

Bioinformatics Practicals In Silico

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Introduction

Bioinformatics is the application of computational technology to handle the rapidly growing repository of information related to molecular biology. Bioinformatics combines different fields of study, including computer sciences, molecular biology, biotechnology, statistics and engineering. It is particularly useful for managing and analysing large sets of data, such as those generated by the fields of genomics and proteomics.

In this report we focus on the bioinformatics tools for mutant analysis through three different projects; mutations in *gai* and *spy* in *Arabidopsis Thaliana*, mutations in *Saccharomyces cerevisiae* and mutations as well as Denovo assembly in *Lactobacillus Helveticus*. We want to sort out new mutation with these tools and learn how to design a bioinformatics test. It includes the quality test, the annotation of our sequenced genomes and various analysis of these results. Thus, everything upstream of the analysis must be properly done, using several software described thereafter. Our machines are too weak in order to analyse the data and performed the bioinformatics steps, thus, we will use a cluster dedicated for this lecture, in Bern Switzerland.

Yeast Genome Analysis

Introduction

Biological introduction The budding Yeast *Saccharomyces cerevisiae* is a common organism used for genetics manipulation. This organism is well conserved among the eukaryote and can be used correlate with human pathways. With a genome with 16 chromosomes (haploid, Mat α) or 32 chromosomes (diploid). 99% of the genome is without introns, make this organism handy to manipulate. 12 million bases pair and contains between 5 800 to 6 572 genes [TODO REF]. The homology with human is estimate to 23%, which is a good candidate for preliminary studies regarding human pathways. The short mating time and growth is also short. Thus, the identification of potential mutant is grandly enhanced. This is a single eukaryotic organism with a division cycle of 90 minutes. Through the process of budding in which smaller daughter cells pinch, or bud, off the mother cell. Due to the microscopic size (5 microM, between bacteria and human cell size) and simple growth environment, yeasts are inexpensive and easy to grow in silico. *Saccharomyces cerevisiae* is also no-pathogen, and forms colonies on agar plates in the laboratory in a few days with no special incubators required (best grow at 30 deg).

tom1

Methods

Arabidopsis Thaliana Genome Analysis

Introduction

GAI Gibberellic-Acid Insensitive is a gene in *Arabidopsis thaliana* in chromosome 1 which is involved in the regulation of plant growth. Precisely, it mediated the input signals and module the growth by decreasing the responsiveness to gibberellin. Gibberellin is a tetracyclic diterpenoid growth factor and influence essentially the stem elongation and other plant developmental processes. If it's mutated (*gai*) and the plant growth better, it's a gain of function gene, in contrary it's a loss of function. The cellular *gai*'s component is in the nucleus and is described as a transcription region of DNA and bind it directly. The mutation in *SPY* (*spy*) is a suppressor of *gai*, conferring to the plant a normal phenotype. GA-deficient *Arabidopsis* mutants display characteristic phenotypes, including dark green leaves and a dwarf growth habit attributable to reduced stem elongation¹. The *gai* mutation affects GA reception or subsequent signal transduction and does not result in GA deficiency. *Gai* encodes a mutant protein that lacks a region of 17 amino acids from close to the N terminus and confers a dominant dwarf, reduced GA-response phenotype. The *gai* allele contains a deletion of 51-bp from within the *GAI* ORF. This in-frame deletion results in the absence of a 17-amino-acid residue segment situated close to the amino terminus of the predicted protein sequence.

SPY For *spy*, three independent recessive mutations at the *SPINDLY* (*SPY*) locus of *Arabidopsis* confer resistance to the gibberellin (GA) biosynthesis inhibitor paclobutrazol. Paclobutrazol or α -tert-Butyl- β -(4-chlorobenzyl)-1H-1,2,4-triazole-1-ethanol, is a plant growth retardant. It is an antagonist of the plant hormone gibberellin. It works by inhibiting gibberellin biosynthesis by inhibiting endoplasmic reticulum monooxygenases. Relative to wild type, *spy* mutants exhibit longer hypocotyls, leaves that are a lighter green colour, increased stem elongation, early flowering, parthenocarp, and partial male sterility. All of these phenotypes are also observed when wild-type *Arabidopsis* plants are repeatedly treated with gibberellin A3 (GA3). The *spy*-1 allele is partially epistatic to the *gai*-2 mutation, which causes GA deficiency. In addition, the *spy*-1 mutation can simultaneously suppress the effects of the *gai*-2 mutation and paclobutrazol treatment, which inhibit different steps in the GA biosynthesis pathway. This observation suggests that *spy*-1 activates a basal level of GA signal transduction that is independent of GA.

