拟除虫菊酯降解基因的克隆及生物信息学分析

Cloning and bioinformatics analysis of gene involved in pyrethroid degradation

Group04

樊双虎 乔铖 边晨凯 王俊欢

2016.6

Background

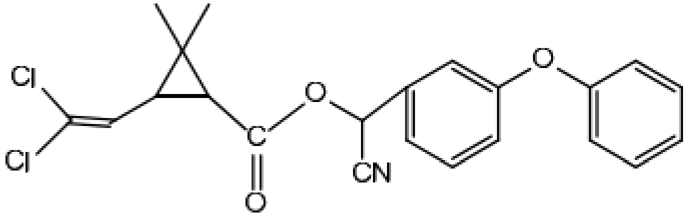
Hydrolase cloning from genomic library

Bioinformatics analysis of causal gene

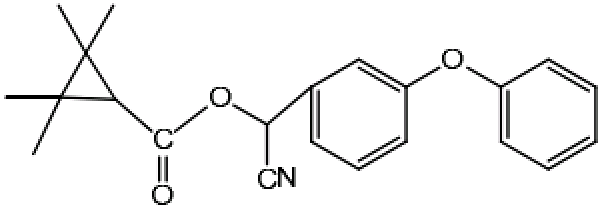
Background

Pyrethroids

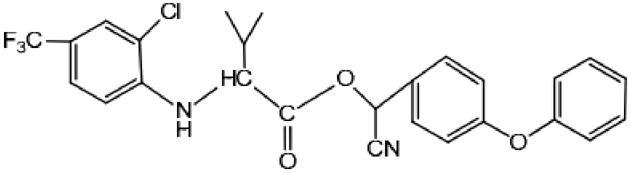
- synthetic analogs of the natural pyrethrin
- accounted for more than 30% of insecticide market
- reproductive toxicity， cytotoxicity and et al



