耐辐射异常球菌*irrE*基因增强细胞耐盐能力的研究

任课老师：罗静初　教授
组员：王丹霞、刘盈盈、刘妍、赵玉兰
报告人：刘盈盈
组号：G10
单位：生物技术研究所，CAAS

主要内容

1. 研究背景
2. 生物信息学分析
3. 生理生化实验结果
4. 展望

全球土壤环境盐碱化严峻

高盐是一种主要的非生物胁迫因素，严重影响农作物的产量和品质。

抗盐特性研究

Salt-stress tolerance of transgenic plant over-producing gene

Gene and source	Transgenic plants	Reference
	Compatible osmolytes	
E.coli mtlD	Tobacco/*Arabidopsis*/wheat	Tarczynski et al,1993/ Thomas et al,1995/ Abebe et al, 2003
Apple *Stpd1*	Japanese persimmon	Gao et al ,2001
A.globiformis codA	*Arabidopsis*/rice	Hayashi et al ,1997/Mohanty et al ,2002
E.coli betA ,betB	Tobacco	Holmstrom et al ,2000
Atriplex hortensis BADH	Wheat	Guo et al ,2000
Vigna aconitifolial L. P5CS	Rice/tobacco	Zhu et al ,1998/Hong et al ,2000
E.coli otsA ,otsB	Rice	Garg et al ,2002
Barlay *HVA1*	mulberry	Lal S et al ,2007
Arabidopsis SOS1	*Arabidopsis*	Shi et al ,2003
Arabidopsis SOD	*Arabidopsis*	Alscher et al ,2002
Tobacco *GST/GPX*	tobacco	Roxas et al ,2000
	Transcription factors	
CBF of LEA-type gene	*Arabidopsis*	Shinozaki et al ,2000/ Pellegrineschi et al,2002

抗逆转录因子成为研究热点

PNAS
Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice

Honghong Hu, Mingqiu Dai, Jialing Yao, Benze Xiao, Xianghua Li, Qifa Zhang, and Lizhong Xiong

PNAS 2006;103;12987-12992; originally published online Aug 21, 2006;
doi:10.1073/pnas.0604882103

Planta (2007) 225:575–588
DOI 10.1007/s00425-006-0373-2

ORIGINAL ARTICLE

The barley ERF-type transcription factor HvRAF confers enhanced pathogen resistance and salt tolerance in *Arabidopsis*

Jinwook Jung · So Youn Won · Seok Cheol Suh · HyeRan Kim · Rod Wing · Yeonhwa Jeong · Ingyu Hwang · Minkyun Kim

Received: 14 April 2006 / Accepted: 4 August 2006 / Published online: 26 August 2006

Abstract We isolated *HvRAF* (*Hordeum vulgare* root abundant factor), a cDNA encoding a novel ethylene response factor (ERF)-type transcription factor, from young seedlings of barley. In addition to the most highly conserved APETALA2/ERF DNA-binding domain, the encoded protein contained an N-terminal MCGGAIL signature sequence, a putative nuclear localization sequence, and a C-terminal acidic transcription activation domain containing a novel mammalian hemopexin domain signature-like sequence. Their homologous
AAK92635 from ric
the ERF proteins
reflecting their funct
yses revealed that *H*
dant in roots than
induced in barley
regimes such as salic
nate, cellulase, and
localization assay, th
targeted to the nucle
with the GAL4 DN

The Plant Cell, Vol. 18, 1292–1309, May 2006, www.plantcell.org © 2006 American Society of Plant Biologists

Functional Analysis of an *Arabidopsis* Transcription Factor, DREB2A, Involved in Drought-Responsive Gene Expression [W][OA]

Yoh Sakuma,[a] Kyonoshin Maruyama,[a] Yuriko Osakabe,[a] Feng Qin,[a] Motoaki Seki,[b] Kazuo Shinozaki,[b,c,d] and Kazuko Yamaguchi-Shinozaki[a,d,e,1]

[a] Biological Resources Division, Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki 305-8686, Japan
[b] Plant Functional Genomics Research Team, RIKEN Genomic Sciences Center, Yokohama, Kanagawa 203-0045, Japan
[c] RIKEN Plant Science Center, Yokohama, Kanagawa 203-0045, Japan
[d] Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Kawaguchi, Saitama 332-0012, Japan
[e] Laboratory of Plant Molecular Physiology, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo 113-8657, Japan

Transcription factors DREB1A/CBF3 and DREB2A specifically interact with *cis*-acting dehydration-responsive element/C-repeat (DRE/CRT) involved in cold and drought stress–responsive gene expression in *Arabidopsis thaliana*. Intact *DREB2A* expression does not activate downstream genes under normal growth conditions, suggesting that DREB2A requires posttranslational modification for activation, but the activation mechanism has not been clarified. DREB2A domain analysis using *Arabidopsis* protoplasts identified a transcriptional activation domain between residues 254 and 335, and deletion of a region between residues 136 and 165 transforms DREB2A to a constitutive active form. Overexpression of

耐盐基因新资源

FABIANNE

A MAPK gene from Dead Sea fungus confers stress tolerance to lithium salt and freezing–thawing: Prospects for saline agriculture

Yan Jin, Song Weining, and Eviatar Nevo*

Institute of Evolution, University of Haifa, Haifa 31905, Israel

Contributed by Eviatar Nevo, November 9, 2005

The Dead Sea is one of the most saline lakes on earth (≈340 g/liter salinity) and is ≈10 times saltier than the oceans. *Eurotium herbariorum*, a common fungal species, was isolated from its water. EhHOG gene, encoding a mitogen-activated protein kinase (MAPK) that plays an essential role in the osmoregulatory pathway in yeast and many other eukaryotes, was isolated from *E. herbariorum*. The deduced amino acid sequences of EhHOG indicated high similarity with homologous genes from *Aspergillus nidulans*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* and contained a TGY motif for phosphorylation by MAPK kinase. When EhHOG was expressed in *S. cerevisiae hog1*Δ mutant, the growth and aberrant morphology of *hog1*Δ mutant was restored under high osmotic stress condition. Moreover, intracellular glycerol content in the transformant increased to a much higher level than that in the mutant during salt-stress situations. *hog1*Δ mutant complemented by EhHOG outperformed the wild type or had higher genetic fitness under high Li^+ and freezing–thawing conditions. The present study revealed the putative presence of a high-osmolarity glycerol response (HOG) pathway in *E. herbariorum* and the significance of *EhHOG* in osmotic regulation, heat stress, freeze stress, and oxidative stress. The Dead Sea is becoming increasingly more saline while the fungi living in it evolutionarily adapt to its high-saline environment, particularly with the extraordinarily high Li^+ concentration. The Dead Sea is potentially an excellent model for studies of evolution under extreme environments and is an important gene pool for future agricultural genetic engineering prospects.

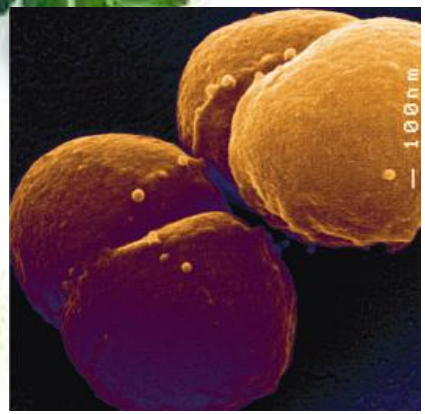
Eurotium herbariorum | HOG | salinity | genetic resources | crop improvement

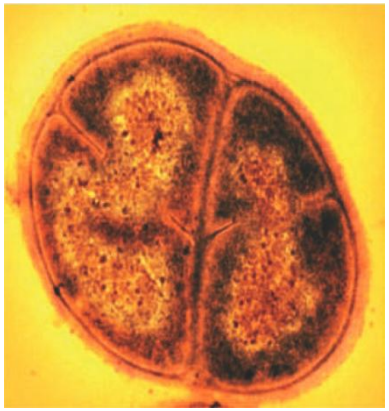
compatible solutes, mainly polyols, have been the major feature of fungi osmoregulation (5).

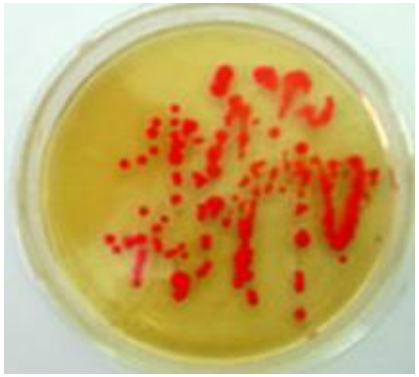
Mitogen-activated protein kinase (MAPK) is a key component of the evolutionarily conserved signal transduction cascades consisting of MAPK/extracellular signal-related kinase (ERK) activated by an MAPK/ERK kinase (MEK), which in turn is activated by an MEK kinase (6). Eukaryotic organisms use different MAPK cascades to regulate various aspects of cellular function (7, 8). MAPKs that specifically transmit environmental stress signals are also known as stress-activated protein kinases (SAPKs). This pathway is called the high-osmolarity glycerol (HOG) response pathway in *Saccharomyces cerevisiae* (9). Members of this MAPK subfamily include Hog1 in *S. cerevisiae*, Spc1 (also called StyI) in *Schizosaccharomyces pombe*, SakA in *Aspergillus nidulans*, and p38/JNK in the mammals. Indeed, *S. cerevisiae hog1* mutants are sensitive to high osmolarity, whereas *spc1* mutations in *S. pombe* result in sensitivity to high osmolarity, heat shock, and oxidative stress. Activation of the HOG pathway increases the transcription of some proteins, including enzymes involved in glycerol synthesis (10). As a result, a high accumulation of glycerol inside the cell occurs and leads to increased internal osmolarity and restores the osmotic gradient between the cells and their environment (11). Therefore, HOG1 gene holds a key position in osmo-adaptation of the yeast *S. cerevisiae*.