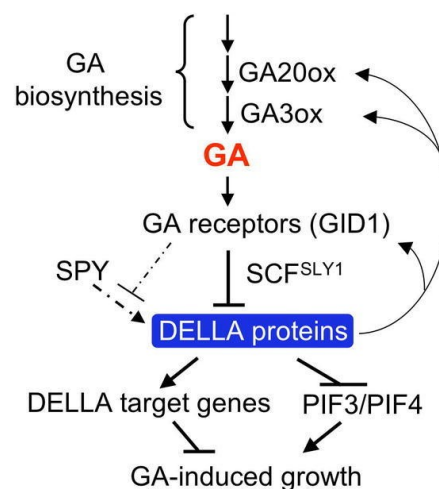


Figure 1: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3243332/figure/i1543-8120-64-1-1-f21/>

Methods

Lactobacillus Helveticus Genome Assembly

Introduction

The diverse bacteria involved in cheese production are essential for the texture and taste development but also, during the ripening process, the microbial changes helps to kill pathogens and reduce spoilage micro-organisms. *Lactobacillus helveticus* is a thermophilic lactic acid bacterium (LAB) used in the dairy industry as a starter or an adjunct culture for cheese manufacture [1]. By releasing **peptidoglycan hydrolases**(PGHs), it has the ability to digest the bacterial cell wall (gram+) inducing death of surrounding bacteria but also its autolysis.

The genomic plasticity of *Lactobacillus helveticus* leads to a high variation in PGHs activity from one strain to another. In a previous study, the activity of a PGH with an estimated size of 30kDa was tested by zymography in nine strains of *Lactobacillus helveticus* of which six were sequenced (see figure 2). Two phenotypes were shown: phenotype A exhibits PGH activity (strains **FAM8102c1c1**, **FAM23285** and **FAM19191**) and phenotype B does not (strains **FAM22016**, **FAM1450** and **FAM1213**).

The aim of this work was to detect potential genomic differences involved in the two different phenotypes by sequencing, assembling and compare the genome of the six strains using a previously annotated reference genome of *Lactobacillus helveticus* (NC_010080). A potential candidate present only in the strains expressing a PGHs activity suggests that it might have been acquired by a viral insertion.

Methods

Sequencing and genome assembly The six *Lactobacillus helveticus* strains **FAM8102c1c1**, **FAM23285**, **FAM19191**, **FAM22076**, **FAM1450**, **FAM1213** were sequenced by Illumina sequencing. The following tasks were performed using the cluster provided by the University of Bern. *FastQC* [2] was used to check the quality of the reads and *Trimmomatic* [3] to filter out bad quality reads. *SOAPdenovo* [4] as well as *Spades* [5] were used to perform the genome assembly with the reads of each strains. For *SOAPdenovo* the k-mer sizes were set to 95, 85, 75 and 65. For *Spades* k-mer sizes were set to 21, 33, 55, 77 and 99 (default values). The four assemblies of SOAPdenovo and the assembly of Spades were compared using Abyss with a maximum number of contigs set to 1000. The best genome assemblies with the bigger N50 and a approximate genome size of 20Mbp (Genome size of *Lactobacillus helveticus*) were then chosen¹.

Genome annotation and pan-genome analysis We used the *PROKKA* pipeline [6] to annotate the genome of the six best assemblies and the reference genome for *Lactobacillus helveticus* NC_010080. *PROKKA* is an automated pipeline that annotates prokaryotic genomes. It locates open reading frames and RNA regions on contigs and translates it to protein sequences, searching for protein homologues in public databases. The resulting standards .gff files containing the annotated genome for each strain are then used by *Roary* [7] to generate a pan-genome of the six strains. The result was then visualized with *Phandango* [8] allowing visualisation of phylogenetic tree, associated metadata and genomic information.

