



中国农业科学院研究生院 (CAAS)

桃树再植病相关的两种蛋白生物信息学分析

(Bioinformatics analysis of two proteins associated with peach tree Replant Disease)

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➤再植病害(replant disease)或连作障碍(replant problem)或是全球性的一项难题，日本学者称之为忌地现象(Yadava and Dould 1980，**即果园刨除老树后栽种新树时**，幼树根系发育不良，生长缓慢，植株矮小，抗性降低，甚至整株死亡的现象。

关于连作障碍产生的原因，国内外的学者从不同角度进行了研究：

- ◆化感作用（自毒物质）
- ◆土壤有害微生物
- ◆土壤微生物群落结构的变化
- ◆土壤理化性质劣变。



自毒物质主要与桃树体内次生代谢产生的氰糖苷（野黑樱苷、扁桃苷）有关。扁桃苷主要分布于桃树种子中，少量存在于叶片；野黑樱苷主要分布于根系中。

- 研究表明果园内残留的桃树根系、种子、叶片中的氰糖苷，经土壤根际微生物分解产生HCN、苯甲酸等有毒物质。
- 另外当桃根系受到机械伤害或昆虫破坏时，储存在液泡中的氰糖苷会被释放出来，被位于质外体的 β -葡萄糖苷酶分解释放HCN、苯甲酸等有毒物质。

(? 是否能在微生物中找出与桃树中 β -葡萄糖苷酶功能相似的酶)

- ◆ 自毒作用物质主要影响根的呼吸作用。通过抑制抗氧化酶SOD等的活性，使根系处于氧胁迫状态，影响植物对营养物质的吸收，从而影响植株的生长。

目的:

- 运用生信手段分析桃树中 β -葡萄糖苷酶, 挖掘出根际微生物中与 β -糖苷酶功能相似的有害微生物。
- 预测桃子SOD蛋白的二级结构、3D结构。

β-葡萄糖苷酶

β-葡萄糖苷酶 (β-glucosidase, EC 3. 2. 1. 21) , 也称为 β-D-葡萄糖糖苷水解酶, 是一类纤维素酶, 能够从含糖化合物中催化水解末端的非还原性 β-D-糖苷键, 释放出 β-D-葡萄糖及相应的单糖、寡糖或复合糖。

β-葡萄糖苷酶属于糖苷水解酶家族。Bernard Henrissat等人根据氨基酸序列的一致性对酶类进行了新的分类, 建立了CAZy数据把氨基酸序列相似度大于30%的列为同一家族。目前根据这一方法已经把糖苷水解酶分成了135个家族。

<http://www.cazy.org/>

1. 序列获取及注释信息分析

- 序列获取:Uniprot 数据库

高级检索蛋白名Beta-glucosidase 物种选择*Prunus Presica*

```
>tr|M5WGE3|M5WGE3_PRUPE Beta-glucosidase OS=Prunus persica OX=3760  
GN=PRUPE_6G061000 PE=3 SV=1
```

```
MDKVKDFNCIYRNPNEPVEARVKDLLSRMTLKEKVGQMTQIERRVSTPDAIRDFSIGSVLS  
AGGSVPFEKALSSDWADMVDGFORSALESRLGIPLIYGIDAVHGNNSVYGATIFPHNVGL  
GATRDADLVKRIGAATALEVRASGIHYTFAPCVAVCRDPRWGRCYESYSEDTEIVRKMTSI  
VTGLQGQPPQGYPKGYPFVLGRNNTIACA KHFVGDGGTHKGLNEGNTISSYDDLRIHM  
APYLN CISDGVSTVMASYSSWNGSKLHADRFLLTEILKDKLGFKGFVISDWEALDQLCEPR  
GADYRFCISSAVNAGIDMVMVPFRYEQFVKDLVYLVEHGNISMSRIDDAVERILRVKFSVSG  
LFEHPFSDRSLLDMVGCKLHRDLAREA VRKSLVLLKNGKDSRKPFLPLDRKAKRILVAGTHA  
DDLGYQCGGWTATWDGRSGRITTGTTVLEAIQKAVGDDTEIIYEQYPSADTLAREDISFAI  
VAVGEGPYAEFRGDNLELAIPFNGTDVISSVADRLPTLVILISGRPLTLEPWLLEKMDALVAA  
WLPGSEGE GIADVIFGDYDFEGLLPVSWFKRVEQLPMNALDNSYDPLYPLGYGLTYNKGK  
SLQ
```

Sequence similaritiesⁱ

Belongs to the glycosyl hydrolase 3 family.

