Large scale function-based genome prospecting for industrial traits applied to 1,3-propanediol production.

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Due to the success of next-generation sequencing, there has been a vast build-up of microbial genomes in the public repositories. FAIR genome prospecting of this huge genomic potential for biotechnological benefitting, require new efficient and flexible methods. In this study, Semantic Web technologies are applied to develop a function-based genome mining approach that follows a knowledge and discovery in database (KDD) protocol. Focusing on the industrial important trait of 1,3-propanediol (1,3-PD) production 187 new candidate species were identified. Furthermore, the genetic architecture of the particular trait was resolved, and persistent domains identified.

genome prospecting | trait, big data | Semantic Web | Knowledge and Discovery in Databases | 1,3-propanediol

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Introduction

Next generation sequencing (NGS) technologies have turned the publicly available genome repositories into data-rich scientific resources that currently contain structural and functional genome information of hundred of thousands bacterial genomes (1) available for further exploitation for medical, environmental and biotechnological purposes. While the biotechnology field has embraced the use of omics technologies in biotech innovation in multiple ways, functional screening of genomic resources for industrially relevant traits at a large scale remains challenging. NGS data generation has caused manual curation to be outpaced rapidly and currently more than 99% of the functional predictions in UniProt are based on automated computational predictions (2). The accuracy of these computationally inferred functional genome annotations is largely unknown due to inconsistent naming of protein functions, use of annotation pipelines of various quality, over-prediction due to the use of generic, non-standardised, annotation acceptance thresholds (3), and lack of data provenance in general. This results in a degree of interoperability lower than required for efficient function based genome prospecting (4). As the predicted gene and protein sequences, underlying the functional annotations are available in the standardised FASTA format, ‘bottom-up’ approaches that start with a gene or protein sequence in an effort to find a corresponding function in genome(s) of interest are

normally used. However, over larger phylogenetic distances, strategies that use sequence-based clustering algorithms are hampered by accumulation of evolutionary signals, lateral gene transfer, gene fusion/fission events and domain expansions (5). Furthermore, when applied at larger scales, such strategies suffer from high computational time and memory requirements that scale quadratically with the number of genome sequences to be compared (6). A robust function-based genome screening requires a sufficiently high degree of interoperability meaning that functional information can be directly compared on the basis of a pre-established syntactic interoperable genome annotation and that any computational functional prediction is directly linked to its provenance. To accomplish this, we have developed SAPP, a semantic annotation infrastructure supporting FAIR computational genomics (7). SAPP uses the Genome Biology Ontology Language (GBOL) as syntax (8) and automatically predicts, tracks and stores structural and functional genome predictions and associated data- and element-wise provenance in RDF, the W3C standard for representing linked data (9). SAPP uses protein domain architectures to systematically describe protein functions (10). Demonstrating a high level of scalability, this set up has already been successfully used in an integrated analysis of the functional landscape of 432 *Pseudomonas* strains (4). In this study, interoperable genome annotations are used in a systematic function-based in silico screening for bacterial species that potentially can convert the bio-refinery by-product glycerol in 1,3-propanediol (1,3-PD) (11, 12). 1,3-PD is an important precursor of biomaterials and it is currently used as a monomer for novel polyester and biodegradable plastics, such as polytrimethylene terephthalate (11). 1,3-PD is a typical product of anaerobic glycerol fermentation and only a limited number of species, mostly enterobacteria, are known to form it. The first step, dehydration of glycerol to 3-hydroxypropionaldehyde (3-HPA) is mediated by a vitamin B12-dependent glycerol dehydratase although an oxygen sensitive, B12-independent alternative enzyme has also been reported (13). The second step reduces the toxic 3-HPA intermediate to 1,3-PD using the (NADH)+H+ dependent 1,3-propanediol-oxydoreductase (PDOR) regenerating NAD+ (12).

For large scale genome prospecting a collection of 84,308

Fig. 1. Search strategy for 1,3-Propanediol candidate species: (A) Key domains in the main pathway for B12 dependent bio-conversion of glycerol to 1,3-propanediol are indicated in red. Key domains for the alternative B12-independent reductive pathway are indicated in orange. Note the generic iron alcohol domain is present in both the oxidative and reductive branch. (B) Generalised functional based search strategy for traits using SAPP. Genome sequences in standard format are converted and complemented with structural and functional annotation. Pattern recognition strategies are deployed to identify domains of interest (dark blue) and complemented with proximity searches (light blue) to find key domain enriched regions.

publicly available bacterial genomes were loaded in the SAPP semantic framework, systematically structurally and functionally annotated and subsequently mined for candidate 1,3-PD producers using a knowledge discovery in databases approach (KDD) (14). Overall, the systematic analysis increased our knowledge on the genetic architecture of this metabolic trait, in terms of the overall domain composition, domain order, distribution, persistence and essentiality. The approach suggested that, in addition to the some 30 producers reported in literature (table 1), at least 187 other genome sequenced species potentially have this trait.

Results

Development of the genome mining workflow. To cope with the influx of biological input data, we followed the Knowledge Discovery in Databases multi step process model (14), which was adjusted to the specific needs of being able to prospect genome data at a large scale. The KDD process includes multiple steps with the first step being to capture relevant prior knowledge. In the next step, a selection of data is used as a training set to develop a screening procedure followed by data mining at a large scale and finally the evaluation and interpretation of the results.

Incorporation of prior knowledge. In 1,3-PD production, two pathways, an oxidative and reductive pathway, are used in parallel for dissimilation of glycerol. In the oxidative pathway, glycerol is first dehydrogenated to dihydroxyacetone by a NAD⁺-linked glycerol dehydrogenase, and then converted

to dihydroxyacetone phosphate by an ATP-dependent dihydroxyacetone kinase (15). Additionally, two alternative reductive pathways, one dependent on vitamin B12, the other not, are used for regenerating NAD⁺ with 1,3-PD as byproduct. For validation of the search strategy and to gain further insights into the genetics and the domains involved in 1,3-PD formation a list of known 1,3 PD producing strains was obtained for cross-validation. From literature some 30 strains have been reported to produce 1,3-PD (Table 1). For training purposes genome sequences from 13 different strains could be obtained from the EBI-ENA data warehouse (1) as not for all of them genome sequences were available. For *Citrobacter freundii* ATCC8090 two draft genome sequences of comparable quality were available and initially both were added to the training set increasing the total number of genomes.

