读书报告

BOOK REPORT

汇报人：吉伟利

汇报日期：2018-06-10

Extremophiles (2016) 20:207–214
DOI 10.1007/s00792-016-0815-0

ORIGINAL PAPER

Exploration and isolation of novel thermophiles in frozen enrichment cultures derived from a terrestrial acidic hot spring

Hiroyuki D. Sakai[1] · **Norio Kurosawa**[1]

从陆地酸性热泉获得的冷冻富集培养物中新型嗜热菌的探索和分离

IF:2.46

Received: 11 December 2015 / Accepted: 28 January 2016 / Published online: 10 February 2016

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Introduction

our results indicate that the last common ancestor of extant life was a thermophile that flourished at a very high temperature.
现存生命的最后一个共同祖先是在极高温度下繁殖的嗜热菌

HT

hyperthermophiles (HT)

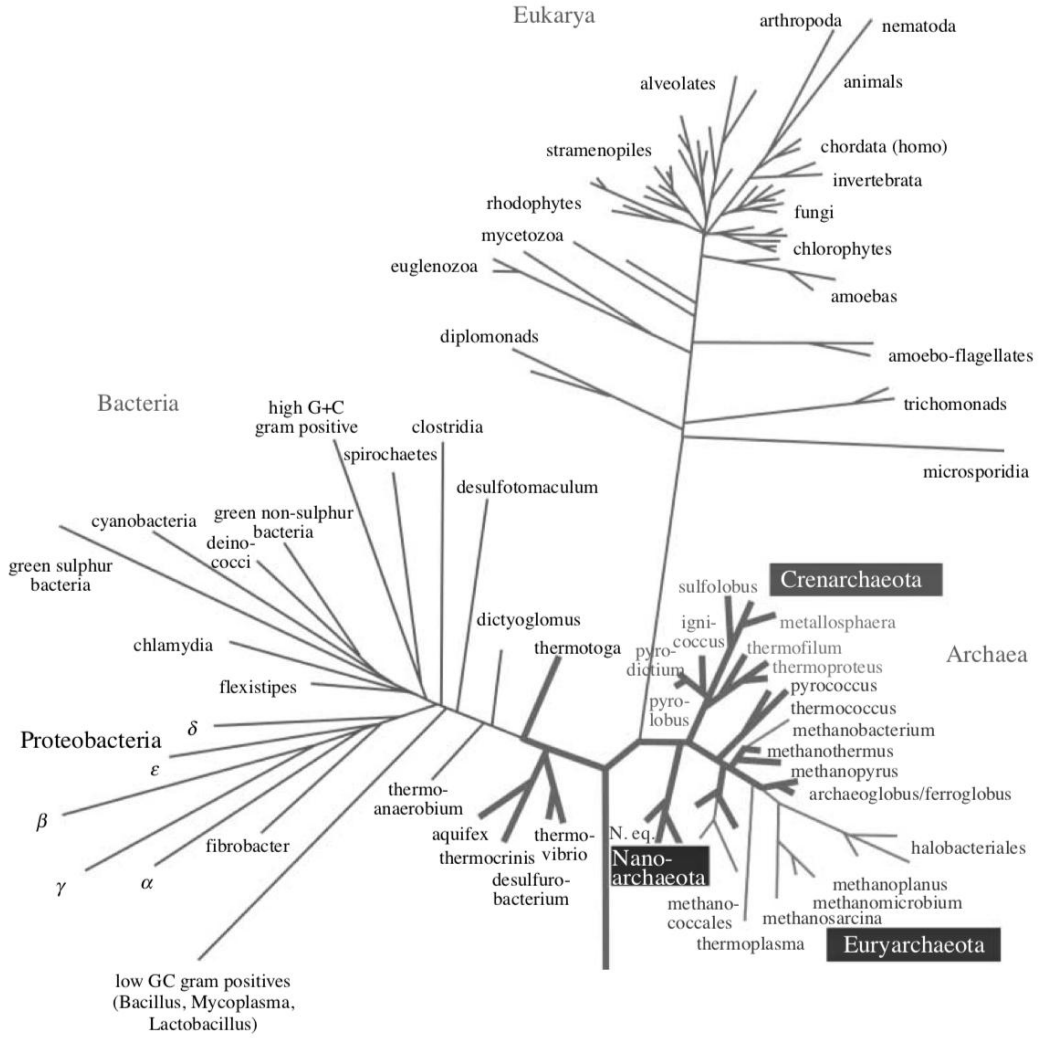


Figure 1. Small subunit ribosomal RNA-based phylogenetic tree. The thick lineages represent HT.

