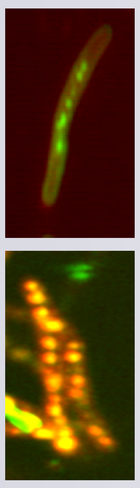
# Rhodococcus response to starving stress: Transcriptomic Analysis.

*Val Fernández Lanza and Fernando de la Cruz.*
*Departamento de Biología Molecular, Universidad de Cantabria (UC)*
&
*Instituto de Biomedicina y Biotecnología de Cantabria(IBBTEC)*

The genus *Rhodococcus* have a very versatile metabolism that allow to growth in several environments and conditions. These bacteria are able to degrate different contaminant compounds like aliphatic and aromatic hydrocarbons, oxygenated and halogenated compounds, nitroaromatics, heterocyclic compounds, nitriles, and various pesticides. Among the metabolic characteristics, *Rhodococcus* can store, under specific conditions, compounds like wax or triacylglycerol, that can be used later as carbon sources [1]. Therefore, *Rhodococcus* are microorganisms with special interest in bioremediation, biotrasformation, biocatalysis and other biotechnological aplications. We are focused on *Rhodococcus jostii*, a non-pathogenic bacteria, isolated from soil with a large chromosome (7.8 Mb) and three endogenous plasmids (1.1 Mb, 44 Kb and 33 Kb). In this study, we want to understand how *Rodococcus jostii*, under starving stress, modify its transcriptome to store triacylglycerol. For this purpose we sequenced two transcriptomes by RNA-Sequencing with a Illumina's Genome Analyzer; one of these sequences obtained from bacteria grown standard conditions and another under starving conditions. This work revealed how *Rhodococcus* is able to modify its metabolism in the absence of nitrogen and sulfites, replacing its main carbon souce (in this case Gluconate), to store triacylglycerols.

# Material & Methods

Extraction, Enrichment & RT-PCR



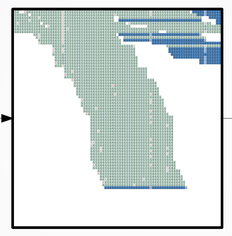
## Sequencing

```
@HWUSI-EAS1597:2:1:28:1102#0/1
GTATGGTCTAGGGGGCCCCCAAGCTTACCGAAATC
+
GG`XRPUb_TQVWON_`_GQXTF[[[YSVGO_YW\
@HWUSI-EAS1597:2:1:28:957#0/1
CCGCCCCTTCCGTTCCGCCGCTTCTCCCGACGAGG
+
BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@HWUSI-EAS1597:2:1:28:82#0/1
CGGGCCCGTGCCACTGTCCCGACTGATCCCGGCCG
+
a`a``a]R]aa`a`a^Z`aa\]_X\@]a_WFW]_X
@HWUSI-EAS1597:2:1:28:1569#0/1
CTCTCTAAAAGCTTTATCTCAACTGTGAGACTGC
+
]_WBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@HWUSI-EAS1597:2:1:28:623#0/1
CTCATCTTGTCAATGCTGCCGTGTTGGCCGACAGT
+
abaabaaaaaa`aa_a_Q_]S]U\_UV`_VS\Y^[
@HWUSI-EAS1597:2:1:28:879#0/1
CAAAGCTAGTGCTTCTCCCTCTGCAGTTTGCTATG
+
\`_\G^bVWHH\_a`a]WGRFYNHI\?]UFYa\aY
```

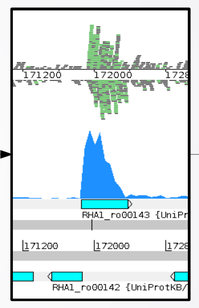
Starving Media:
3 Random Read's Sets
of 70% of total Sequencing.

Standard Media:
3 Random Read's Sets
of 70% of total Sequencing.