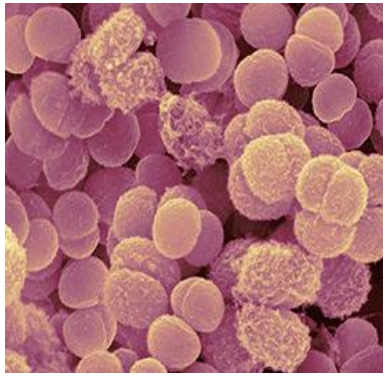
The presence of HOG1 homologous genes has been reported in fungal species (12, 13) and mammals (14), indicating that this pathway is conserved among eukaryotes. However, no information is available for the HOG pathway or the molecular mechanism of stress tolerance in *E. herbariorum*. Here, we cloned the

耐辐射异常球菌(*Deinococcus radiodurans*)





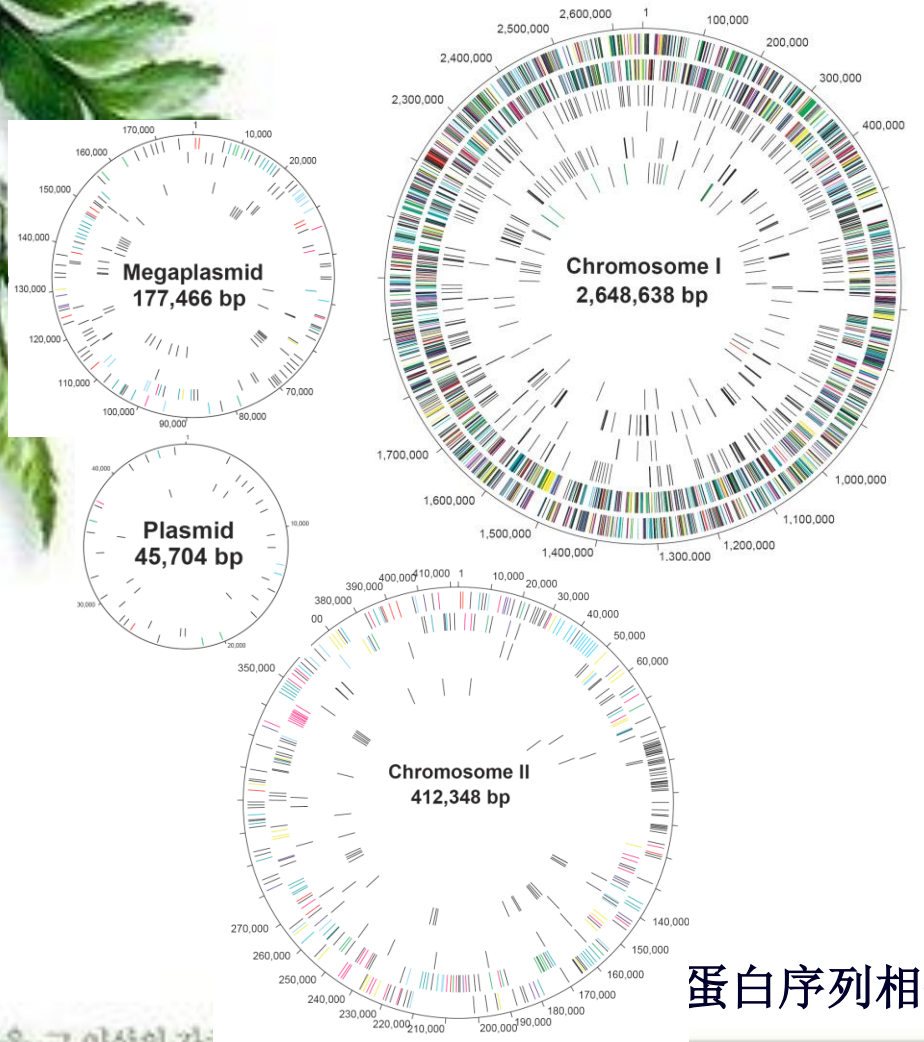




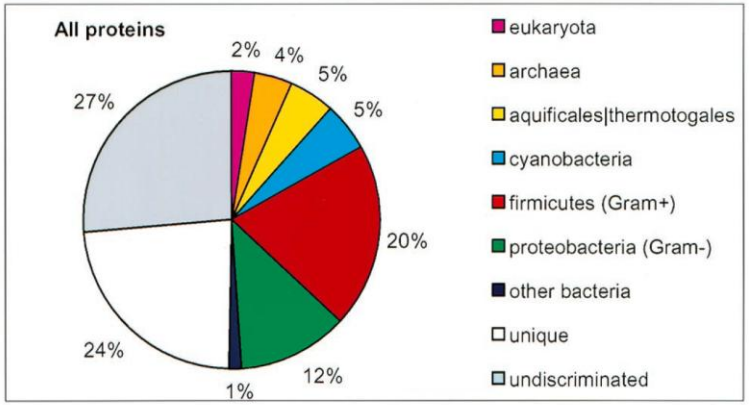
- 革兰氏阳性球菌
- 1956年分离
- 培养物为红色
- 二分体或四分体形式
- 无孢子形成
- 无致病性

主要内容

1. 研究背景
2. 生物信息学分析
3. 生理生化实验结果
4. 展望



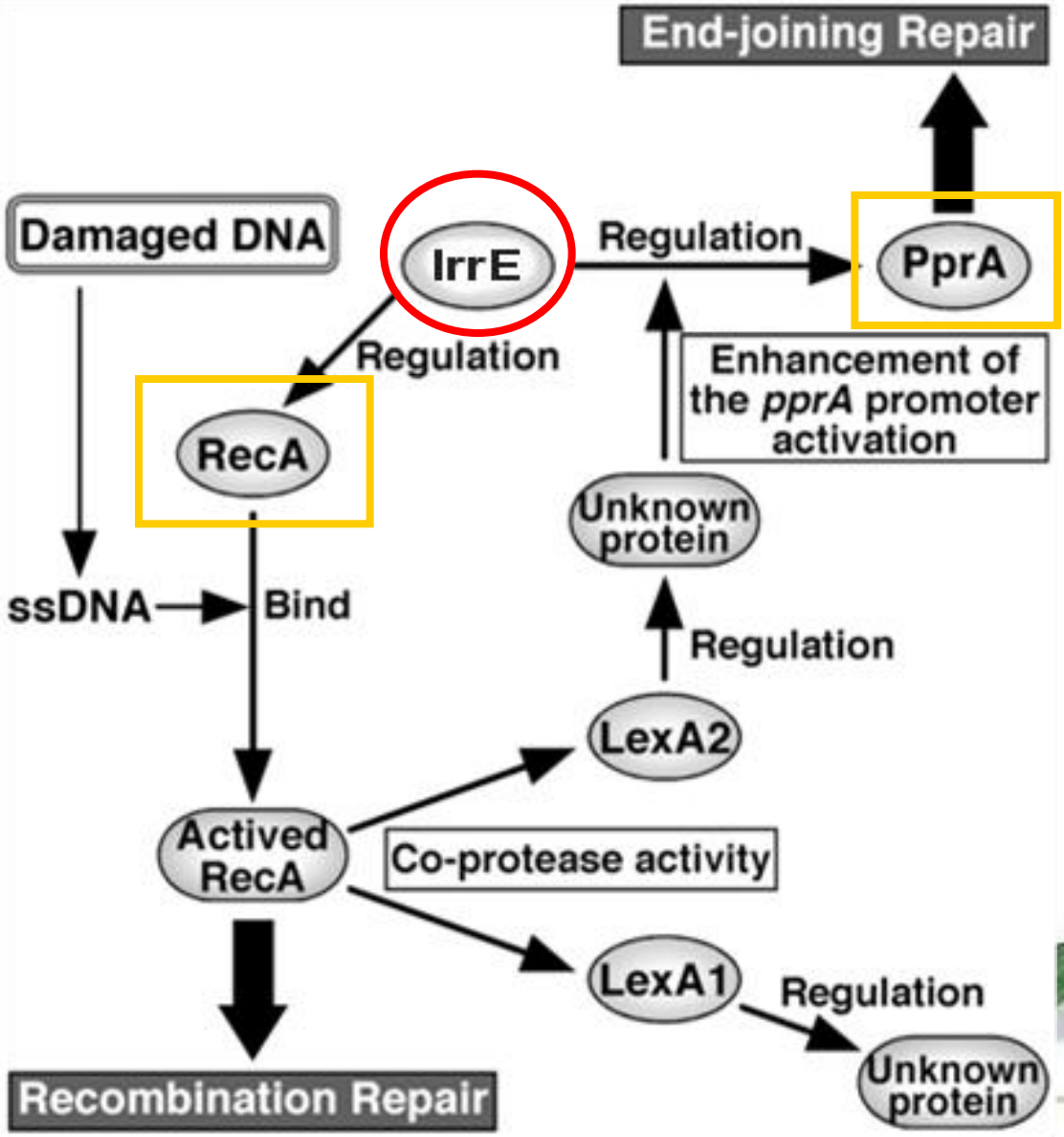
1999年完成耐辐射异常球菌全基因组测序;
其基因组含有2个染色体2个质粒;
预测含有3187个ORF