Data transformation. To obtain a high degree of interoperability the selected genome sequences were *de novo* structurally and functionally annotated using the Semantic annotation Platform with Provenance (SAPP) (7) using Prodigal (16) for gene prediction and InterProScan (17) for protein annotation. Protein domain architectures were used to systematically describe the encoded protein functions (10). Structural and functional genome annotations were subsequently transformed into Linked Data fragments using the Resource Description Framework (RDF) as a data-metadata model (7, 18).

A function-based search strategy for the oxidative branch. The first two enzymes essential for the oxidative

Table 1. Overview of known 1,3-PD producers.

Organism	Genome assembly ID	B12 (in silico prediction)	DAK configuration	1,3-PD (mol/mol)	Acetate (mol/mol)	Reference
<i>Clostridium perfringens</i> str. 13	GCA_000009685.1	Dependent	DAK1/DAK1_2			
<i>Clostridium butyricum</i> E4	GCA_000182605.1	Independent	DAK1/ DAK1_2	0.58	0.03	Petitdémange et al., 1995
<i>Klebsiella michiganensis</i> strain M5al ==oxytoca	GCA_000308735.2	(in-) dependent	DAK1	0.41	0.03	Yang et al., 2007
<i>Citrobacter freundii</i> ATCC 8090	GCA_000312465.1	Dependent	DAK1	0.65	0.22	Barbirato et al., 1998
<i>Citrobacter freundii</i> ATCC 8090	GCA_000734905.1	Dependent	DAK1	0.65	0.22	Barbirato et al., 1998
<i>Clostridium pasteurianum</i> ATCC 6013= DSM 525	GCA_000330945.1	Dependent	DAK1/ DAK1_2	0.5	0.12	Taconi et al., 2009
<i>Halanaerobium saccharolyticum</i> DSM 6643	GCA_000350165.1	Dependent	DAK1/ DAK1_2	0.41	0.26	Kivisto et al., 2012
<i>Clostridium diolis</i> DSM 15410	GCA_000409695.1	Independent	DAK1/ DAK1_2	0.58	0.12	Otte et al., 2009
<i>Klebsiella pneumoniae</i> ATCC 25955	GCA_000409715.1	Dependent	DAK1/	0.65	0.22	Barbirato et al., 1998
<i>Clostridium butyricum</i> VPI3266 = DSM 10702	GCA_000409755.1	Independent	DAK1/ DAK1_2	0.6	0.01	Gonzalez-Pajuelo et al., 2004
<i>Raoultella planticola</i> DR3 = DSM ATCC 33531	GCA_000735435.1	Independent (no alc)	DAK1	N.D.	0.15	Jarvis et al., 1997
<i>Klebsiella pneumoniae</i> DSM 2026 = ATCC 15380	GCA_000949515.1	Dependent	DAK1	0.62	0.09	Mu et al., 2006
<i>Clostridium beijerinckii</i> NRRL B-593	GCA_002006325.1	Independent (no alc)	DAK1_2	0.59	0	Gungormusler et al., 2011
<i>Trichococcus</i> strain ES5	GCA_900067165.1[NS1]		DAK1/ DAK1_2	0.66		Streps 2020
<i>Caloramator viterbensis</i> DSM 13723T				0.69	0.21	Seyfried et al 2002
<i>Clostridium butyricum</i> AKR102a				0.52	N.D.	Wilkens et al., 2012
<i>Clostridium butyricum</i> CNCM 1211				0.64	0.11	Barbirato et al., 1998
<i>Clostridium butyricum</i> DSM 5431				0.51		Rehman et al., 2008
<i>Clostridium butyricum</i> E5				0.54	0.08	Petitdémange et al., 1995
<i>Clostridium butyricum</i> F2b				0.63	0.13	Papanikolaou et al., 2004
<i>Clostridium butyricum</i> VPI 1718				0.68	0.04	Chatzifragkou et al., 2011
<i>Clostridium multifementans</i> DSM 5431				0.4	0.01	Biebl et al., 1992
<i>Klebsiella pneumoniae</i> DSM4799				0.45	N.D.	Jun et al., 2010
<i>Klebsiella pneumoniae</i> HR526				0.4	N.D.	Xu et al., 2009
<i>Klebsiella pneumoniae</i> XJ-Li				0.61	0.05	Zhan et al., 2007
<i>Lactobacillus brevis</i> N1E9.3.3				0.46	0.07	Vivek et al., 2016
<i>Lactobacillus buchneri</i> B 190				0.27	0.18	Schutz et al., 1984
<i>Lactobacillus panis</i> PM1						Tae Sun Kang (2014)
<i>Pantoea agglomerans</i> CNCM-1210				0.61	0.16	Barbirato et al., 1998
<i>Shimwellia blattae</i> ATCC 33430				0.45 g/g		Rodríguez 2016
<i>Clostridium beijerinckii</i> DSM 791				0.55 (gg 1)		Pradima 2017

pathway involved in the production of 1,3 PD are glycerol dehydrogenase which is dependent on NADH and produces dihydroxyacetone (DHA) and DHA-kinase, which subsequently phosphorylates DHA. These key reactions are represented by four Pfam domains; Fe-ADH (PF00465) for glycerol dehydrogenase and DAK1, (PF02733) or DAK1_2, (PF13684) both capturing the kinase domain of the dihydroxyacetone kinase family in combination with DAK2, (PF02734), capturing phosphatase domain of the dihydroxyacetone kinase family (Figure 1). A pattern recognition analysis was performed (see methods section for details) for DAK2 domain neighbours in the training set database followed by a Boolean DAK1/DAK2 or DAK1_2/DAK2 proximity search in the search results. Pattern recognition showed that the 14 genomes of the training data set encoded at least one dihydroxyacetone kinase family protein with either a DAK1/DAK2 or DAK1_2/DAK2 protein architecture; 13 strains coded for DAK1/DAK2, 8 strains for DAK1_2/DAK2 and 7 strains encoded for both configurations (Table 1). In addition, other domains were identified that clustered together with the DAK configuration such as an Alkaline shock protein, a phosphotransferase system component and an alcohol dehydrogenase domain. Another copy of this domain is an important factor for the 1,3 PD pathway (Table 2).

