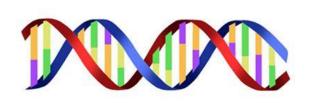
水稻Xa23基因分析与 功能结构预测



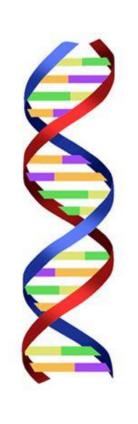
CAAS17MS3 G04-G06, G10

召集人:路璐

报告人: 孙宏达

2018. 6. 23





研究背景

2

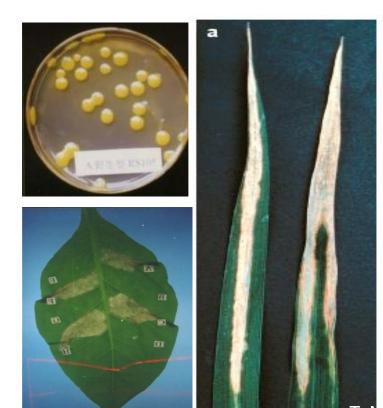
基因分析

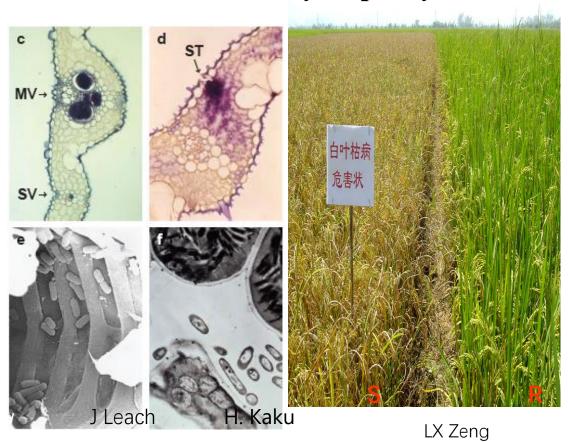
2

结构预测

作为全球最重要的粮食作物之一,水稻养活着世界上半数人口,因此水稻生产的可持续发展直接关系到粮食安全。水稻白叶枯病严重影响水稻的产量,且由于该病是维管束病害,化学防治效果不理想,目前防治该病最为经济有效的方法是培育并种植抗病品种(Reddy et al., 1989)。

Xanthomonas oryzae pv. oryzae- Xoo





病原物的主要毒性因子

T3SS effectors:

TALEs

PthXo1 Os8N3

AvrXa7 Os11N3

.

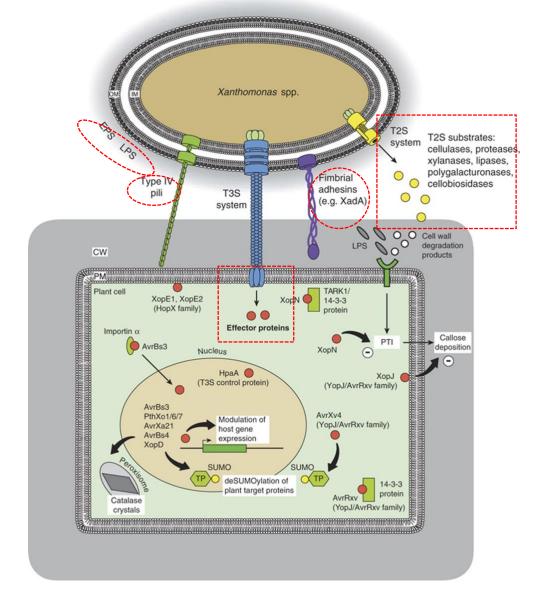
non-TALEs >18

 $XopY_{Xoo}$

OsCERK1,OsRLCK185

TALEs:

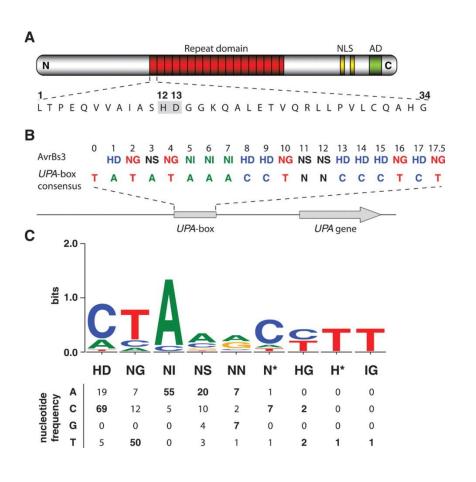
transcription activator-like effectors



Model of known virulence factors from Xanthomonas spp.