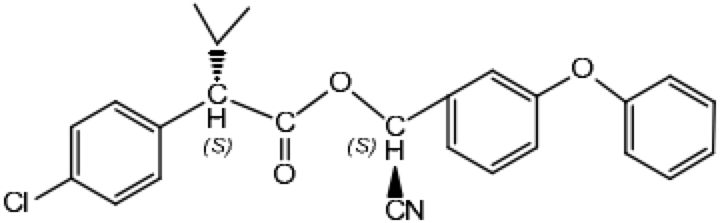
Cypermethrin



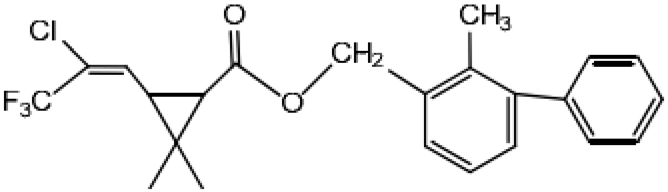
Fenpropathrin



Tau-fluvalinate

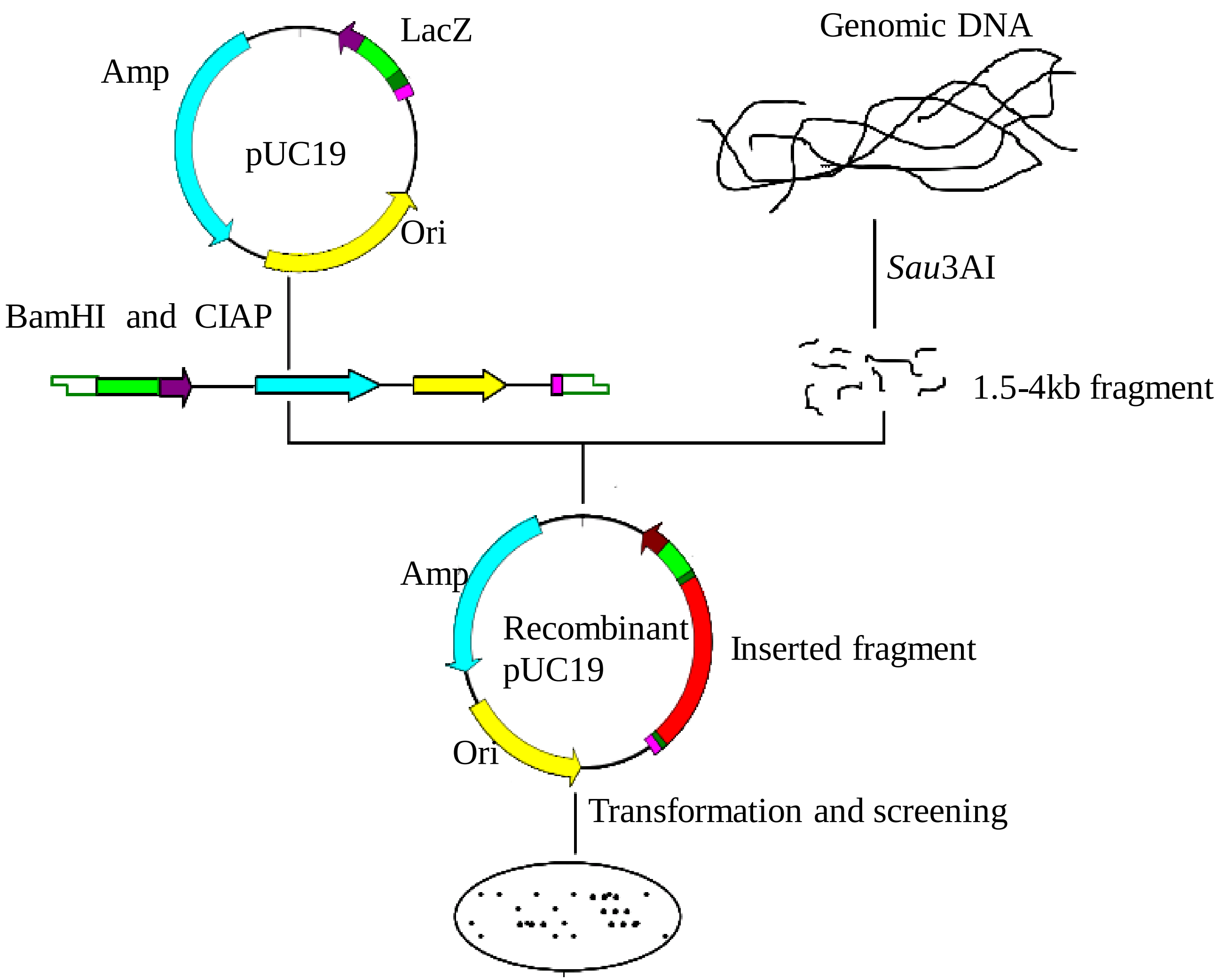


Esfenvalerate