Introduction

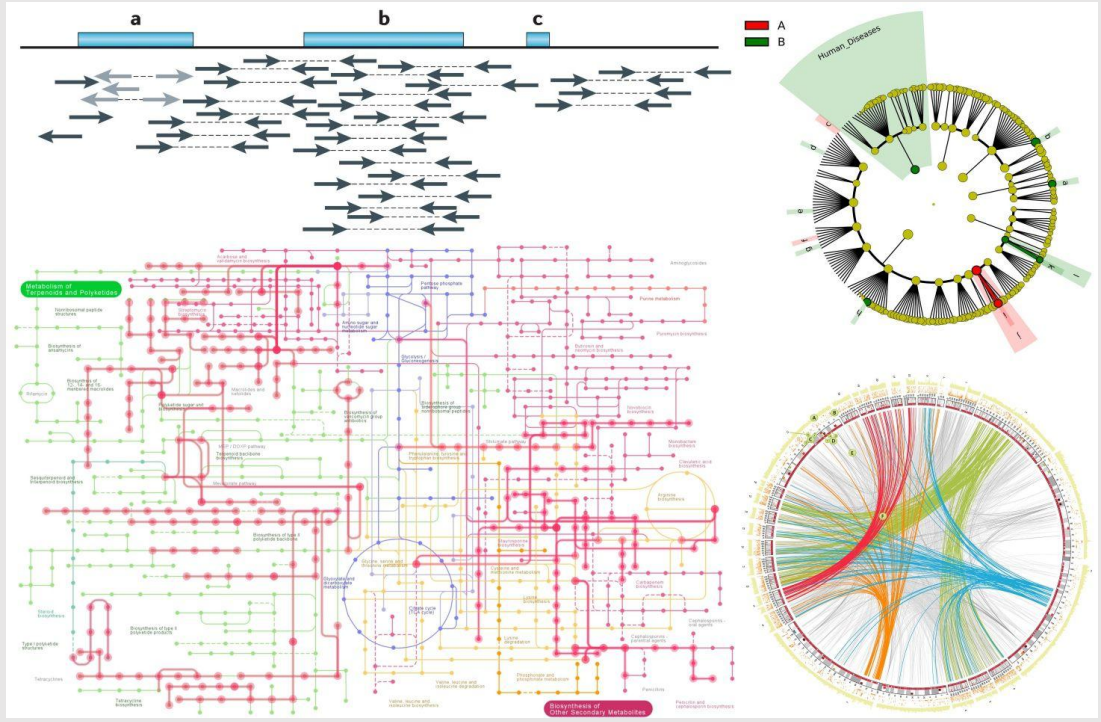
Taq聚合酶

美国黄石公园里热泉中发现并成功分离嗜热细菌海栖热袍菌(*Thermus aquaticus*)

Taq聚合酶是从嗜热细菌海栖热袍菌（*Thermus aquaticus*）中分离出的DNA聚合酶

Introduction

宏基因组



可培养的微生物

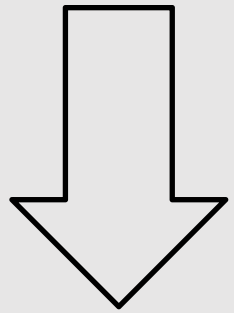
为什呢这么多培养不出来？

Introduction

阻碍嗜热菌的生长：

缓慢的自然生长速率
培养基成分引起的抑制
群体中其它细胞产生的抗菌物质
缺乏群体感应信号
所提供的营养物质的过度浓缩
专性厌氧或兼性厌氧
严格的生长条件（高分压CO_2）
需要两种其它辅助菌株

怎么办呢？



frozen enrichment cultures (ENFE)

2

Materials and methods

Sampling

采样地点	Ohwaku-dani, Hakone, Japan （35°14'22 'N， 139°01'07‘’E）
样品类型	表面沉积物 地热通风口下游约50cm处的小型热池
温度pH	68°C，pH 1.4
采样时间	2014年6月
样品运送	将大约200 mL样品收集在无菌玻璃瓶中，并在环境温度下运送到实验室

Enrichment culture

三种不同温度

56℃、68℃、83℃

四种不同pH

1.4　4.0

6.0　8.0

Modified Brock′ s basal salt

0.01, 0.10,

1.00 % (w/v)

