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PAMDB: a comprehensive *Pseudomonas aeruginosa* metabolome database

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ABSTRACT

The *Pseudomonas aeruginosa* Metabolome Database (PAMDB, http://pseudomonas.umaryland.edu) is a searchable, richly annotated metabolite database specific to *P. aeruginosa*. *P. aeruginosa* is a soil organism and significant opportunistic pathogen that adapts to its environment through a versatile energy metabolism network. Furthermore, *P. aeruginosa* is a model organism for the study of biofilm formation, quorum sensing, and bioremediation processes, each of which are dependent on unique pathways and metabolites. The PAMDB is modelled on the *Escherichia coli* (ECMDB), yeast (YMDB) and human (HMDB) metabolome databases and contains >4370 metabolites and 938 pathways with links to over 1260 genes and proteins. The database information was compiled from electronic databases, journal articles and mass spectrometry (MS) metabolomic data obtained in our laboratories. For each metabolite entered, we provide detailed compound descriptions, names and synonyms, structural and physiochemical information, nuclear magnetic resonance (NMR) and MS spectra, enzymes and pathway information, as well as gene and protein sequences. The database allows extensive searching via chemical names, structure and molecular weight, together with gene, protein and pathway relationships. The PAMBD and its future iterations will provide a valuable resource to biologists, natural product chemists and clinicians in identifying active compounds, potential biomarkers and clinical diagnostics.

INTRODUCTION

Metabolomics is the system wide analysis of small molecule metabolites generated as a result of a specific cellular or physiological condition (1). Together with transcriptomics and proteomics, it provides a comprehensive, systems biology analysis of global biological processes at the cellular level (2). The significant expansion in the number of organism or discipline specific metabolomic databases in the previous decade is a result of the increasing importance of metabolite identification in the pathogenesis of disease, tools for biomarker discovery, and in the biotechnology and bioremediation industry. These include the pioneering Human Metabolome Database (HMDB) (3), the Yeast Metabolome Database (YMDB) (4), the *Escherichia coli* Metabolome Database (ECMDB) (5), the Small Molecule Pathway Database (SMPDB) (6) and the Toxin/Toxin-Target Database (T3DB) to name a few (7).

It has become increasingly evident that organism-specific databases are a necessity to advance the field of metabolomics given that distinct species have very distinct metabolomes. Furthermore, metabolomic studies provide considerably more phenotypic information than the transcriptomic or proteomic studies alone. This is particularly important in the case of *Pseudomonas aeruginosa,* which has an extensive repertoire of energy metabolism pathways and secreted secondary metabolites, including quorum sensing (QS) molecules, that represents a unique and chemically diverse metabolome (8). Recent NMR and GC–MS analysis of the metabolites in *P. aeruginosa* and a mutant unable to make QS signaling molecules revealed that the QS system plays a central role in regulating carbohydrate, polyamine and lipid metabolism (9). *Pseudomonas aeruginosa* is also a model organism for biofilm formation and iron acquisition, both of which are dependent upon unique metabolites produced by this organism (10–12). Many of these metabolites also have broad applicability to biotechnology: rhamnolipids have been proposed as bioremediation agents (13,14), and 2-heptyl-4-hydroxyquinolone *N*-

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oxide (HQNO) has been used to probe cytochrome function from humans to bacteria (15,16). Moreover, *P. aeruginosa* metabolites are at the center of the growing field of polymicrobial interactions involving bacterial (17–20) and fungal organisms (21,22). As these fields develop, *P. aeruginosa* metabolites will increasingly permeate the literature, requiring a comprehensive resource for understanding the genetic and biochemical basis for their production.

