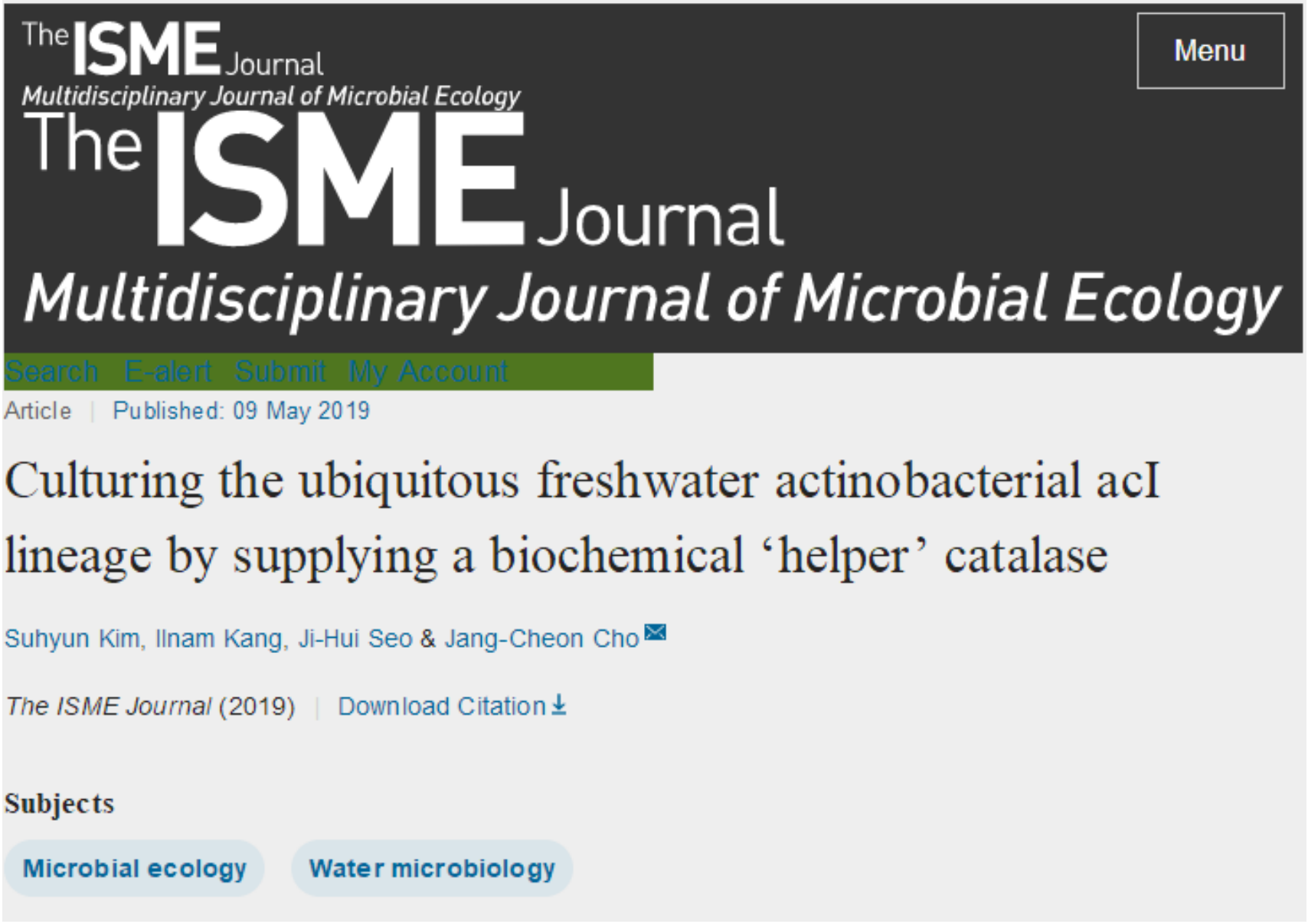
读书报告

汇报人：牛铭铭

2019.07.07



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通过提供生化“辅助”过氧化氢酶培养无处不在的淡水放线菌acI谱系

CONTENTS

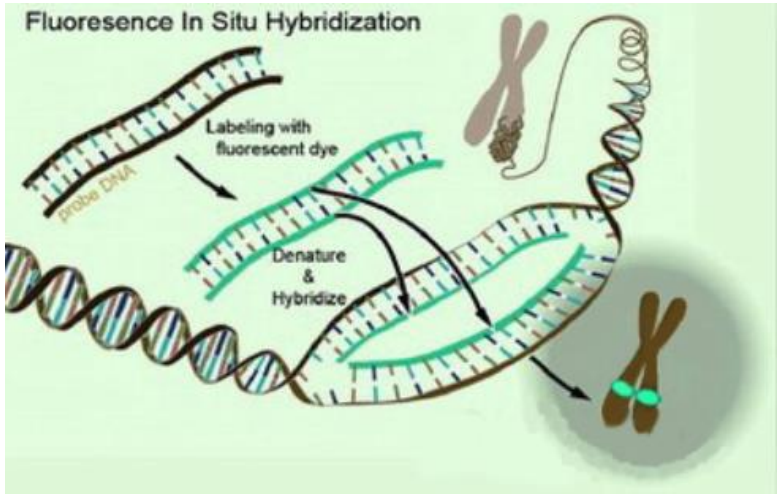
Introduction

1 Introduction

acI lineage

放线菌门的acI谱系是淡水环境中数量最多、分布最广的细菌群。

1 Introduction



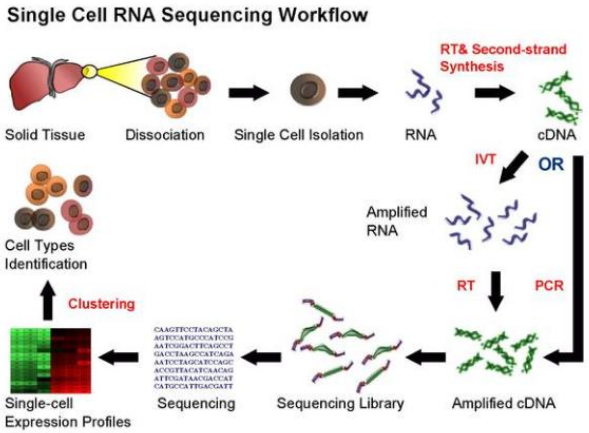
荧光原位杂交技术

16S rRNA基因序列分析

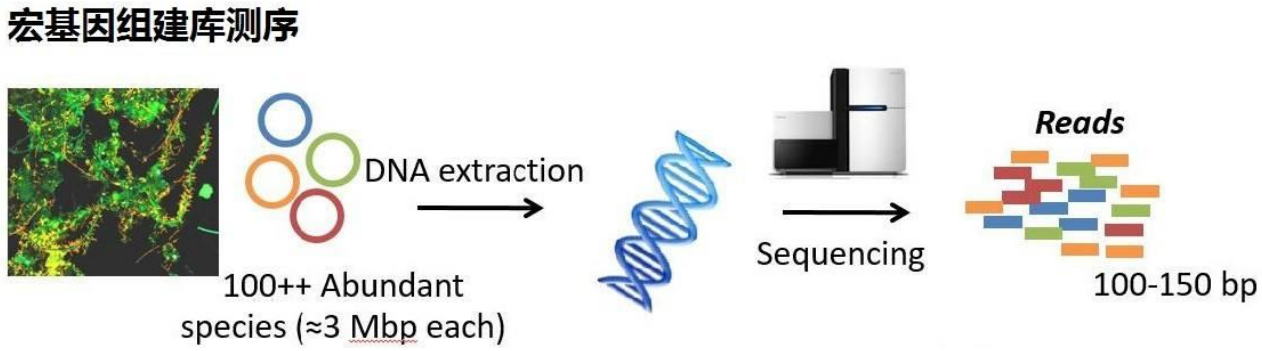
研究表明，acI谱系及其亚组根据季节，深度和栖息地特征表现出特定的分布，并且有> 10个单系部落属于三个亚系（acI-A，-B和-C）(Newton RJ *et al.*, 2011)。

1 Introduction

acI基因组体积小(1.2–1.6 Mb)，富含获取和利用碳水化合物及含氮量高的有机化合物的基因。（Garcia SL *et al.*,2013；Ghylin TW *et al.*, 2014；Ghai R *et al.*, 2014）。

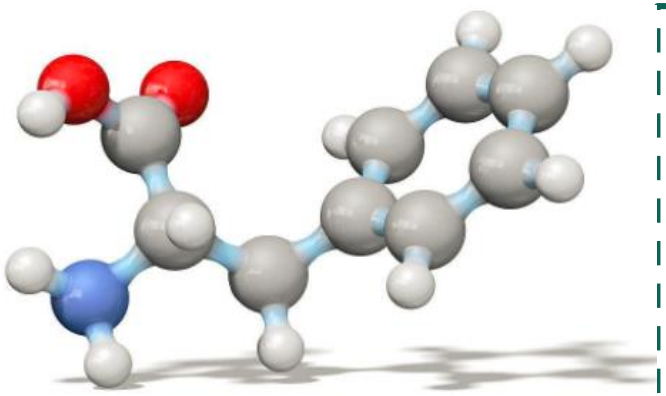


单细胞基因组测序
(SAGs)



宏基因组测序
(MAGs)

1 Introduction



acI谱系能够利用不同的底物，包括亮氨酸，胸苷，葡萄糖，乙酸盐，N-乙酰基葡糖胺和氨基酸混合物，但底物利用模式取决于底物，栖息地和亚系（Eckert EM *et al.*,2012； Salcher MM *et al.*,2013）。

acI谱系依赖于共存的微生物来提供各种维生素和氨基酸。（Garcia SL *et al.*,2018）。

1 Introduction

尽管在各种培养努力后从acI谱系的混合或无菌培养物中获得了丰富的基因组数据，但所有初始培养物都未能成为稳定生长的纯培养物（Kang I *et al.*,2017; Neuenschwander SM *et al.*, 2018）。