Development of a function-based search strategy for the reductive branch. For NAD⁺ regeneration and 1,3-PD production two alternative pathway are known, a well-studied B12-dependent pathway and a lesser studied oxygen sensitive B12-independent pathway (figure 1). Pattern recognition analysis that was used to obtain the DAK configuration was used to obtain the domain persistency and copy number of key domain classes in the two alternative pathways among the

genomes of the training set. The iron containing aldehyde reductase/alcohol dehydrogenase domain (PF00465), is present in both the oxidative and reductive branch but functions in the reductive branch as an aldehyde reductase. The three dehydratase Pfam domains signifying the B12-dependent pathway, PF02287 (small subunit), PF02288 (medium subunit), and PF02286 (large subunit) have a domain class persistency of 0,6 with, when present, on average per genome 1,7 (large and small subunit) or 3,7 copies (medium subunit) in the training set. The diol dehydratase reactivase ATPase-like domain PF08841 has a persistency of 0,6 and a copy number of 1. Note that only four strains in the training data set were molecularly characterized. The degree of persistency of these key domains of the B12-dependent pathway in the training set therefore suggests that multiple 1,3-PD-producing strains of the training set may actually not use the B12-dependent pathway for 1,3 PD-production. Alternatively, a B12-independent glycerol dehydratase function is reported to be involved in 1,3-PD production (13) and consists of a glycine radical (PF01228) and a pyruvate formate lyase-like domain (PF02901). Both domains are present in all 13 genomes of the training data set with a relative high copy number of 6. The persistency of the radical SAM superfamily domain, a key part of the B12-independent glycerol dehydratase reactivase enzyme (19), was also 1 with an average copy number of 32 indicating that these three domains are ubiquitous and promiscuous and normally also used to support other functionalities (Table 3). As the high copy number of persistent signifying domains of both the B12-dependent and B12-independent reductive branch suggests that they play roles in multiple processes, a Boolean multi-compound proximity search was developed for the reductive branch. In this approach, the physical co-localization

Table 2. Reference set DAK analysis is this needed, or should we modify the text to make it fit?

Domain	PatternFrequency	support	JaccardIndex	Domain description
PF02734	32	1	1	DhaL domain
PF02733	19	0.59	0.93	DhaK domain
PF13684	8	0.25	0.75	Uncharacterised protein, DhaK domain
PF03780	8	0.25	0.41	Alkaline shock protein Asp23
PF03610	16	0.5	0.11	Phosphotransferase system, mannose-type IIA component
PF00465	8	0.25	0.05	Alcohol dehydrogenase, iron-type

of the persistent signifying domain classes in the respective genomes is included in the search. From the list of natural producers with a molecularly characterized 1,3-PD operon, the size of 1,3-PD operon was estimated to encompass approximately 18,000 bases. Furthermore, signifying domains could be found on both strands due to the use of internal promoters.

For the B12-independent pathway search criteria were based on prior knowledge obtained from a single characterized strain (13). Here four domains were specified (PF00465, PF01228, PF02901, PF04055) extending 20,000 base pairs up and downstream of the alternative dehydratase domains. Furthermore, taking internal promoters into account, domains were allowed to be present on both strands when genes are facing in the opposite direction in case of bidirectional promoter.

The B12-dependent pathway should contain at least five signature domains (PF00465, PF02286, PF02287, PF02288, PF08841) and were analyzed with the same restrictions as applied to the independent pathway.

Including the signifying domains used in the query, the core of the B12-dependent 1,3-PD operon may consist of eight domains. Other highly persistent domains (Support level > 0.5) enriched within the syntenic region are ‘Cobalamin adenosyltransferase’, (PF01923), ‘Haem-degrading’ (PF03928), ‘Alcohol dehydrogenase, iron-type’ (PF00465), and the ‘Micro-compartment protein’ (PF00936), (see Table 4).

Data preparation. To be able to mine a large set of publicly available genomes for the presence of the vitamin B12 variant 1,3-PD operon, 84,308 bacterial genomes containing 51 phyla, 64 classes, 145 orders, 335 families, 1,126 genera and 2,661 species were downloaded from the EBI-ENA data warehouse. The SAPP annotation platform was subsequently used for *de novo* structural and functional annotation of these genomes resulting in a semantic database of 300,196,266 predicted protein encoding genes linked to the corresponding protein sequences, predicted protein domain architectures and provenance.

Data Mining for candidate 1,3 PD producers. Two parallel pathways must be present to convert glycerol to 1,3-PD. Data mining for candidate 1,3-PD producers was therefore split in two steps. First, the search space was reduced by searching for the presence of a possible oxidative branch. In the second step, the reduced search space was searched for the presence of a possible reductive branch. As the molecular knowledge obtained for B12-independent pathway is based

on the characterization of a single *Clostridium butyricum* strain, data mining for the reductive branch focused on finding candidate B12-dependent 1,3 PD producers.

Oxidative branch: Following the function-based search strategy outlined above and summarised in figure 1, a proximity search with the two DAK configurations in parallel resulted in a 41% reduction of the search space. The thus reduced search space contained 49,110 genomes assigned to 3,526 species from 976 genera. The most abundant species were *Listeria monocytogenes* (5521), *Streptococcus pneumoniae* (6305), *Escherichia coli* (7577) and *Staphylococcus aureus* (10232). The reduced search space was used as an input for a proximity search for the presence of the B12-dependent reductive pathway.

Reductive branch Identification of B12-dependent strains according to the criteria obtained from the training set and based upon the results obtained from the oxidative branch, resulted in a list of 247 species (5,538 strains). Strains of *Klebsiella pneumoniae* (1,637) and *Listeria monocytogenes* (1,788), both pathogenic species were over-represented as well as *Escherichia coli* (1,067). An overview of identified species can be found in Supplementary table S1. The most frequently observed additional syntenic domains showing a persistency of 0.75 and above are shown in Table 6.

For further analysis, strains were collapsed in species groups and each species group was treated as one. At species level, domain connectivity (figure 2) and persistency of the B12-dependent 1,3-PD trait showed a high degree of similarity with the training set (Table 6).