Filter byⁱ

 Reviewed (252)
Swiss-Prot

Popular organisms

A. thaliana (7)

E. coli K12 (2)

B. subtilis (1)



Slime mold (1)

SALTY (2)

Other organisms

BLAST

1 to 250 of 252 Show 250

Entry	Entry name	Protein names	Gene names	Organism	Length	Cross-reference (PDB)					Cross-referenc	
<input checked="" type="checkbox"/> P40406	NAGZ_BACSU	 Beta-hexosaminidase	nagZ ybbD, yzbA, BSU01660	Bacillus subtilis (strain 168)	642	3BMX	X-ray	1.40	A/B	1-642	[>]	3BMX 3LK6 3NVD 4GYJ 4GYK
						3LK6	X-ray	2.88	A/B/C/D	27-642	[>]	
						3NVD	X-ray	1.70	A/B	1-642	[>]	
						4GYJ	X-ray	1.65	A/B	18-642	[>]	
						4GYK	X-ray	1.80	A/B	18-642	[>]	
<input checked="" type="checkbox"/> P75949	NAGZ_ECOLI	 Beta-hexosaminidase	nagZ ycfO, b1107, JW1093	Escherichia coli (strain K12)	341							

M5WGE3在Uniprot库中家族信息

UniProtKB - A7LXU3 (BGH3B_BACO1)

Display

Help video

BLAST

Align

Format

Add to basket

History

Entry

Publications

Feature viewer

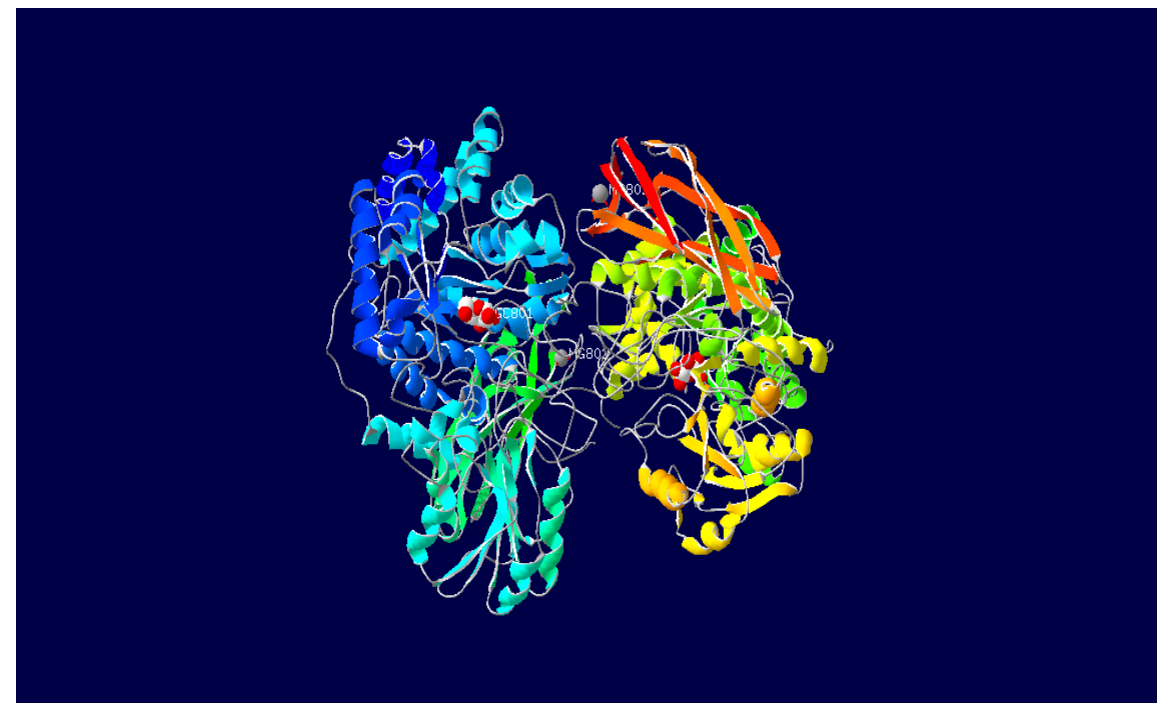
Feature table

Protein | **Beta-glucosidase BoGH3B**

Gene | **BACOVA_02659**

Organism | *Bacteroides ovatus* (strain ATCC 8483 / DSM 1896 / JCM 5824 / NCTC 11153)

Status |  Reviewed - Annotation score:  - Experimental evidence at protein level¹



5jp0 (卵形拟杆菌)