Pseudomonas aeruginosa is ubiquitous in the environment, growing both planktonically and in polysaccharide-encased biofilms. Because it is so adaptable, it can occupy extremely diverse ecological niches and cause a variety of infections in the human host (23). Critical to its adaptability is the plasticity of both its metabolome and its genome. Unlike most other bacteria where the vast majority (>90%) of genes are 'core', just 70% of the genes in *P. aeruginosa* are considered to be 'core' while the other 30% are considered 'accessory' (24–26). The significance of *P. aeruginosa* in clinical infection and the rise of multi-drug resistance has led to numerous multi-omic studies that have provided significant information on the metabolic adaptability of *P. aeruginosa* at the gene and protein level (16,27–32). Extensive *P. aeruginosa* gene and protein data is available in several excellent resources including the *Pseudomonas* Genome Database that evolved from the *Pseudomonas* Community Annotation Project (PseudoCAP), which now has over 200 complete *Pseudomonas* genomes (33), PseudoCyc, a pathway-genome database with 121 pathways and >800 enzymatic reactions (34), and the SYSTOMONAS database, a resource for systems biology analysis of *Pseudomonas* (35). These resources are in addition to the information available through the widely used KEGG database (36,37).

The metabolic adaptability of *P. aeruginosa* has been well studied and there exists an extensive literature on secreted quorum sensing molecules. Unfortunately, the integration of this important metabolomic information along with other unique *P. aeruginosa* metabolites into a searchable database is lacking. Recent metabolome studies of *P. aeruginosa* by our group and others have identified extensive gaps in all of the above-mentioned databases with regard to the level of *P. aeruginosa* metabolite coverage, metabolite descriptions, metabolic pathways, referential spectra, and many other essential pieces of information needed to conduct or interpret *P. aeruginosa* metabolomic studies. To begin addressing this deficiency, we have used the database development and annotation framework developed for the *E. coli* (ECMDB) (38) and yeast (YMDB) (39) metabolome databases together with in-house developed bioinformatics toolkits to generate a *P. aeruginosa* metabolomic database (PAMDB) for the reference strain PAO1. This first version of PAMDB captures the needs for those studying the biochemistry of *P. aeruginosa* by gathering the physiochemical properties, reference NMR and MS spectra, sub-cellular localization, pathways, enzyme reactions, and links to PseudoCAP indexed sequence information for a given metabolite in a central database. This richly annotated database provides the first *P. aeruginosa* specific metabolite resource comprising a cross-disciplinary searchable platform for microbiologists, biochemists, and medicinal chemists interested in compounds as potential biomarkers, clinical diagnostics, and the discovery of novel therapeutic or bioremediation strategies.

DATABASE DESCRIPTION

PAMDB is a comprehensive, robustly-annotated, and highly integrative metabolome database for *P. aeruginosa* researchers that provides analytical as well as molecular information on metabolites and their biological and chemical properties. It includes detailed physical and chemical information on both general and *P. aeruginosa* specific metabolites as well as their related proteins (e.g. enzymes and transporters), reactions and pathways (Figure 1). The information is collected and assembled from a variety of reliable and open data sources, such as HMDB (40), ECMDB (5), KEGG (37), PubChem (41), Chemical Entities of Biological Interest (ChEBI) (42), ChemSpider (43), the Pseudomonas Genome database (33), PseudoCyc (34,44) and BioCyc (44). In addition, a significant amount of new information has been independently compiled through extensive literature research and via biochemical/metabolomic experiments conducted in our own laboratories (such as the recently identified congeners of 2-alkyl-4(1*H*)-quinolones and the heme derived metabolites biliverdin IXα (BVIXα), BVIXβ and BVIXδ (45,46). The data in PAMDB has been carefully assessed, populated, and validated through the efforts of bioinformaticians, biochemists, analytical chemists and microbiologists within our groups.