Additional constraints. As the candidate species are depending on vitamin B12 for the production of 1,3-PD it could be beneficial to additionally select for the ability to synthesize vitamin B12. Secondly, the best known natural B12 dependent 1,3-PD producers are considered to be pathogenic or opportunistic pathogenic microorganisms such as *Klebsiella pneumoniae* (20).

Following the strategy outlined above a function-based screening was developed for the distribution of the trait for vitamin B12 synthesis among the candidate species. Using a multi-domain approach containing all domain signatures required for vitamin B12 synthesis a vitamin B12 (21) biosynthesis pathway was detected in 86 of the 187 candidate species using a multi-domain approach containing all domain signatures required for vitamin B12 synthesis. In order to obtain the bio-safety level of each candidate species, the BacDive database was queried (22). Forty-seven candidate species were classified at bio-safety level 2 and higher

Table 3. B12-independent

Domain	PatternFrequency	Support	JaccardIndex	Domain description
PF00465	4	1	0.03	Alcohol dehydrogenase, iron-type
PF01228	4	1	0.07	Glycine radical domain
PF02901	4	1	0.11	Pyruvate formate lyase domain
PF04055	4	1	0.01	Radical SAM
PF00465	4	1	0.03	Alcohol dehydrogenase, iron-type
PF13353	3	0.75	0.03	4Fe-4S single cluster domain
PF03319	1	0.25	0.08	Ethanolamine utilization protein EutN/carboxysome structural protein Ccml
PF06130	1	0.25	0.12	Phosphate propanoyltransferase

Table 4. Frequent pattern analysis on reference genomes for B12-dependent 1,3 PD and a minimum support value of 0.5

Domain	PatternFrequency	Support	JaccardIndex	Domain description
PF02288	12	1	0.86	Diol/glycerol dehydratase/dehydratase reactivating factor
PF08841	12	1	1	Diol dehydratase reactivase ATPase-like domain
PF02286	12	1	1	Diol/glycerol dehydratase, large subunit
PF02287	12	1	1	Propanediol/glycerol dehydratase, small subunit
PF01923	8	0.67	0.4	Adenosylcobalamin biosynthesis, ATP:cob(I)alamin adenosyltransferase-like
PF03928	8	0.67	0.59	Haem-degrading
PF00465	8	0.67	0.08	Alcohol dehydrogenase, iron-type
PF00936	6	0.5	0.35	Microcompartment protein, bacteria

Table 5. Oxidative on genome resource

Domain	PatternFrequency	support	JaccardIndex	Domain description
PF02734	74929	1	1	DhaL domain
PF02733	38482	0.51	0.91	DhaK domain
PF13684	32142	0.43	0.97	Uncharacterised protein, DhaK domain
PF03610	27381	0.37	0.15	Phosphotransferase system, mannose-type IIA component
PF03780	23828	0.32	0.34	Alkaline shock protein Asp23

Table 6. Pattern frequency analysis for the reductive branch on genomes obtained from the oxidative analysis. Protein domains in **bold** are reoccurring from the training set.

Domain	PatternFrequency	Support	JaccardIndex	Domain description
PF08841	8193	1	0.48	Diol dehydratase reactivase ATPase-like domain
PF03928	8188	1	0.16	Haem-degrading
PF02288	8193	1	0.62	Diol/glycerol dehydratase/dehydratase reactivating factor
PF02287	8193	1	0.61	Propanediol/glycerol dehydratase, small subunit
PF02286	8193	1	0.62	Diol/glycerol dehydratase, large subunit
PF01923	8178	1	0.13	Adenosylcobalamin biosynthesis, ATP:cob(I)alamin adenosyltransferase-like
PF00465	8193	1	0.03	Alcohol dehydrogenase, iron-type
PF15953	8098	0.99	0.76	Microcompartment protein PduM
PF06130	8127	0.99	0.44	Phosphate propanoyltransferase
PF03319	8126	0.99	0.26	Ethanolamine utilization protein EutN/carboxysome structural protein Ccml
PF00936	8135	0.99	0.32	Microcompartment protein, bacteria
PF00171	8125	0.99	0.01	Aldehyde dehydrogenase domain
PF00871	6278	0.77	0.06	Aliphatic acid kinase, short-chain
PF13375	6152	0.75	0.16	RnfC Barrel sandwich hybrid domain
PF10662	6119	0.75	0.22	Ethanolamine/propanediol utilisation protein, EutP/PduV
PF10531	6158	0.75	0.03	Soluble ligand binding domain
PF01512	6179	0.75	0.07	NADH-ubiquinone oxidoreductase 51kDa subunit, FMN-binding domain
PF00288	6118	0.75	0.03	GHMP kinase N-terminal domain

and 48 species were considered to be non-pathogenic (bio-safety level 1) while 92 species remained unclassified. The bio-safety level 1 label was combined with the ability for vitamin B12 synthesis with resulted in the following candidate species; *Anaeromusa acidaminophila*, *Anaerosalibacter massiliensis*, *Blautia obeum*, *Clostridium drakei*, *Clostridium magnum*, *Geobacillus thermoglucosidasius*, *Intestinimonas butyriciproducens* and *Thermincola ferriacetica*, (see Table suppl S1).

Discussion

Bioprospecting entails the systematic search for economically valuable genetic and biochemical resources from nature (23). For genome prospecting, the *in silico* mining of sequenced genomes and metagenomes for new biotechnologically relevant proteins and enzymes is a continuously evolving field. Two approaches can be applied, a “top-down” approach that begin by searching for a function and is followed by identification of the corresponding gene(s) and a

Fig. 2. Protein domain connectivity order. Arrows indicate the order in which protein domains were identified in operonic structures. The darker and thicker the arrow indicates that this connection was more observed.