## Alignment.



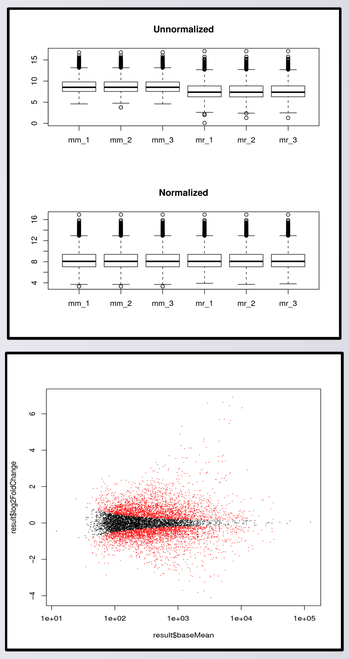
## Counts per Gene.




## Matrix's counts.

| Gen | mm | mr | mm | mr | mm | mr |
|---|---|---|---|---|---|---|
| RHA1_ro03132 | 4047 | 1577 | 5081 | 1683 | 4117 | 1578 |
| RHA1_ro00993 | 8430 | 3202 | 9474 | 3469 | 8564 | 3196 |
| RHA1_ro04342 | 491 | 190 | 565 | 190 | 496 | 190 |
| RHA1_ro02378 | 5724 | 1330 | 6427 | 1330 | 5727 | 1330 |
| RHA1_ro01671 | 403 | 245 | 441 | 280 | 406 | 245 |
| RHA1_ro03949 | 4050 | 4062 | 4644 | 4272 | 4074 | 4062 |
| RHA1_ro02879 | 1533 | 751 | 1752 | 889 | 1602 | 749 |
| RHA1_ro07232 | 1206 | 782 | 1385 | 785 | 1280 | 785 |
| RHA1_ro02046 | 9034 | 4943 | 10128 | 5310 | 9222 | 4951 |
| RHA1_ro01230 | 1332 | 329 | 1453 | 328 | 1332 | 328 |
| RHA1_ro05572 | 15756 | 9644 | 18231 | 10395 | 16245 | 9660 |
| RHA1_ro04815 | 962 | 648 | 1033 | 648 | 979 | 648 |
| RHA1_ro06442 | 51861 | 32538 | 56706 | 34249 | 52682 | 32552 |
| RHA1_ro04654 | 1803 | 680 | 1839 | 682 | 1804 | 682 |
| RHA1_ro02743 | 1293 | 805 | 1574 | 875 | 1329 | 805 |
| RHA1_ro04774 | 2703 | 1785 | 3277 | 1925 | 2752 | 1785 |
| RHA1_ro02457 | 6867 | 1784 | 7685 | 1927 | 7091 | 1787 |
| RHA1_ro06959 | 3262 | 707 | 3650 | 707 | 3294 | 707 |
| RHA1_ro00880 | 15612 | 13936 | 17892 | 14991 | 15986 | 13956 |
| RHA1_ro04674 | 5636 | 4111 | 6473 | 4379 | 5863 | 4134 |
| RHA1_ro06610 | 18270 | 10842 | 19608 | 11447 | 18414 | 10837 |
| RHA1_ro06177 | 48536 | 33691 | 53525 | 35184 | 49312 | 33695 |
| RHA1_ro04718 | 38491 | 18803 | 43184 | 20219 | 39302 | 18823 |
| RHA1_ro02929 | 3167 | 3370 | 3468 | 3485 | 3209 | 3380 |
| RHA1_ro07275 | 1890 | 908 | 2302 | 770 | 2189 | 893 |