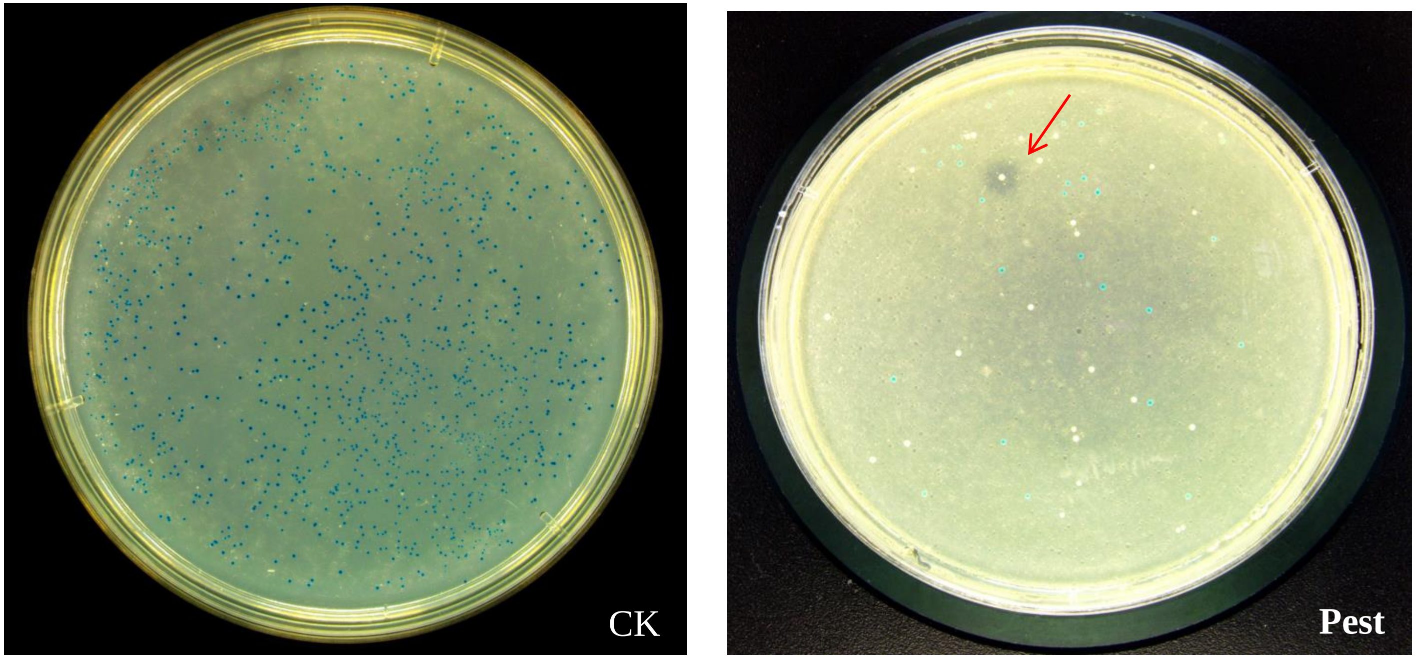


Bifenthrin

Hydrolase cloning from genomic library



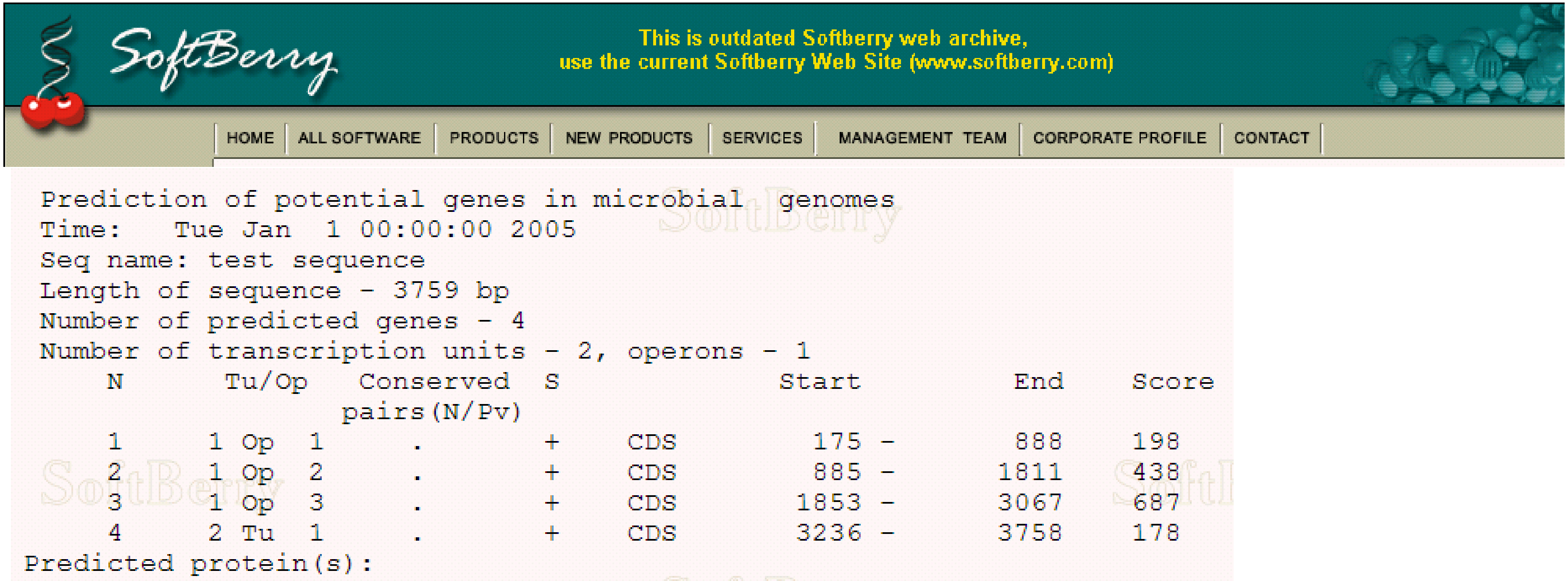
The construction and screening of genomic library of *Paracoccus* PYD