蛋白序列相似性分析

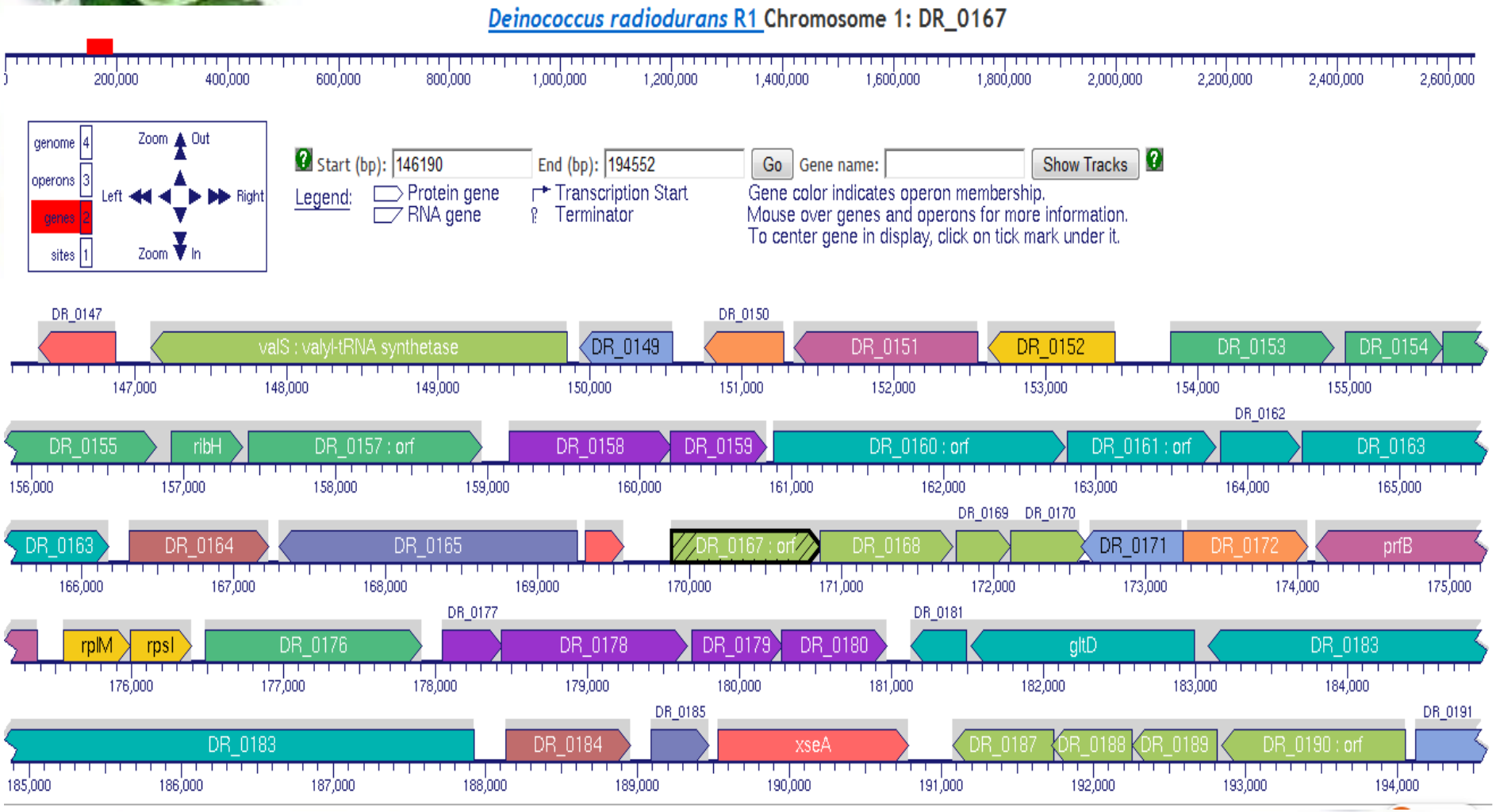
Kira S. Makarova *et al.*, 1999, ***MICROBIOL. MOL. BIOL. REV.***

