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PRODORIC (release 2009): a database and tool platform for the analysis of gene regulation in prokaryotes

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ABSTRACT

PRODORIC is a database that provides annotated information on the regulation of gene expression in prokaryotes. It integrates a large compilation of gene regulatory data including transcription factor binding sites, promoter structures and gene expression patterns. The whole dataset is manually curated and relies on published results extracted from the scientific literature. The current extended version of PRODORIC contains gene regulatory data for several new microorganisms. Major improvements were realized in the design of the web interface and the accessibility of the stored information. The database was further improved by the implementation of various new tools for the elucidation of gene regulatory interactions. Thus, the PRODORIC platform represents a framework for the interactive exploration, prediction and evaluation of gene regulatory networks in prokaryotes. PRODORIC is accessible at http://www.prodoric.de.

INTRODUCTION

In the last decade, the analysis and modeling of prokaryotic gene regulatory networks as basis of a systems biology approach to infection and biotechnological processes became of central interest (1,2). In this context network reconstruction requires reliable datasets of gene regulatory interactions, which are usually only available in the scientific literature. The fast accumulation of published gene regulatory data enhanced by the availability of numerous finished genomes and by high-throughput technologies fostered the development of structured repositories in the form of public databases.

Several specialized gene regulation databases with focus on one model organism or several organism groups were established (3–8). The PRODORIC database was released in 2003 as a universal data source covering gene regulation in prokaryotes with focus on pathogenic bacteria (9). In a manual curation process relevant data is extracted by constantly screening of the scientific literature. The main part of PRODORIC contains a unique collection of transcription factor binding sites (TFBSs) and their interacting transcription factors. Besides these regulatory interactions, promoter structures with transcriptional initiation sites and sigma factor binding sites were included. Moreover, gene expression data derived from published microarray experiments were integrated. An integral part of PRODORIC are aligned profiles of TFBSs for a certain regulator represented as positions weight matrices (PWMs) and sequence logos (10,11). Provided PWMs are useful tools for pattern matching, and thus for the prediction of unknown putative TFBSs in DNA sequences of interest. For this purpose PRODORIC is associated with the prediction tool Virtual Footprint that allows a PWM based scanning of sequences or even whole genomes for new regulator targets (12).

Here, we summarize the modifications and improvements of PRODORIC made in the recent years. This comprises a significant increase of data content and updates of our tools. Moreover, PRODORIC was further developed towards a database and bioinformatics tool platform

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The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors

Table 1. Statistics of the PRODORIC content (september 2008)

Organism	TFBSs	Genes	Regulons	PWMs[a]	Promoters	Profiles[b]
Escherichia coli	1670	1045	90	88 (76)	740	64 (4666)
Bacillus subtilis	785	738	88	71 (53)	493	34 (2488)
Pseudomonas aeruginosa	197	264	33	22 (19)	164	16 (1292)
Staphylococcus aureus	106	39	9	–	29	–
Rhodobacter sphaeroides	38	33	4	3 (3)	28	–
Streptococcus pyogenes	18	13	5	3 (2)	11	19 (416)
Bradyrhizobium japonicum	14	22	3	3 (3)	11	–
Synechococcus sp.	13	6	2	–	13	–
Rhizobium meliloti	12	4	8	–	11	2 (198)
Others	68	67	25	7 (7)	86	13 (402)
Sum	2921	2231	267	197 (163)	1586	148 (9462)

[a] The non-redundant number of position weight matrices (number in parentheses).
[b] The sum of genes that are linked to the profiles (number in parentheses).

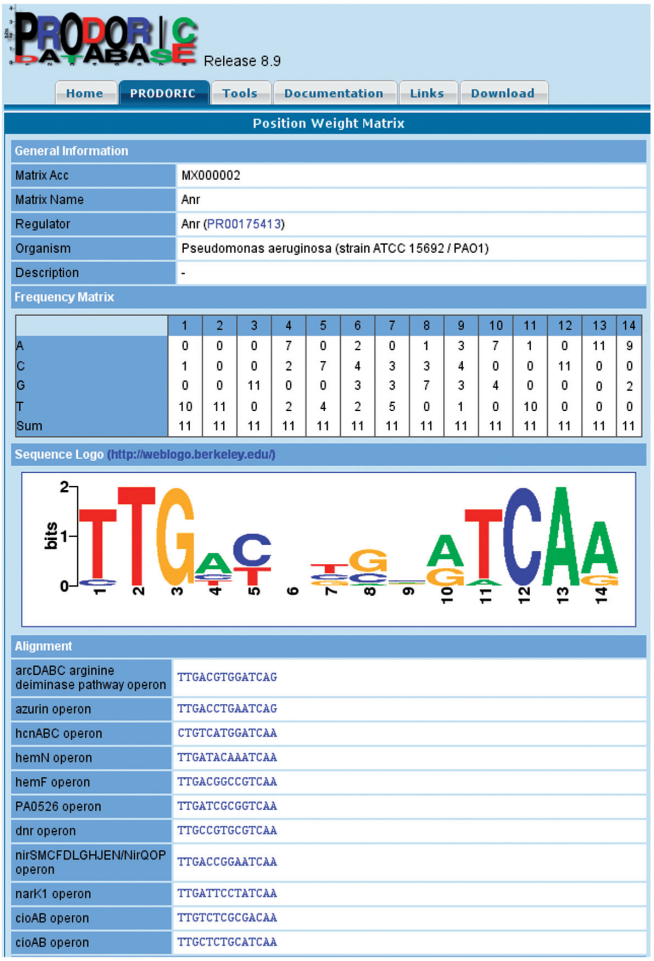
combining data and software for the interactive browsing, prediction and evaluation of gene regulatory networks in prokaryotes.

