EvoMining 2.0: A customizable computational pipeline for evolutionary reconstructions during genome mining

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## Abstract

## Introduction

## Results and Discussion

### Section 1 EvoMining 2.0 Updated model and pipeline

EvoMining is a visual genome mining tool that following evolutionary principles has the milestone of prioritize non standard secondary metabolite pathways. The algorithm follows enzyme families from central pathways on their recruitment as components of natural products biosynthetic gene clusters (BGCs) within a genomic database.

EvoMining inputs are a (1) a custom genomic database (genomic-DB), (2) a central pathways database (central-DB) and (3) a natural product database (natural-DB) composed of genes that belongs to experimentally tested BGCs. These three databases are provided and can be modified, replaced and expanded by the user. In this work genomic-DB are collection of up to date genomes in RAST format from taxonomically related organisms such as Actinobacteria, Cyanobacteria, Pseudomonas and Archaea. Selection of this taxa obeys to the possibility of comparing well known NPs producing organisms such as Actinobacteria and Cyanobacteria in contrast with Archaea that has been poorly investigated. The central-DB contains nine central pathways from Actinobacteria previously curated (Barona-Gómez, Cruz-Morales, and Noda-García 2012), plus an update of seed metabolic enzymes identified after manual curation congruent with the central EvoMining paradigm. The natural-DB currently comprises all sequences that belongs to some BGCs from The Minimum Information about a Biosynthetic Gene cluster (MIBiG) (Medema et al. 2015).

As output EvoMining identifies on the genomic-DB those expanded families from the central-DB that has at least a recruited member onto the natural-DB, proceeding then to the reconstruction of the evolutionary history of the enzyme family. Given an enzyme from the central-DB, the product of EvoMining analysis is a color coded tree of the expanded enzyme family that provides information about the metabolic fate. Specifically, enzymes from central metabolism are differentiated from known Natural Products enzymes and those expansions with potential activity into secondary metabolism are emphasised as putative novel recruitments. Further analysis of these hits allows visualization of the genomic vicinity guiding to the discovery of novel BGCs. In addition to the updates associated to the workflow of EvoMining, the version to be released will include the possibility of defining the dynamics of the gene content of any given BGC to explore the chemical plasticity related to EvoMining hits. This allows to prioritize which clusters possess more metabolite variations, therefore unmasking biosynthetic darkmatter (Medema and Fischbach 2015, Blin et al. 2017).

EvoMining code and components (blast, muscle, FastTree, newick utilities, Gblocks,apache and SVG perl module) are wrapped on the docker container nselem/newevomining downloadable at the Docker hub. Code is available at at github: nselem/EvoMining and manual at <https://github.com/nselem/EvoMining/wiki>. EvoMining tool will allow researchers to examine their own genomes and their own enzyme families in the search of expansions involved on nobel secondary metabolism.

EvoMining will identify those expanded families of the central-DB within the genomic-DB that has at least a recruited member onto the natural-DB, proceeding then to the reconstruction of the evolutionary history of the enzyme family. Given an enzyme from the central-DB, the product of EvoMining analysis is an interactive color coded tree of the enzyme expanded family where best bidirectional hits (BBH) of central-DB are differentiated from Natural Products members and those expansions close to a Natural Product sequence that are not BBH with central-DB enzymes are emphasised as putative nobel recruitments into secondary metabolism.

Genomic DB

|  |  |  |  |
| --- | --- | --- | --- |
| Feature | EvoMining 1 | EvoMining 2 | Novel insights |
| Code |  |  |  |
|  | Consulting web site | Standalone docker tool |  |
| Databases |  |  |  |
|  | Fixed | Customizable |  |
| Genomic-DB |  |  |  |
|  | Actinobacteria 230 genomes | Actinobacteria 1245 genomes | EvoMining predictions are lineage dependnet |
|  |  | Cyanobacteria 416 genomes |  |
|  |  | Archaea 876 genomes |  |
| Central-DB |  |  |  |
|  | Actinobacteria 106 families | Actinobacteria 106 families |  |
|  |  | Cyanobacteria 81 families |  |
|  |  | Archaea 80 families |  |
|  |  | RetroEvoMining XX num |  |
| Natural-DB |  |  |  |
|  | Manual curation | MiBIG |  |