*irrE*是耐辐射球菌中重要的DNA 修复保护途径开关基因

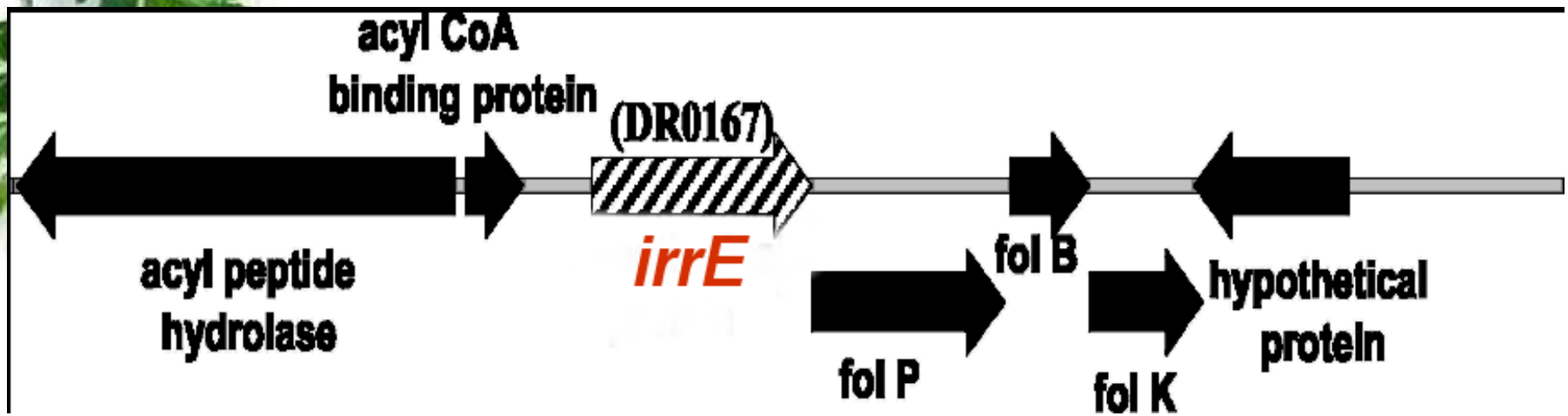


Katsuya Satoh, Microbiology, 2006

DR_0167在染色体上的位置



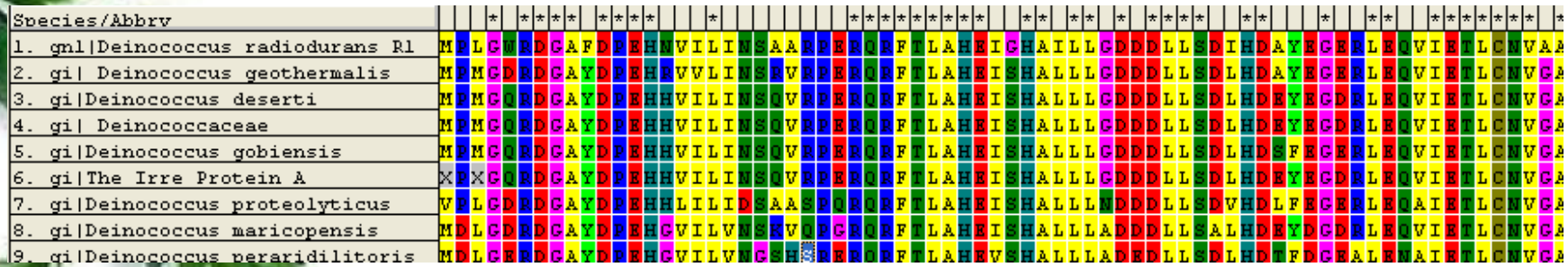
*irrE*基因的研究进展



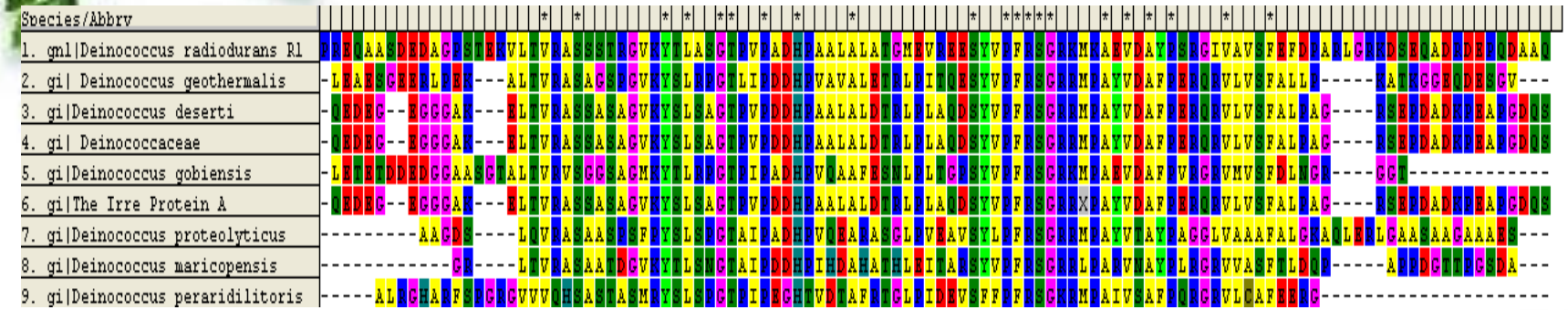
***DR*菌一号染色体上显示DR0167的位置**

EARL ET AL. JOURNAL OF BACTERIOLOGY, Nov. 2002

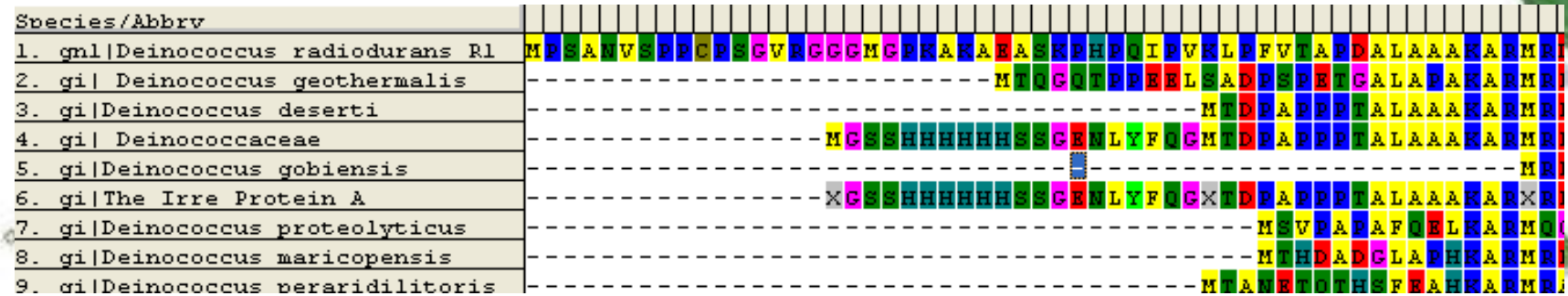
多序列比对


Species/Abbrv
1. gnl|Deinococcus radiodurans R1
2. gi| Deinococcus geothermalis
3. gi|Deinococcus deserti
4. gi| Deinococcaceae
5. gi|Deinococcus gobiensis
6. gi|The Irre Protein A
7. gi|Deinococcus proteolyticus
8. gi|Deinococcus maricopensis
9. gi|Deinococcus peraridilitoris

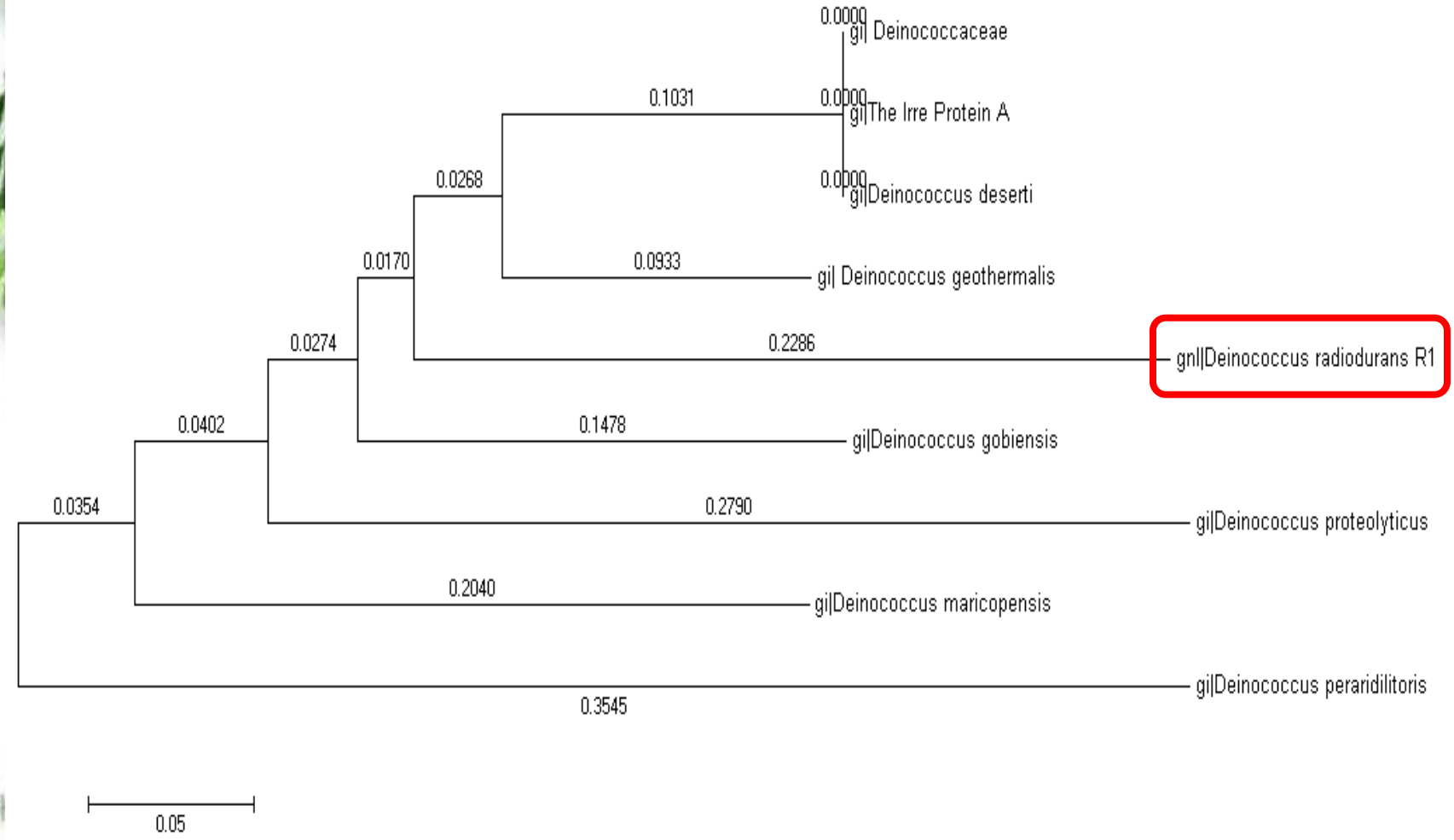
N端


Species/Abbrv
1. gnl|Deinococcus radiodurans R1
2. gi| Deinococcus geothermalis
3. gi|Deinococcus deserti
4. gi| Deinococcaceae
5. gi|Deinococcus gobiensis
6. gi|The Irre Protein A
7. gi|Deinococcus proteolyticus
8. gi|Deinococcus maricopensis
9. gi|Deinococcus peraridilitoris

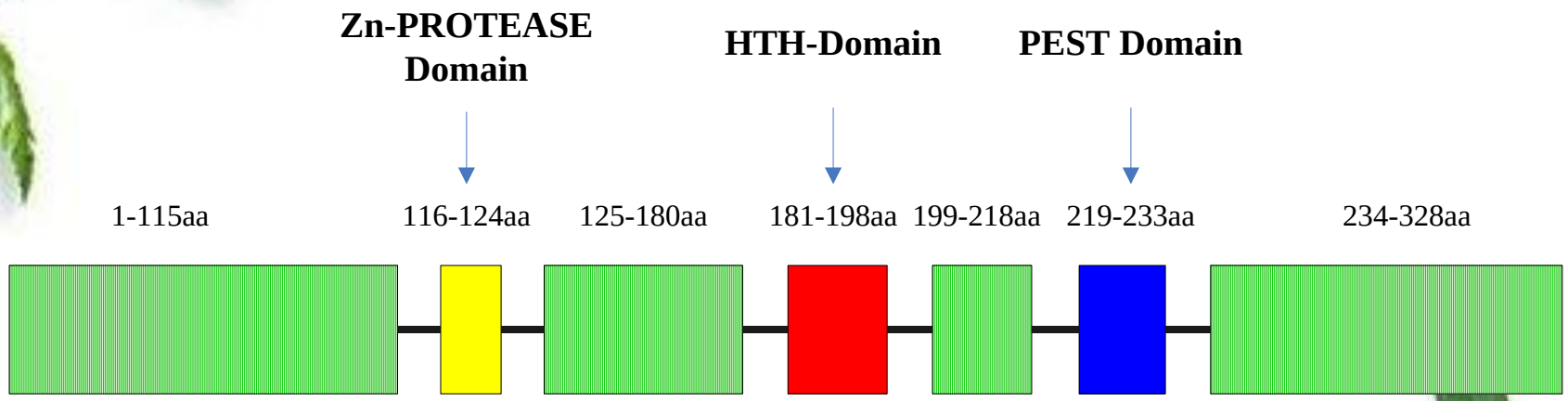
C端


Species/Abbrv
1. gnl|Deinococcus radiodurans R1
2. gi| Deinococcus geothermalis
3. gi|Deinococcus deserti
4. gi| Deinococcaceae
5. gi|Deinococcus gobiensis
6. gi|The Irre Protein A
7. gi|Deinococcus proteolyticus
8. gi|Deinococcus maricopensis
9. gi|Deinococcus peraridilitoris