缺乏纯培养物阻碍了对该谱系的生理学和生态学的进一步研究。

“要真正了解它们，你必须培养它们”（Charnock *et al*，2017）

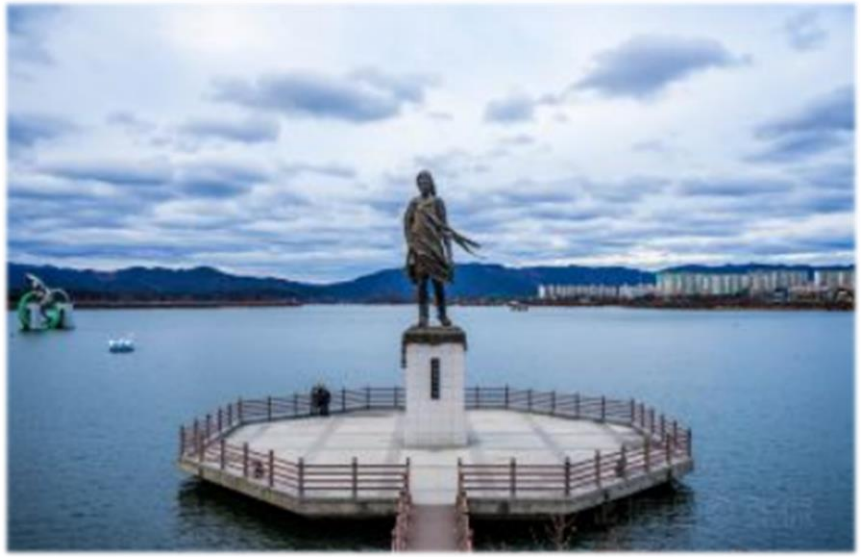
1 Introduction

首次建立了稳定生长的acI谱系纯培养物；

分析了acI菌株的表型特征，并确定了仅基于基因组信息难以预测的代谢特征以及符合先前基因组指导推断的特征。

Materials and Methods

2 样品采集



采样地点：韩国春川昭阳湖

采样深度：水下1m

水样处理：0.2μm滤膜过滤，高压灭菌1.5h，充气3h。

acI strains:

IMCC25003 / IMCC26103

2 acI细菌的初步分离

Table S1 Media used in this study and their composition.

Components of media		
Components (abbreviation)	Compound(s)	Final concentration
Ammonium (N)	NH_4Cl	10 µM
Phosphate (P)	KH_2PO_4	10 µM
Trace metals (TM)	$FeCl_3 \cdot 6H_2O$	117 nM
	$MnCl_2 \cdot 4H_2O$	9 nM
	$ZnSO_4 \cdot 7H_2O$	800 pM
	$CoCl_2 \cdot 6H_2O$	500 pM
	$Na_2MoO_4 \cdot 2H_2O$	300 pM
	Na_2SeO_3	1 nM
	$NiCl_2 \cdot 6H_2O$	1 nM
Vitamin mixture (V)	Thiamine·HCl	59 nM
	Niacin	81 nM
	Ca-Pantothenate	84 nM
	Pyridoxine	59 nM
	Biotin	409 pM
	Folic acid	453 pM
	Vitamin B12	70 pM
	Myo-inositol	555 nM
	p-Aminobenzoic Acid	7 nM
Carbon mixture (CM)	Pyruvate	50 µM
	D-Glucose	5 µM
	N-Acetyl-D-glucosamine	5 µM
	D-Ribose	5 µM
	Methyl alcohol	5 µM
20 proteinogenic amino acid mixture (AA)	Each amino acid	100 nM, each

Media definition	
Media	Definition
FAM	0.2 µm-filtered and autoclaved freshwater medium supplemented with N, P, and TM
FAMV	FAM supplemented with V
FAMV+CM	FAMV supplemented with CM
FAMV+AA	FAMV supplemented with AA
FAMV+CM+AA	FAMV supplemented with CM and AA

2

Table S2 Trials to establish pure culture of strain IMCC25003.

Trial	Media composition	Additional substrate	Reference
1st attempt	FAMV		
	FAMV+CM		
	FAMV+AA	0.5×, 1×, 5×, and 10× of CM	
	AFM[a]+V+CM+AA		
	FM[b]+V+CM+AA		
2nd attempt	FAMV+CM+AA	20 μM acetate	[14]
		20 μM oxaloacetate	[6]
		20 μM putrescine	[6, 15]
		20 μM glycerol	[6]
		20 μM xylose	[15, 16]
		1 mg L^{-1} proteose peptone No. 3	
		1 mg L^{-1} yeast extract	
3rd attempt	FAMV+CM+AA	1:20 diluted spent medium[c]	[15, 17], This study
4th attempt	FAMV+CM+AA	10 U mL^{-1} catalase	

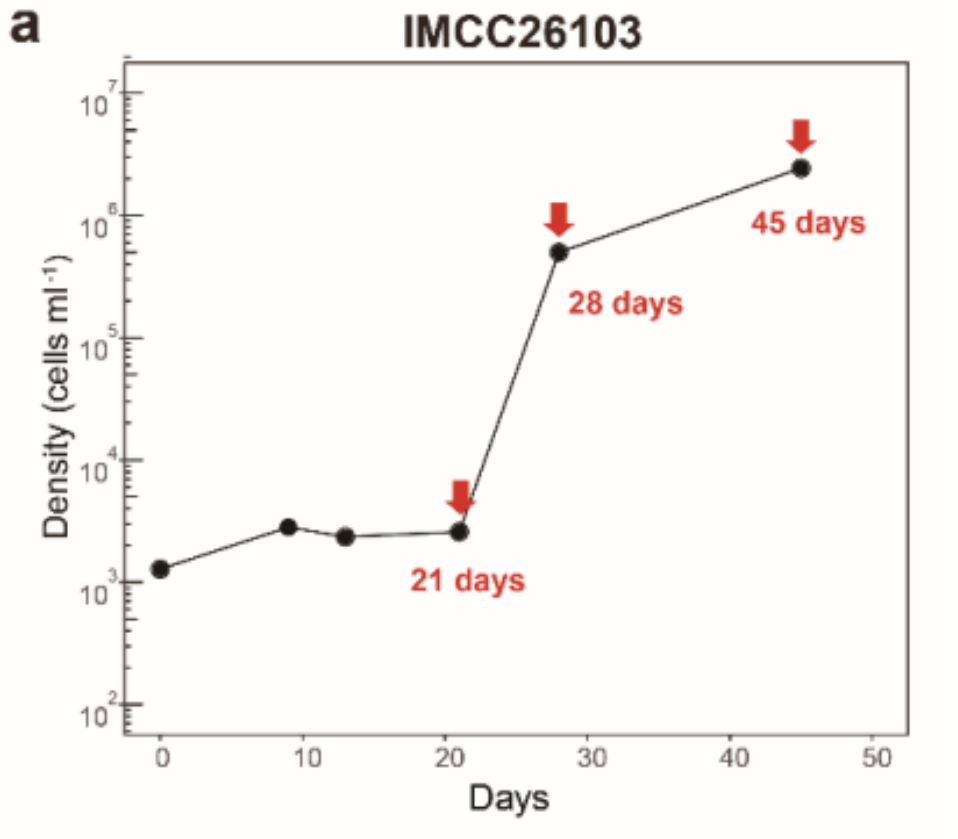
[a]AFM, Artificial freshwater medium [18]. [b]FM, 0.1 μm-filtered but non-autoclaved freshwater medium. [c]Spent medium, a spent medium of the genus *Limnohabitans* filtrated through 0.1 μm pore-size membrane after cultivation of *Limnohabitans* sp. IMCC26003. For the media abbreviations, refer to Supplementary Table S1.