Extraction of the genes for each phenotypes Grep was applied to the files generated by *Roary* to extract the nine PHG's [1] labelled "Lhv_" with *PROKKA* (table 2). The set of genes

¹Due to the temporary unavailability of the cluster, this operation has been performed by L. Falquet and the results were provided to the students afterwards.

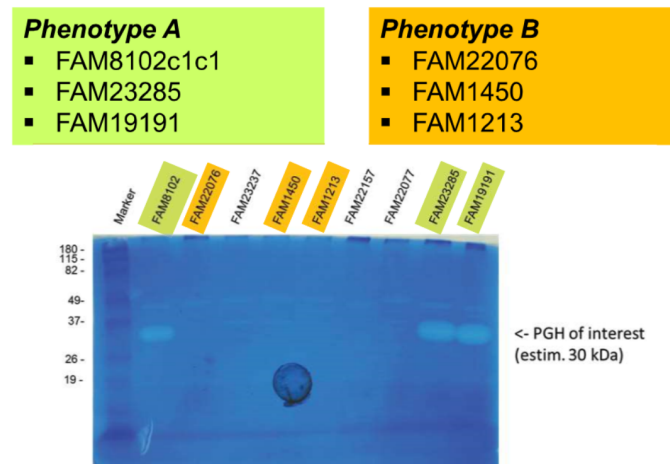


Figure 2: Phenotype A is expressing an active peptidoglycan hydrolase and phenotype B is not.

found in strains expressing phenotype A was then compared to the set of gene showing phenotype B. In table 1 we have the two PGHs present only in the three strains expressing the PGHs activity. The nucleotide sequences were then converted to amino acid sequences for further comparison.

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1 CLUSTAL format alignment by MAFFT L-INS-i (v7.310)
2
3
4 FAM19191_1K_003 MTSRQLGVDVAVYQGTSMYAHNAGAKFGIAKLTEGTNYVNPKAHYQIKSLHANHMYVHA
5 FAM23285_1K_004 MTSRQLGVDVAVYQGTSMYAHNAGAKFGIAKLTEGTNYVNPKAHYQIKSLHANHMYVHA
6 FAM8102_1K_0056 MTSRQLGVDVAVYQGTSMYAHNAGAKFGIAKLTEGTNYVNPKAHYQIKSLHANHMYVHA
7 .....
8
9 FAM19191_1K_003 YHFATFGYSVSRKLEGKAFVKRAKAENISKKRFLWLDWESGSGNCVTGGKAASTKAILA
10 FAM23285_1K_004 YHFATFGYSVSRKLEGKAFVKRAKAENISKKRFLWLDWESGSGNCVTGGKAASTKAILA
11 FAM8102_1K_0056 YHFATFGYSVSRKLEGKAFVKRAKAENISKKRFLWLDWESGSGNCVTGGKAASTKAILA
12 .....
13
14 FAM19191_1K_003 FMKVCHDAGYKVGLYSGASLLRNNIDTKQIVKKYGTCTIWSASYPTDLAYTPNFNYFPSMD
15 FAM23285_1K_004 FMKVCHDAGYKVGLYSGASLLRNNIDTKQIVKKYGTCTIWSASYPTDLAYTPNFNYFPSMD
16 FAM8102_1K_0056 FMKVCHDAGYKVGLYSGASLLRNNIDTKQIVKKYGTCTIWSASYPTDLAYTPNFNYFPSMD
17 .....
18
19 FAM19191_1K_003 GVAIWQFCDNWKGLGVDGNISLIDLHKDSAGKKVTKPAEKPKEKPEKKTGVVYAPVINRN
20 FAM23285_1K_004 GVAIWQFCDNWKGLGVDGNISLIDLHKDSAGKKVTKPAEKPKEKPEKKTGVVYAPVINRN
21 FAM8102_1K_0056 GVAIWQFCDNWKGLGVDGNISLIDLHKDSAGKKVTKPAEKPKEKPEKKTGVVYAPVINRN
22 .....
23
24 FAM19191_1K_003 PNWMIQLMDGNHGYTGKYIKTNTRWKYFDVKTIGMKCYKLGTDKQWVPAKFLKVE
25 FAM23285_1K_004 PNWMIQLMDGNHGYTGKYIKTNTRWKYFDVKTIGMKCYKLGTDKQWVPAKFLKVE
26 FAM8102_1K_0056 PNWMIQLMDGNHGYTGKYIKTNTRWKYFDVKTIGMKCYKLGTDKQWVPAKFLKVE
27 .....