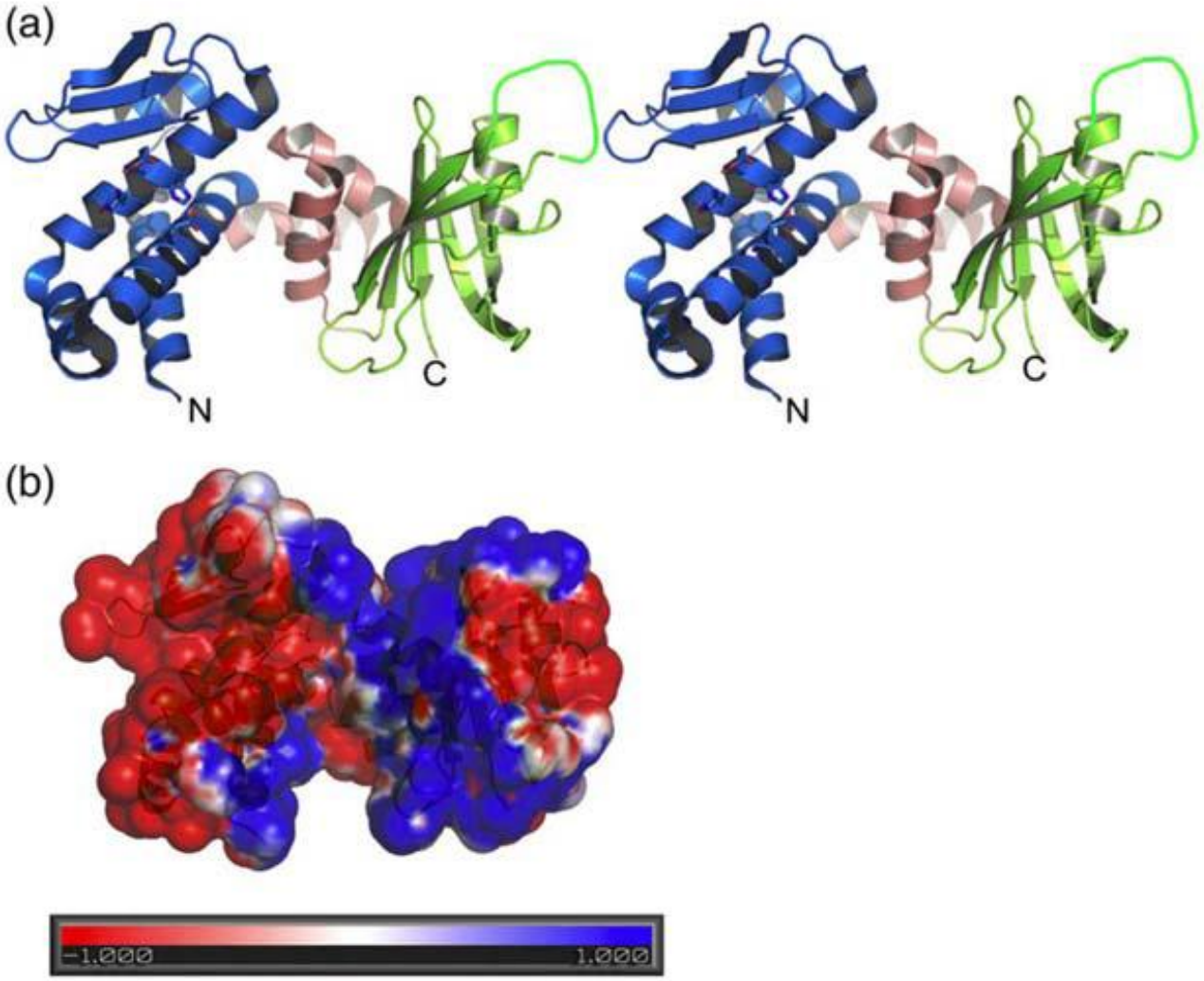
构建irrE蛋白系统发育进化树



IrrE蛋白结构域



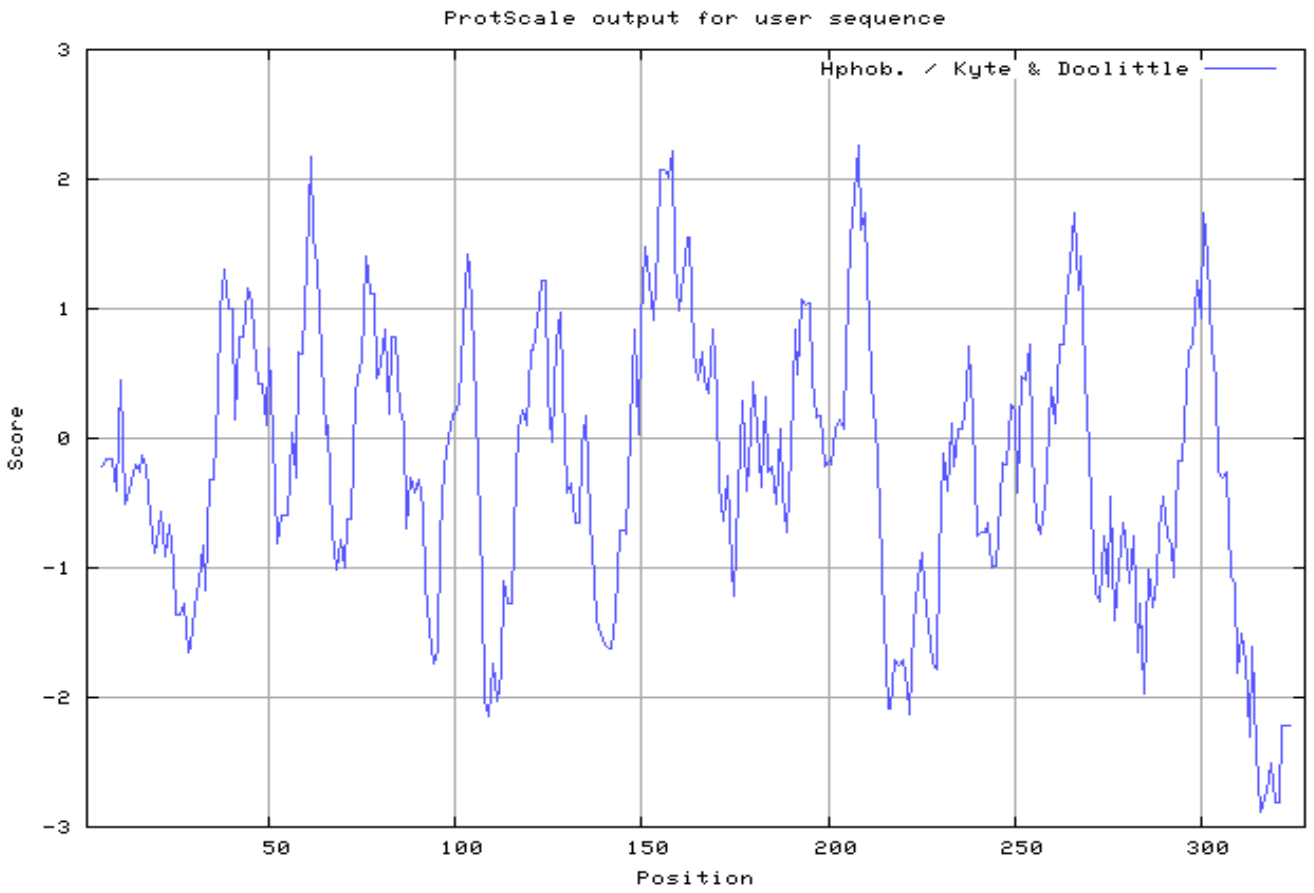
Guanjun Gao, 2005



沙漠异常球菌IrrE 蛋白晶体结构(Vujicic-Zagar *et al.*, 2009)
(a) IrrE 结构立体示意图；(b) IrrE 蛋白的表面电势