The clones containing hydrolase genes on LB/Tributyrin plate

Sequencing clone P132 : an inserted fragment of 3.6kb

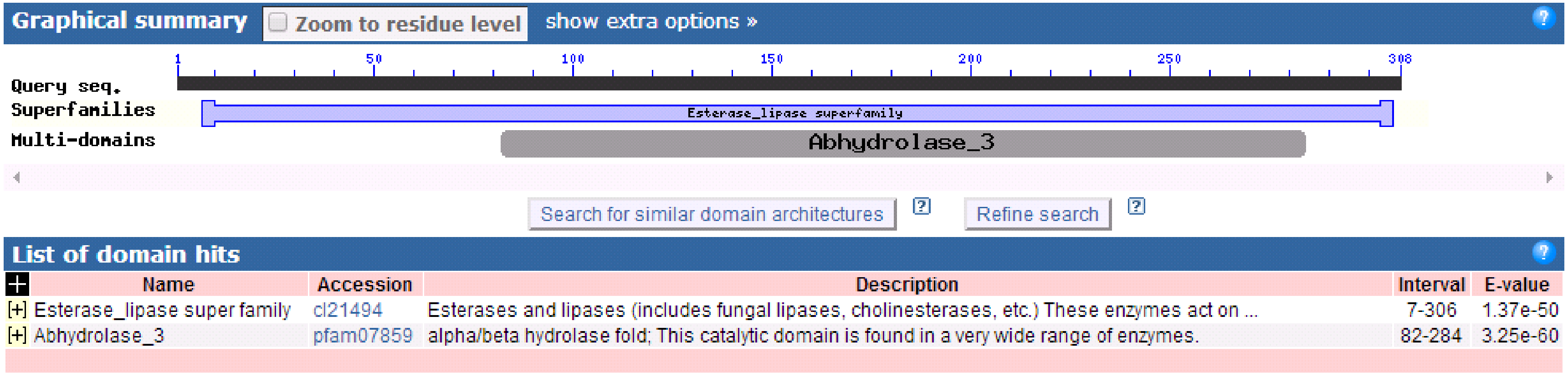
ORF prediction



This is outdated Softberry web archive,
use the current Softberry Web Site (www.softberry.com)

```
 Prediction of potential genes in microbial genomes
 Time:   Tue Jan  1 00:00:00 2005
 Seq name: test sequence
 Length of sequence - 3759 bp
 Number of predicted genes - 4
 Number of transcription units - 2, operons - 1
     N      Tu/Op   Conserved  S             Start          End     Score
                   pairs(N/Pv)

     1     1 Op  1      .      +    CDS        175 -         888     198
     2     1 Op  2      .      +    CDS        885 -        1811     438
     3     1 Op  3      .      +    CDS       1853 -        3067     687
     4     2 Tu  1      .      +    CDS       3236 -        3758     178
Predicted protein(s):
```