家族中有三维结构

Sequence Plot



Psipred 预测二维结构



三维结构预测

2. NCBI Blast寻找目标微生物

- BLAST:以M5WGE3进行blastp

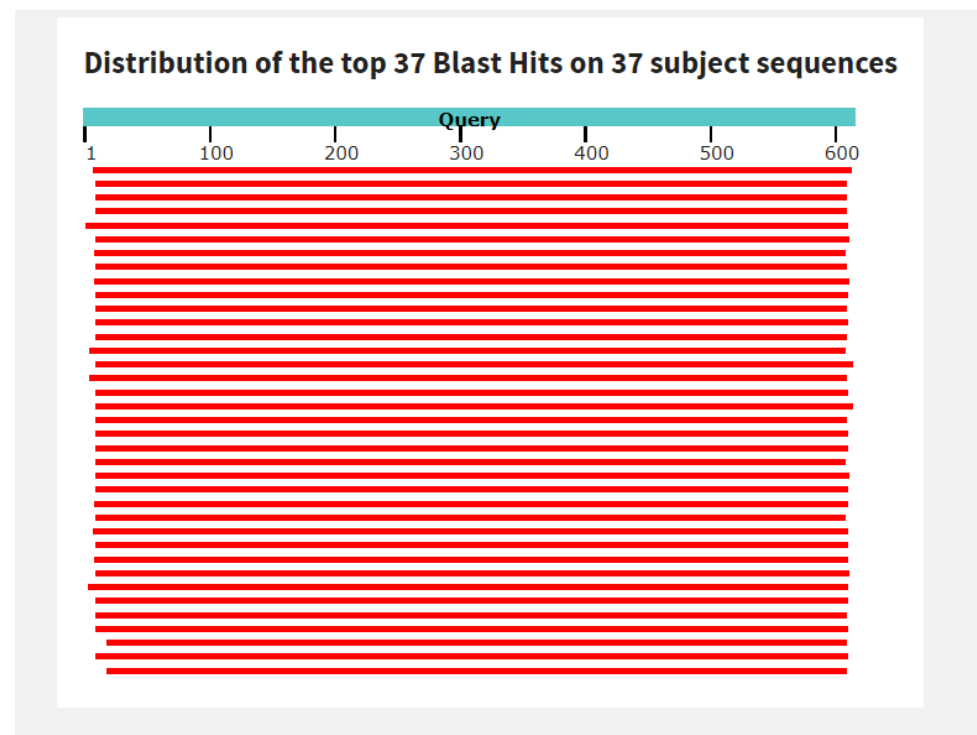
Search Parameters	
Program	blastp
Word size	2
Expect value	0.05
Hitlist size	100
Gapcosts	14,2
Matrix	PAM250
Filter string	F
Genetic Code	1
Window Size	40
Threshold	11
Composition-based stats	2

Database	
Posted date	May 5, 2021 3:17 AM
Number of letters	58,380,296,694
Number of sequences	168,568,479
Entrez query	None

共检索到67个结果 identity最小69%

Fliter:

Bacteria (taxid:2) 检索到37



• 根据朱炜（2009）与桃树再植病相关的六株微生物为：

假单胞杆菌(*Pseudomonas sp*)

根癌农杆菌(*Agrobacterium tumefaciens*)

细黄链霉菌(*Streptomyces microflavus*)

淡紫灰链霉菌(*Streptomyces lavendulae*)

灰色链霉菌(*Streptomyces griseus*)

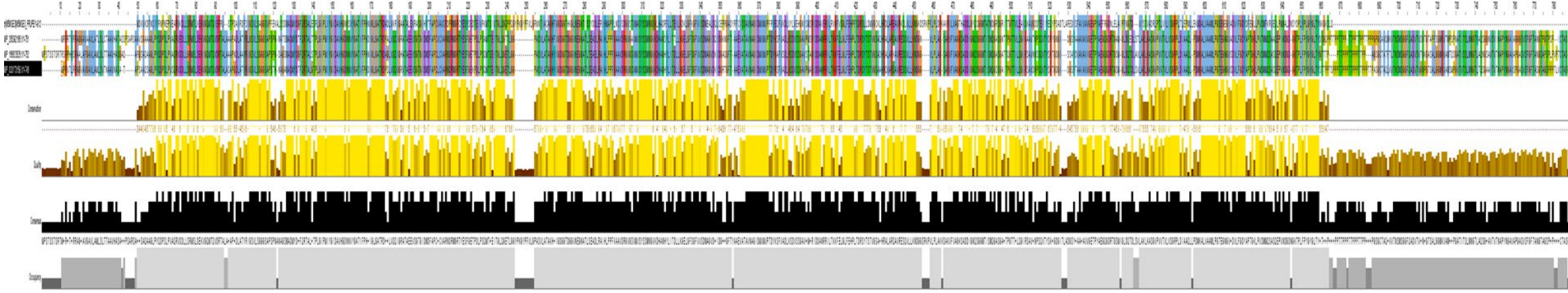
? 是否在Blast中存在同种/属的微生物

CLUSTAL O (1.0.4) Multiple Sequence Alignment

Sequence alignment output showing sequence identifiers (e.g., 8888, 8889) and alignment positions (1-1000) with symbols like |, -, and * indicating alignment status.

Color-coded alignment output where each column represents a position in the sequence and each row represents a sequence. Symbols are color-coded to indicate matches, mismatches, and gaps.

Reference sequence alignment output showing sequence identifiers and alignment positions with symbols like |, -, and *.