- IrrE 蛋白是异常球菌属特异蛋白。对IrrE 蛋白的氨基酸序列进行BLASTp 比对分析，结果显示，IrrE 蛋白由3 个模体（motif）组成。其中包括1 个锌离子依赖型多肽酶模体（1-162 位氨基酸），1 个螺旋-转角-螺旋（HTH）模体（163-204 位氨基酸）和1 个具有小分子结合能力的GAF模体（205-328 位氨基酸）

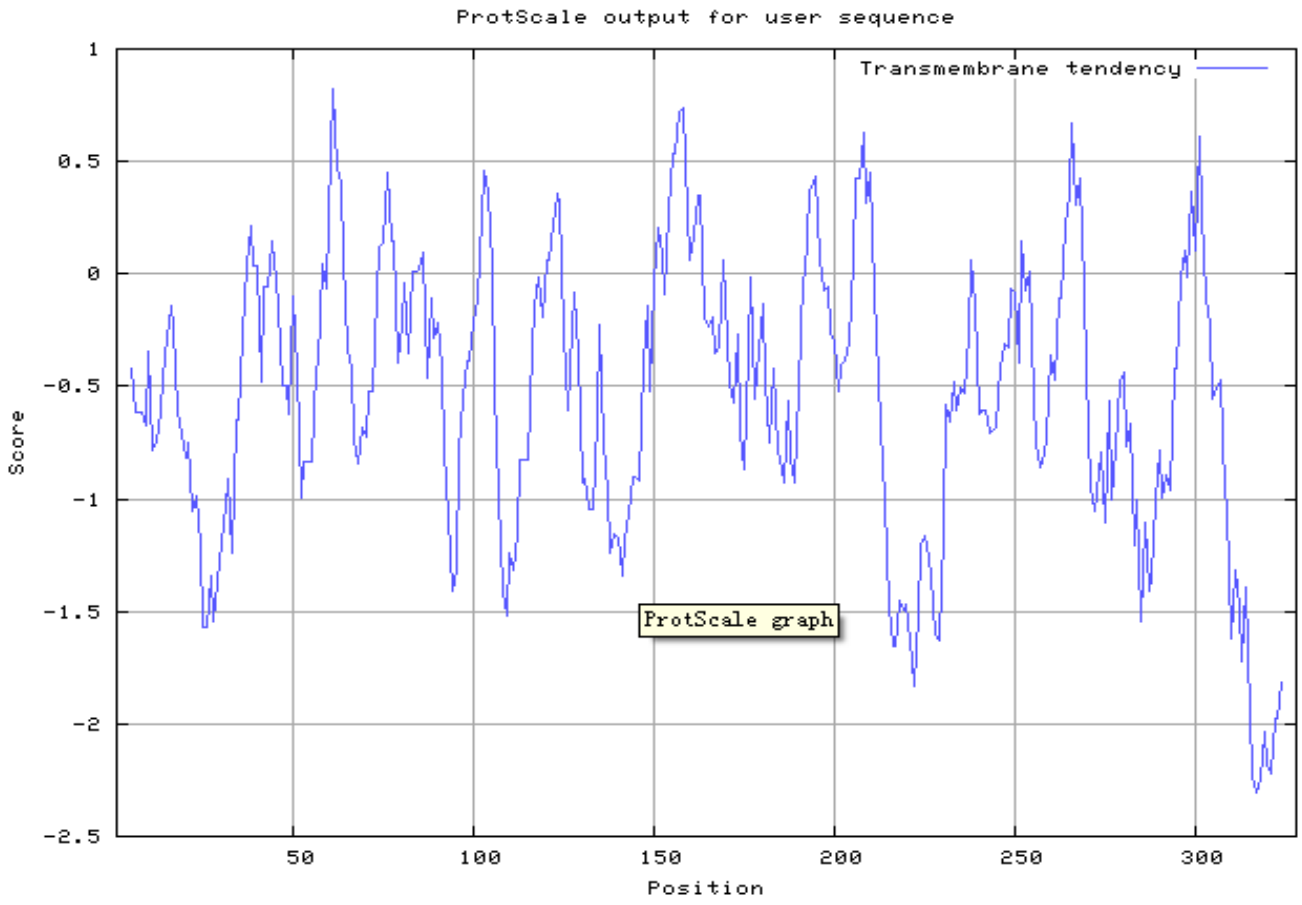
疏水性分析



Using the scale **Hphob. / Kyte & Doolittle**, the individual values for the 20 amino acids are:

```
Ala:  1.800  Arg: -4.500  Asn: -3.500  Asp: -3.500  Cys:  2.500  Gln: -3.500
Glu: -3.500  Gly: -0.400  His: -3.200  Ile:  4.500  Leu:  3.800  Lys: -3.900
Met:  1.900  Phe:  2.800  Pro: -1.600  Ser: -0.800  Thr: -0.700  Trp: -0.900
Tyr: -1.300  Val:  4.200  : -3.500  : -3.500  : -0.490
```

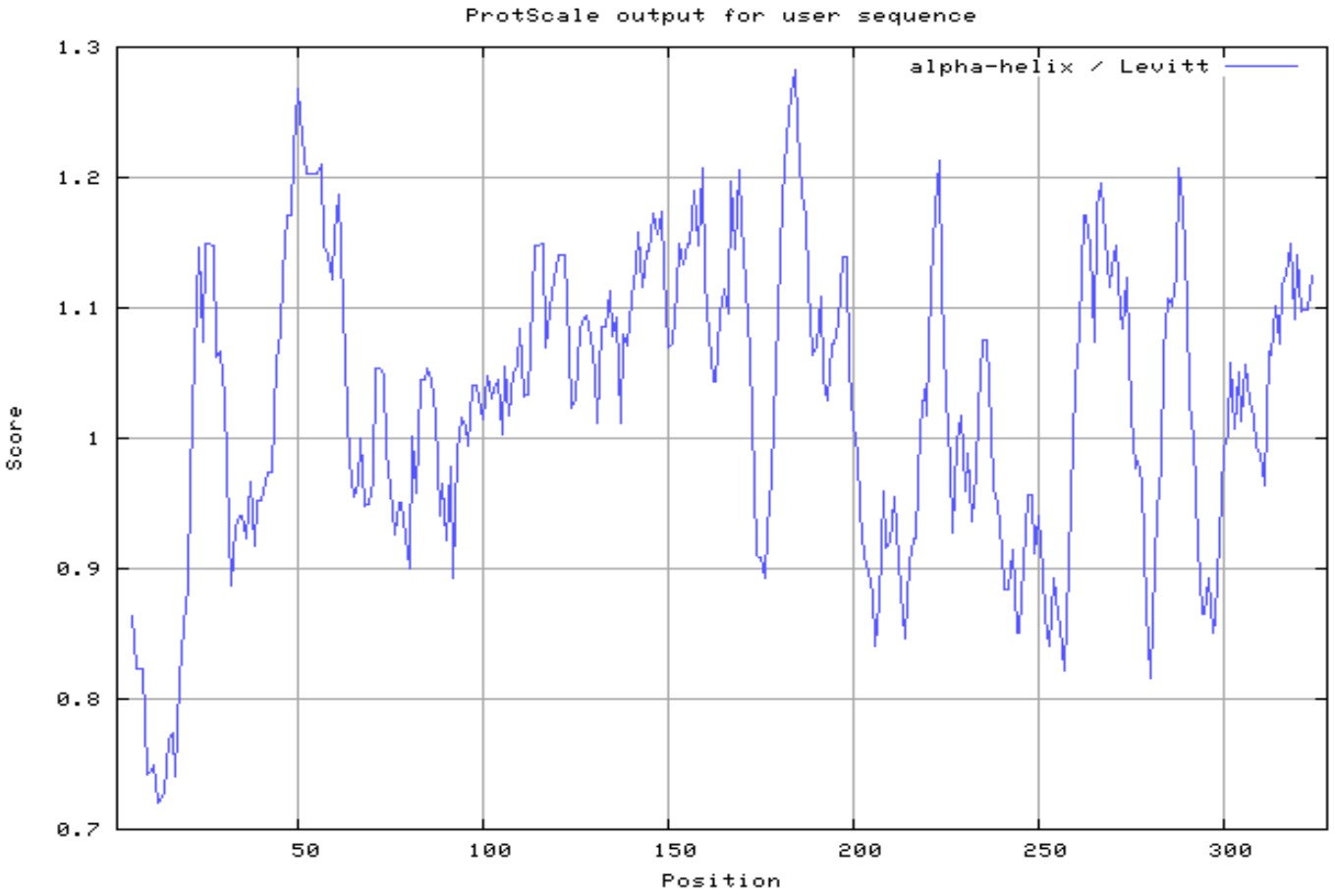
跨膜区域



Using the scale **Transmembrane tendency**, the individual values for the 20 amino acids are:

```
Ala:  0.380  Arg: -2.570  Asn: -1.620  Asp: -3.270  Cys: -0.300  Gln: -1.840
Glu: -2.900  Gly: -0.190  His: -1.440  Ile:  1.970  Leu:  1.820  Lys: -3.460
Met:  1.400  Phe:  1.980  Pro: -1.440  Ser: -0.530  Thr: -0.320  Trp:  1.530
Tyr:  0.490  Val:  1.400  : -2.445  : -2.370  : -0.715
```

