Enzyme and Microbial Technology 43 (2008) 1–12

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Review

Paradigm in biodegradation using *Pseudomonas putida*—A review of proteomics studies

Kai-Chee Loh*, Bin Cao

Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore

ARTICLE INFO

Article history:
Received 29 November 2007
Received in revised form 20 February 2008
Accepted 4 March 2008

Keywords:
Proteomics
Pseudomonas putida
Biodegradation
Catabolic pathway
Physiological response

ABSTRACT

Pseudomonas putida has been extensively studied as a paradigm in environmental biotechnology due to its capabilities in catabolizing various aromatic compounds. In addition to the fate of these aromatic compounds, the physiological status of the bacterial cells involved is another important aspect in biodegradation processes. In recent years, proteomics that deals with the high-throughput analysis of gene products directly at the protein level has been shown as a powerful tool to explore bacterial physiology in biodegradation processes. Through proteomics approaches, the understanding of global metabolic and regulatory alterations in response to various environmental stimuli or phenotypic changes after metabolic engineering has been facilitated. In this review, we summarize the proteomics tools in environmental applications and the proteomics studies of *P. putida* in bioremediation. The technological and methodological advances in *P. putida* proteome research and the physiological responses to different environmental conditions revealed by proteomics as well as *P. putida* catabolic pathway elucidation through proteomics are discussed.

Contents

1. Introduction

Ubiquitous bacteria Pseudomonads belong to the *gamma* subclass of the Proteobacteria. They are able to colonize various environments, including soil, water and plant rhizosphere and play important roles in metabolic activities in the environment. Due to their strong capabilities in degradation and biotransformation of biogenic and xenobiotic pollutants, Pseudomonads have great potential for different biotechnological applications, particularly in the areas of bioremediation and biocatalysis.

Abbreviations: BLAST, basic local alignment search tool; CBB, Coomassie Brilliant Blue; CF, chromatofocusing; 2-DE, two-dimensional gel electrophoresis; DIGE, fluorescence difference gel electrophoresis; ESI, electrospray ionization; ICAT, isotope-coded affinity tag; IEF, isoelectric focusing; IPG, immobilized pH gradient; iTRAQ, isobaric tags for relative and absolute quantification; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; *m*/*z*, mass-to-charge ratio; NCBI, National Center for Biotechnology Information; pI, isoelectric point; PMF, peptide mass fingerprinting; PSD, post-source decay; PTM, post-translational modification; RP, reverse phase; ToF, time-of-flight.

* Corresponding author. Tel.: +65 6516 2174; fax: +65 6779 1936.
E-mail address: chelohkc@nus.edu.sg (K.-C. Loh).

0141-0229/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2008.03.004

Table 1
Current status of genome-sequencing projects for *Pseudomonas* species

Organism	Size (Mbp)	GenBank
Genome-sequencing completed		
Pseudomonas aeruginosa PA7	6.60	CP000744.1
P. aeruginosa PAO1	6.26	AE004091.2
P. aeruginosa UCBPP-PA14	6.54	CP000438.1
P. entomophila L48	5.89	CT573326.1
Pseudomonas fluorescens Pf-5	7.07	CP000076.1
P. fluorescens PfO-1	6.44	CP000094.1
Pseudomonas mendocina ymp	5.10	CP000680.1
Pseudomonas putida F1	6.00	CP000712.1
P. putida GB-1	6.10	CP000926.1
P. putida KT2440	6.18	AE015451.1
Pseudomonas stutzeri A1501	4.60	CP000304.1
Pseudomonas syringae pv. Phaseolicola 1448A	6.11	CP000058.1
P. syringae pv. Syringae B728a	6.09	CP000075.1
P. syringae pv. Tomato DC3000	6.54	AE016853.1

P. putida plasmid	Size (bp)	Number of encoded proteins	GenBank
Genome-sequencing completed			
pPP81	2,534	4	AJ289784
pWW0	116,580	139	AJ344068
pDTG1	83,042	90	AF491307
pYQ39	2,297	3	AF273219
NAH7	82,232	84	AB237655
pWW53	107,929	86	AB238971

Organism	Institution
Genome-sequencing in progress	
P. aeruginosa 2192	Broad Institute
P. aeruginosa C3719	Broad Institute
P. aeruginosa OPPA8	Allegheny-Singer Research Institute
P. aeruginosa PACS2	University of Washington
P. aeruginosa PKS6	Allegheny-Singer Research Institute
P. fluorescens SBW25	Sanger Institute
P. pseudoalcaligenes MTCC5210	Central Leather Research Institute
P. putida PRS1	TIGR
P. putida W619	DOE Joint Genome Institute
P. syringae pv. tomato T1	The Sainsbury Laboratory