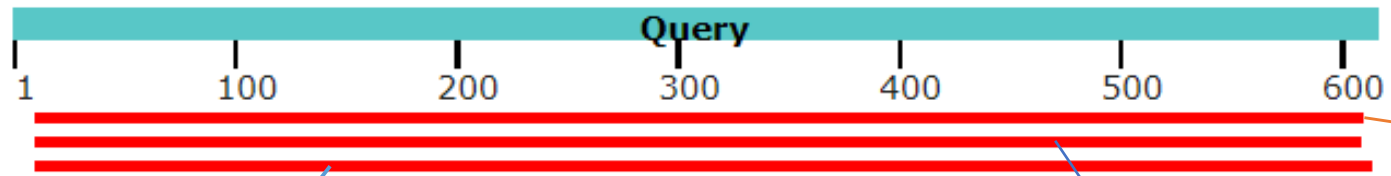


多序列比对

对37个细菌建树



Distribution of the top 3 Blast Hits on 3 subject sequences



Blast

Streptomyces sp.
NRRL F-5123

Streptomyces sp. KK5PA1

Streptomyces sp. URHA0041

预测出三种细菌(与朱炜 (2009) 研究吻合)中与桃树中 β -葡萄糖苷酶功能相似的酶, 可能是产生自毒物质的有害微生物。

Superoxide dismutase 酶

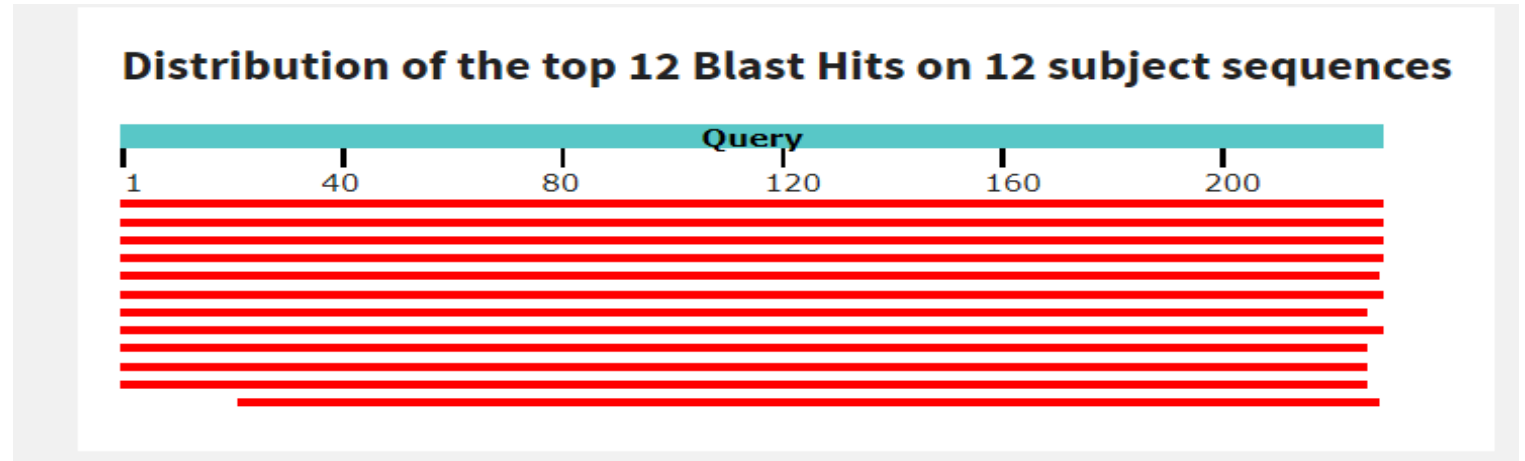
- Uniprot 库中获取:
- >sp|Q9SM64|SODM_PRUPE Superoxide dismutase [Mn], mitochondrial OS=Prunus persica OX=3760 GN=SOD PE=2 SV=1

MALRTLVSRRATLGLGFRQQLRGLQTFSLPDL
PYNYGALPAISGDIMQLHHQNHQTYVTNY
NKALEQLHDAISKGDAPTVAKLHSAIKFNGGG
HINHSIFWKNLAPVREGGGEPPKGS LGWAIDT
NFGSLEALVQKMNAEGAALQGSGVWLALD
KELKKLVETTANQDPLVTKGPTLVPLLGIDVW
EHAYYLQYKNVRPDYLNKNIWKVINWKYASEVY
EKESP

I. NCBI中Blast

Filter: identity>70% 12条

Search Parameters	
Program	blastp
Word size	2
Expect value	0.05
Hitlist size	100
Gapcosts	14,2
Matrix	PAM250
Filter string	F
Genetic Code	1
Window Size	40
Threshold	11
Composition-based stats	2



12 sequences selected ?