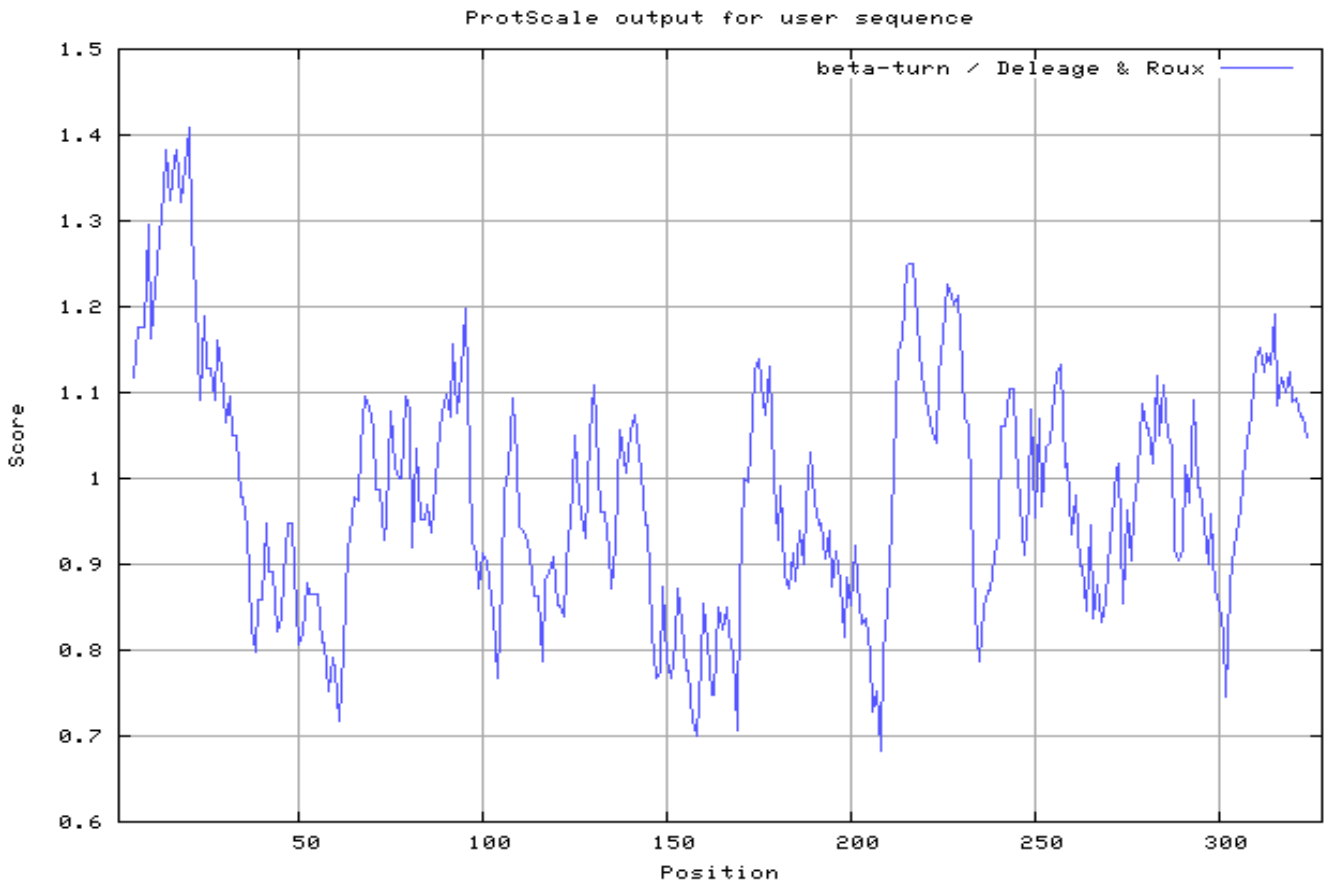
α-螺旋



Weights for window positions 1,..,9, using **linear weight variation model**:

1	2	3	4	5	6	7	8	9
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
edge				center				edge

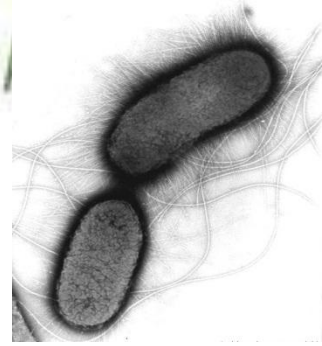
β-折叠



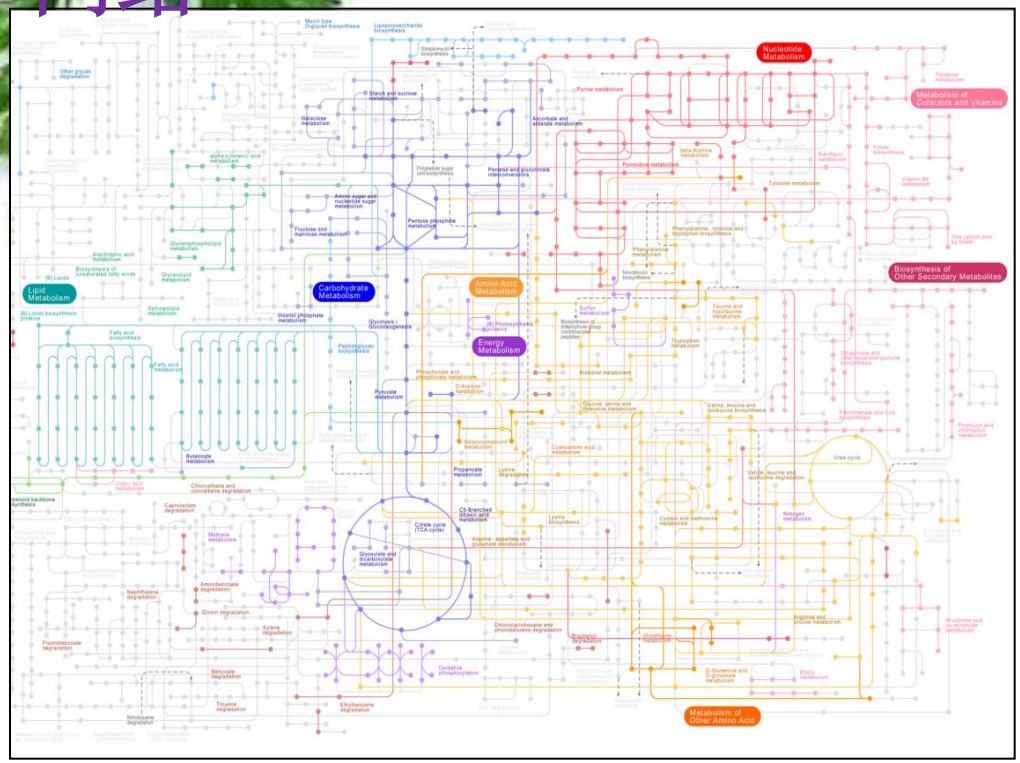
Using the scale **beta-turn / Deleage & Roux**, the individual values for the 20 amino acids are:

Ala:	0.788	Arg:	0.912	Asn:	1.572	Asp:	1.197	Cys:	0.965	Gln:	0.997
Glu:	1.149	Gly:	1.860	His:	0.970	Ile:	0.240	Leu:	0.670	Lys:	1.302
Met:	0.436	Phe:	0.624	Pro:	1.415	Ser:	1.316	Thr:	0.739	Trp:	0.546
Tyr:	0.795	Val:	0.387	:	1.385	:	1.073	:	0.944		