### Section 2 Update and insights from genomic databases

EvoMining discovery of arsenopoliketydes was set on a genomic database of Actinobacteria, a phylum known by being source of natural products biosynthetic gene clusters. Nevertheless, EvoMining genomic model i.e. that secondary metabolism BGCs often includes enzymes from enzyme families that have been expanded and recruited to perform new metabolic functions is not exclusive of Actinobacteria. As a consecuence EvoMining can be applied to other bacterial taxonomical groups beside Actinobacteria but potential genomic databases should at least posses expansions of enzyme families from central metabolism.

As candidates to new genomic databases were selected genus Pseudomonas, phylum Cyanobacteria and kingdom Archaea. Pseudomonas and Cyanobacteria were chosen because of their known presence of biosynthetic gene clusters, there are respectively 60 and 58 BGCs reported on MiBIG. Archaea is the opposite case, there is not archaeal BGC reported on MiBIG. Archaea resembled Bacteria in the facts that i) Archaea uses horizontal gene transfer as a genic interchange mecanism, ii) Archaeal genomes contains operons (**???**) and iii) in general there is introns absence{Reference to Computational Methods for Understanding Bacterial and Archaeal Genomes}. Archaeas do have introns, but they are mainly located on genes that encodes ribosomal and transfer RNA (**???**). General lack of introns allows automatic genome annotation just like Bacteria, operon gene organization permits functional inference to a certain degree and HGT contribute to expansions on Archaeal genomes. Some phylum on Archaea has an open pangenome, and as we will show some Archaea has central pathway expansions. This lead us to think that evoMining is suitable to analize Archaeal genomes, even more if evolutionary logic behave on Archaea as on bacteria, since EvoMining is an evolutionary oriented method, not entirely based on previous knowledge of BGC's sequences, new BGC's classes may be found on Archaea.

Previous EvoMining genome-DB comprises 230 genomes from Actinobacteria with 106 central families as the corresponding central-DB. Candidate genomic databases were explore to determine wether or not other taxa posses expansions of enzymatic families devoted to central metabolism. Some families in the core genome of a taxonomical group may not exists on another, because of that, before exploring enzyme family expansions besides Actinobacteria on new genomic databases, it is needed a previous identification of central families themselves on the other taxa. A common set of central enzyme families was determined for *Actinobacteria*, *Cyanobacteria*, *Pseudomonas* and *Archaeas* by finding best bidirectional hits (BBH) of original 339 seeds belonging to the 106 central Actinobacteria enzyme families on the following genomes: 1)Cyanobacteria phylum: \* Cyanothece sp. ATCC 51142*,* Synechococcus sp. PCC 7002 CP000951.1*,* Synechocystis sp. PCC 6803 AP012205.1*, 2)Pseudomonas generus* Pseudomonas fluorescens pf0-1*,*Pseudomonas protegens Pf5*,*Pseudomonas syringae pv. actinidiae ICMP 18884 NZ\_CM0027 51.1*,*Pseudomonas fulva 12-X NC\_015556.1\* 3) Archaea kingdom *Natronomonas pharaonis*,*Methanosarcina acetivorans*, *Sulfolobus solfataricus* and *Nanoarchaeum equitans Kin4-M*. To avoid missing BBH due to genome quality selected genomes were chosen to be on one contig. As an outcome of this process a central database with 42 enzyme families common to all taxonomical groups was assembled. Candidate genomic databases were integrated by 876 Archaea genomes, 416 Cyanobacteria genomes, and 219 Pseudomonas genomes. Actinobacteria database was expanded from 230 to 1244 Actinobacteria genomes. Finally, with the central and genomic databases as described, taxas *Actinobacteria*, *Cyanobacteria*, *Pseudomonas* and *Archaeas* were explored in search of central enzyme families expansions. (Figure 2A)