Conserved domain analysis: the ORF **Pyh** has a hydrolyase domain

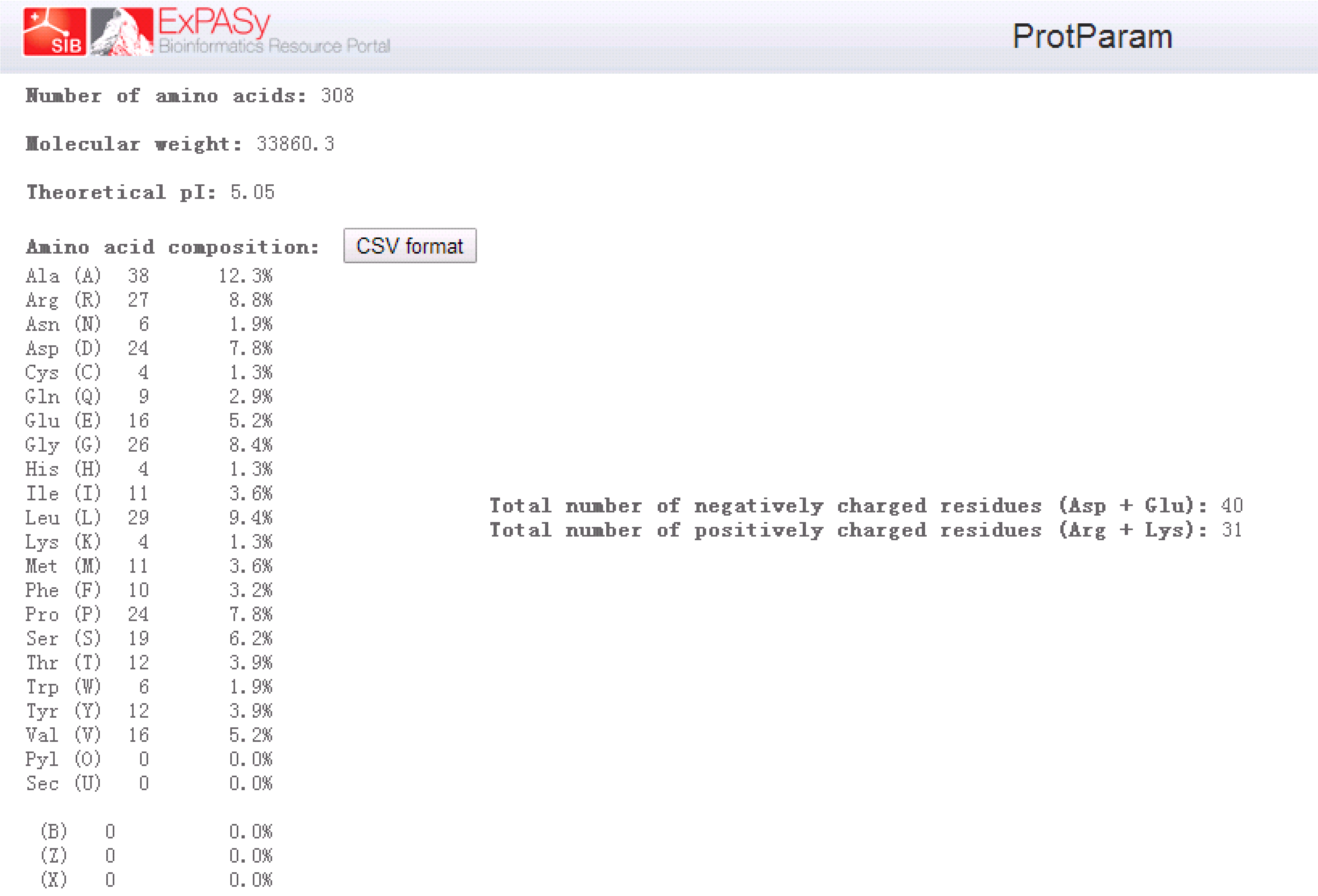


Name	Accession	Description	Interval	E-value
[+] Esterase_lipase super family	cl21494	Esterases and lipases (includes fungal lipases, cholinesterases, etc.) These enzymes act on ...	7-306	1.37e-50
[+] Abhydrolase_3	pfam07859	alpha/beta hydrolase fold; This catalytic domain is found in a very wide range of enzymes.	82-284	3.25e-60

Biochemical assay : the clone P132 can degrade pyrethroid

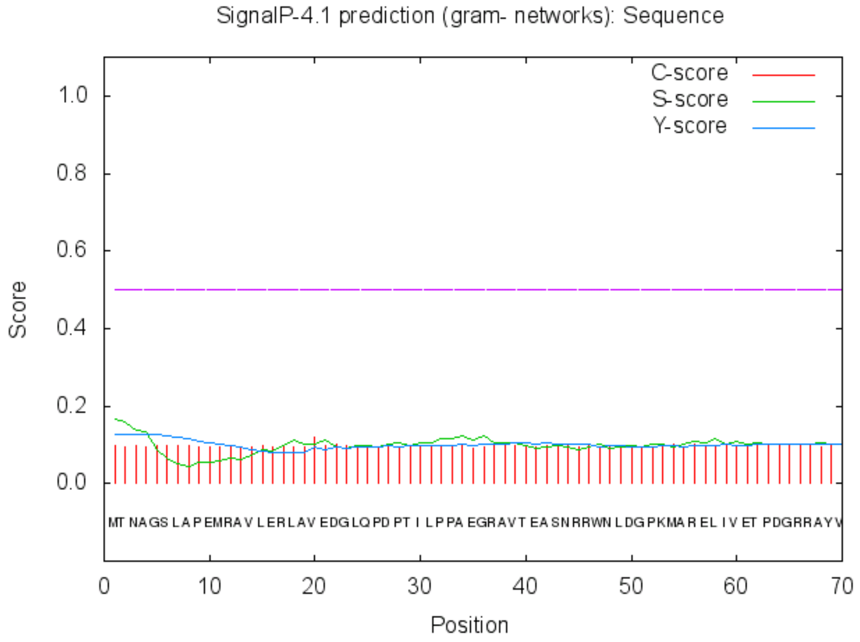
Bioinformatics analysis of *pyh*

Character ization of the primary structure

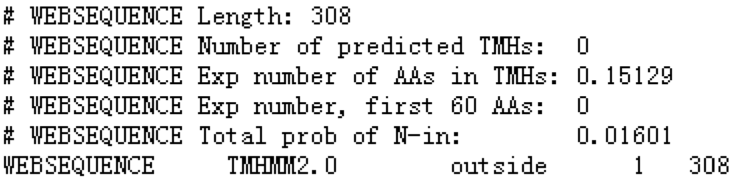


```
Number of amino acids: 308

Molecular weight: 33860.3

Theoretical pI: 5.05

Amino acid composition:
Ala (A)  38      12.3%
Arg (R)  27       8.8%
Asn (N)   6       1.9%
Asp (D)  24       7.8%
Cys (C)   4       1.3%
Gln (Q)   9       2.9%
Glu (E)  16       5.2%
Gly (G)  26       8.4%
His (H)   4       1.3%
Ile (I)  11       3.6%
Leu (L)  29       9.4%
Lys (K)   4       1.3%
Met (M)  11       3.6%
Phe (F)  10       3.2%
Pro (P)  24       7.8%
Ser (S)  19       6.2%
Thr (T)  12       3.9%
Trp (W)   6       1.9%
Tyr (Y)  12       3.9%
Val (V)  16       5.2%
Pyl (O)   0       0.0%
Sec (U)   0       0.0%

 (B)   0          0.0%
 (Z)   0          0.0%
 (X)   0          0.0%

Total number of negatively charged residues (Asp + Glu): 40
Total number of positively charged residues (Arg + Lys): 31
```