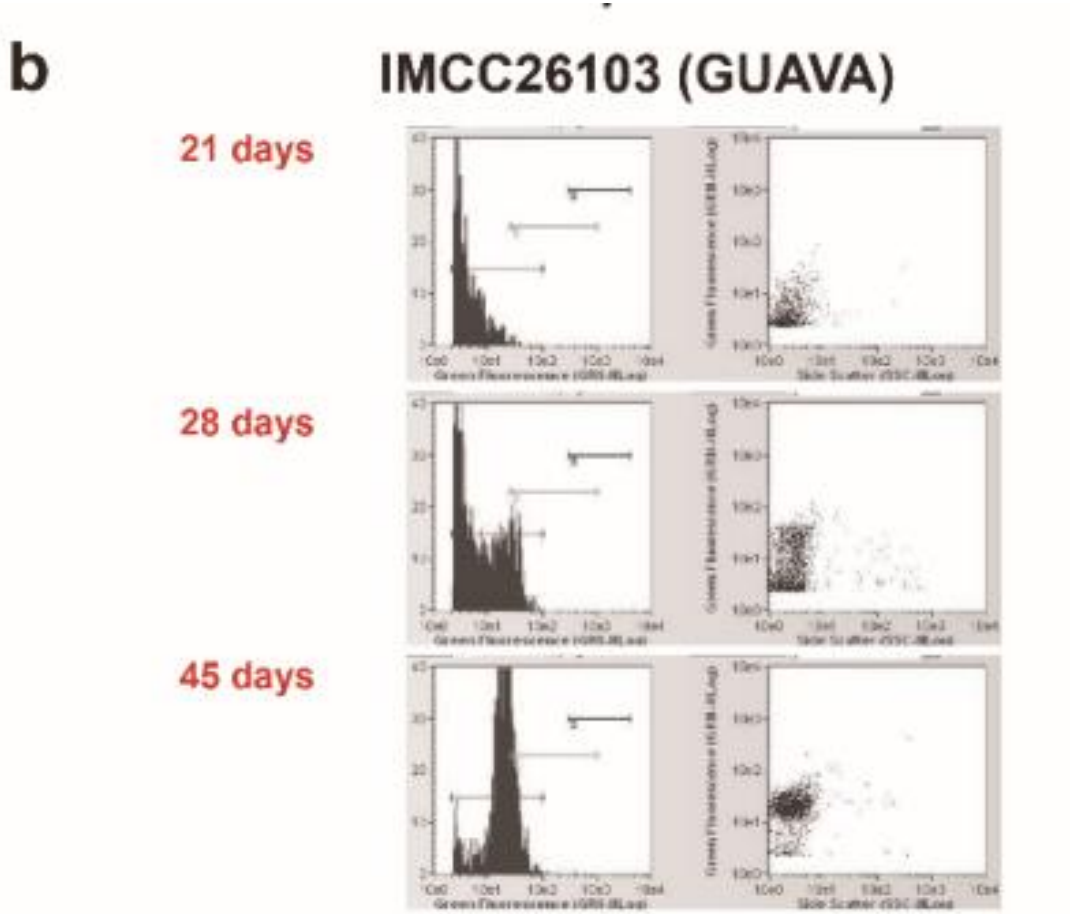
18°C for 5weeks

Results and Discussion

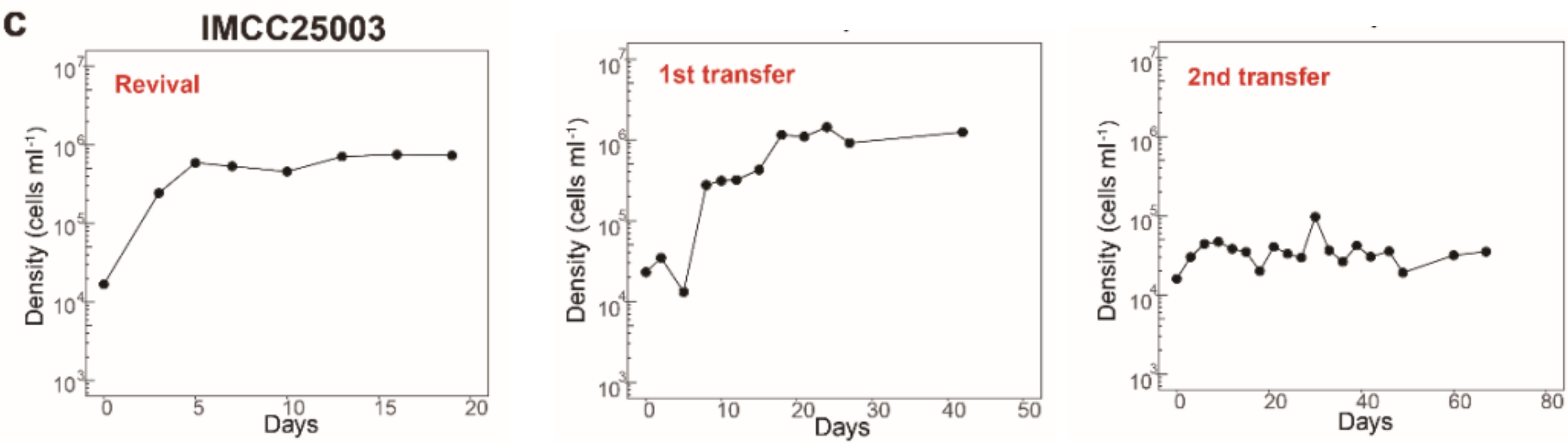
3 acI 菌株的复苏和传代

1st attempt





3 acI 菌株的复苏和传代

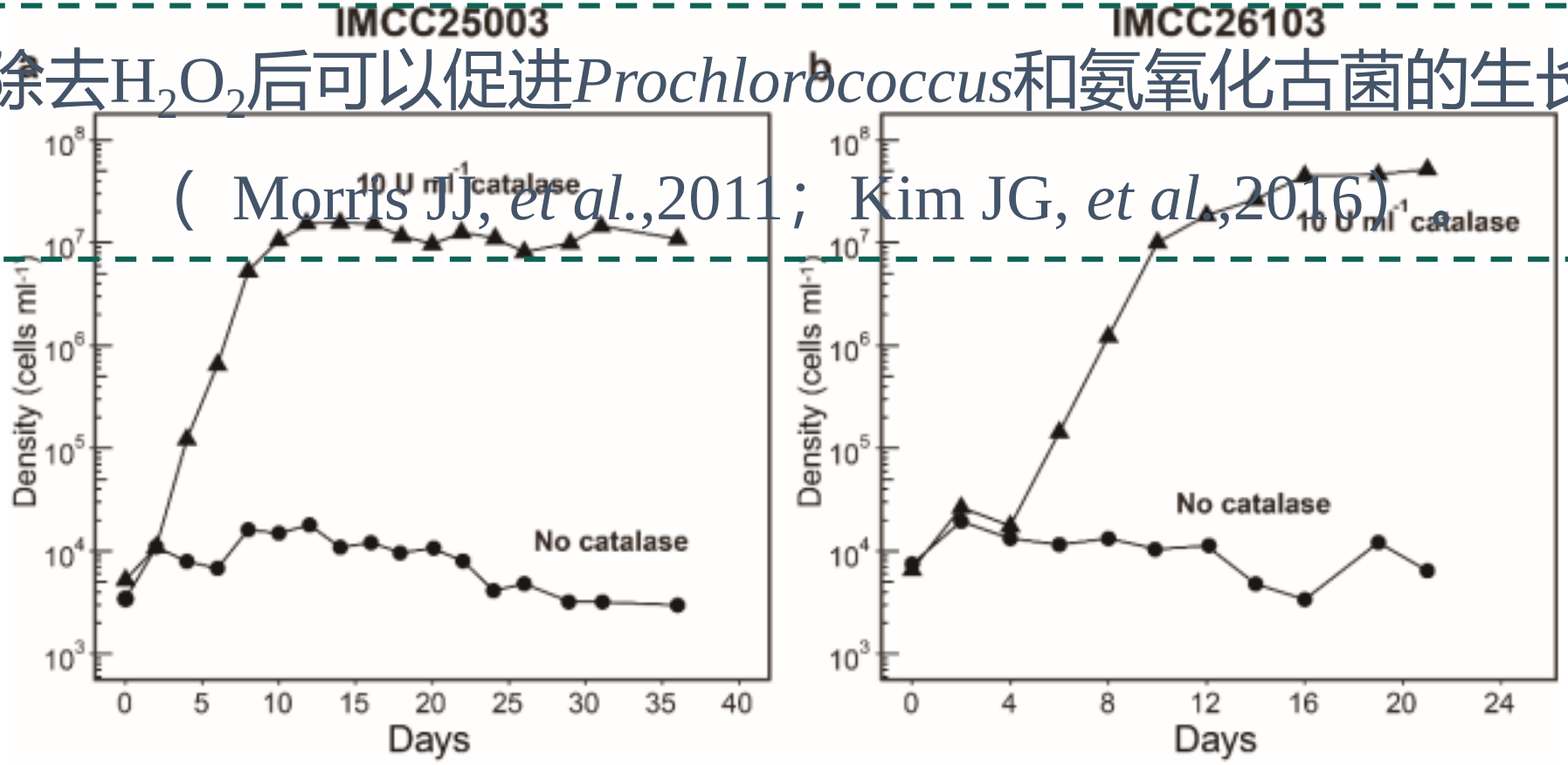


3 通过添加过氧化氢酶成功维持acI菌株的稳定生长

提出假设：控制生长抑制剂可能比提供生长促进剂更重要。

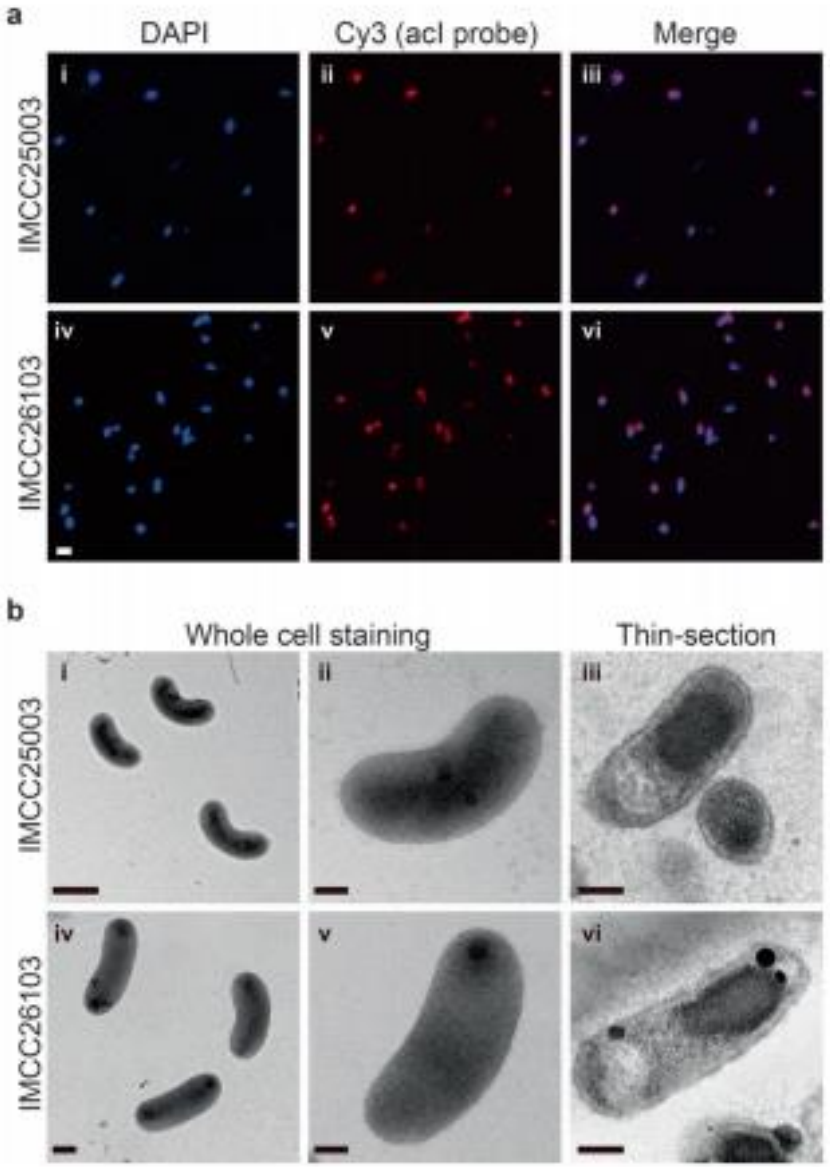
除去H_2O_2后可以促进*Prochlorococcus*和氨氧化古菌的生长。