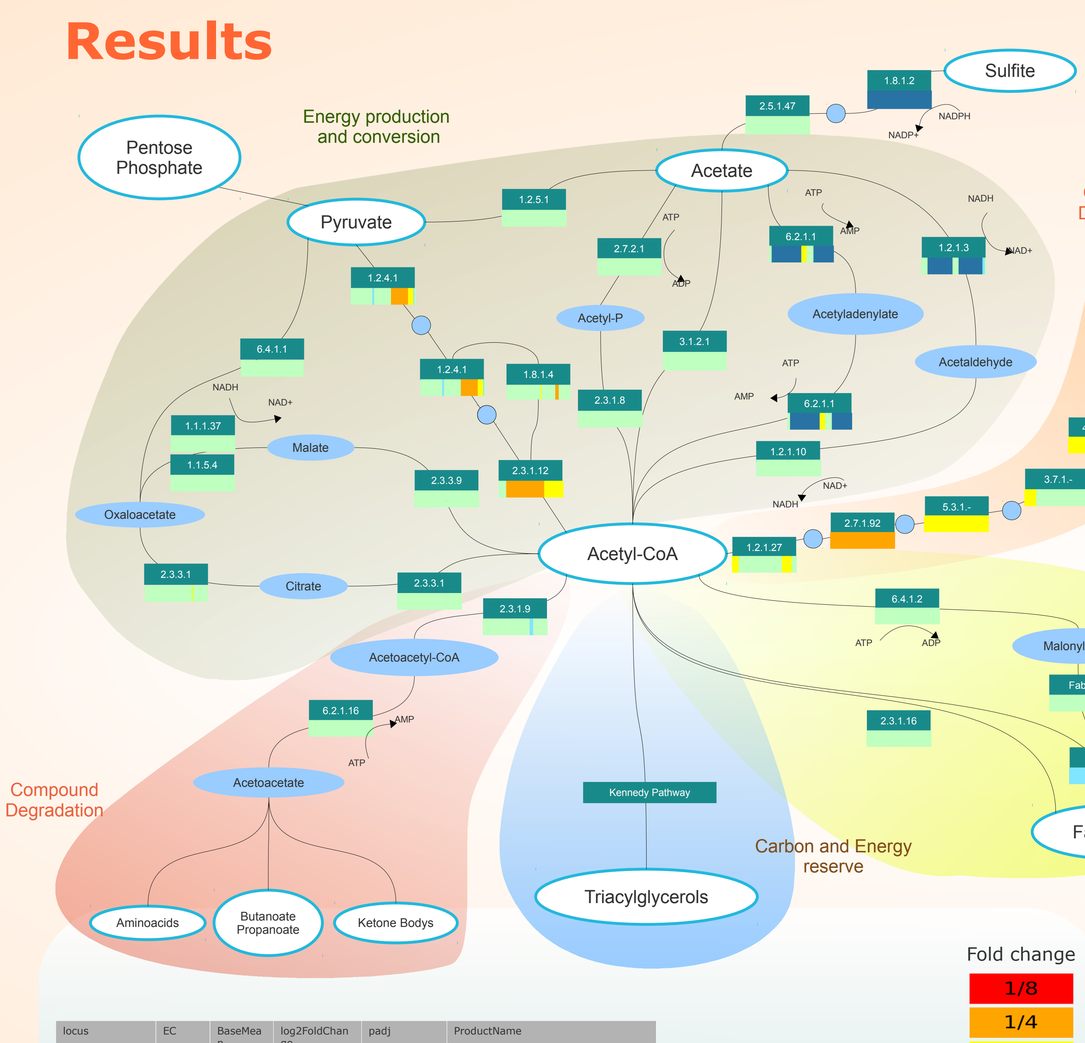
## Statistical Treatment.