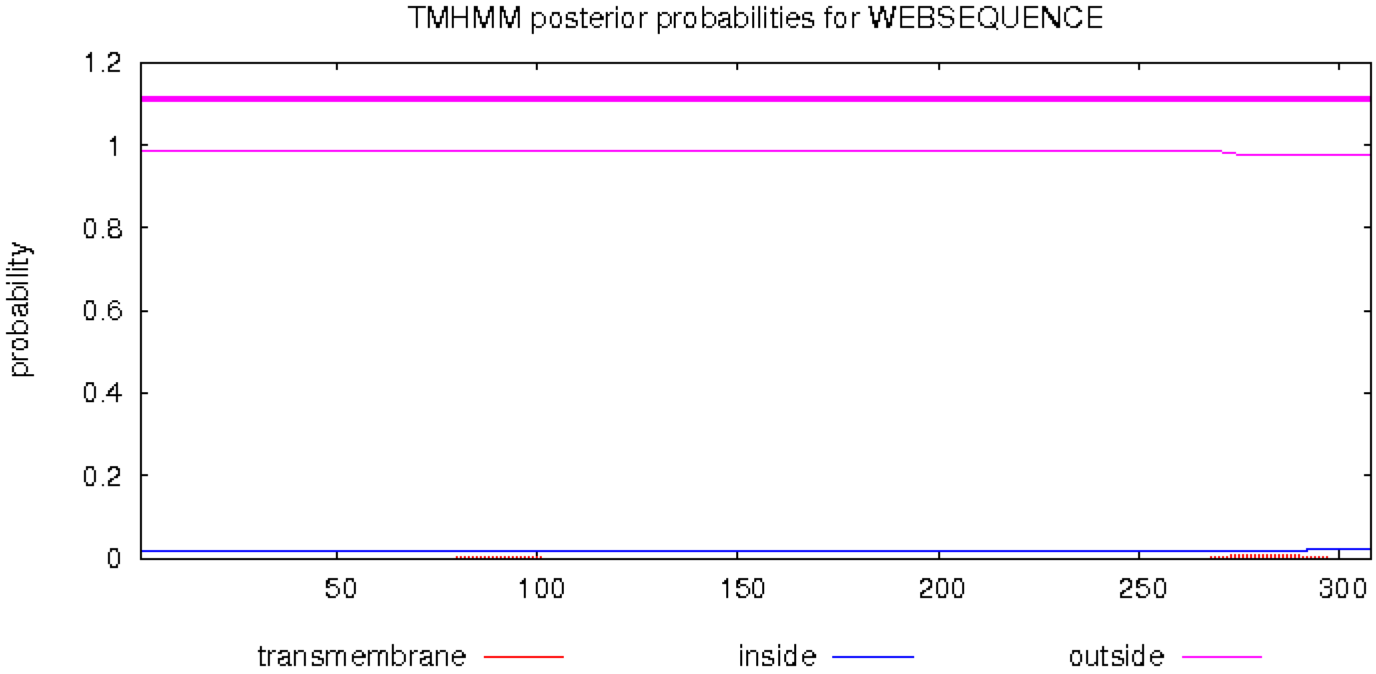
Signal peptide prediction of Pyh protein: NO