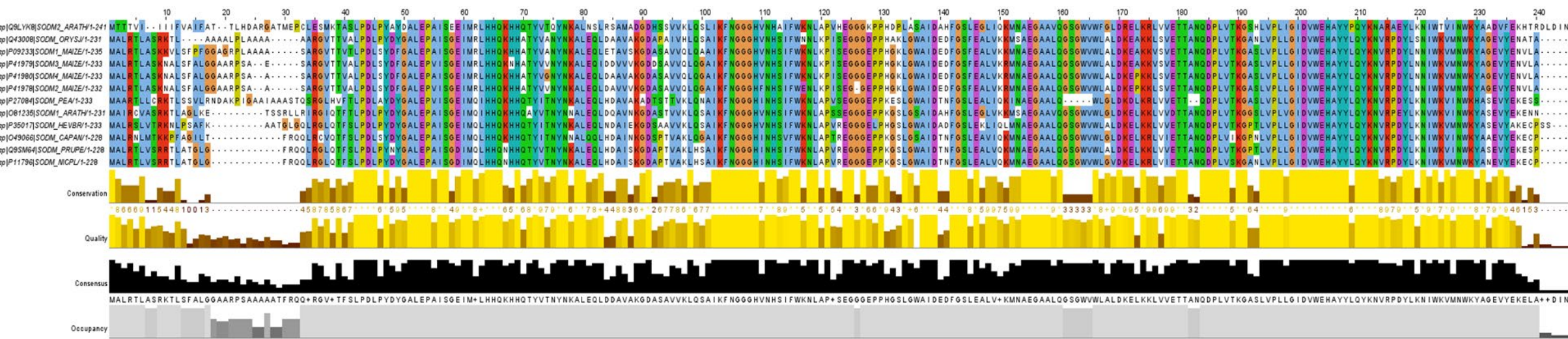
Organism	Blast Name	Score	Number of Hits	Description
Mesangiospermae	flowering plants		12	
• Pentapetalae	eudicots		7	
• rosids	eudicots		5	
• fabids	eudicots		3	
• Prunus persica	eudicots	368	1	Prunus persica hits
• Hevea brasiliensis	eudicots	329	1	Hevea brasiliensis hits
• Pisum sativum	eudicots	297	1	Pisum sativum hits
• Arabidopsis thaliana	eudicots	313	2	Arabidopsis thaliana hits
• Nicotiana glauca	eudicots	361	1	Nicotiana glauca hits
• Nicotiana glauca	eudicots	361	1	Nicotiana glauca hits
• Capsicum annuum	eudicots	337	1	Capsicum annuum hits
• Zea mays	monocots	295	4	Zea mays hits
• Oryza sativa Japonica Group	monocots	294	1	Oryza sativa Japonica Group hits

II. 在uniprot中blast结果

[Collapse table](#)

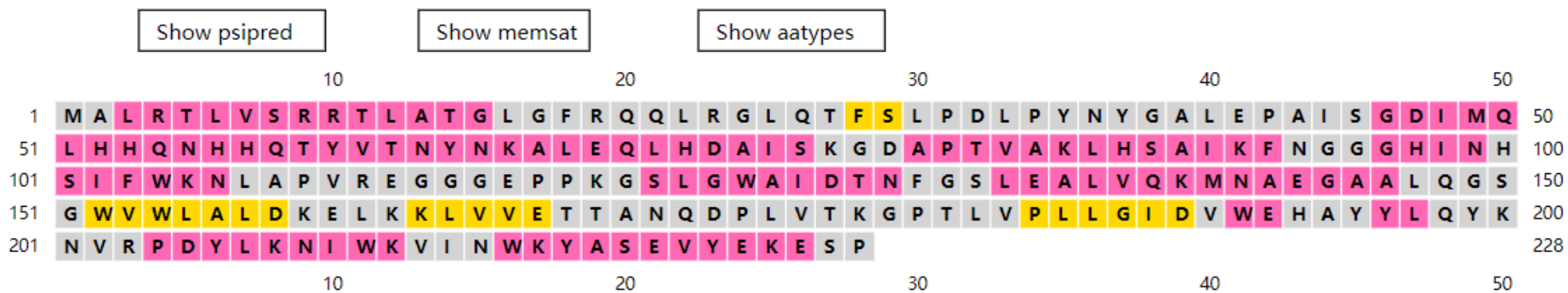
Entry	Protein names	Match hit	Identity
Q9SM64	Superoxide dismutase [Mn], mitochondrial (<i>Prunus persica</i>)		100.0%
P11796	Superoxide dismutase [Mn], mitochondrial (<i>Nicotiana plumbaginifolia</i>)		95.2%
O49066	Superoxide dismutase [Mn], mitochondrial (<i>Capsicum annuum</i>)		84.2%
P35017	Superoxide dismutase [Mn], mitochondrial (<i>Hevea brasiliensis</i>)		85.0%
O81235	Superoxide dismutase [Mn] 1, mitochondrial (<i>Arabidopsis thaliana</i>)		77.7%
Q43008	Superoxide dismutase [Mn], mitochondrial (<i>Oryza sativa</i> subsp. <i>japonica</i>)		76.7%
P27084	Superoxide dismutase [Mn], mitochondrial (<i>Pisum sativum</i>)		74.9%
P09233	Superoxide dismutase [Mn] 3.1, mitochondrial (<i>Zea mays</i>)		74.5%
P41980	Superoxide dismutase [Mn] 3.4, mitochondrial (<i>Zea mays</i>)		75.1%
P41979	Superoxide dismutase [Mn] 3.3, mitochondrial (<i>Zea mays</i>)		72.9%
P41978	Superoxide dismutase [Mn] 3.2, mitochondrial (<i>Zea mays</i>)		73.4%
Q9LYK8	Superoxide dismutase [Mn] 2, mitochondrial (<i>Arabidopsis thaliana</i>)		70.7%
...	.		