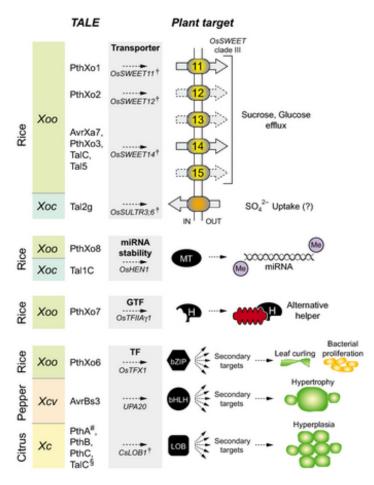
Daniela Büttner, and Ulla Bonas FEMS Microbiol Rev 2010;34:107-133

TALEs在寄主体内的靶标



Breaking the code of DNA binding specificity of TAL-type III effectors

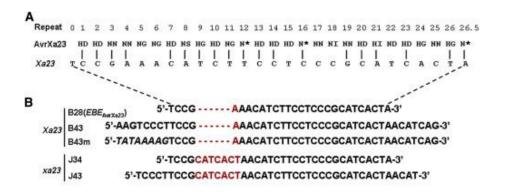
Jens Boch et al. Science 2009;326:1509-1512

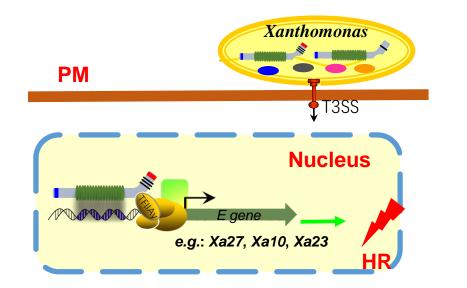


TAL effectors – pathogen strategies and plant resistance engineering

Jens Boch et al. New Phytologist 2014;204:823-832

研究背景





Wang et al. Mol Plant 2015



NCBI 检索

Nucleotide:

xa23-JG30

Oryza sativa Indica Group executor R protein XA23 (xa23) gene, xa23-JG30 allele, complete cds

1,726 bp linear DNA

Accession: KP123635.1 GI: 721363854

<u>Protein</u> <u>PubMed</u> <u>Taxonomy</u>

GenBank FASTA Graphics

XA23

Xa23-CBB23

Oryza sativa Indica Group executor R protein XA23 (Xa23) gene, Xa23-CBB23 allele, complete cds

1,720 bp linear DNA

Accession: KP123634.1 GI: 721363841

<u> Protein PubMed Taxonom</u>y

GenBank FASTA Graphics

NCBI 检索

Global Align (Needleman-Wunsch):

Xa23与xa23的核苷酸序列比对

物种	登录号	得分	相同核苷酸	相同和相似核苷酸	空位
Species	Accession	Score	Identity	Similarity	Gaps
Xa23 / xa23	KP123634.1	8578.5	1719/1726(99.6%)	1719/1726(99.6%)	6/1726(0.3%)

两个都是XA23基因,但是启动子是有区别的,开放阅读框都是一样的,因为启动子有区别,所以第一个在水稻中是不表达,表现为不抗病,第二个是表达的,表现为抗病。

秦腾飞. 水稻白叶枯病菌无毒基因avrXa23的克隆及与AvrXa23互作的水稻蛋白的功能分析[D]. 中国农业科学院, 2016.