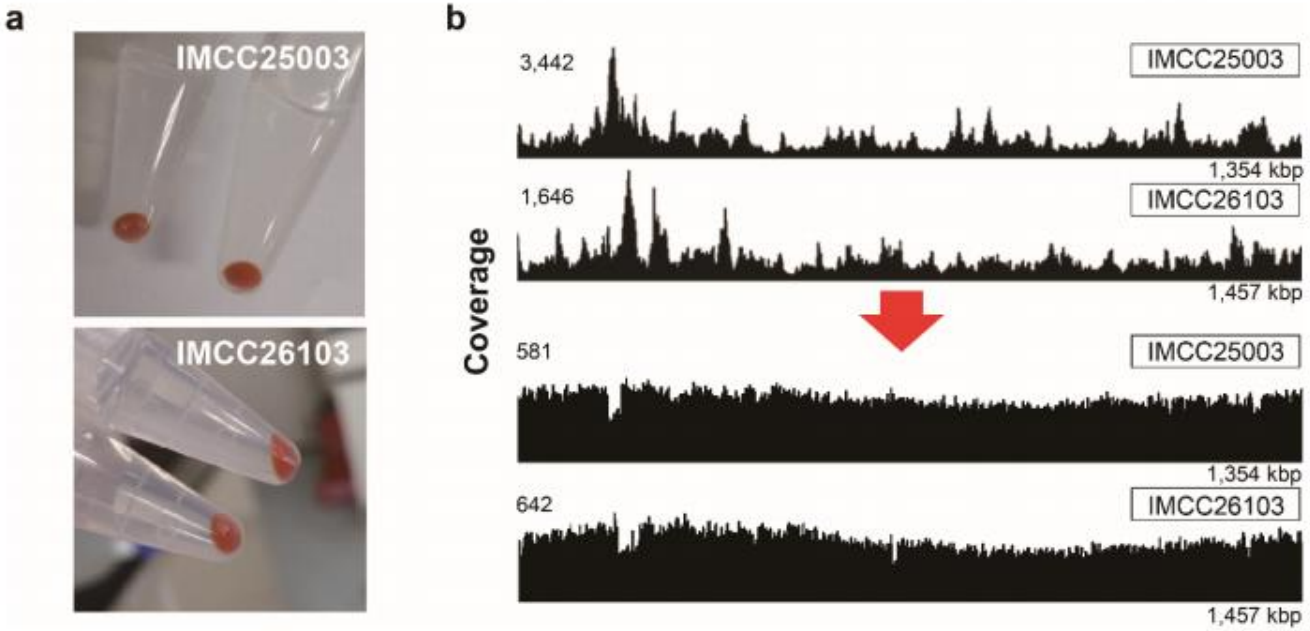
（ Morris JJ, *et al*.,2011； Kim JG, *et al*.,2016）



过氧化氢酶

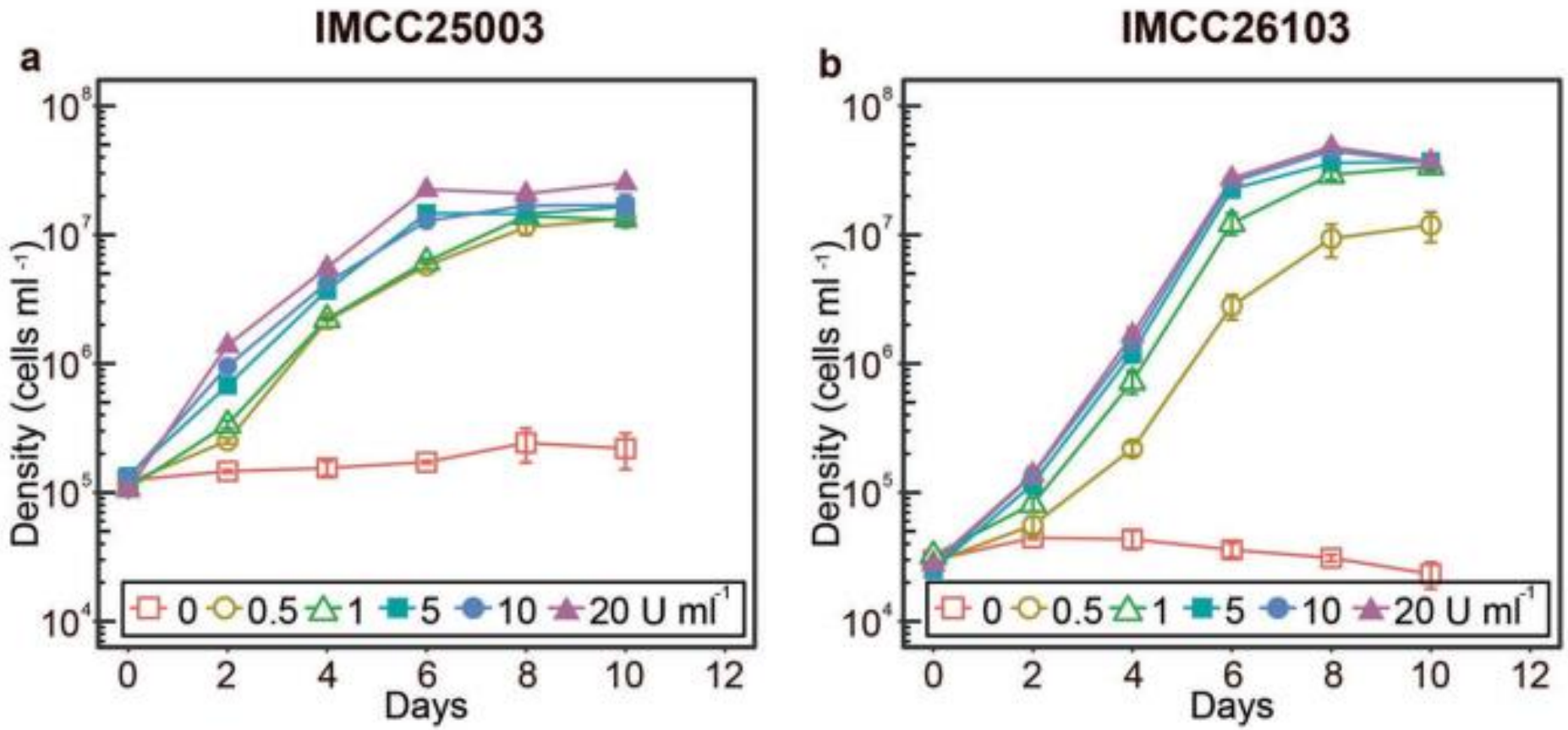
3 培养物纯度的确认


a
DAPI
Cy3 (acl probe)
Merge
IMCC25003
IMCC26103
i
ii
iii
iv
v
vi
b
Whole cell staining
Thin-section
IMCC25003
IMCC26103
i
ii
iii
iv
v
vi


a
IMCC25003
IMCC26103
b
Coverage
3,442
IMCC25003
1,354 kbp
1,646
IMCC26103
1,457 kbp
581
IMCC25003
1,354 kbp
642
IMCC26103
1,457 kbp