DATABASE CURATION AND CONTENT

PRODORIC relies completely on published results with experimental validation and is not complemented with computational predicted data. The transformation of free-text data from the primary literature into structured information is constantly done manually by a team of curators. During the process of literature screening we observed that even refined PubMed searches with keywords like 'gene regulation' and 'prokaryotes' are not sensitive enough since important classification terms like 'DNaseI footprint' or 'electromobility shift assay' are not generally part of PubMed abstracts. Since these terms are often associated with figure captions we optimized the literature preselection and data mining tasks by use of the PDF search engine CaptionSearch (13).

The main content of PRODORIC was significantly increased to an overall number of nearly 3000 TFBSs. The number of promoter and operon structures as well as expression profiles increased concurrently (Table 1). The main portion of regulatory interactions is expectedly covered by the two model organisms *Escherichia coli* and *Bacillus subtilis*. Interestingly, these are followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* revealing the relevance of data from pathogenic bacteria. An other striking group of bacteria annotated recently are phototrophic bacteria like *Rhodobacter sphaeroides* and *Synechococcus sp.* DNA sequence elements like TFBSs or transcriptional start sites are usually mapped to fixed genomic positions. Consequently, PRODORIC is limitted to sequenced organisms with elucidated genome sequence. Therefore, finished genomes are imported from flat files into PRODORIC in a frequent process, so the number of available organisms has increased to 696 different bacterial genomes with a total of 1304 replicons.

For the purpose of pattern matching and prediction of potentially new transcription factor targets, a significant number of new PWMs were generated from aligned profiles of TFBSs (Figure 1). This PWM library provides the data basis for the PRODORIC associated prediction tool Virtual Footprint.



Position Weight Matrix

General Information

Matrix Acc	MX000002
Matrix Name	Anr
Regulator	Anr (PR00175413)
Organism	Pseudomonas aeruginosa (strain ATCC 15692 / PAO1)
Description	-