“bottom-up” approach that start with a gene of interest in an effort to find a corresponding function in genome(s) of interest. For bottom-up approaches many sequence similarity tools such as Blast (24) exist. Bottom-up studies usually start with a selection of (meta)genome sequences followed by sequence similarity-based clustering and selection of candidate sequences and re-annotation of interesting candidates thereby avoiding ambiguity related problems in current functional annotations. For bacterial species however, *a priori*, gene fusion-fission events can be expected (25) hampering sequence similarity-based detection and clustering of multi-domain proteins encoding genes, while the same domains may also be present in multiple proteins. When searching for polygenic traits these problems are aggravated and thus a function-based approach searching for key functions may be more effective especially when there is insufficient understanding of the genetic architecture of trait in terms of the minimal number of genes required for the trait and function of the domains encoded by the different genes. In order to overcome ambiguity related problems in functional annotations, SAPP identifies and annotates protein function based on domain architecture. Protein domains have been shown to provide an accurate representation of the functional capabilities of a protein (10). To overcome ambiguity related problems in functional annotations SAPP identifies and annotates protein function based on domain architecture. As profile hidden Markov Models (HMM), which favour in their scoring functionally important sites, are used for the identification of protein domains, statistically robust annotation profiles can be obtained over large phylogenetic distances. KDD is a multi-step process. Crucial steps are data standardisation and feature extraction for pattern searching. By using

protein domain architectures as proxy for protein functions, a high degree of standardisation is obtained. As protein domain architectures can be directly transformed in highly interoperable strings of Pfam domain identifiers, a “top-down” functional screening can be done efficiently.

Applying the KDD approach on Linked Data allows for validation of initial results in multiple ways and for iteration after modification. Driven by a strong need to be able to integrate and analyse biological datasets across databases, there has been a considerable increase in the adoption of Semantic Web technologies in the life-sciences (26). However, a SPARQL endpoint for phenotype data is only available via WikiData (27). Other resources for phenotype data such as BacDive, here used to obtain bio-safety levels of candidate species, do provide API’s to mine their data but currently cannot be directly queried using SPARQL. With the growing importance of Semantic Web technologies for the life sciences interoperability levels on all aspect will increase, enhancing mining possibilities, aiding the discovery of new traits in an unprecedented pace.

By using molecular knowledge obtained from molecular characterisations of the 1,3-PD operon of four species and validating and iterating the search pattern on a training data set of genome sequences of twelve known 1,3-PD producers, a large collection of 84,308 publicly available bacterial could be efficiently mined in a top-down approach yielding 187 new candidate species some of which could be confirmed by literature (28–32).

Conclusions

Through transformation of genomic data into a FAIR linked-data format, iterative function-based approaches can be de-

veloped to mine the large genome repositories. By presenting functional annotation as unambiguous protein domain architectures a high level of interoperability is obtained allowing for the development of efficient function-based top-down searches not limited to supervised trait identification.

Materials and methods

Data annotation and mining. Bacterial genomes were downloaded from the ENA database using the enaBrowser-Tools (1). All downloaded microorganisms were converted into a semantic repository using the annotation platform based on functional analysis (SAPP) (7). All genomes were *de novo* structurally re-annotated using Prodigal (16) and functionally annotated using Pfam from InterProScan (17). Each genome was stored as an individual graph database using the HDT library and were processed using a domain pattern recognition software that was developed to aid in the data processing (33, 34). This application constructs operon-like structures around one or more protein domains from genomic data and discovers patterns in the present protein domains using a Frequent Pattern Mining and a Latent Dirichlet Allocation approach.

Pattern recognition. The application that was used for pattern recognition has been developed in Java 8 and uses Scala version 2.11. The annotated genomes are provided as RDF data in HDT format allowing to easily scale beyond the size limitations of current graph databases. Spark Core has been implemented to enable parallelization for the processing of the genomes. The Frequent Pattern Mining and Latent Dirichlet allocation algorithms from the Apache SPARK MLlib Machine Learning Library, are applied to analyse patterns in the domain architecture of operons Meng2016. The support value of a pattern with one domain is a measure for the local persistency of that specific domain. The support value is defined as the number of regions where the domain occurs divided by the total number of regions.

Oxidative branch identification. To identify strains containing the oxidative branch as shown in figure 1, DAK domains were processed using the domain pattern recognition software. Genes containing DAK1 (PF02733) or DAK1_2 (PF13684) in combination with DAK2, (PF02734), 95% gene confidence according to prodigal and neighbour linking was applied to identify strains capable for growth on glycerol.

Reductive branch identification. Strains that contained the oxidative branch were further investigated for the reductive branch using domains according to the B12 dependent or independent pathway. Region identification was performed using PF02287 and 20,000 base pairs up and downstream. Regions containing all domains of interest were further investigated for domain occurrence and synteny.

Reductive branch identification. Strains that contained the oxidative branch were further investigated for the reductive branch using domains according to the B12 dependent or

independent pathway. Region identification was performed using PF02287 and 20,000 base pairs up and downstream. These regions were modified to only retain genes that form an operon-like structure. The biggest intergenic region allowed between two genes on the same strand was 50 nucleotides. To also include the possibility of a bidirectional promoter, a gap of 500 (or less) nucleotides is allowed between stretches of genes on the reverse and forward strand. To prevent identical regions created around a gene which contains multiple copies of the PF02287 or different genes containing PF02287, regions that (partly) overlap are merged into one. The operon-like structure was preserved.

B12 synthesis identification. Phylogenetic information was complemented with phenotype information through BacDive (22). The BacDive resource was parsed and each entry record was transformed into RDF allowing integration of genotype and phenotype information. SPARQL queries were used to retrieve information with regards to pathogenicity and temperature.

ACKNOWLEDGEMENTS

This research was supported by the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement (323009) and by the Gravitation grant (024.002.002) of the Netherlands Ministry of Education, Culture and Science. The work conducted by the U.S. Department of Energy Joint Genome Institute (DOE-JGI), a DOE Office of Science User Facility, was supported by the Office of Science of the DOE under Contract No. DE-AC02-05CH11231. IBISBA 1.0 - Project ID: 730976 - Funded under: H2020-EU.1.4.1.2. - Integrating and opening existing national and regional research infrastructures of European interest. This work was carried out on the Dutch national e-infrastructure with the support of the SURF foundation.