启动子比对

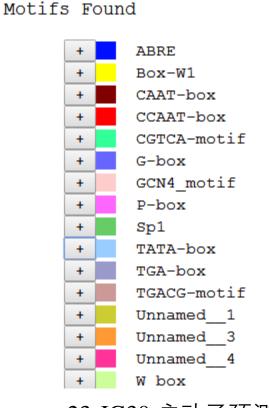


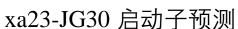
相较于xa23的等位基因,Xa23的等位基因的开放阅读框与它完全一致,但因其在启动子区域缺乏AvrXa23的转座子激活类元件(TALE)的结合元件,所以Xa23在水稻、烟草和马铃薯中会引起强烈的过敏反应。

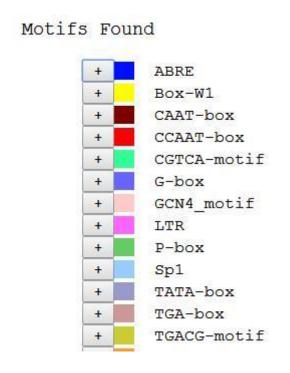
Wang C, Zhang X, Fan Y, Gao Y, Zhu Q, Zheng C, Qin T, Li Y, Che J, Zhang M, Yang B, Liu Y, Zhao K. XA23 is an executor R protein and confers broad-spectrum disease resistance in rice. Mol Plant 2014, : .

启动子预测

PlantCare(http://bioinformatics.psb.ugent.be/webtools/plantcare/html/):







Xa23-CBB23 启动子预测

NCBI 检索

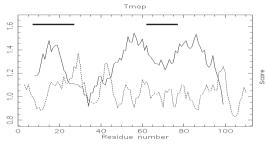
PubMed:

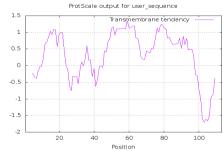
- 1、Xa23位于水稻的第11号染色体上,物理位置为: 24046274-24046729,编码113个氨基酸,5'UTR有56个氨基酸,3'UTR有222个氨基酸;
- 2、Xa23是一个执行R蛋白,对水稻白叶枯病具有广谱抗性,主要对 Philippine 的 P1-P10小种,中国的C1-C7小种和日本的T1-T3小种具有抗性。

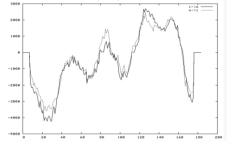
Wang C, Zhang X, Fan Y, Gao Y, Zhu Q, Zheng C, Qin T, Li Y, Che J, Zhang M, Yang B, Liu Y, Zhao K. XA23 is an executor R protein and confers broad-spectrum disease resistance in rice. Mol Plant 2014, : .

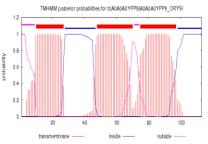
氨基酸序列性质

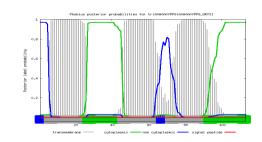
跨膜分析:









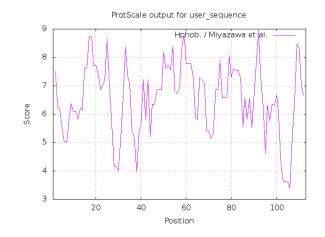


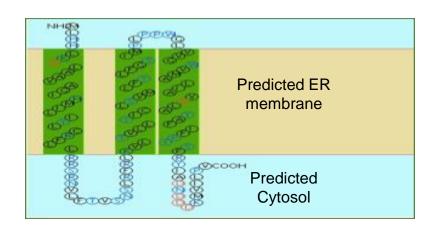
工具	Tmap	ProtScale	TMpred	TMHMM	Phobius
跨膜螺旋个数	3	3	3	3	3

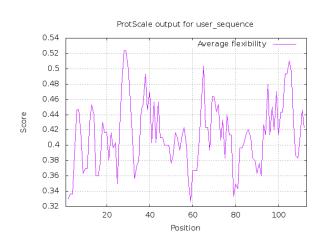
氨基酸序列性质

跨膜分析:

Core	Value	Start	End	E-Value
18	4.499	11	25	2.07E-05
55	6.869	44	69	4.81E-09
83	4.634	72	93	1.28E-05







亲/疏水 (Hphob. / Miyazawa et al)