Frequency Matrix

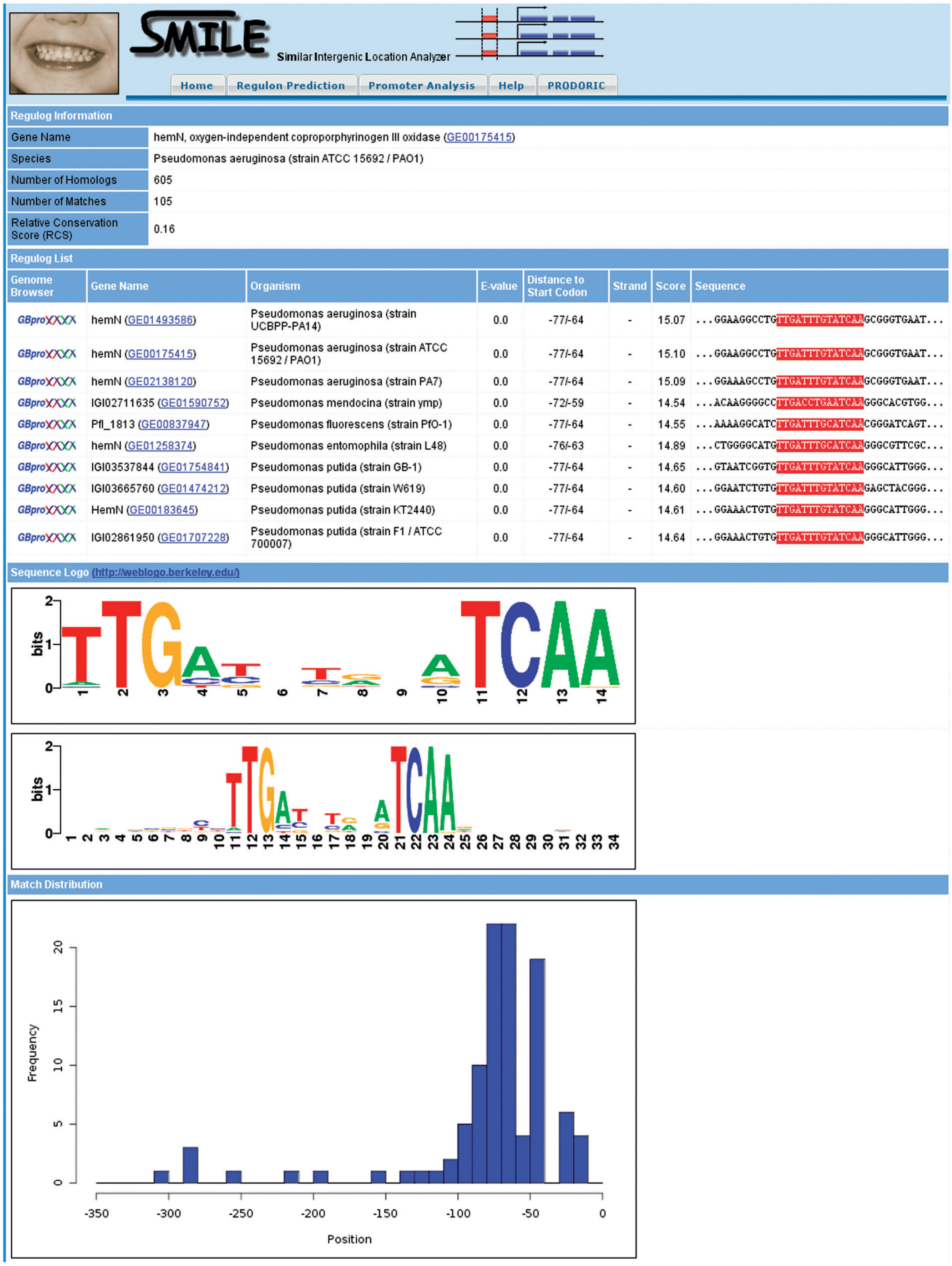
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	0	0	0	7	0	2	0	1	3	7	1	0	11	9
C	1	0	0	2	7	4	3	3	4	0	0	11	0	0
G	0	0	11	0	0	3	3	7	3	4	0	0	0	2
T	10	11	0	2	4	2	5	0	1	0	10	0	0	0
Sum	11	11	11	11	11	11	11	11	11	11	11	11	11	11

Sequence Logo (http://weblogo.berkeley.edu/)

Alignment

arcDABC arginine deiminase pathway operon	TTGACGTGGATCAG
azurin operon	TTGACCTGAATCAG
hcnABC operon	CTGTCATGGATCAA
hemN operon	TTGATACAAATCAA
hemF operon	TTGACGGCCGTCAA
PA0526 operon	TTGATCGCGGTCAA
dnr operon	TTGCCGTGCGTCAA
nirSMCFDLGHJEN/NirQOP operon	TTGACCGGAATCAA
narK1 operon	TTGATTCCTATCAA
cioAB operon	TTGTCTCGCGACAA
cioAB operon	TTGCTCTGCATCAA

Figure 1. Position weight matrix view of PRODORIC for the binding site of the Anr transcription faction from *Pseudomonas aerugionosa*.



Regulog Information

Gene Name	hemN, oxygen-independent coproporphyrinogen III oxidase (GE00175415)
Species	Pseudomonas aeruginosa (strain ATCC 15692 / PAO1)
Number of Homologs	605
Number of Matches	105
Relative Conservation Score (RCS)	0.16