Bibliography

1. Nicole Silvester, Blaise Alako, Clara Amid, Ana Cerdeño-Tarraga, Laura Clarke, Iain Cleland, Peter W. Harrison, Suran Jayatilaka, Simon Kay, Thomas Keane, Rasko Leinonen, Xin Liu, Josué Martínez-Villacorta, Manuela Menchi, Kethi Reddy, Nima Pakseresh, Jeena Rajan, Marc Rossello, Dmitriy Smirnov, Ana L. Toribio, Daniel Vaughan, Vadim Zalunin, and Guy Cochrane. The European Nucleotide Archive in 2017. *Nucleic Acids Research*, 46 (D1):D36–D40, jan 2018. ISSN 13624962. doi: 10.1093/nar/gkx1125.
2. The UniProt Consortium. UniProt: the universal protein knowledgebase. *Nucleic Acids Research*, 46(5):2699–2699, mar 2018. ISSN 0305-1048. doi: 10.1093/nar/gky092.
3. Alexandra M. Schnoes, Shoshana D. Brown, Igor Dodevski, and Patricia C. Babbitt. Annotation Error in Public Databases: Misannotation of Molecular Function in Enzyme Superfamilies. *PLoS Computational Biology*, 5(12):e1000605, dec 2009. ISSN 1553-7358. doi: 10.1371/journal.pcbi.1000605.
4. Jasper J. Koehorst, Jesse C.J. J. Van Dam, Ruben G.A. A. Van Heck, Edoardo Saccenti, Vitor A.P. Martins P. Martins Dos Santos, Maria Suarez-Diez, and Peter J. Schaap. Comparison of 432 *Pseudomonas* strains through integration of genomic, functional, metabolic and expression data. *Scientific Reports*, 6(1):38699, 2016. ISSN 2045-2322. doi: 10.1038/srep38699.
5. Sarah K Kummerfeld and Sarah A Teichmann. Protein domain organisation: adding order. *BMC Bioinformatics*, 10(1):39, jan 2009. ISSN 1471-2105. doi: 10.1186/1471-2105-10-39.
6. Dennis P. Wall, Parul Kudtarkar, Vincent A. Fusaro, Rimma Pivovarov, Prasad Patil, and Peter J. Tonellato. Cloud computing for comparative genomics. *BMC Bioinformatics*, 11, 2010. ISSN 14712105. doi: 10.1186/1471-2105-11-259.
7. Jasper J. Koehorst, Jesse C.J. J. Van Dam, Edoardo Saccenti, Vitor A.P. P. Martins Dos Santos, Maria Suarez-Diez, and Peter J. Schaap. SAPP: functional genome annotation and analysis through a semantic framework using FAIR principles. *Bioinformatics*, 34(8): 1401–1403, apr 2018. ISSN 1367-4803. doi: 10.1093/bioinformatics/btx767.
8. J.C.J. van Dam, J.J. Koehorst, J.O. Vik, V.A.P. Martins dos Santos, P.J. Schaap, and M. Suarez-Diez. The Empusa code generator and its application to GBOL, an extendable ontology for genome annotation. *Scientific Data*, 6(1), 2019. ISSN 20524463. doi: 10.1038/s41597-019-0263-7.
9. Christian Bizer, Tom Heath, and Tim Berners-Lee. Linked Data. In *Semantic Services, Interoperability and Web Applications*, pages 205–227. IGI Global, 2011. doi: 10.4018/978-1-60960-593-3.ch008.
10. Jasper J. Koehorst, Edoardo Saccenti, Peter J. Schaap, Vitor A. P. Martins dos Santos, and Maria Suarez-Diez. Protein domain architectures provide a fast, efficient and scalable alternative to sequence-based methods for comparative functional genomics. *F1000Research*, 5(1987):1987, jun 2017. ISSN 2046-1402. doi: 10.12688/f1000research.9416.3.
11. R. K. Saxena, Pinki Anand, Saurabh Saran, and Jasmine Isar. Microbial production of 1,3-propanediol: Recent developments and emerging opportunities. *Biotechnology Advances*, 27(6):895–913, 2009. ISSN 07349750. doi: 10.1016/j.biotechadv.2009.07.003.