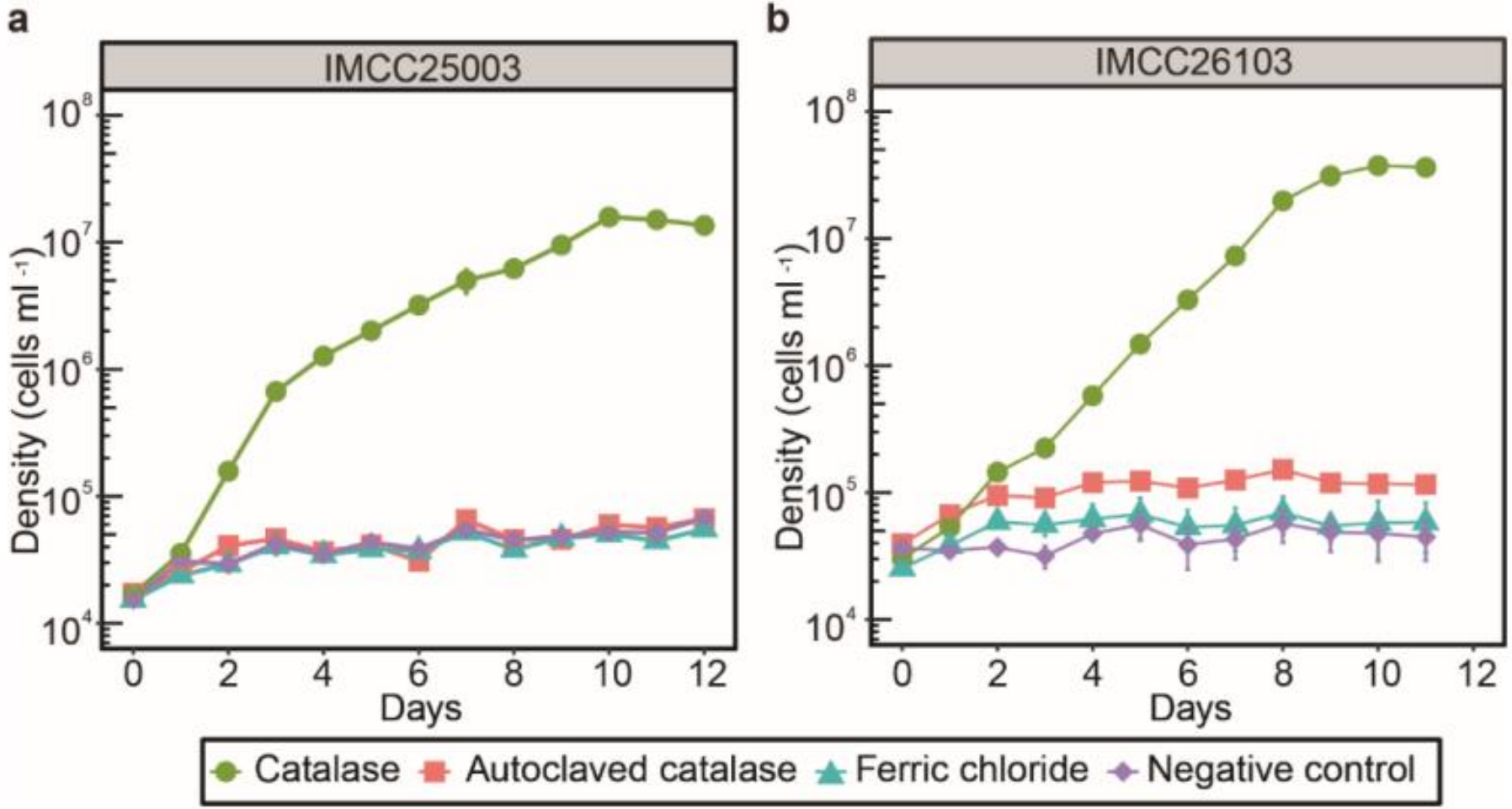
3 过氧化氢酶依赖的acI菌株的生长特征

1、过氧化氢酶浓度



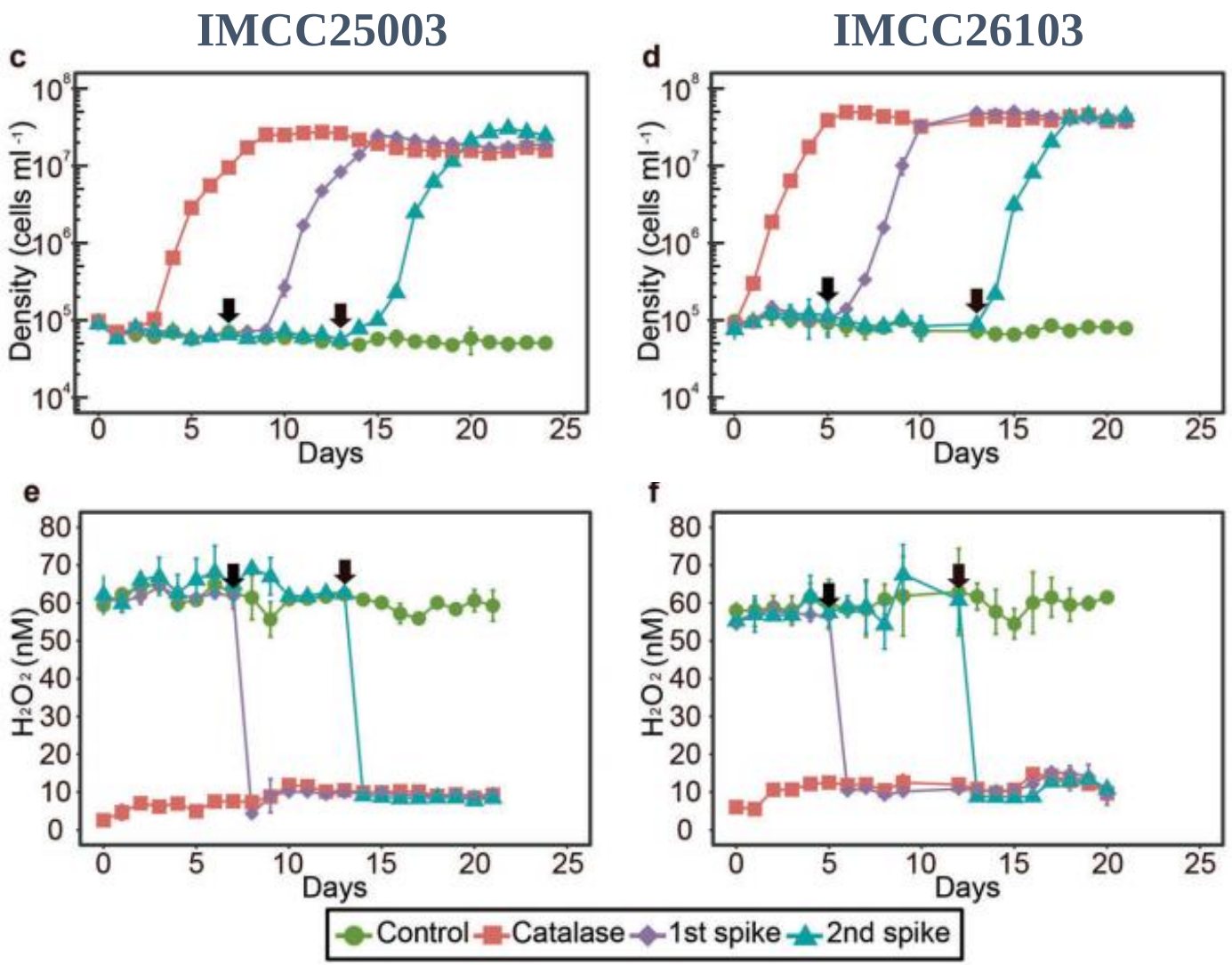
3 过氧化氢酶依赖的acI菌株的生长特征

2、铁离子和灭活的过氧化氢酶



3 过氧化氢酶依赖的acI菌株的生长特征

3、细胞密度和H2O2浓度



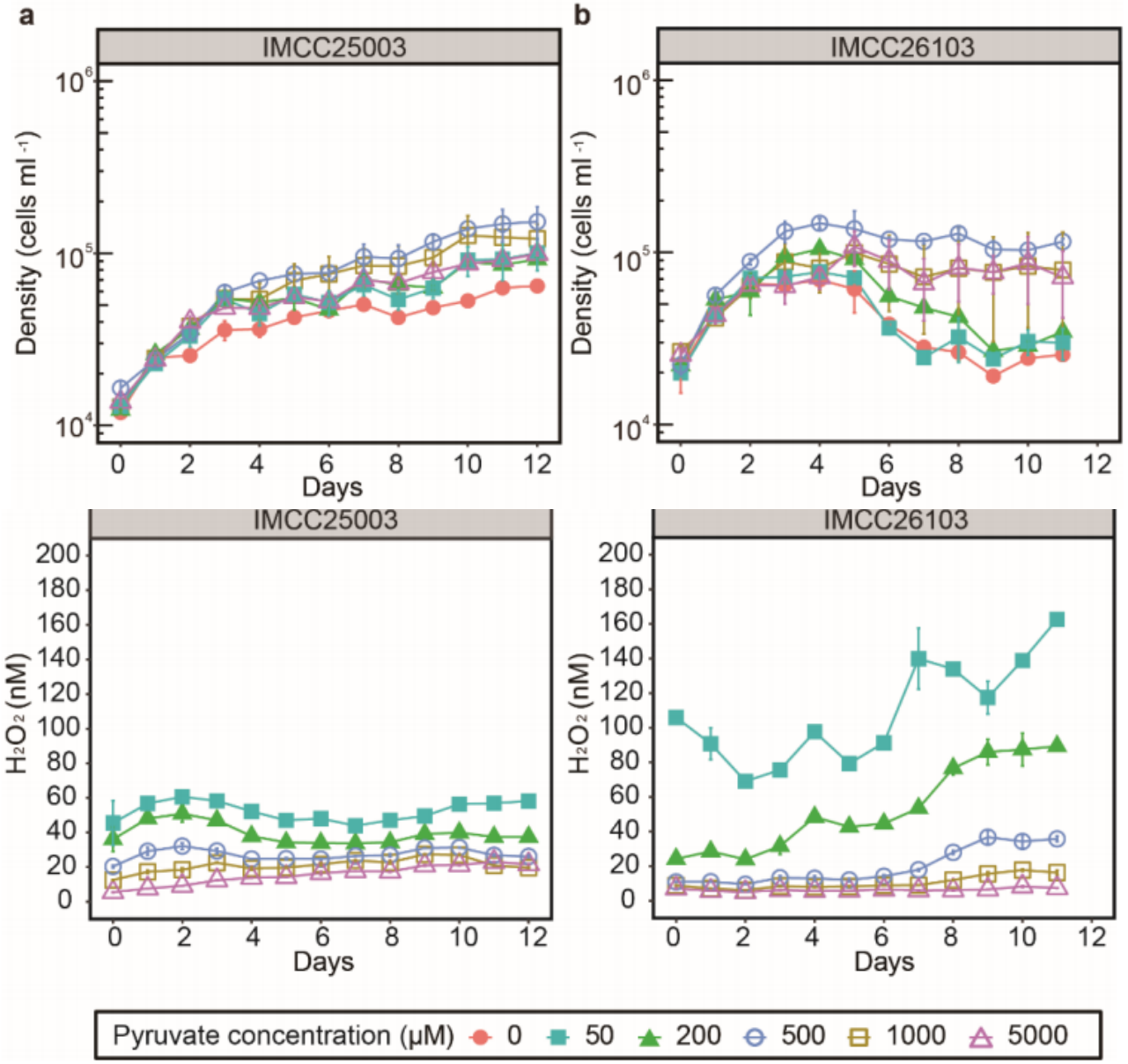
过氧化氢酶去除H_2O_2对于维持acI菌株的生长可能是重要的。

3 过氧化氢酶依赖的acI菌株的生长特征

4、丙酮酸

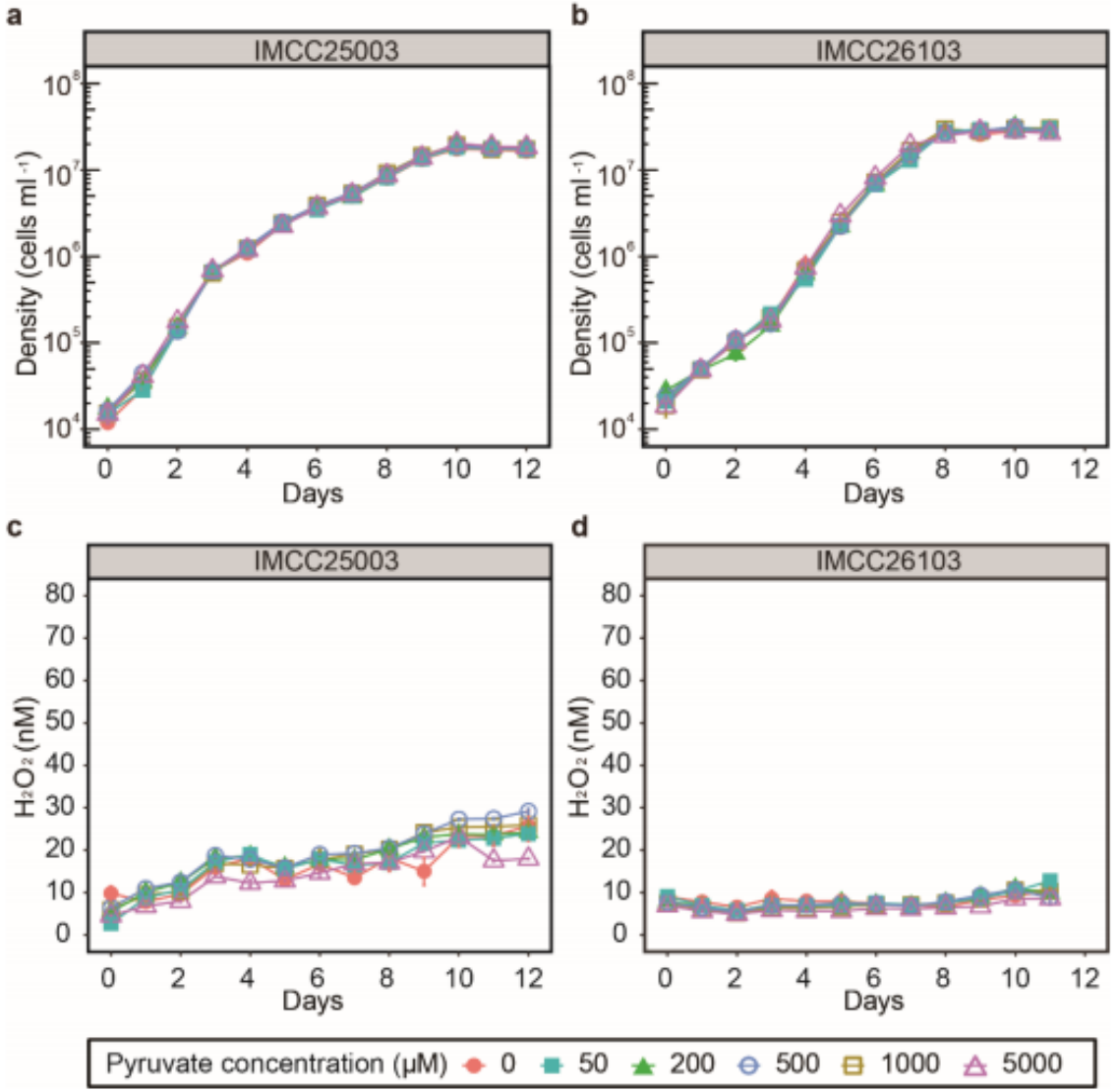
菌株生长增强水平：

丙酮酸<过氧化氢酶



3 过氧化氢酶依赖的acI菌株的生长特征

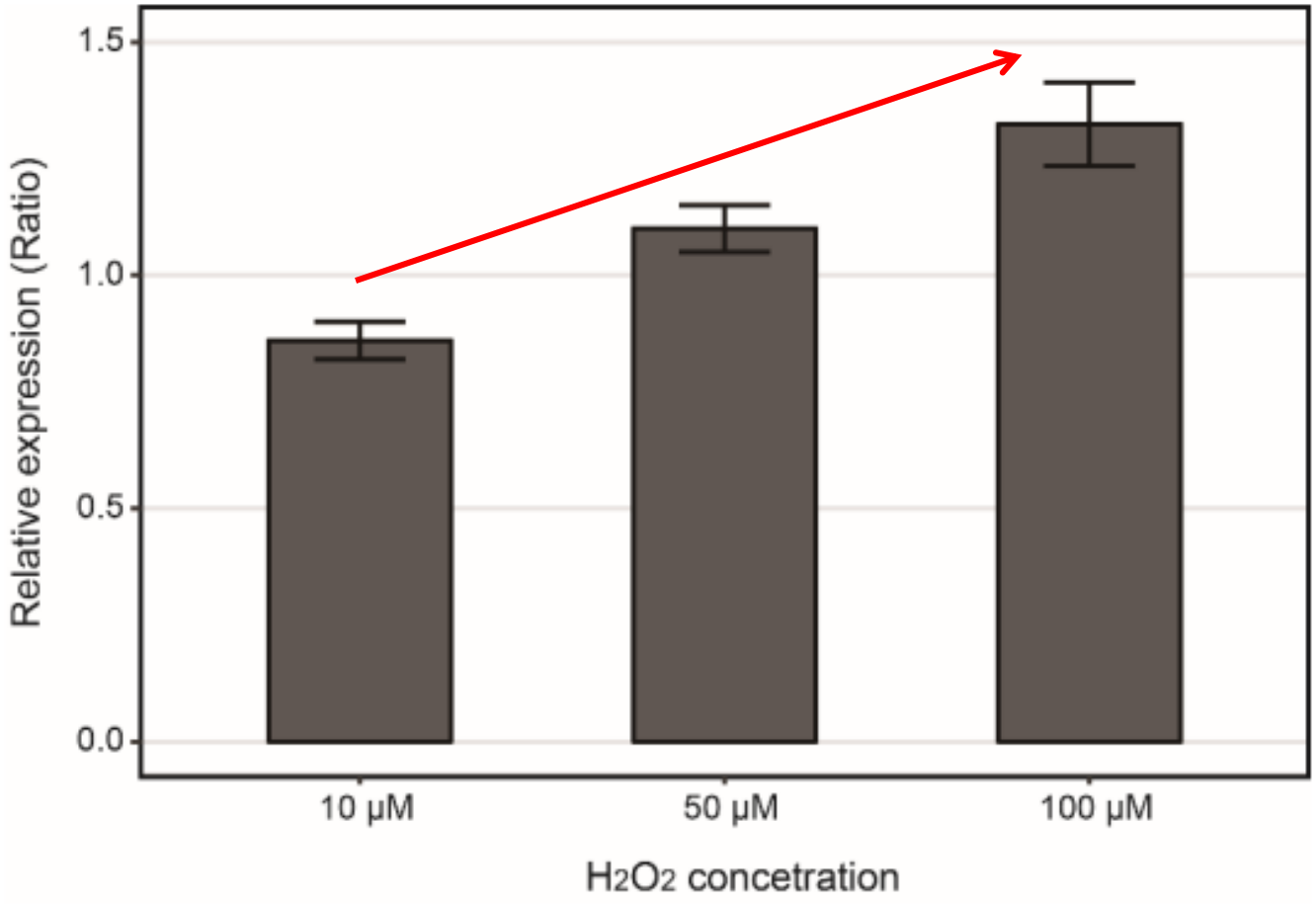
5、丙酮酸+过氧化氢酶