PAMDB currently contains >4370 *P. aeruginosa* metabolites, and >9620 related reactions, which involve ∼1267 proteins including enzymes and transporters. These proteins, reactions and metabolites are described in over 939 associated pathways. Of the compounds listed in the database >470 are linked to their respective $^{1}H/^{13}C$ NMR spectra and >1930 to reference MS/MS spectra. All of the entries in the database are specific to *P. aeruginosa* PAO1. A detailed comparison with two other widely available *P. aeruginosa* resources: the PseudoCyC (34) pathway genome database, and the pathway/genome databases collection, BioCyc (44), is provided in Table 1. As can be seen from this comparison, PAMDB provides the most extensive central resource for *P. aeruginosa* metabolites, metabolism, biochemistry, pathways and reference spectral information.

PAMDB integrates metabolites, their corresponding reactions, proteins involved and the complete metabolic pathways in a single database and is constructed similarly to the long-established metabolome database series developed by Wishart and colleagues (e.g. HMDB, YMDB and ECMDB). The database is organized by categories: metabolites, proteins, reactions and pathways. Each entry in one category is seamlessly linked to any relevant entries in the other categories by hyperlinks, which provides the user a global picture of the biological context of a specific metabolite including reaction mechanisms, specific enzymes, transporters and non-redundant pathways. The database can be readily browsed by metabolite, protein, reaction, or pathway from a drop-down link on the navigation bar. Each browsing view provides the data in a series of summaries that contain navigation links to more detailed information on the metabolite, protein, reaction or pathway.