Supplementary

12. Wei Jiang, Shizhen Wang, Yuanpeng Wang, and Baishan Fang. Key enzymes catalyzing glycerol to 1,3-propanediol. *Biotechnology for Biofuels*, 9(1):57, dec 2016. ISSN 1754-6834. doi: 10.1186/s13068-016-0473-6.
13. C. Raynaud, P. Sarcabal, I. Meynial-Salles, C. Croux, and P. Soucaille. Molecular characterization of the 1,3-propanediol (1,3-PD) operon of *Clostridium butyricum*. *Proceedings of the National Academy of Sciences*, 100(9):5010–5015, 2003. ISSN 0027-8424. doi: 10.1073/pnas.0734105100.
14. Petar Ristoski and Heiko Paulheim. Semantic Web in data mining and knowledge discovery: A comprehensive survey. *Journal of Web Semantics*, 36:1–22, jan 2016. ISSN 15708268. doi: 10.1016/j.websem.2016.01.001.
15. R. G. Forage and E C Lin. DHA system mediating aerobic and anaerobic dissimilation of glycerol in *Klebsiella pneumoniae* NCIB 418. *Journal of bacteriology*, 151(2):591–9, aug 1982. ISSN 0021-9193.
16. Doug Hyatt, Gwo-Liang Chen, Philip F Locascio, Miriam L Land, Frank W Larimer, and Loren J Hauser. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC bioinformatics*, 11(1):119, jan 2010. ISSN 1471-2105.
17. Robert D. Finn, Teresa K. Attwood, Patricia C. Babbitt, Alex Bateman, Peer Bork, Alan J. Bridge, Hsin Yu Chang, Zsuzsanna Dosztanyi, Sara El-Gebali, Matthew Fraser, Julian Gough, David Haft, Gemma L. Holliday, Hongzhan Huang, Xiaosong Huang, Ivica Letunic, Rodrigo Lopez, Shennan Lu, Aron Marchler-Bauer, Huaiyu Mi, Jaina Mistry, Darren A. Natale, Marco Necci, Gift Nuka, Christine A. Orengo, Youngmi Park, Sebastien Pesseat, Damiano Piovesan, Simon C. Potter, Neil D. Rawlings, Nicole Redaschi, Lorna Richardson, Catherine Rivoire, Amaia Sangrador-Vegas, Christian Sigrist, Ian Sillitoe, Ben Smithers, Silvano Squizzato, Granger Sutton, Narmada Thanki, Paul D. Thomas, Silvio C.E. Tosatto, Cathy H. Wu, Ioannis Xenarios, Lai Su Yeh, Siew Yit Young, and Alex L. Mitchell. InterPro in 2017-beyond protein family and domain annotations. *Nucleic Acids Research*, 45(D1):D190–D199, 2017. ISSN 13624962. doi: 10.1093/nar/gkw1107.
18. Graham Klyne and Jeremy J Carroll. Resource Description Framework (RDF): Concepts and Abstract Syntax. *W3C Recommendation*, 10:1–20, 2004.
19. Jonathan M. Demick and William N. Lanzilotta. Radical SAM activation of the B12-independent glycerol dehydratase results in formation of 5'-deoxy-5'-(methylthio)adenosine and not 5'-deoxyadenosine. *Biochemistry*, 50(4):440–2, feb 2011. ISSN 1520-4995. doi: 10.1021/bi101255e.
20. Yu Wang, Fei Tao, C. Li, Lixiang Li, and Ping Xu. Genome Sequence of *Klebsiella pneumoniae* Strain ATCC 25955, an Oxygen-Insensitive Producer of 1,3-Propanediol. *Genome Announcements*, 1(4):4–5, aug 2013. ISSN 2169-8287. doi: 10.1128/genomeA.00587-13.
21. E. Raux, H. L. Schubert, and M. J. Warren*. Biosynthesis of cobalamin (vitamin B12): a bacterial conundrum. *Cellular and Molecular Life Sciences*, 57(13):1880–1893, dec 2000. ISSN 1420-682X. doi: 10.1007/PL00000670.
22. Carola Söhrngen, Adam Podstawka, Boyke Bunk, Dorothea Gleim, Anna Vetscininova, Lorenz Christian Reimer, Christian Ebeling, Cezar Pendarovski, and Jörg Overmann Leibniz. BacDive - The Bacterial Diversity Metadatabase in 2016. *Nucleic Acids Research*, 44(D1):D581–D585, 2016. ISSN 13624962. doi: 10.1093/nar/gkv983.
23. Anthony Artuso. Bioprospecting, benefit sharing, and biotechnological capacity building. *World Development*, 30(8):1355–1368, aug 2002. ISSN 0305750X. doi: 10.1016/S0305-750X(02)00040-2.
24. Stephen F Altschul, Thomas L Madden, Alejandro A Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J Lipman. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs, sep 1997. ISSN 03051048.
25. Sophie Pasek, Jean-Loup Risler, and Pierre Brézellec. Gene fusion/fission is a major contributor to evolution of multi-domain bacterial proteins. *Bioinformatics*, 22(12):1418–1423, 2006.
26. K.-H. Cheung, Eric Prud'hommeaux, Yimin Wang, and Susie Stephens. Semantic Web for Health Care and Life Sciences: a review of the state of the art. *Briefings in Bioinformatics*, 10(2):111–113, mar 2009. ISSN 1467-5463. doi: 10.1093/bib/bbp015.
27. Elvira Mittra, Andra Waagmeester, Sebastian Burgstaller-Muehlbacher, Lynn M Schriml, Andrew I Su, and Benjamin M Good. Wikidata: A platform for data integration and dissemination for the life sciences and beyond. *bioRxiv*, 2015. doi: 10.1101/031971.
28. Frauke Luers, Markus Seyfried, Rolf Daniel, and Gerhard Gottschalk. Glycerol conversion to 1,3-propanediol by *Clostridium pasteurianum*: Cloning and expression of the gene encoding 1,3-propanediol dehydrogenase. *FEMS Microbiology Letters*, 154(2):337–345, 1997. ISSN 03781097. doi: 10.1016/S0378-1097(97)00351-0.
29. Anniina Kivistö, Ville Santala, and Matti Karp. 1,3-Propanediol production and tolerance of a halophilic fermentative bacterium, *Halanaerobium saccharolyticum* subsp. *saccharolyticum*. *Journal of Biotechnology*, 158(4):242–247, apr 2012. ISSN 01681656. doi: 10.1016/j.jbiotec.2011.10.013.
30. Stefan Pflügl, Hans Marx, Diethard Mattanovich, and Michael Sauer. 1,3-Propanediol production from glycerol with *Lactobacillus diolivorans*. *Bioresource Technology*, 2012. ISSN 09608524. doi: 10.1016/j.biortech.2012.05.121.
31. Nurul H. Khan, Tae Sun Kang, Douglas A. S. Grahame, Monique C. Haakensen, Korn-sulee Ratanapariyanuch, Martin J. Reaney, Darren R. Korber, and Takuji Tanaka. Isolation and characterization of novel 1,3-propanediol-producing *Lactobacillus panis* PM1 from bioethanol thin stillage. *Applied Microbiology and Biotechnology*, 97(1):417–428, jan 2013. ISSN 0175-7598. doi: 10.1007/s00253-012-4386-4.
32. Heba M. Amin, Abdelgawad M. Hashem, Mohamed S. Ashour, and Rajini Hatti-Kaul. 1,2 Propanediol utilization by *Lactobacillus reuteri* DSM 20016, role in bioconversion of glycerol to 1,3 propanediol, 3-hydroxypropionaldehyde and 3-hydroxypropionic acid. *Journal of Genetic Engineering and Biotechnology*, 11(1):53–59, jun 2013. ISSN 1687157X. doi: 10.1016/j.jgeb.2012.12.002.
33. Javier D Fernández, Miguel A Martínez-Prieto, Claudio Gutiérrez, Axel Polleres, and Mario Arias. Binary RDF Representation for Publication and Exchange (HDT). *Web Semantics: Science, Services and Agents on the World Wide Web*, 19:22–41, 2013.
34. Eric Prud'hommeaux and Andy Seaborne. SPARQL Query Language for RDF. *W3C Recommendation*, 2009(January):1–106, 2008. doi: citeulike-article-id:2620569.