As it could be expected, central families expansions increases whith genome size, nevertheless at different velocities and not linearly, the growth rate is different on each genomic group. Archaea, Cyanobacteria, Actinobacteria and Pseudomonas expansions behave similar until a genome size of 5,000,000 bp, after that genome size Pseudomonas number of elements on the 42 expanded central families grows faster than Actinobacteria, which in turn surpasses Cyanobacteria and Archaea, but Archaea does not have until now many genomes bigger than 5000000 bp. *Streptomycetales* and *Nostocales* orders from Actinobacteria and Cyanobacteria respectively are among the orders with bigger genome size and also bigger Number of expansions. Some of the biggest Cyanobacteria genomes may be a contamination (Hassalya bisoydea).

Now that a common set of central enzymes are determined, and that it is shown that all genomic database posses expansions of central families, even at different rates, differences of the families across selected taxa can be explored.

#### 2b Diversity on central Families according to the lineage

Expansions and recruitments of the 42 common central families were quantified by EvoMining on *Actinobacteria*, *Cyanobacteria*, *Pseudomonas* and *Archaeas* (Figure 2B). Families like Wether a family is expanded or not depends on the taxa selected as genomic-DB

This results showed that expansion of a central family is clade specific, a family can be expanded on Actinobacteria but not on Cyanobacteria, even more a family can be part of the core of Actinobacteria but not exist on Archaea. Expansions were find to be similar in these 4 taxonomical groups,

On cyanobacteria PriA an enzyme with no previously knwon secondary metabolism on Actinobacteria has been recruited into saxitoxin cluster.  
Para aumentar la base de datos tomamos retroevomining y obtuvimos 2 en comun.. falta considerar las super duplicadas... Finalmente tomamos una enzima del shell en lugar de el core. (Puede ser del shell de streptomyces)

### Section 3 Update and insights from central DB

core/ shell genome Expansions other central  
To limitate the search for enzymes of recent recruitment into natural products

As we have explored other genomic databases, central database customization is also implemented, on this case we have use TauD en enzyme from taurine assimilation system on enterobacteria, on the genomic database Pseudomonas. Enzymes does not necessary have to be central, in the sense that they belong to the core of its taxonomic group, but at least should have expansions.

### Section 4 Update and insights from Natural products DB

Backward EvoMining

### From Backward EvoMining natural products DB

235 Enzymes from BGC clusters were tested 55 reduced  
56 intermediate  
124 Super

3.2(Backward Evening)  
Coelicolor clusters

where Orgs stands for organisms with at least one copy and copies is the total number of copies on that family on the genomic-DB

Esto se hará solo sobre Streptomyces de los 1246

HERE TABLE WITH CLUSTERS

Coelicolor

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Full...partial | Main.product | Biosynthetic.class | Organism | X..Backward.EvoMining.Hits | Open.closed |
| BGC0000038 | Full | coelimycin | Polyketide | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0000194 | Full | actinorhodin | Polyketide | Streptomyces coelicolor A3(2) | NA | NA |
| GC0000315 | Full | calcium-dependent antibiotic | NRP | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0000551 | Full | sapB | RiPP | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0000595 | Full | SCO-2138 | RiPP | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0000849 | Full | gamma-butyrolactone | Other | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0000940 | Full | desferrioxamine B | Other | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0000324 | Partial | coelibactin | NRP | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0000325 | Partial | coelichelin | NRP | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0000660 | Partial | albaflavenone | Terpene | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0000663 | Partial | hopene | Terpene | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0000910 | Partial | melanin | Other | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0000914 | Partial | methylenomycin | Other | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0001063 | Partial | undecylprodigiosin | NRP / Polyketide | Streptomyces coelicolor A3(2) | NA | NA |
| BGC0001181 | Partial | geosmin | Terpene | Streptomyces coelicolor A3(2) | NA | NA |

Presence Absence EvoMining was run over enzymes with expansion number between .1 and .6 Figuras/GenomicDBS.svg