O81235 有三维结构



III. 多序列比对

Sequence Plot



Get PNG

Get SVG

PSIPRED Cartoon

IV. Psipred 二级结构预测

V. 三级结构预测

Oligo-State
Homo-tetramer (matching prediction)

Ligands
4 x MN

GMQE 0.81 **QMEAN** 0.08

4 x MANGANESE (II) ION
 MN.1: 4 residues within 4Å:
 4 PLIP interactions:
 MN.2: 4 residues within 4Å:
 4 PLIP interactions:
 MN.3: 4 residues within 4Å:
 4 PLIP interactions:
 MN.4: 4 residues within 4Å:
 4 PLIP interactions:

Global Quality Estimate

QMEAN		0.08
C β		-0.09
All Atom		1.69
solvation		1.63
torsion		-0.49

Local Quality Estimate
Residue Number

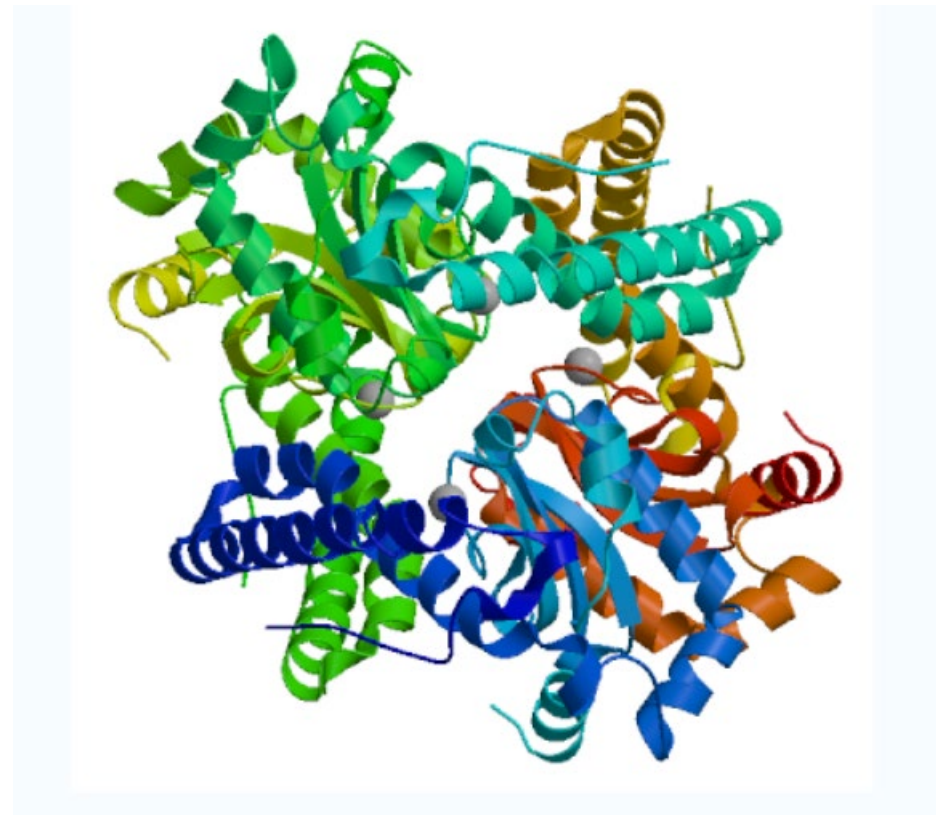
Comparison
Protein Size (Residues)

Template 4c7u.1.A **Seq Identity** 84.08% **Coverage**

Description
SUPEROXIDE DISMUTASE [MN] 1, MITOCHONDRIAL
Crystal structure of manganese superoxide dismutase from Arabidopsis thaliana