Table S1. Overview of species identified to have the potential of producing 1,3 PD

Anaeromusa acidaminophila	Eubacteriaceae bacterium CHKCI004	Lactobacillus kimchicus
Anaerosalibacter massiliensis	Eubacterium sp. 14-2	Lactobacillus kisonensis
Aneurinibacillus migulanus	Eubacterium sp. An11	Lactobacillus mellifer
Aneurinibacillus sp. UBA3580	Eubacterium sp. An3	Lactobacillus namurensis
Bacillaceae bacterium MTCC 10057	Eubacterium sp. UBA3279	Lactobacillus panis
Bacillus azotoformans	Eubacterium sp. UBA3326	Lactobacillus paracollinoides
Bacillus massiliosenegalensis	Eubacterium sp. UBA7134	Lactobacillus pobuzihii
Bacillus sp. 7504-2	Firmicutes bacterium UBA3567	Lactobacillus rapi
Bacteroides fragilis	Firmicutes bacterium UBA3570	Lactobacillus rennini
Blautia obeum	Firmicutes bacterium UBA3573	Lactobacillus reuteri
Blautia schinkii	Flavonifractor plautii	Lactobacillus rossiae
Blautia sp. UBA2945	Flavonifractor sp. An306	Lactobacillus silagei
Carnobacteriaceae bacterium UBA7837	Flavonifractor sp. An82	Lactobacillus siliginis
Carnobacterium alterfunditum	Fusobacteriaceae bacterium UBA2433	Lactobacillus similis
Carnobacterium funditum	Fusobacterium hwasookii	Lactobacillus spicheri
Cetobacterium somerae	Fusobacterium nucleatum	Lactobacillus versmoldensis
Citrobacter amalonaticus	Fusobacterium sp. CM1	Listeria innocua
Citrobacter freundii complex	Fusobacterium sp. CM22	Listeria ivanovii
Citrobacter koseri	Fusobacterium sp. HMSC064B11	Listeria monocytogenes
Citrobacter rodentium	Fusobacterium sp. HMSC073F01	Listeria seeligeri
Citrobacter sp. 30_2	Fusobacterium sp. OBRC1	Listeria welshimeri
Citrobacter sp. A1	Fusobacterium ulcerans	Metakosakonia massiliensis
Citrobacter sp. CFSAN044567	Fusobacterium varium	Mycobacterium vaccae
Citrobacter sp. KTE151	Geobacillus sp. (strain Y4.1MC1)	Paraclostridium benzoelyticum
Citrobacter sp. KTE30	Geobacillus thermoglucosidasius	Paraclostridium bifermentans
Citrobacter sp. KTE32	Geosporobacter ferrireducens	Pediococcus acidilactici
Citrobacter sp. L17	Halanaerobium hydrogeniformans	Pediococcus claussenii
Clostridia bacterium UC5.1-2H11	Halanaerobium saccharolyticum	Pediococcus pentosaceus
Clostridiaceae bacterium BRH_c20a	Ilyobacter polytropus	Pelosinus fermentans
Clostridiales bacterium DRI-13	Intestinimonas butyriciproducens	Pelosinus sp. HCF1
Clostridiales bacterium PH28_bin88	Klebsiella aerogenes	Phytobacter ursingii
Clostridiales bacterium VE202-03	Klebsiella michiganensis	Pluralibacter gergoviae
Clostridiales bacterium mt11	Klebsiella oxytoca	Propionibacterium freudenreichii
Clostridium baratii	Klebsiella pneumoniae	Propionimicrobium sp. BV2F7
Clostridium botulinum	Klebsiella quasipneumoniae	Pseudopropionibacterium propionicum
Clostridium drakei	Klebsiella sp. 10982	Quasibacillus thermotolerans
Clostridium estertheticum	Klebsiella sp. 1_1_55	Salmonella choleraesuis
Clostridium lundense	Klebsiella sp. AA405	Sebaldella termitidis
Clostridium magnum	Klebsiella sp. AS10	Serratia marcescens
Clostridium pasteurianum	Klebsiella sp. HMSC09D12	Shigella boydii
Clostridium perfringens	Klebsiella sp. HMSC16A12	Shigella flexneri
Enterobacter cloacae complex	Klebsiella sp. HMSC22F09	Shigella sonnei
Enterobacter sp. 10-1	Klebsiella sp. KGM-IMP216	Sporomusa acidovorans
Enterobacter sp. Bisph2	Klebsiella sp. KTE92	Sporomusa sphaeroides
Enterobacter sp. GN02600	Klebsiella sp. LTGPAF-6F	Sporomusaceae bacterium UBA5961
Enterobacteriaceae bacterium UBA2606	Klebsiella sp. M5al	Sporomusaceae bacterium UBA5989
Enterobacteriaceae bacterium UBA3163	Klebsiella sp. OBRC7	Sporomusaceae bacterium UBA7701
Enterobacteriaceae bacterium UBA3516	Klebsiella sp. PO552	Sporomusaceae bacterium UBA864
Enterobacteriaceae bacterium UBA4747	Klebsiella sp. S1	Streptococcus australis
Enterobacteriaceae bacterium UBA5935	Klebsiella variicola	Streptococcus sanguinis
Enterobacteriaceae bacterium UBA869	Klebsiella variicola CAG:634	Streptococcus sp. AS14
Enterobacteriaceae bacterium UBA898	Kluyvera intermedia	Streptococcus sp. F0442
Enterococcus avium	Lachnospiraceae bacterium 3-1	Streptococcus suis
Enterococcus malodoratus	Lachnospiraceae bacterium 7_1_58FAA	Thermincola ferriacetica
Enterococcus mundtii	Lachnospiraceae bacterium A2	Thermincola potens
Enterococcus pseudoavium	Lactobacillus brevis	Thermoanaerobacter sp. (strain X513)
Enterococcus raffinosus	Lactobacillus collinoides	Thermoanaerobacter sp. (strain X514)
Enterococcus sp. 3H8_DIV0648	Lactobacillus coryniformis	Thermoanaerobacter sp. A7A
Enterococcus sp. HMSC066C04	Lactobacillus curvatus	Thermoanaerobacterium thermosaccharolyticum
Enterococcus sp. HMSC072H05	Lactobacillus diolivorans	Tissierellia bacterium S5-A11
Enterococcus sp. HMSC29A04	Lactobacillus fuchuensis	Eubacterium hallii
Escherichia coli	Lactobacillus ginsenosidimutans	
Escherichia fergusonii	Lactobacillus graminis	