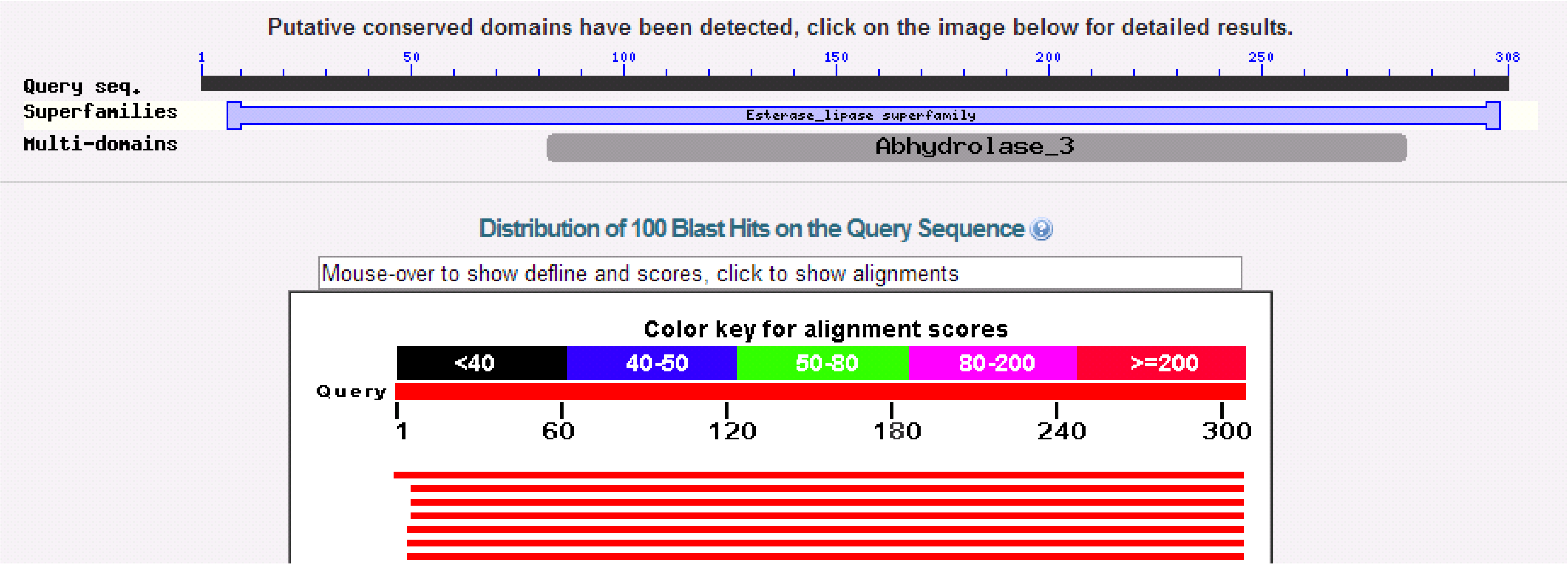
Transmembrane regions prediction: NO



```
# WEBSEQUENCE Length: 308
# WEBSEQUENCE Number of predicted TMHs:  0
# WEBSEQUENCE Exp number of AAs in TMHs: 0.15129
# WEBSEQUENCE Exp number, first 60 AAs:  0
# WEBSEQUENCE Total prob of N-in:        0.01601
WEBSEQUENCE     TMHMM2.0        outside      1   308
```



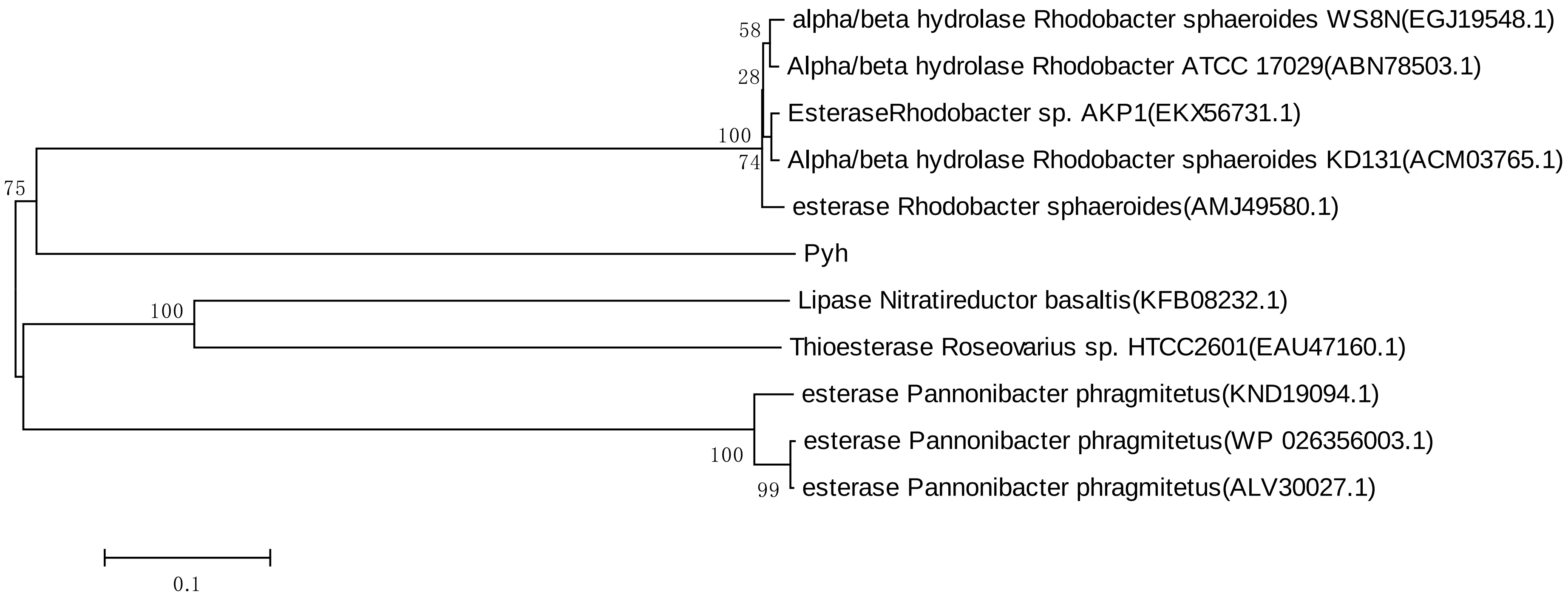
Phylogenetic analysis of Pyh and other hydrolyases from different strains



Putative conserved domains have been detected, click on the image below for detailed results.