Model-Template Alignment

Model	Sequence	Residue
Model_01:A	HALRRTLVSRRTLATGLGFRQCLRGLQTFSLPDLFPYNYGALEPAISGDIMQLHHQNHHCYVVTNYNKALEQLHDAI	75
Model_01:B	HALRRTLVSRRTLATGLGFRQCLRGLQTFSLPDLFPYNYGALEPAISGDIMQLHHQNHHCYVVTNYNKALEQLHDAI	75
Model_01:C	HALRRTLVSRRTLATGLGFRQCLRGLQTFSLPDLFPYNYGALEPAISGDIMQLHHQNHHCYVVTNYNKALEQLHDAI	75
Model_01:D	HALRRTLVSRRTLATGLGFRQCLRGLQTFSLPDLFPYNYGALEPAISGDIMQLHHQNHHCYVVTNYNKALEQLHDAI	75
4c7u.1.A	-----TFSLPDLFPYNYGALEPAISGIMQLHHQNHHCYVVTNYNKALEQLDCAV	72
Model_01:A	SKGDAFTVAKLHSAIKFNGGGGHINHSIFWNNDAFVREGGGSEFFKGLGWAIDTNGSLEALVQKMNNAEGARLQGS	150
Model_01:B	SKGDAFTVAKLHSAIKFNGGGGHINHSIFWNNDAFVREGGGSEFFKGLGWAIDTNGSLEALVQKMNNAEGARLQGS	150
Model_01:C	SKGDAFTVAKLHSAIKFNGGGGHINHSIFWNNDAFVREGGGSEFFKGLGWAIDTNGSLEALVQKMNNAEGARLQGS	150
Model_01:D	SKGDAFTVAKLHSAIKFNGGGGHINHSIFWNNDAFVREGGGSEFFKGLGWAIDTNGSLEALVQKMNNAEGARLQGS	150
4c7u.1.A	SKGDAFTVAVBLCSAIFKNGGGGHINHSIFWNNDAFVREGGGSEFFKGLGWAIDTNGSLEALVQKMNNAEGARLQGS	147
Model_01:A	GWVWLALPKELKLVVETTTNODPLVTRGGLVPLLEGGIVVMEHAYQLQKNNVSPDYLRKNIWKVLSNWKYASEVYER	225
Model_01:B	GWVWLALPKELKLVVETTTNODPLVTRGGLVPLLEGGIVVMEHAYQLQKNNVSPDYLRKNIWKVLSNWKYASEVYER	225
Model_01:C	GWVWLALPKELKLVVETTTNODPLVTRGGLVPLLEGGIVVMEHAYQLQKNNVSPDYLRKNIWKVLSNWKYASEVYER	225
Model_01:D	GWVWLALPKELKLVVETTTNODPLVTRGGLVPLLEGGIVVMEHAYQLQKNNVSPDYLRKNIWKVLSNWKYASEVYER	225
4c7u.1.A	GWVWLALPKELKLVVETTTNODPLVTRGGLVPLLEGGIVVMEHAYQLQKNNVSPDYLRKNIWKVLSNWKYASEVYER	222
Model_01:A	-----	228
Model_01:B	-----	228
Model_01:C	-----	228
Model_01:D	-----	228
4c7u.1.A	-----	224



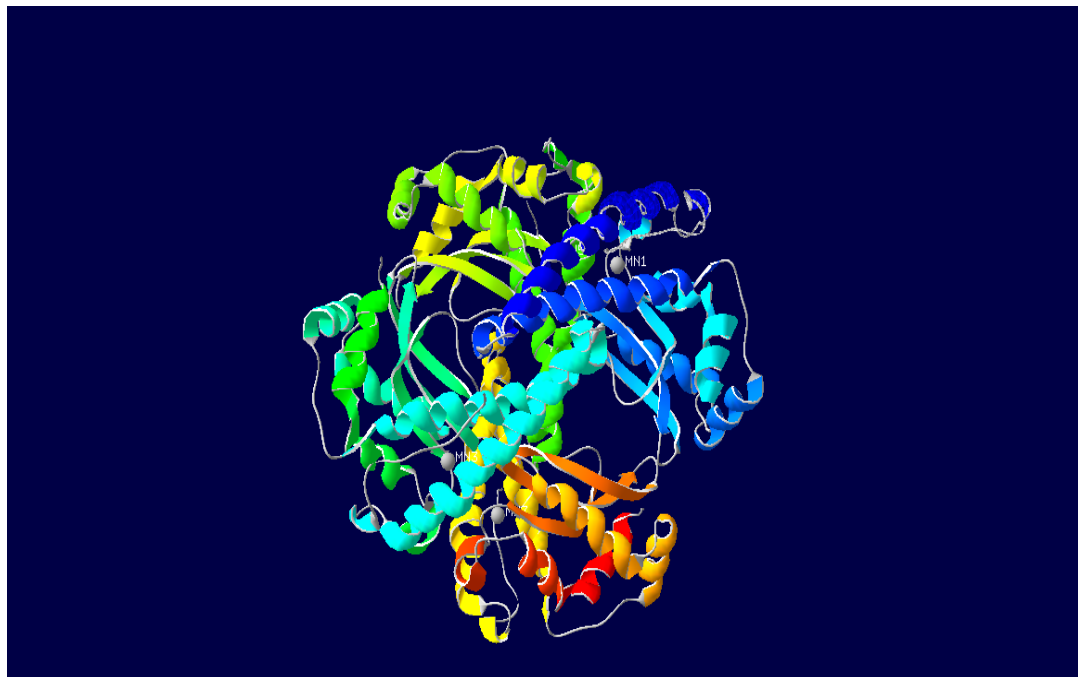
✓ 4c7u.1.A SUPEROXIDE DISMUTASE [MN] 1, MITOCHONDRIAL
Crystal structure of manganese superoxide dismutase from Arabidopsis thaliana