三种不同浓度的YE

50 % (w/v) H_2SO_4 or 1.0 M NaOH

调节pH

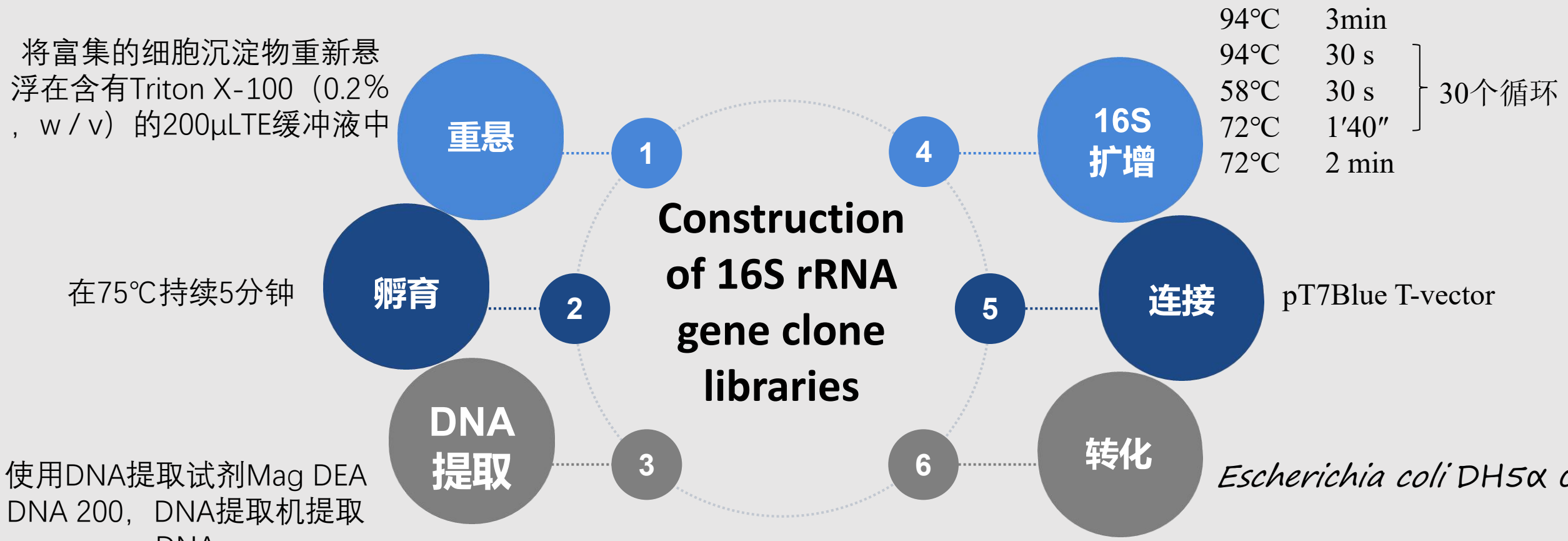
$(NH_4)_2SO_4$	1.3g
KH_2PO_4	0.25g
$MgSO_4.7H_20$	0.25
$CaC1_2.2H_20$	0.08
yeast extract	1.0

数字表示所用的富集条件（例如，**68_1.4_001**表示富集文化在68°C，pH 1.4和存在0.01%（w / v）YE）下孵育。

Enrichment culture

在亚培养后，将5mL处于指数后期的培养物在15,880×g和25℃下离心20分钟。 将细胞沉淀储存在-25℃直至DNA提取，并且将1mL补充有10%（v / v）DMSO的培养物储存在-80℃作为用于分离微生物的接种物。

Construction of 16S rRNA gene clone libraries of the enrichment cultures



对每个插入的16S rRNA基因的5’区域大约750bp进行测序

Identification of 16S rRNA gene clones and phylogenetic analysis

分离物：68_1.4_0.1 and 83_6.0_0.01

引物： A21F (5’-TTCCGGTTG ATCCYGCCGGA)
U1492R (5’-GGYTACCTTGTT ACGACTT)

3 Results

Microbial communities of enrichment cultures

	Supplemented with 0.01 % (w/v) yeast extract			Supplemented with 0.10 % (w/v) yeast extract		
	pH 1.4	pH 4.0	pH 6.0	pH 1.4	pH 4.0	pH 6.0
53 °C	+[a], B[b]	+, B	–, N	++, A	+, B	–, N
68 °C	+, A	+, A	+, A	+++, A	++, A	+, A
83 °C	+, A	+, A	+, A	++, A	+, A	+, A