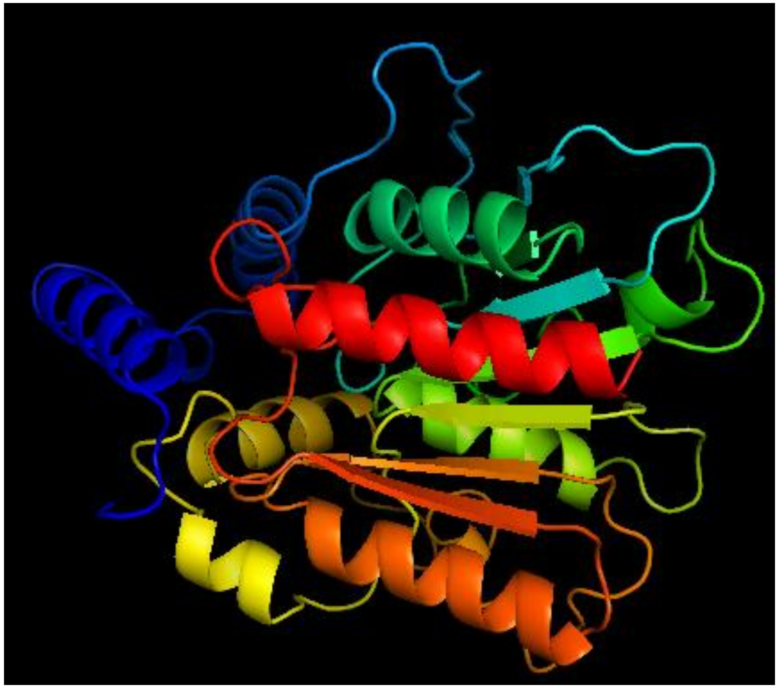
Distribution of 100 Blast Hits on the Query Sequence

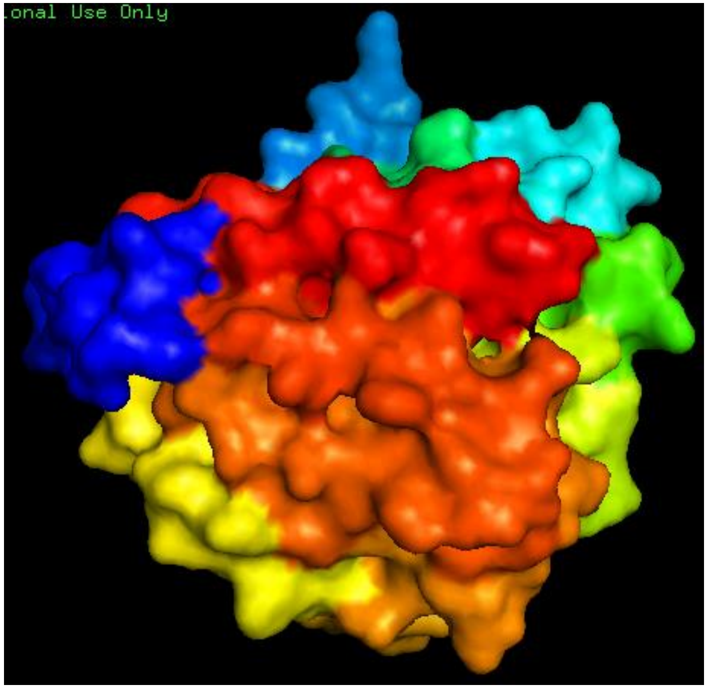
Mouse-over to show defline and scores, click to show alignments



58
alpha/beta hydrolase Rhodobacter sphaeroides WS8N(EGJ19548.1)
28
Alpha/beta hydrolase Rhodobacter ATCC 17029(ABN78503.1)
EsteraseRhodobacter sp. AKP1(EKX56731.1)
100
74
Alpha/beta hydrolase Rhodobacter sphaeroides KD131(ACM03765.1)
75
esterase Rhodobacter sphaeroides(AMJ49580.1)
Pyh
100
Lipase Nitratireductor basaltis(KFB08232.1)
Thioesterase Roseovarius sp. HTCC2601(EAU47160.1)
esterase Pannonibacter phragmitetus(KND19094.1)
esterase Pannonibacter phragmitetus(WP 026356003.1)
100
99
esterase Pannonibacter phragmitetus(ALV30027.1)
0.1

The 3D structure of **Pyh**





Thank you!