Regulog List

Genome Browser	Gene Name	Organism	E-value	Distance to Start Codon	Strand	Score	Sequence
GBpro	hemN (GE01493586)	Pseudomonas aeruginosa (strain UCBPP-PA14)	0.0	-77/-64	-	15.07	...GGAAGGCCTGTTGATTTGTATCAAGCGGGTGAAT...
GBpro	hemN (GE00175415)	Pseudomonas aeruginosa (strain ATCC 15692 / PAO1)	0.0	-77/-64	-	15.10	...GGAAGGCCTGTTGATTTGTATCAAGCGGGTGAAT...
GBpro	hemN (GE02138120)	Pseudomonas aeruginosa (strain PA7)	0.0	-77/-64	-	15.09	...GGAAAGCCTGTTGATTTGTATCAAGCGGGTGAAT...
GBpro	IGI02711635 (GE01590752)	Pseudomonas mendocina (strain ymp)	0.0	-72/-59	-	14.54	...ACAAGGGGCCTTGACCTGAATCAAGGGCACGTGG...
GBpro	Pfl_1813 (GE00837947)	Pseudomonas fluorescens (strain PfO-1)	0.0	-77/-64	-	14.55	...AAAAGGCATCTTGATTTGCATCAACGGGATCAGT...
GBpro	hemN (GE01258374)	Pseudomonas entomophila (strain L48)	0.0	-76/-63	-	14.89	...CTGGGGCATGTTGATTTGCATCAAGGGCGTTCGC...
GBpro	IGI03537844 (GE01754841)	Pseudomonas putida (strain GB-1)	0.0	-77/-64	-	14.65	...GTAATCGGTGTTGATTTGTATCAAGGGCATTGGG...
GBpro	IGI03665760 (GE01474212)	Pseudomonas putida (strain W619)	0.0	-77/-64	-	14.60	...GGAATCTGTGTTGATTTGTATCAAGAGCTACGGG...
GBpro	HemN (GE00183645)	Pseudomonas putida (strain KT2440)	0.0	-77/-64	-	14.61	...GGAAACTGTGTTGATTTGTATCAAGGGCATTGGG...
GBpro	IGI02861950 (GE01707228)	Pseudomonas putida (strain F1 / ATCC 700007)	0.0	-77/-64	-	14.64	...GGAAACTGTGTTGATTTGTATCAAGGGCATTGGG...

Sequence Logo (http://weblogo.berkeley.edu/)

Match Distribution

Figure 2. SMILE analysis using the Anr binding site in the promoter of the *hemN* gene. The results show both a high evolutional and positional conservation between the orthologous promoters (the list of matches was shortened).

DATABASE ACCESS

There are principally four different ways to access PRODORIC:

(i) Submitting a database query via the supplied web forms.
(ii) Browsing through the content by the use of genome browser GBpro.
(iii) Exploring the regulatory network as visualized graph with the ProdoNet tool.
(iv) Accessing the database via webservices [Simple Object Access Protocol (SOAP) interface].

The previously developed PRODORIC web interface was significantly improved with regard to its design, handling and web browser support. Database queries with genes, proteins, TFBSs and PWMs can be submitted via web forms. We added new sections for searching promoters, expression profiles and whole regulons. Besides the regular web forms, various improved possibilities for interactive browsing of the database contents were implemented. The new version of the genome browser GBpro offers an improved presentation of gene regulatory features both as genome map and formatted sequence. In this context, the application of inline frames enabled a more convenient browsing through the database contents. We recently developed ProdoNet, a new visualization tool for the exploration of PRODORIC contents in an interactive graph view (14). This tool enables the detection and visualization of underlying gene regulatory networks to uncover the multiple levels of gene regulation like regulatory circuits and various network motifs. Moreover, ProdoNet allows for the mapping and visualization of sets of co-expressed genes to gene regulatory network graphs. A different method to query the database without using the webpages was implemented recently via the establishment of webservices using SOAP. These webservices enable a platform-independent access to PRODORIC and offer an interactive way for data integration which was realized first for the SYSTOMONAS and ROSY platforms (15,16). A more detailed description of the SOAP interface and application examples are available on the PRODORIC website.

PREDICTION OF GENE REGULATORY NETWORKS

Although the PRODORIC core database excludes computationally predicted data, we follow the approach of a database assisted interactive prediction and validation of gene regulatory networks. Produced results are usually most accurate since they are based on the most recent set of data. For this purpose we developed Virtual Footprint, a tool for the prediction of potentially new transcription factor targets (12). Various search patterns can be defined by PWMs, IUPAC consensus strings or regular expressions. Complex bipartite patterns consisting of two subpatterns separated by a spacer are also possible. The integrated PWM library derived from the PRODORIC dataset was extended to 197 patterns corresponding to 163 different transcription factors (Table 1). The Virtual Footprint program allows the analysis of complete genomes with one PWM, which is called ‘regulon analysis’. In the other program mode ‘promoter analysis’, all available patterns are applied on one sequence. The new PRODORIC release 2009 was supplemented with a new tool called SMILE (similar intergenic location analyzer). Using this novel tool, the evolutionary conservation of Virtual Footprint matches can be further investigated by a comparative analysis of orthologous promoter sequences similar to a regulog analysis (17). In SMILE both sequence and positional conservation within an orthologous group of matches can be analyzed. This approach enables the evaluation of putative transcription factor targets and helps to rule out false-positive predictions (Figure 2).

CONCLUSIONS

PRODORIC is a manual curated data resource and bioinformatics tool platform about gene regulation and gene expression covering all sequenced prokaryotes. The whole system is supplemented with various browsing, prediction and validation tools representing a framework for the interactive analysis and visualization of gene regulatory networks. The manual curation process of PRODORIC will be continued. Mapping of gene regulatory interactions on sequenced genomes will be one of the most challenging task. The availability of reliable gene regulatoy networks will be essential for modeling approaches in systems biology.

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