Pseudomonas putida KT2440, one of the best-characterized Pseudomonads, is the plasmid-free derivative of a toluene-degrading bacterium designated *P. putida* mt-2. It was also the first host–vector biosafety system for gene cloning in Gram-negative soil bacteria. Although *P. putida* KT2440 has been extensively used as a host for cloning and gene expression, this strain is mainly known for its ability to catabolize aromatic compounds and has served as a model organism for many biodegradation studies involving recalcitrant aromatic compounds. The sequencing of the genomes for a number of *Pseudomonas* species, including *P. putida* KT2440 and some catabolic plasmids, such as pWW0 from *P. putida* mt-2, have recently been completed. As indicated in Table 1, many more of these genome-sequencing projects for several other *Pseudomonas* species or strains are still in progress (www.ncbi.nih.gov).

With the availability of the genome data, functional genomics analysis of *P. putida* has become possible. *P. putida* has been characterized on the genome-wide scale in terms of its transcriptome and proteome using DNA microarray, two-dimensional gel electrophoresis (2-DE) or liquid chromatography (LC) coupled with mass spectrometry (MS) and bioinformatics. The information obtained has facilitated the understanding of global metabolic and regulatory alterations in response to various environmental conditions or phenotypic changes after metabolic engineering. In this review, the technological and methodological advances in *P. putida* proteome research are discussed in detail. In addition, physiological responses to different environmental conditions revealed by proteome analysis and applications of proteomics methods to elucidate catabolic pathways are also discussed.

2. Proteomics methodologies

2.1. Overview

Proteomics is one of the newest emerging technologies in functional genomics and arguably the most daunting omics approach. In using proteomics, one is not only interested in identifying and quantifying the differentially expressed proteins, but also in determining their localization, modifications, interactions, activities and importantly, their functions. The first proteome analysis was implemented by the introduction of 2-DE in the 1970s [1]. 2-DE enabled researchers to resolve protein mixtures based on isoelectric focusing (IEF) in the first dimension and molecular weight (M_r) based on sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension, followed by visualization and quantification of the protein spots in the gels. Although microsequencing techniques had occasionally been used to identify some proteins after separation, 2-DE was essentially restricted to being a descriptive technology. The landmark breakthrough in proteomics was the invention of two soft ionization methods for biomolecules in mass spectrometric analysis, namely matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). In these technologies, proteins or peptides are ionized using MALDI or ESI, which are then analyzed by a time-of-flight (ToF) or ion trap mass analyzer to generate the peptide mass fingerprints (PMF). In some cases, two of these analyzers could be connected in tandem to generate m/z profiles for the fragment ions, from which valuable sequence information could be obtained. The MALDI and ESI technologies and the improvements made in mass spectrometry instrumental accuracy, sensitivity and automation now render proteomics analysis amenable to high-throughput approaches. A summary of some of the representative gel-based and gel-free proteomics approaches are schematically illustrated in Fig. 1.

2.2. Gel-based proteomics

With a history of around 30 years now, 2-DE remains the most widely used proteomics tool for resolving protein mixtures from cells, tissues, and biofluids. In the first proteome study, O'Farrell [1] was able to resolve and detect about 1100 proteins from lysed *E. coli* cells on a single 2D map. Depending on the gel size and pH gradient used, 2-DE has been demonstrated to have a resolving capacity of more than 5000 proteins simultaneously in a single gel at less than 1 ng of protein *per* gel spot.

Immobilized pH gradient techniques for IEF separation is a major development in 2-DE history. This greatly improved gel reproducibility, rendering quantitative comparison between gels feasible [2,3]. Furthermore, the introduction of zoom gels with narrow-range pH gradient enhanced the capability and detection sensitivity of 2-DE. A study of the *E. coli* proteome demonstrated that more than 70% of the entire proteome could be displayed using six overlapping narrow pI range strips (pH 4–5, 4.5–5.5, 5–6, 5.5–6.7, 6–9 and 6–11); however, only around 40% could be revealed in the wide pI range (pH 4–10) analysis [4]. Another strategy to enhance the capability of 2D gels is to employ sample pre-fractionation methods, such as sequential extraction with increasingly stronger solubilization solutions and preparative IEF separation. In Molloy et al. [5], *E. coli* proteins were extracted sequentially with Tris-base, urea/CHAPS/DTT and then a combination of urea, thiourea and zwitterionic surfactants, where even membrane proteins have been identified.

Coomassie Brilliant Blue (CBB) staining and silver staining are the predominant protein detection methods in 2-DE. However, their low sensitivity or limited linear dynamic range may impede accurate quantitative analysis. Recently, the application of radioac-