## Methodology

EvoMining algorithm and all its dependencies: blast, muscle, Gblocks, Quicktree and newick utilities were packaged on a docker container available at dockerhub. Every EvoMining container has the code inside which turns EvoMining in a totally open standalone genome mining tool, Dockerfile and code are available at github. EvoMining inputs are a genomic database (genomic-DB), a seed family enzymes database from central or shell metabolism (central-DB), and a set of sequences experimentally tested to be part of natural products biosynthetical gene clusters (np-DB), optionally a database containing antiSMASH analysis of the genomic DB may be integrated to the pipeline. To standardize protein annotation and take advantage of functional annotation genomes on genomic-DB were processed by the Rapid Annotation using Subsystem Technology (RAST) platform. Sequences of the central-DB and np-DB must be on fasta format. MIBiG repository was used and is included on EvoMining docker image as default np-DB, metadata such as producer organism and kind of compound are also integrated to the np-DB.

Once databases are set analysis of expansions-recruitment of enzyme families from central-DB within the context of the genomic and natural product databases is performed by EvoMining. The first step, initialized by the user trough the web interface, is to retrieve from the genomic-DB all those sequences that belong to the expanded families enzymes of the central-DB. To this end a blastp search (default:e-value 0.001, score 100) using as queries seed enzymes from the central-DB is run against the genome-DB. Next it is established which sequences are the most conserved respect to the central-DB, to this end best bidirectional hits between the expanded families and central-DB are identified and stored in a temporal database (conserved-db). Next a heat plot is displayed pinpointing by family which organisms posses an expansion on its copy number, on this work an expansion is understood as an organisms that posses a number of sequences on certain family above the average plus an standard deviation. To identify expanded enzyme families that may also have been recruited into secondary metabolism, i.e. which enzyme families has some members into the natural product database, a new blastp search (e-value 0.001) is run onto the np-DB using as queries all sequences on expanded families.

At this point families that exhibits expansions and recruitments may be selected by the user to posterior phylogenetical analysis. Selected expanded-recruited enzyme families are then aligned with muscle, automatically curated by Gblocks, and phylogenetically reconstructed by Quicktree. Once the tree is available EvoMining gives evolutionary insights by coloring the expanded family differentiating its history as follows: in red enzyme sequences stored on the conserved-DB, in blue sequences from np-db, on cyan antiSMASH known hits if antiSMASH-DB was provided, and in green EvoMining predictions. An EvoMining prediction are all those enzymes divergent enough from central metabolism that were not red marked and similar enough to secondary metabolism that are closer to a np-DB sequence (blue) than to a conserved-db sequence (red). All enzymes not marked on red or cyan close to a blue enzyme and that are not on sister leaves with a red enzyme are green colored. This algorithm leaves black leaves on many cases.

To study the nature of expansions on other taxonomic groups beside Actinobacteria, three genomic databases were integrated by 1245 Actinobacteria, 416 Cyanobacteria, 876 Archaea, all genomes were annotated by RAST and mined by AntiSMASH stand alone docker parameters XXXXX. For this work Actinobacteria central-DB is composed by 295 Actinobacteria sequences of central enzymes organized in ninety-three families involved in nine pathways, including amino acid biosynthesis, glycolysis, pentose phosphate pathway, and tricarboxylic acids cycle. Seed sequences were taken mainly from 1) *Streptomyces coelicolor A3*, 2) *Mycobacterium tuberculosis H37Rv* and 3) *Corynebacterium glutamicum*. To integrate Cyanobacteria central-DB best bidirectional hits of Actinobacteria central-DB were selected among three organisms distributed on Cyanobacteria phylum 1)\* Cyanothece sp. ATCC 51142*, 2)* Synechococcus sp. PCC 7002 CP000951.1*, 3)* Synechocystis sp. PCC 6803 AP012205.1*. Same procedure was taken to assemble Archaea central-DB, selecting organisms from diverse sections of Archaea kingdom, on this case 1)* Natronomonas pharaonis*, 2)* Methanosarcina acetivorans*, 3)* Sulfolobus solfataricus\* and 4) *Nanoarchaeum equitans Kin4-M*. Expansions of enzyme families shared between this three taxonomic groups were calculated with EvoMining default parameters. Graphics for the data analysis on this work were generated by r, to guarantee reproducibilty a rmarkdown document is available at: github/nselem/phd. Finally, EvoMining evolutionary analysis was conducted on PriA enzyme family on the three genomic database with default EvoMining parameters.