丙酮酸对生长的抑制作用可忽略不计。

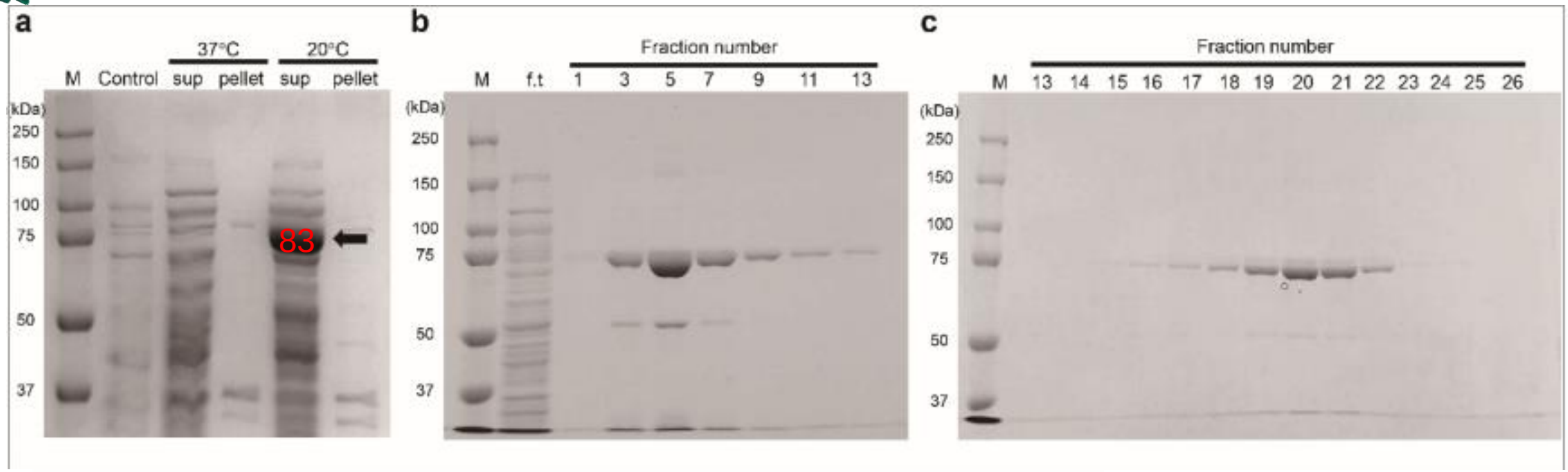
过氧化氢酶对于acI菌株的生长是必需的，其机制涉及但不限于H_2O_2分解。

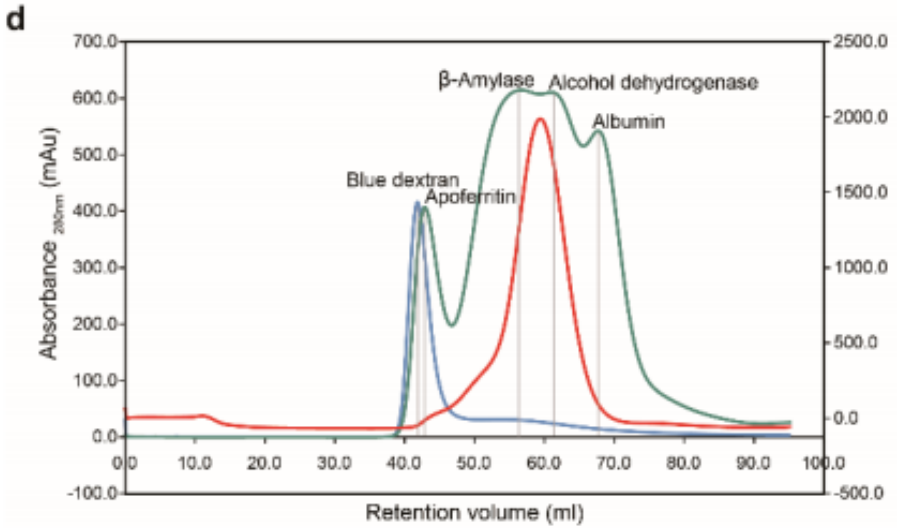
3 acI谱系的KatG的表征——qPCR

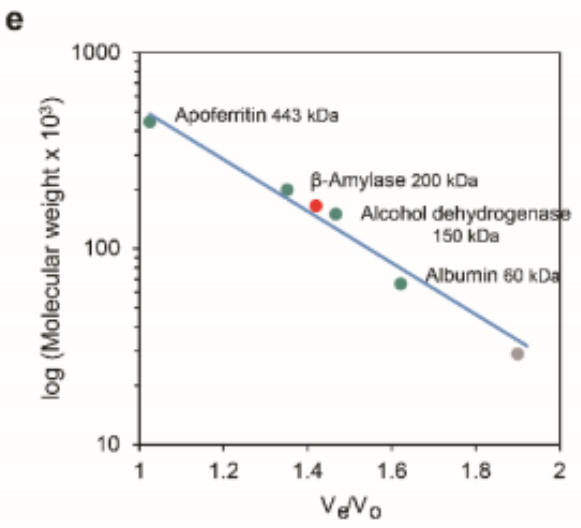


IMCC25003中katG的表达

3 acI谱系的KatG的表征——克隆、表达、纯化

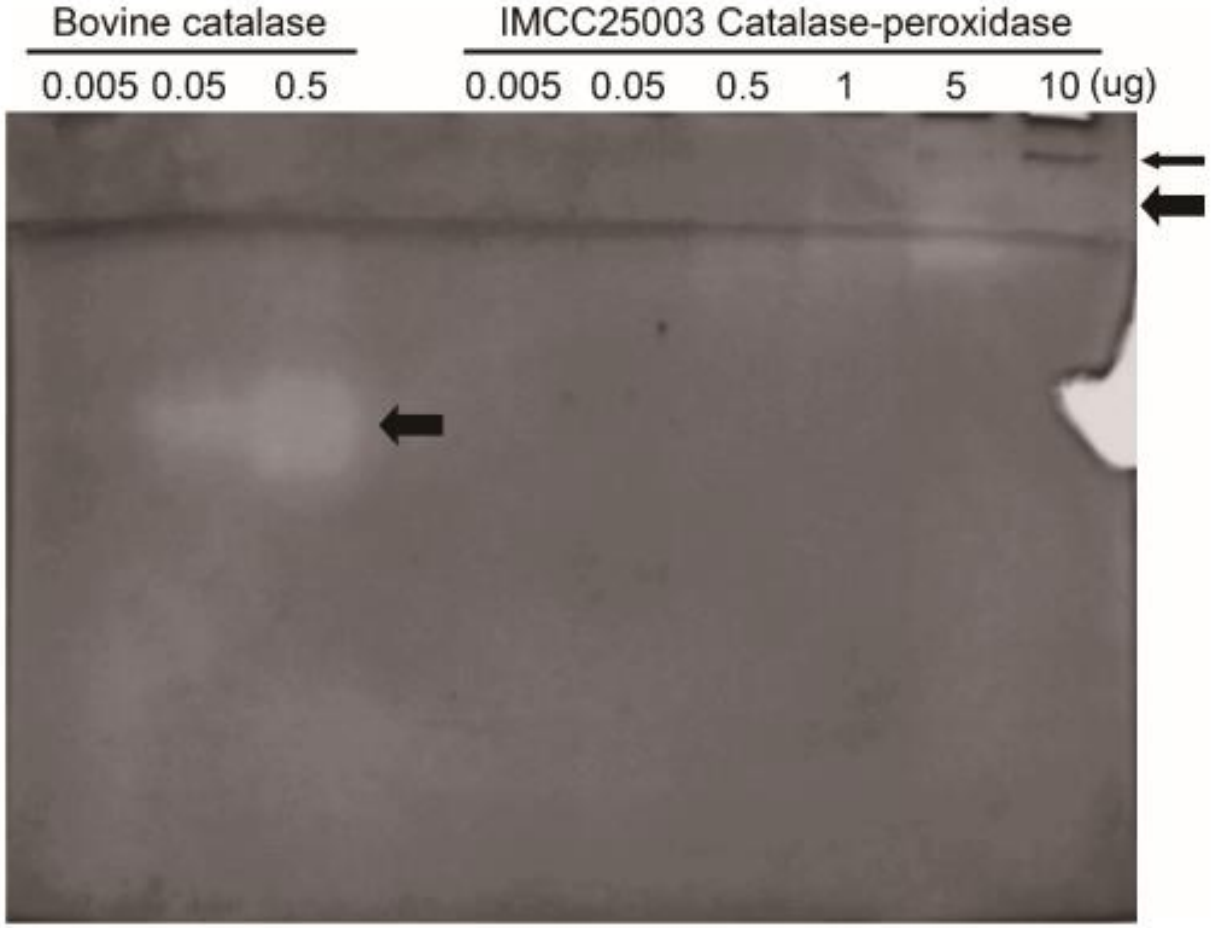






IMCC25003 KatG是由两个亚基组成的同源二聚体。

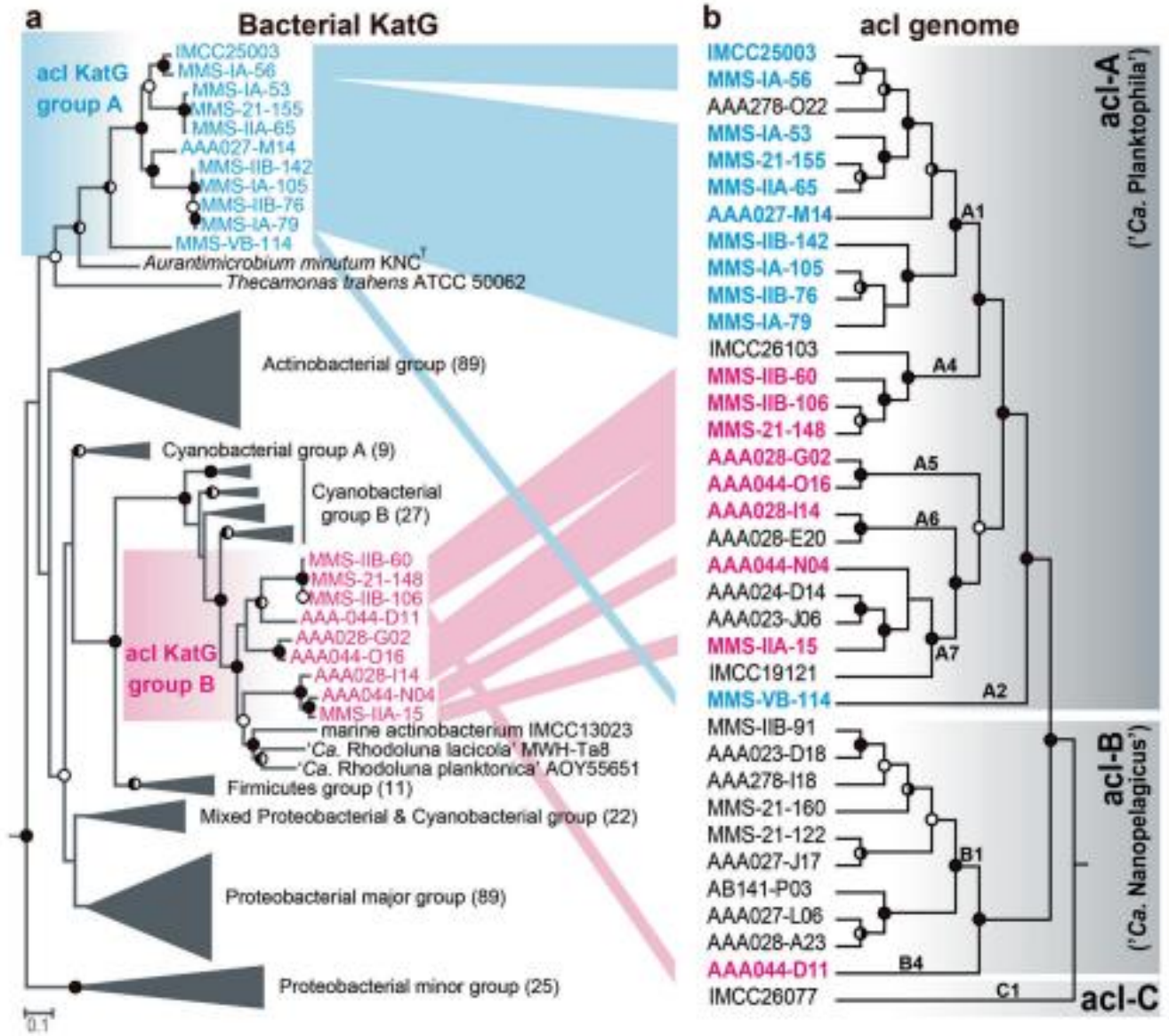
3 acI谱系的KatG的表征——酶活性测定



酶活性：

KatG<KatE

3 acI谱系的KatG的表征——系统发育分析