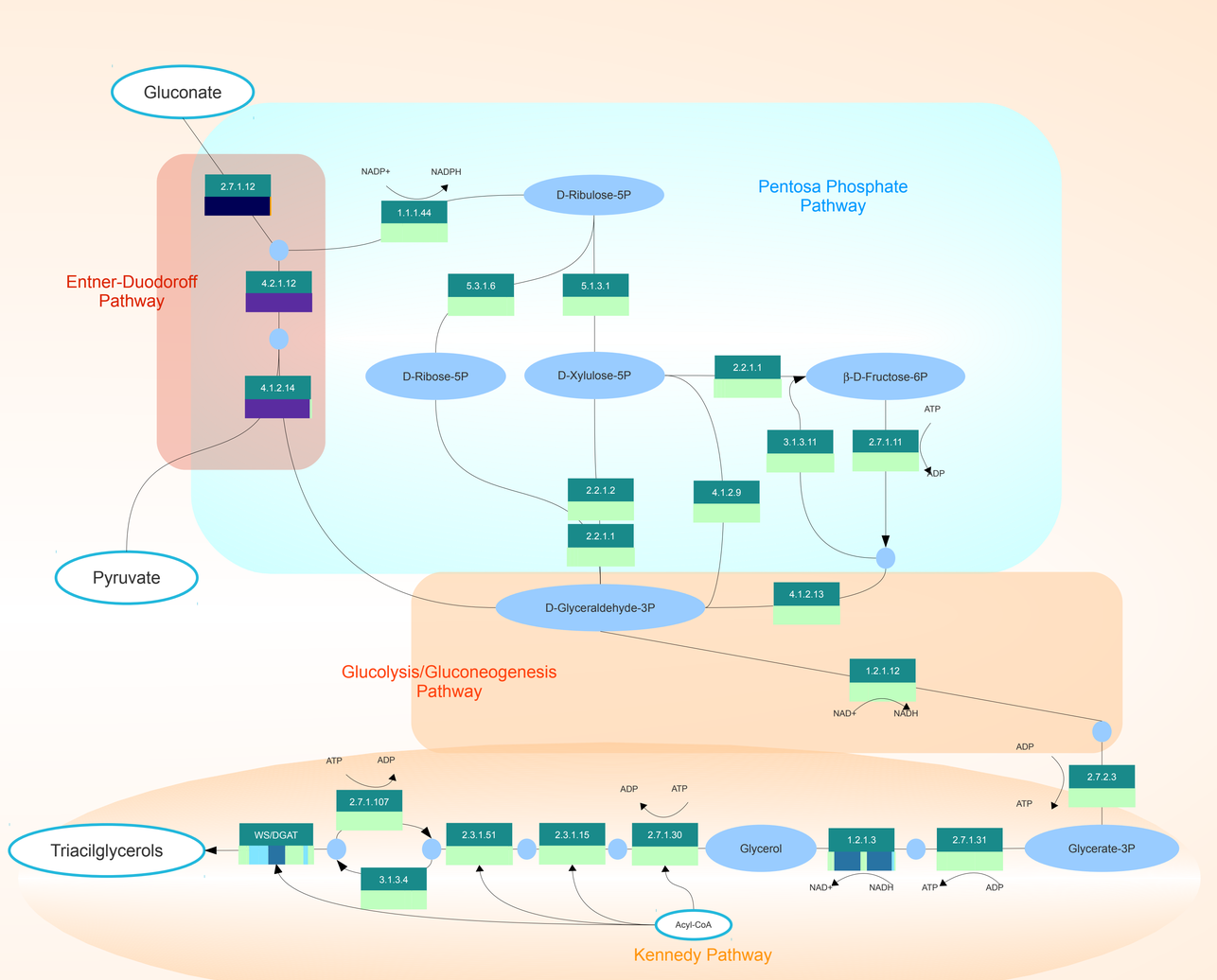



First *R. jostii* strain RHA1 was cultivated aerobically at 30°C in Streptomyces medium (Standard Media, S.M.) and then in mineral salts medium M9 (Starving Media, S.M.) according to Sambrook *et al*. 1989 . Sodium gluconate (20% w/v) were used as sole carbon source in S.M. N-limiting conditions were specified, the concentration of ammonium chloride in the M.M. was reduced to 10 mM to allow lipid accumulation [1] . Cells were grown in S.M., 25ml of cultive and harvested during exponential (D.O. 0.6) and stationary growth phases. The pellet in stationary growth phase was washed with M.M. solution and then moved into 25ml of that medium for several hours.