To amplify the concept of central-DB to shell-DB allowing to explore expansions beyond just enzymes belonging to the core genome of a taxonomic group a shell enzyme was explored. TauD, an enzyme that belongs to the shell genome on Pseudomonas was selected as seed of central-DB. Pseudomonas genome-DB was integrated as follows (KARINA como integraste la base de Pseudomonas, how where nps predicted). Genomes on genome-DB were RAST annotated and on this case no antiSMASH mining was conducted. EvoMining expansion-recruitment and phylogenetical analysis with default parameters was conducted for TauD enzyme family.

Backward Enzyme sequences from S. coelicolor and S. lividans biosynthetic gene clusters were retrieved from MiBiG to test how will behave as seeds from backward EvoMining  
Average copy number  
 All databases are downloadable at Zenodo (Doi numbers genomic-DB: STREPTOMYCES, Actinobacteria, Cyanobacteria, Pseudomonas, Archaea, central-DB: Actinobacteria, Cyanobacteria, Pseudomonas, Archaea, lividans/coelicolor BGC ) #### Discussion During the decade between 1970 and 1980, Archaea was recognized as new life domain, a kingdom different from Bacteria and Eucarya in an exciting first great application of 16S phylogeny(**???**,(**???**)) . Main differences between this kingdoms are that Archaeal DNA is not arranged in a nucleus as in Eucarya and Archaeal celular walls are not composed from peptidoglycans as in Bacteria. Archaeal proteins may be higlhy valuable to biotechnology industry for their great stability due to extreme temperature, PH and salt content conditions on Archeal habitats. Despite no Archaeal Natural products biosynthetic gene clusters (BGC's) has been reported on MiBIG, Archaea do have BGC's, some of them seems to be acquired by horizontal gene transfer (HGT) like methano nrps {search reference}. Other Archeal natural products known are archaeosins, Diketopiperazines, Acyl Homoserine Lactones, Exopolysaccharides, Carotenoids, Biosurfactants, Phenazines and Organic Solutes but this knowledge is not comparable to Bacterial BGC's knowledge(**???**).

Natural products biosynthetic gene clusters search is actually performed using either *high-confidence/low-novelty or low-confidence/high-novelty* bioinformatic approaches (Medema and Fischbach 2015). High confidence methods compares query sequences with previously known BGC's such as nrps or PKS, examples of this algorithms are antiSMASH and clusterfinder (**???**?). EvoMining searches on expansions from central metabolic pathways enzyme families, it has been classified as low confidence/high novelty method. EvoMining has proved useful on Actinobacteria phylum where its use lead to Arseno-compounds discovery (Cruz-Morales et al. 2016). Also on Actinobacteria antiSMASH analysis on 1245 genomes found 774 different classes of natural products, the same analysis on 876 Archaeal genomes, a full kingdom, identifies only 35 BGC's classes. So either Archaea does not have natural products BGC's or this are not yet known. Next paragraph deals with a possible approach about how natural products BGC's can be find.

EvoMining is a trade off between conserved known central metabolic function and enough expansions divergence on sequence and on clusters to divergence

## Figures

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[@dufresne\_algorithmique\_2016,@blin\_recent\_nodate,@kurtboke\_revisiting\_2017,@miller\_interpreting\_2017,@schniete\_expanding\_2017,@kim\_recent\_2017,@robertsen\_toward\_2017,@juarez-vazquez\_evolution\_nodate,@chavali\_bioinformatics\_nodate,@tracanna\_mining\_2017,@ren\_breaking\_2017,@choudhary\_current\_2017,@alanjary\_antibiotic\_2017,@chevrette\_sandpuma:\_2017,@wohlleben\_antibiotic\_2016,@weber\_secondary\_2016]

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