在acI谱系中KatG发生了复杂的进化过程。

3 acI菌株的表型特征

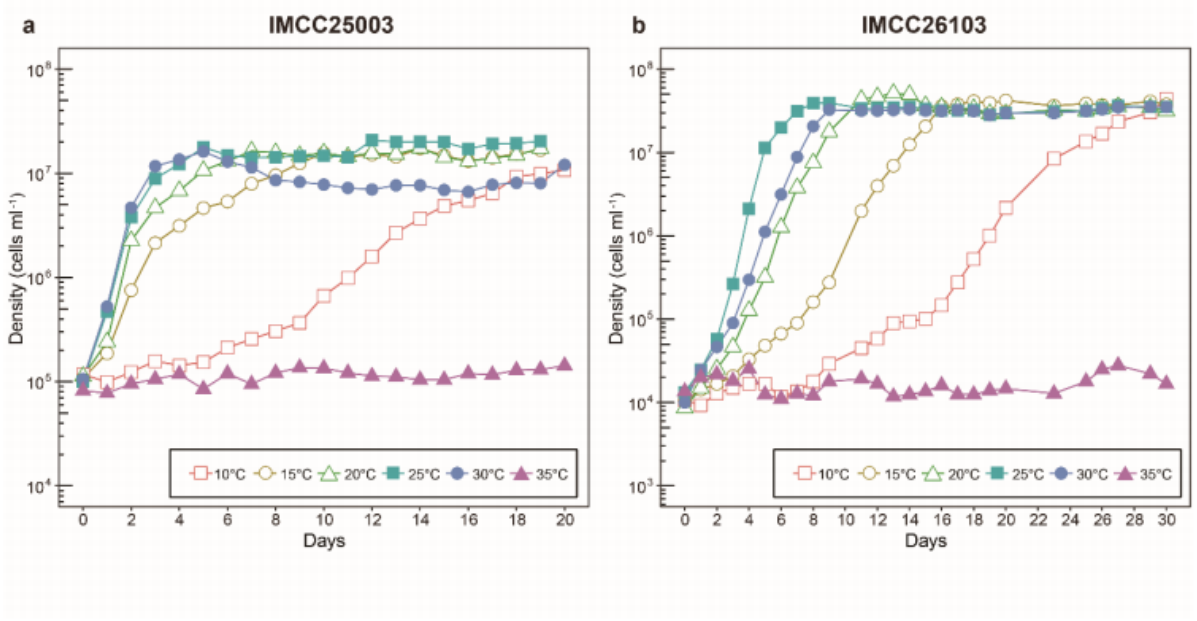


Fig. S13 Growth curves of strain IMCC25003 (**a**) and strain IMCC26103 (**b**) at different temperatures.

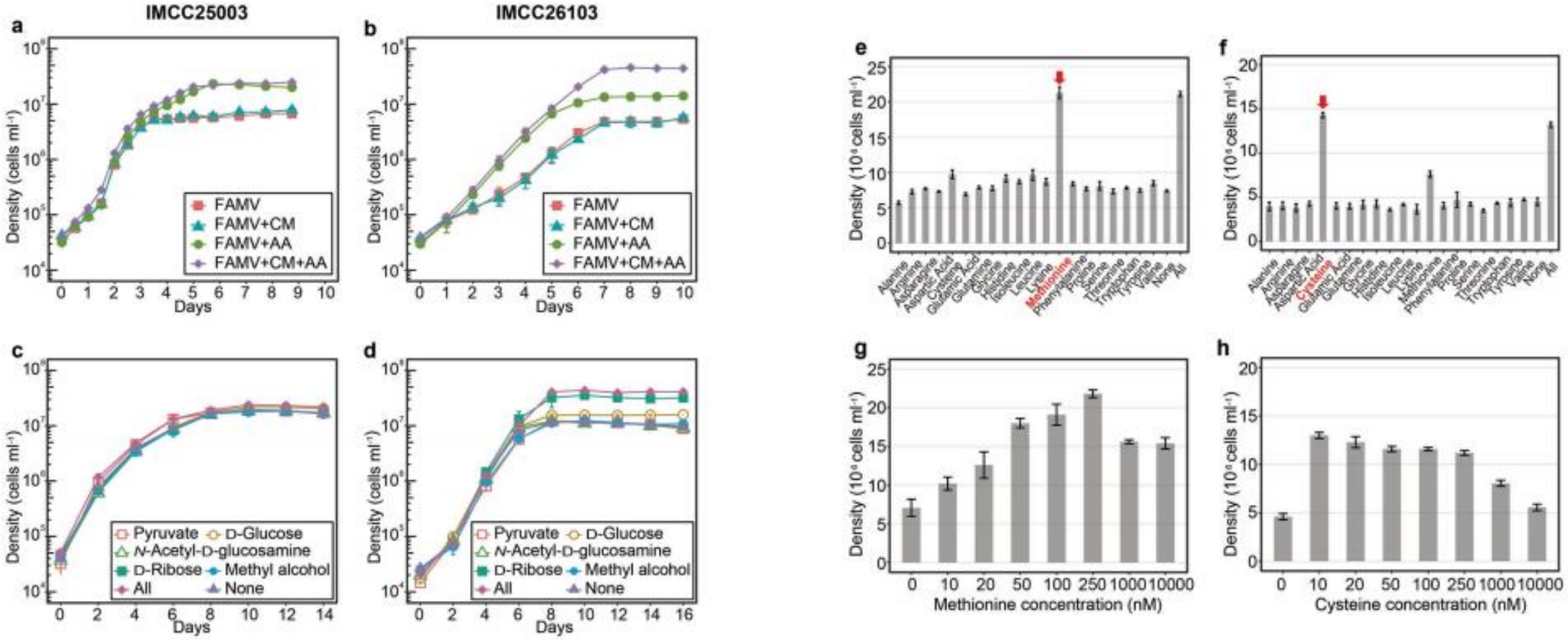
温度耐受

Table S5 Fatty acids composition (%) of two acI strains.

Fatty acid	IMCC25003	IMCC26103
Saturated fatty acids		
C10:0		0.46
C12:0	1.64	7.93
C14:0	18.22	7.85
C16:0	23.11	28.45
C17:0		1.05
C18:0	2.14	10.49
Unsaturated fatty acids		
C15:1 ω6*c*	1.11	
C17:1 ω8*c*	2.31	1.35
C18:1 ω9*c*	2.10	25.80
summed feature 3 (16:1 ω7*c*/16:1 ω6*c*)	45.79	12.28
summed feature 5 (18:2 ω6,9*c*/18:0 ante)		0.99
summed feature 8 (18:1 ω7*c*, 18:1 ω6*c*)	3.58	3.36

脂肪酸

3 acI菌株的表型特征



碳、氮源利用

Conclusion

4 Conclusion

通过向培养基中补充过氧化氢酶以降低H_2O_2浓度，成功维持了acI谱系（最丰富的淡水浮游细菌群）纯培养物的活跃生长，从而能够进一步分析仅从基因组序列无法推断的生理特性。

这种简单的过氧化氢酶补充方法可以促进具有新型基因组的浮游细菌的培养，从而有助于研究普遍存在且数量丰富的淡水寡营养细菌的生态作用。

请各位老师同学批评指正！