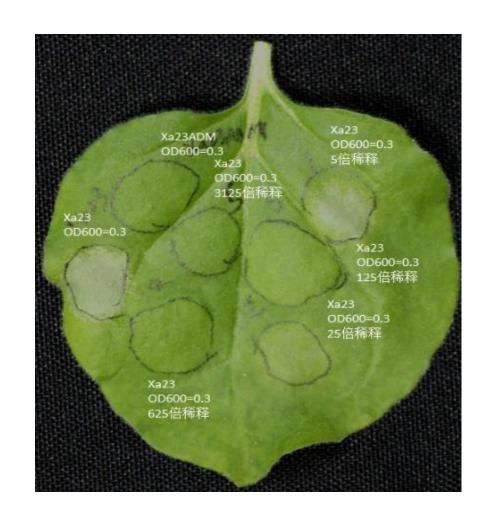
XA23
MLHH*LKELAAVAGIHMILIYLCRFLL*RRSRNVLFTVSNSLRFRL *KVLTVLLYICLSVMLFYLFGSIM*PLPPWGL*VVGWVMALIAVEL AYAFIFPYSF*RYIADNDDDKMVILPV

柔性和刚性 (Average flexibility)

Charged residue +; Charged residue -

XA23在烟草中的瞬时表达

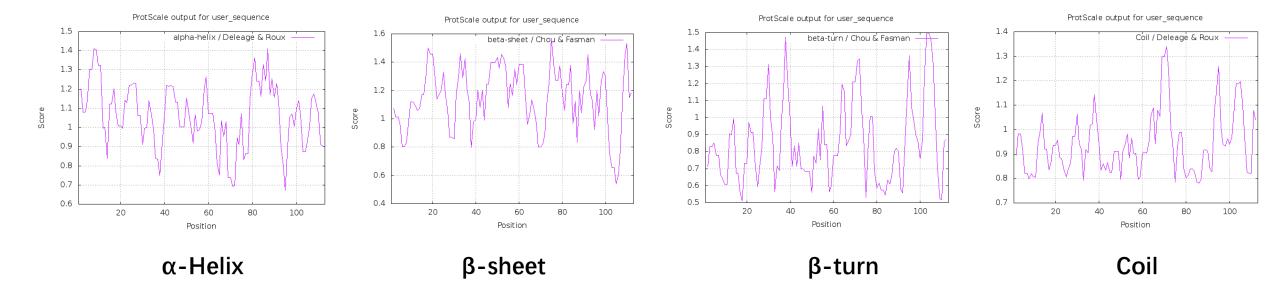
OD600=0.8 p35s-Xa23-Flag 24h	LB Xa23 His	RB
		HR
XA23	RYIAMVILPV	+
XA23AD	RYIAMVILPV	-
XA232	RYIAMVILPV	+
XA231	RYIAMVILPV	+
XA23DE	RYIAMVILPV	+
XA23D+	RYIAMVILPV	+



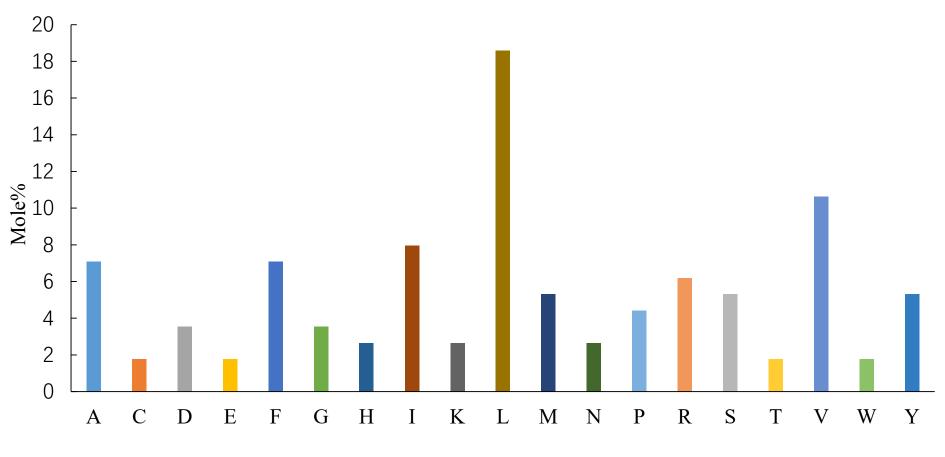
Charged residue + Charged residue -

氨基酸序列性质

二级结构特征分析:



氨基酸组成情况

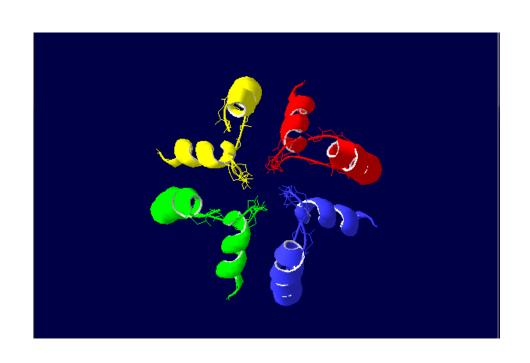


疏水氨基酸: 74.34%

蛋白质结构预测



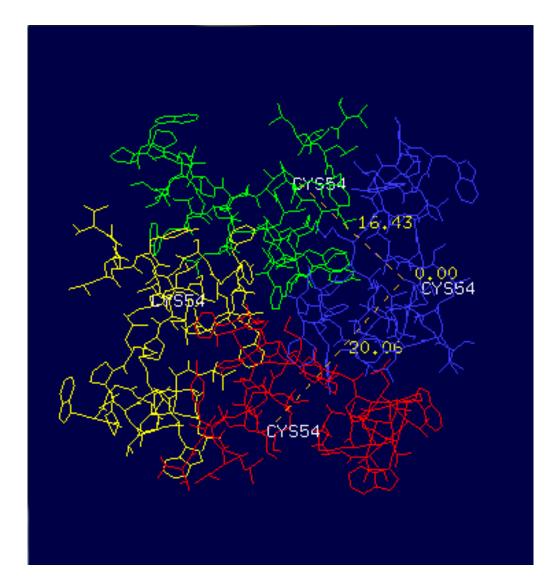




Tool: Swiss-pdbViewer

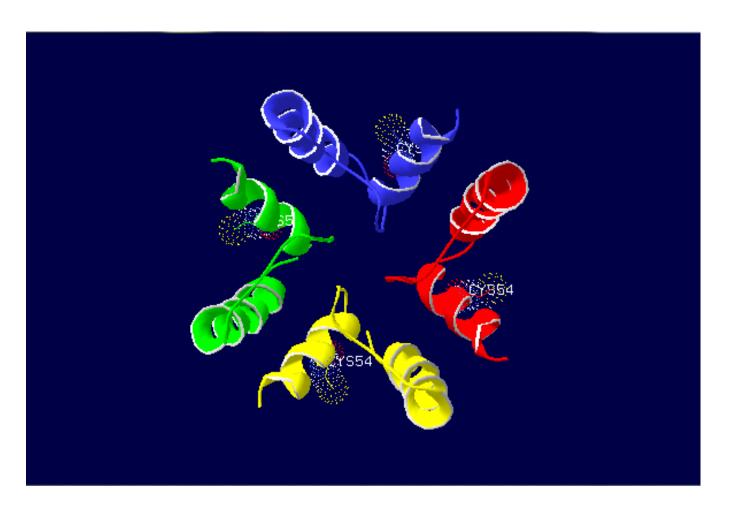
观察其带状结构发现这个立体蛋白质构型是由四个完全相同的亚基构成,每个亚基包含两个 α 螺旋。

蛋白质结构预测



在该结构中查找二硫键发现 并没有二硫键的存在,改为 查找与二硫键形成相关的半 胱氨酸(Cys)结果在四个 亚基上均发现有一个半胱氨 酸存在且彼此间相聚较远。

蛋白质结构预测



在观察各个氨基酸分布时发现 各个氨基酸在四个亚基上呈现 对称分布。

SWISS-MODEL预测该蛋白 为离子通道蛋白。

请批评指正, 谢谢!