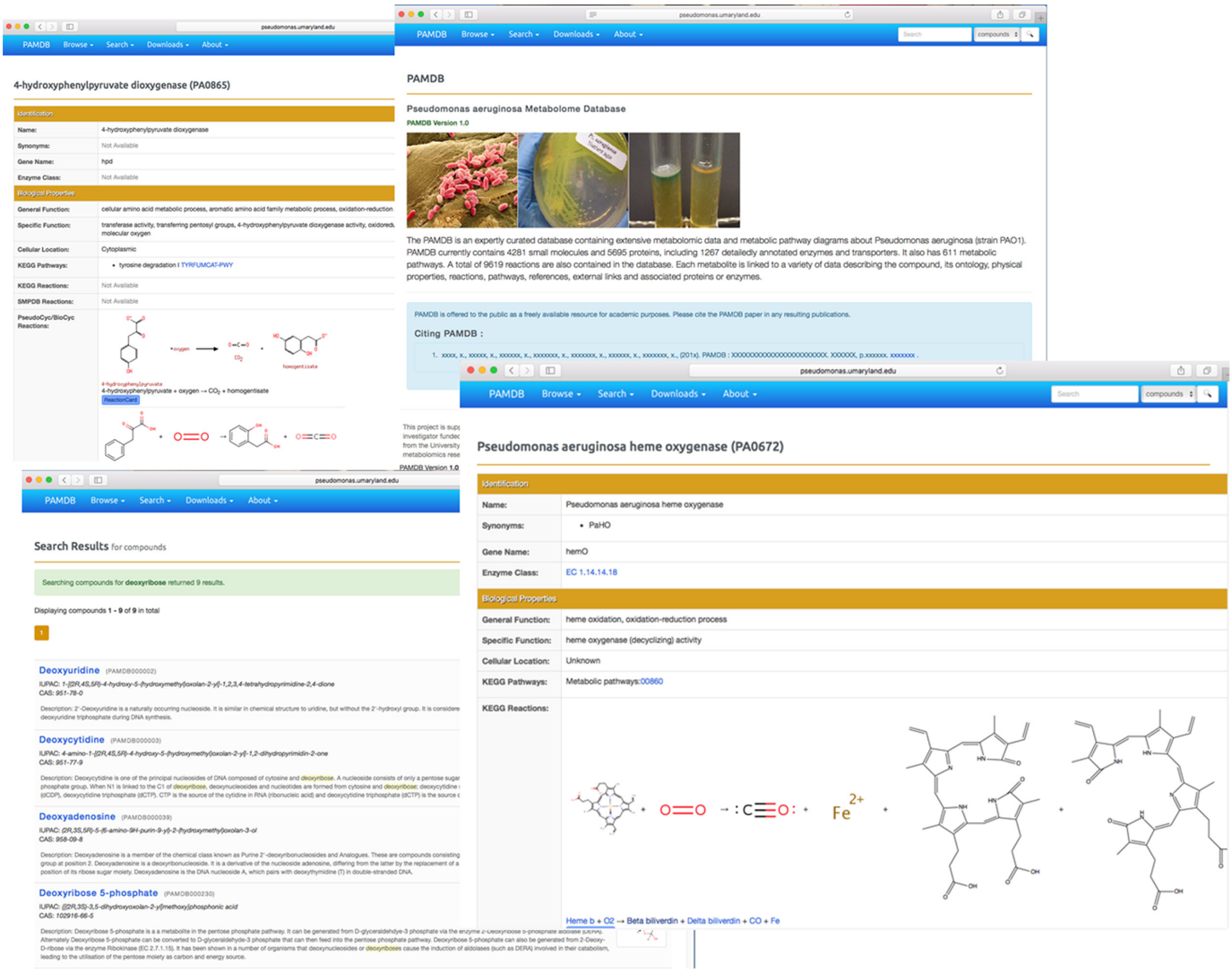

Figure 1. A screenshot compilation of several PAMDB search and data display tools for metabolites, proteins and reactions. Clockwise from top left. (1) Metabolite information page; (2) PAMDB front-page; (3) Enzyme information page; (4) Search result page. Each page has a navigation bar with a full text search box providing intuitive browsing capabilities.

Table 1. Comparison of the content and coverage of the available *P. aeruginosa* specific databases

Database feature	*PAMDB*	*PseudoCyc*	*BioCyc*	*KEGG*
Number of metabolites	4375	812	1285	3118
Related proteins (including enzymes and transporters)	1453	979	1146	1762
Metabolic reactions	9619	976	1725	~2500
Metabolic pathways	938	198	334	121
Number of MS spectra	1930	No	No	No
Number of NMR spectra	470	No	No	No
Number of external database links	Up to 12	Up to 8	Up to 8	Up to 12
Structure search	Yes	No	No	Yes
Graph search	Yes	No	No	No
MS spectra search	Yes	No	No	No
Sequence search	Yes	Yes	Yes	Yes

* As of 21 September 2017. For BioCyc, the *Pseudomonas aeruginosa* PAO1 database is compared. For KEGG, *Pseudomonas aeruginosa* PAO1 pathways associated proteins and compounds are compared, and the reaction number is estimated based on the global KEGG database statistics.

In addition to the browsing function, the navigation bar has a search tool allowing metabolites to be searched by their chemical formula or molecular weight. A text search function is also included to allow a flexible keyword search in all categories (i.e. metabolite protein, reaction or pathway). Similar to the previously mentioned databases (HMDB, ECMDB, YMDB), PAMDB employs a well-tested, user-friendly interface allowing one to readily extract information on specific metabolites including chemical names, chemical taxonomy, proteins, enzyme reactions and all available MS or NMR spectra.

PAMDB also features a one-click search for finding molecules sharing similar structures. This feature enables users to quickly find related compounds as well as metabolite derivatives. This function is implemented with a path-based Daylight-like fingerprint algorithm from (http://www.daylight.com/dayhtml/doc/theory/index.pdf) the Open Babel chemical toolbox (47), which indexes small molecule fragments based on linear segments of up to 7 atoms. Pairwise similarities between a query molecule and all the molecules in PAMDB are calculated and sorted by Tanimoto coefficient. Users can use it to quickly determine what metabolites from the PAO1 metabolome are related to a compound of their interest. Meanwhile, users can also draw a molecular structure or paste a SMILES string of a metabolite of interest into the JSME molecular editor (48) embedded in PAMDB, and the structure similarity will be scored and ranked by the Open Babel Daylight-like fingerprinting algorithm described above.