0.87 0.79 84.08 X-ray, 2.0Å homo-tetramer ✓ 4 x MN

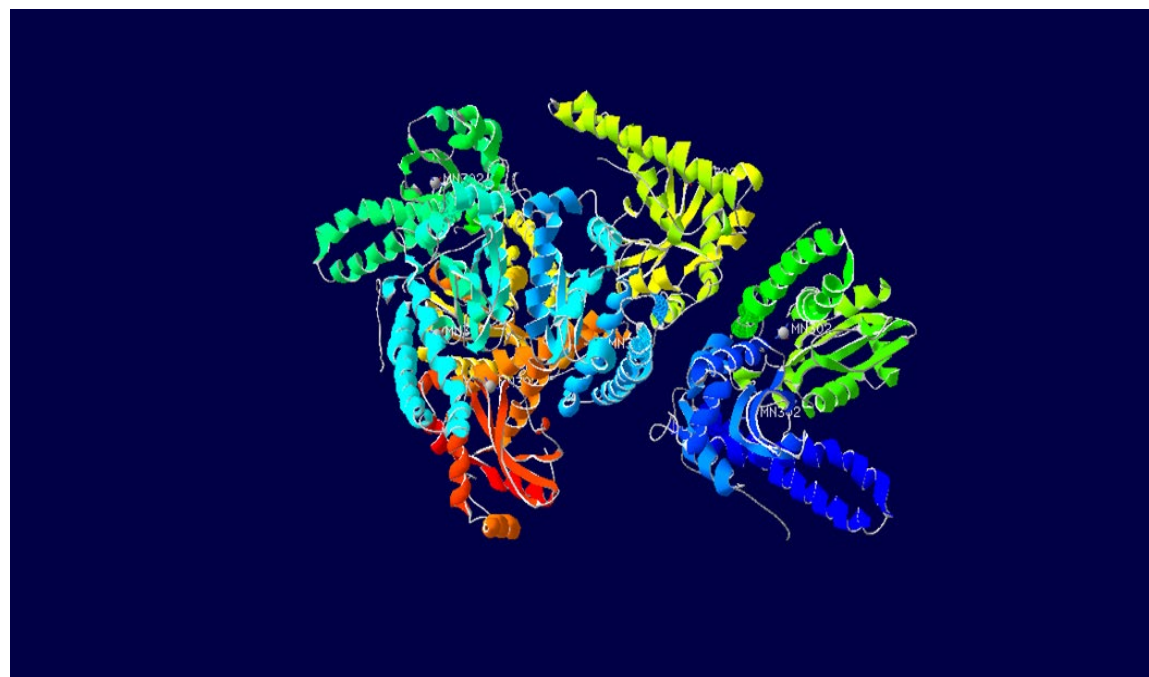
Template

Swiss model 预测

三级结构预测



桃子预测结果



模板拟南芥

总结与展望

- ✓ 预测到三种产生自毒物质的有害根际微生物；未来的工作可从这三种细菌入手分析它们产生毒性物质的过程，寻找一些防治它们的途径。
- ✓ 预测出SOD蛋白的结构；但需要进一步研究SOD酶受到胁迫时的结构，以及增强SOD酶表达量用来抵御再植病。

致谢

- 真挚的感谢罗静初老师，为我们讲授生物信息学课程。
- 感谢4G08组可爱的人们，这一学期的互相帮助、互相鼓励。
- 感谢研途中遇到的每一个人！

References (部分)

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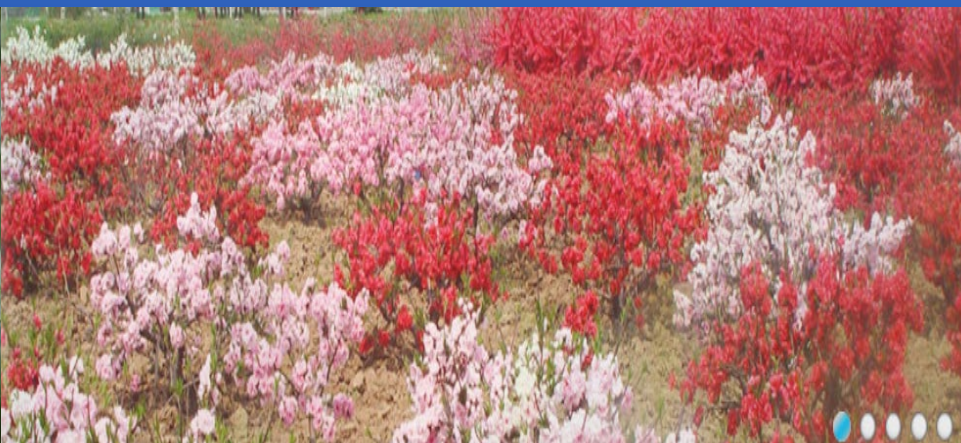
中国农业科学院研究生院 (CAAS)

Thank you!

请老师、同学批评指正



中国桃网



国家桃种质资源圃 (郑州) 全景



中国桃网