We made 3 sets of pseudo-replicates selecting randomly a 70% of the total sequences. The reads were alignment with Bowtie [3], with the defaults parameters, against references genomes (Genbanks CP000431-4, Chromosome and 3 plasmids). First, we calculated the reads per kilobase in each gene. Then we did quantile normalization according [4] using the R software and the *limma* package . The differential expresion was computed with the package DESeq.

# Results




Fold change

| locus | EC | BaseMean | log2FoldChange | padj | ProductName |
|---|---|---|---|---|---|
| RHA1_ro04332 | 6.2.1.1 | 22301.8 | 2.78797 | 0 | acetate--CoA ligase |
| RHA1_ro11190 | 6.2.1.1 | 15283.6 | 2.57246 | 0 | acetate--CoA ligase |
| RHA1_ro01619 | 6.2.1.1 | 998209 | 0.100911 | 0.523464 | acetate--CoA ligase |
| RHA1_ro08189 | 6.2.1.1 | 1822.33 | 0.0659582 | 0.591599 | probable acetate--CoA ligase |
| RHA1_ro08877 | 6.2.1.1 | 1296.14 | 0.0533019 | 0.712259 | acetate--CoA ligase |
| RHA1_ro10183 | 6.2.1.1 | 1915.17 | 0.0455767 | 0.711271 | acetate--CoA ligase |
| RHA1_ro02673 | 6.2.1.1 | 1134.29 | -0.383906 | 0.00442024 | probable acetoacetate--CoA ligase |
| RHA1_ro06598 | 6.2.1.1 | 4114.51 | -1.8393 | 0 | probable acetate--CoA ligase |





In this kind of representation, width of the bars represent the relative expression between isoenzimes whereas colour represent the differential expression of this isoenzime

We used the information of the pathways described in KEGG data base to analyse our results. We focused on pathways implicated in the biosynthesis and storage of triacylglycerols. We are interested in key metabolic intermediates, such as pyruvate, acetyl-CoA, and glycerol-3-phosphate. To represent the expression of the isoenzimes, we developed a method which represent, at the same time, the relative expression between the isoenzimes and the differential expression between conditions.

# Discussion

In overview, we can observe that the anabolism of Rhodococcus is downregulate. In addition, the oxidative phosphorilation machinery are upregulate to supply system the reductive power necessary to produce fatty acid. Some of the pathways involved in the catabolism of mainly metabolites are dowregulated. We observed that, Rhodococcus modifies its transcriptome, upregulation of the pathways related with pyruvate where acetate is involved. In the other hand, the Entner-Doudoroff pathway is heavily upregulate. The change of the carbon sources may be the reason for this activation, but data no present in this poster suggest that this event may be implicated in starving conditions. Finally, we observed that, in the Kennedy's pathway, main route of synthesis of triacylglycerols, the upregulation of WS/DGAT could be enough to stimulate the production of these fatty acids.

## References:

[1] Alvarez, H.M., R. Kalscheuer, and A. Steinbuchel, *Accumulation and mobilization of storage lipids by Rhodococcus opacus PD630 and Rhodococcus ruber NCIMB 40126.* Appl Microbiol Biotechnol, 2000. **54**(2): p. 218-23.
[2] Sambrook J, Fritsch EF, Maniatis T, *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989.
[3] Langmead B, Trapnell C, Pop M, Salzberg SL. *Ultrafast and memory-efficient alignment of short DNA sequences to the human genome*. Genome Biol 10:R25.
[4] James H. Bullard, Elizabeth Purdom, Kasper D. Hansen, and Sandrine Dudoit. *Evaluation of statistical methods for normalization and differential expression in mRNA-seq experiments*. BMC bioinformatics, 11(1):94+, February 2010.

## ACKNOWLEDGMENTS:

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