A BLAST (49) sequence search function is also provided in PAMDB to allow users to find enzymes or transporters of interest by simply providing a sequence in FASTA (50) format. Metabolically important enzymes and transporters from other organisms can be straightforwardly mapped to the *P. aeruginosa* genome to reveal important homologs in *P. aeruginosa* related to a metabolite or involved in a biological process.

Moreover, to facilitate analysis of a user's mass spectrometry data, PAMDB provides mass spectra search functions, including tandem mass spectra analysis, which allows users to rapidly search and identify unknown biological compounds with their own peak list using a peak matching algorithm described by Dworzanski *et al.* (51).

SYSTEM IMPLEMENTATION

The design of PAMDB followed the architecture of Model–view–controller (MVC), in which internal data logics are separated from user input and data presentation. PAMDB is implemented as a natively compiled C++ FastCGI frontend attached to a structured query language (SQL) driven relational database. The raw information stored in the database is dynamically extracted from the database and rendered into web pages by PAMDB hypertext markup language (HTML) interface responder. Up to 1000 most recent queries are dynamically cached in memory for fast content reloading. PAMDB is hosted on a server equipped with an Intel Xeon 3 GHz quad-core CPU and 4GB of RAM.

CURATION AND QUALITY ASSURANCE

Names, formulas and identifiers of *P. aeruginosa* metabolites were first extracted from published manuscripts, textbooks and high quality open biological compound libraries. Annotations for metabolites already available from existing high quality data sources such as HMDB, ECMDB and ChEBI, data were selectively extracted and imported into PAMDB using in-house developed scripts. If information on a metabolite was not available in any data source, this metabolite was manually entered into PAMDB by a curator using information manually compiled from various resources such as the literature, raw experimental data and cheminformatic predictions. All manually entered metabolite annotations were later reviewed by a senior researcher with expertise in the field.

In addition to the literature-derived and database-derived metabolites, PAMDB has also been populated with metabolites identified during the course of our studies on iron and heme metabolism in *P. aeruginosa*, including the heme metabolites biliverdin IXα (BVIXα), BVIXβ and BVIXδ (46). Additionally, a novel mono-unsaturated 1-hydroxy-2-nonenyl-1,4-dihydroquinolin-4-one (C9:1 QNO) metabolite potentially associated with antimicrobial potency in co-cultures and markers for *P. aeruginosa* infections in cystic fibrosis patients, has been included in the PAMDB in addition to previously characterized 2-alkyl-4-quinoline (AQ) metabolites (45,52,53).

To ensure the quality of all imported data, rule based filters were applied to validate entries during data import and post-filter out potential common errors preventively, and errors found during manual checks. In addition, random inspections on entries were performed by senior curators of our joint PAMDB editorial board, comprising experts from relevant fields within biochemistry, microbial genetics and analytical chemistry.

CONCLUSION

In summary, PAMDB is a richly annotated, comprehensive metabolomics database that brings together chemical, physical and biological data for over 4370 metabolites found in *P. aeruginosa*. In comparison to other *P. aeruginosa* specific databases, PAMDB offers up to four times greater metabolite coverage, five times more biochemical reactions, and three times more biochemical pathways. It also provides a large number of query functions that support searches via text, chemical structure, molecular weight, MS and NMR peak lists, and protein sequences. Each metabolite entry in PAMDB includes detailed compound descriptions, synonyms, chemical taxonomies, physicochemical data as well as extensive hyperlinks and referential data. In addition to providing a reference for scientists in the area of microbial metabolomics, we predict PAMDB will garner widespread interest from researchers in the fields of bioremediation, clinical diagnostics and biomarkers, natural products and medicinal chemistry.

PAMDB has been developed to be a discovery platform for not only the *Pseudomonas* community but also for microbiologists, biochemists, and medicinal chemists interested in obtaining new insights into microbial metabolism,

biofilm formation, biomedicine and in bioremediation. As resources permit this database will continue to be updated as new compounds and pathways are discovered and become available through publication and dissemination.

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