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Figure 3: Alignment of amino acid sequences of group 2372 for the three strains.

Results

Gene	Annotation	Avg group size nuc	FAM19191_1K	FAM23285_1K	FAM8102_1K
group_2348	Lhv.2053 Lysin (<i>L.crispatus</i>) pseudo-gene in <i>L.helveticus</i>	1121/ 41 kDa	FAM19191_1K.00069	FAM23285_1K.00060	FAM8102_1K.00069
group_2372	Lhv.2053 Lysin (<i>L.crispatus</i>) pseudo-gene in <i>L.helveticus</i>	893/ 33 kDa	FAM19191_1K.00397	FAM23285_1K.00499	FAM8102_1K.00565

Table 1: Genes present only in the three strains with a PGH activity.

According to figure 2, the PGH involved is approximately 30kDa thus matches with group 2372. Looking at the alignment of the amino acid sequences (Figure 3) we see that the sequences are identical thus showing a great conservation between the three strains.

Gene	Annotation	FAM1213_1K	FAM1450_1K	FAM19191_1K	FAM22076_1K	FAM23285_1K	FAM8102_1K
group_1103	Lhv_0549 N-acetylmuramidase	FAM1213_1K_01187	FAM1450_1K_00785	FAM19191_1K_01147	FAM22076_1K_00934	FAM23285_1K_01072	FAM8102_1K_01185
group_1218	Lhv_1433 Lysin	FAM1213_1K_01833	FAM1450_1K_00044	FAM19191_1K_01884	FAM22076_1K_01582	FAM23285_1K_01903	FAM8102_1K_01986
group_3457	Lhv_0649 Lysozyme	FAM1213_1K_00895	FAM1450_1K_00838	FAM19191_1K_01232	FAM22076_1K_00917	FAM23285_1K_01191	FAM8102_1K_01268
group_852	Lhv_1295 Enterolysin M23 family peptidase	FAM1213_1K_00043	FAM1450_1K_01113	FAM19191_1K_00150	FAM22076_1K_00164	FAM23285_1K_00217	FAM8102_1K_00225
group_862	Lhv_1059 LysM peptidoglycan-binding domain-containing protein	FAM1213_1K_00147	FAM1450_1K_00238	FAM19191_1K_00248	FAM22076_1K_00274	FAM23285_1K_00308	FAM8102_1K_00381
group_993	Lhv_1433 Lysin	FAM1213_1K_00691	FAM1450_1K_01203	FAM19191_1K_01800	FAM22076_1K_00088	FAM23285_1K_01748	FAM8102_1K_01891
group_995	Lhv_0191 Amidase	FAM1213_1K_00700	FAM1450_1K_00303	FAM19191_1K_00506	FAM22076_1K_00064	FAM23285_1K_00566	FAM8102_1K_00638
group_1862	Lhv_2053 Lysin (L.crispatus) pseudogene in L.helveticus		FAM1450_1K_00045	FAM19191_1K_01885	FAM22076_1K_01583	FAM23285_1K_01904	FAM8102_1K_01987
group_1899	Lhv_2053 Lysin (L.crispatus) pseudogene in L.helveticus		FAM1450_1K_00267	FAM19191_1K_00615	FAM22076_1K_00716	FAM23285_1K_00607	FAM8102_1K_00746
group_1344	Lhv_1307 Enterolysin M23 family peptidase			FAM19191_1K_00162	FAM22076_1K_00152	FAM23285_1K_00229	FAM8102_1K_00237
group_1345	Lhv_0190 N-acetylmuramidase			FAM19191_1K_00507	FAM22076_1K_00063	FAM23285_1K_00565	FAM8102_1K_00639

Table 2: PGHs in common between all strains. Extracted from the files generated by *Roary* and labeled "Lhv_" by *PROKKA*.

Discussion We can see that PGHs are present in all strains (table 2), therefore the phenotype observed in the figure 2 is not due to an absence of PGH.

Using BLASTp [9] with default parameters, the protein was searched to be a particular lysin ([WP_101853908.1](#)) encoded by the pneumococcal bacteriophage Cp-1 [10].

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