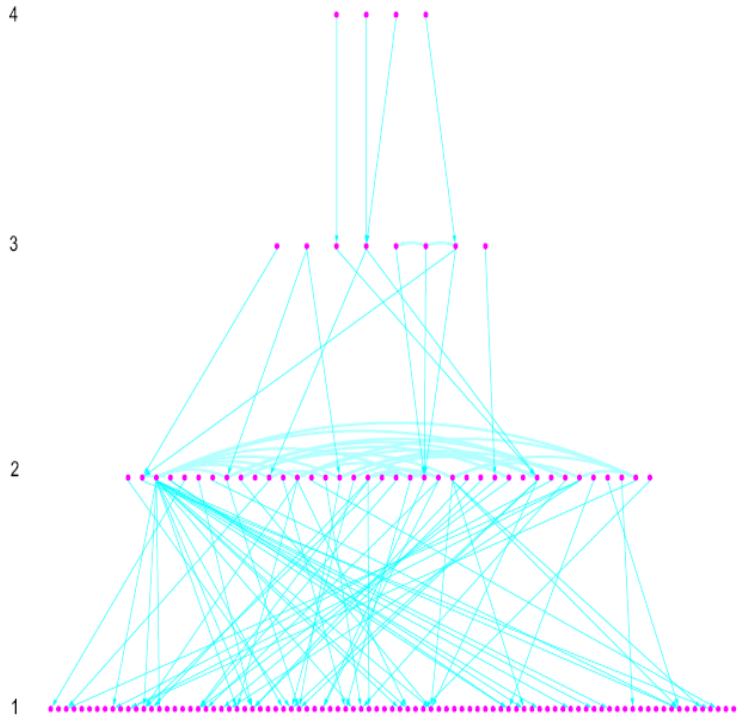
研究基础



大肠杆菌的代谢途径与调控网络



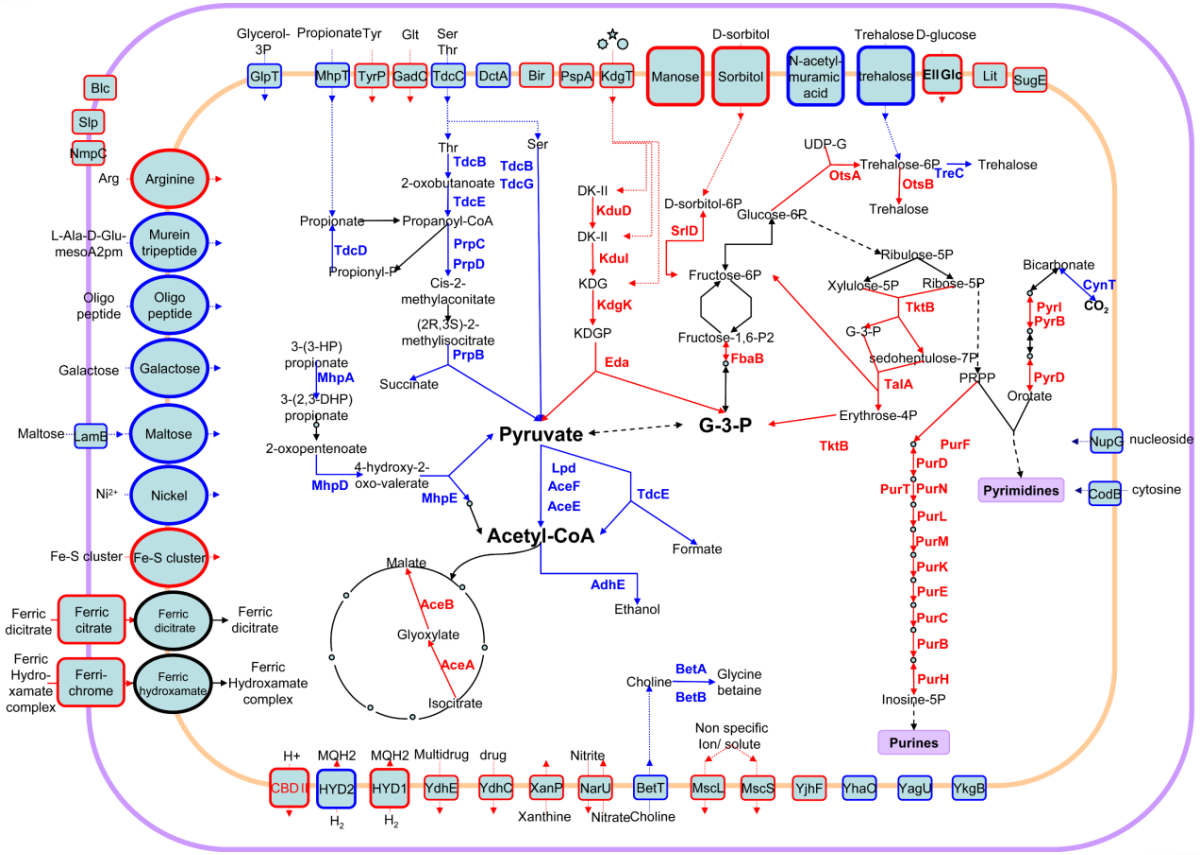
Regulatory hierarchy in *E. coli*



模式生物大肠杆菌

Haiyuan Yu and Mark Gerstein, 2006, *PNAS*

irrE蛋白的全局调控

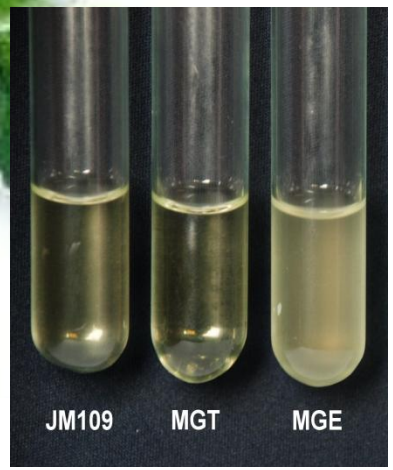


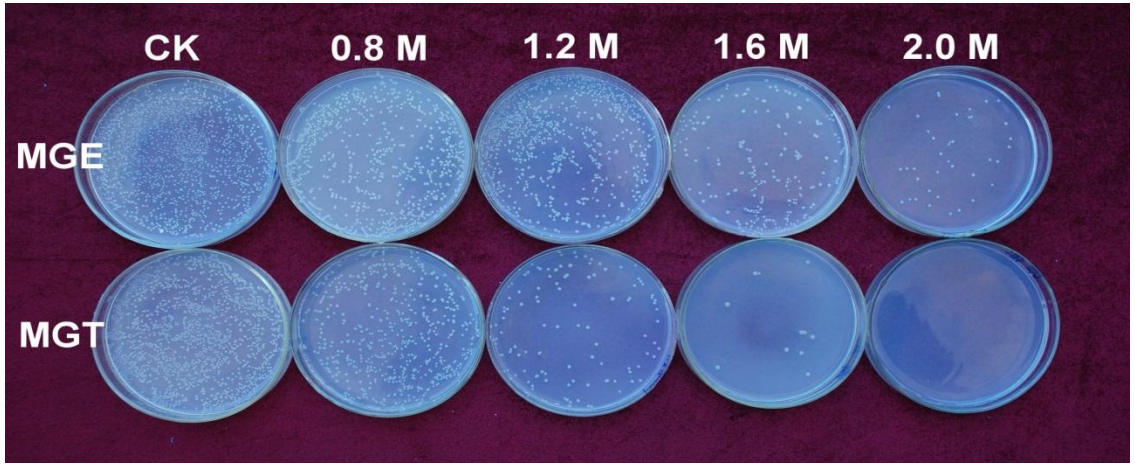
- 216个基因表达上调，149个基因表达下调，
- 其中大肠杆菌中连续90个基因（b0260-b0349）表达受到IrrE的显著抑制。
- 涉及海藻糖代谢，核苷酸代谢，氨基酸代谢，底物利用，酸抗性，呼吸相关酶和转运蛋白等多种代谢功能

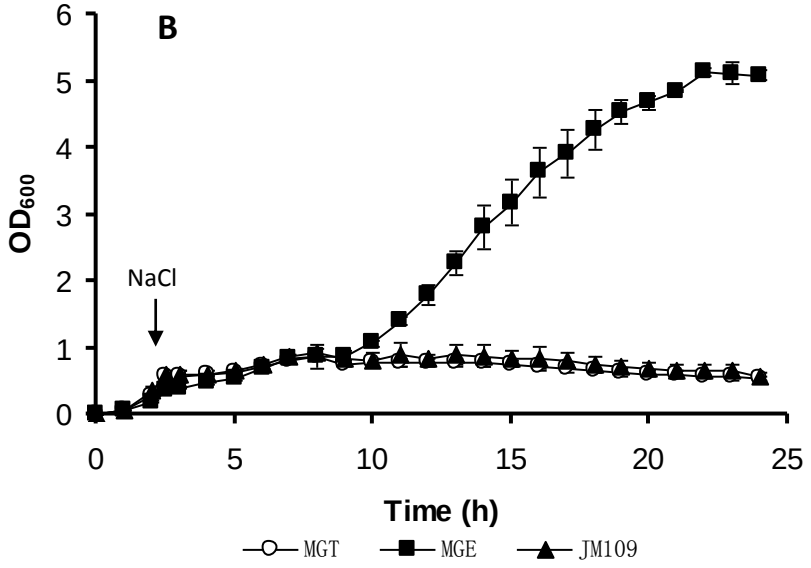
主要内容

1. 研究背景
2. 生物信息学分析
3. 生理生化实验结果
4. 展望

IrrE提高大肠杆菌的耐盐胁迫抗性







A. 0.8 M 氯化钠的LB培养基， 培养15小时

B. IrrE重组大肠杆菌1M氯化钠胁迫下的生长曲线

C. 不同盐浓度冲击下IrrE重组大肠杆菌的生长

主要内容

1. 研究背景
2. 生物信息学分析
3. 生理生化实验结果
4. 展望

应用 展望

- 目前对于耐辐射异常球菌的应用仅限于利用基因工程手段改造该菌，应用于降解辐射污染环境中的重金属和有机污染物。研究 irrE 基因的功能以及调控网络中的作用，对于探索耐辐射异常球菌的极端抗性、揭示DNA损伤修复的分子机制具有理论和实际应用意义，将irrE 基因转移到植物中.

- 感谢罗老师对我们的谆谆教导
- 感谢2012级生物信息学硕士班全体同学
- 感谢G10组的成员