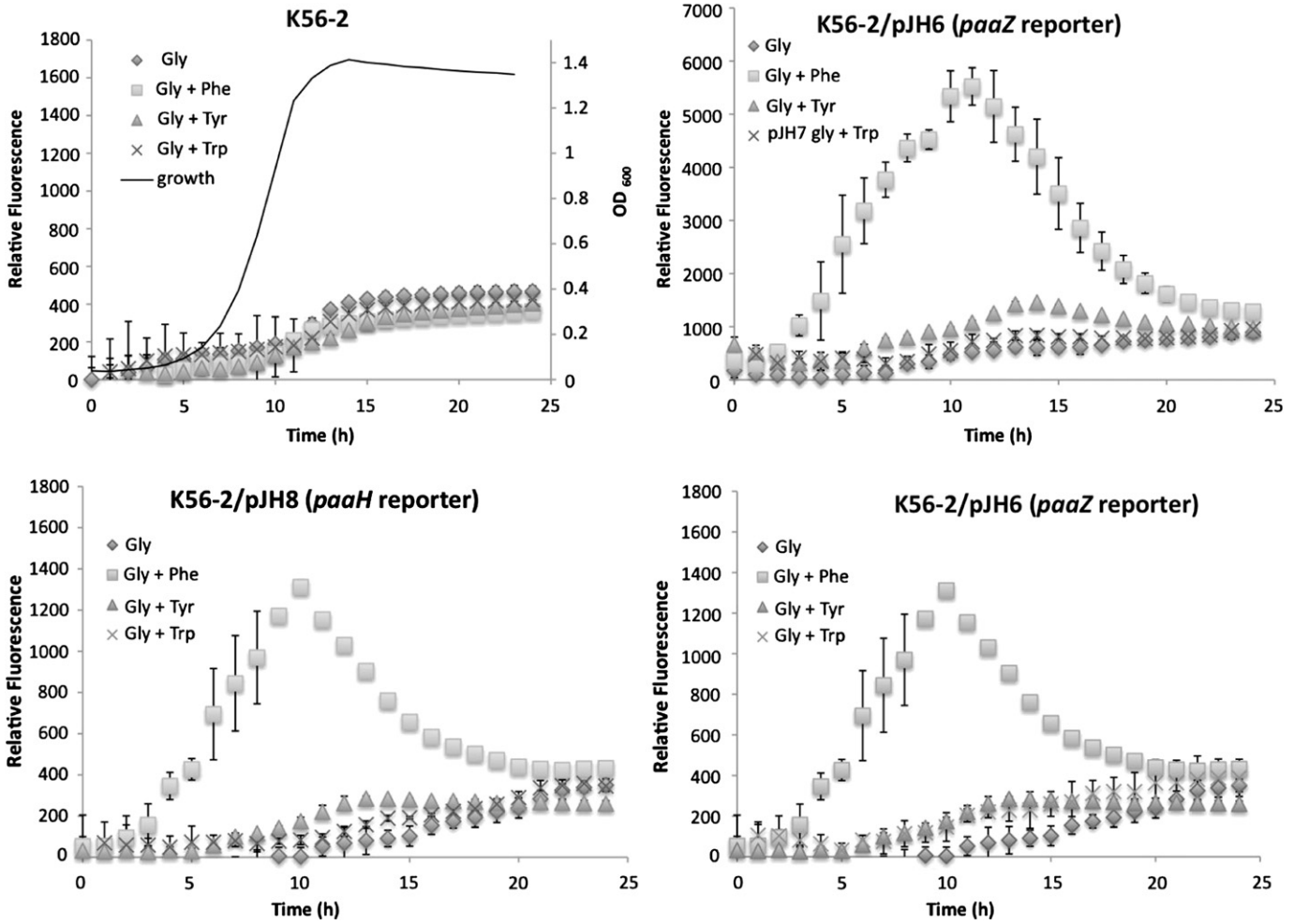

Fig. 1. Phenylalanine activates PA catabolic gene promoters. *B. cenocepacia* K56-2 wild type P_{paaA}, P_{paaZ} and P_{paaH} reporter strains were inoculated in 96-well plates in MOPS minimal medium containing 10 mM glycerol and each of the aromatic amino acids phenylalanine, tyrosine or tryptophan at the same concentrations present in SCFM. Plates were incubated in a Biotek Synergy 2 plate reader at 37 °C with shaking and optical density (OD_{600}) and fluorescence were automatically recorded every hour. Top left panel, a representative growth curve of *B. cenocepacia* K56-2 in MOPS 10 mM glycerol is shown. Top right panel, note the different scale of the *y*-axis. Error bars represent the standard deviation of three independent experiments.