[a] Symbols +++, strong growth was observed ($OD_{600} > 0.3$); ++, relatively strong growth was observed ($OD_{600} = 0.1 - 0.3$); +, weak growth was observed ($OD_{600} = 0.01–0.1$); –, no growth was observed ($OD_{600} < 0.01$)

[b] Letters A, archaeal 16S rRNA gene was detected; B, bacterial 16S rRNA gene was detected; N, no PCR was conducted due to no observed growth

Table 1 Microbial growth of enrichment cultures and 16S rRNA gene detection

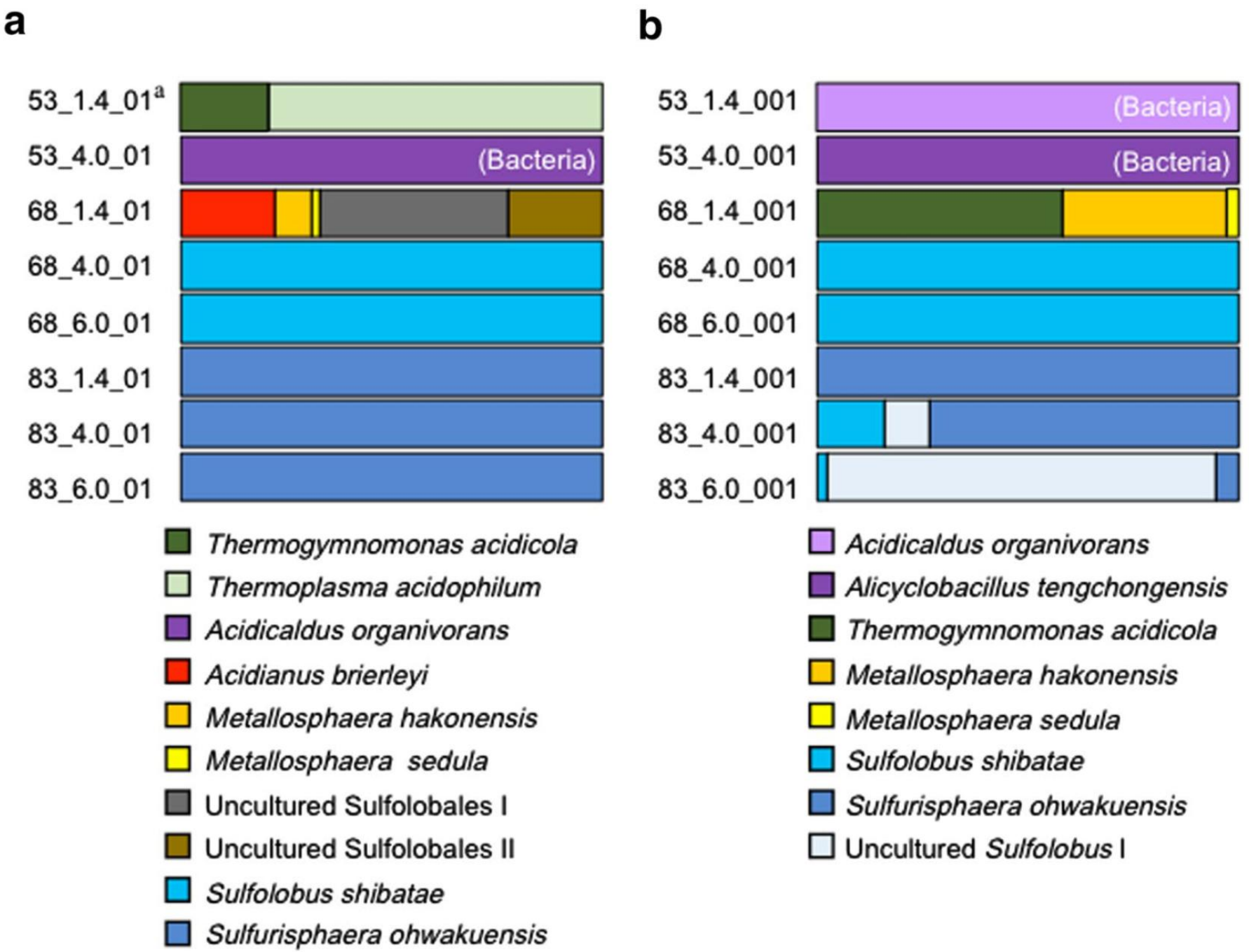


Fig. 1 Microbial community structures of growth-observed enrichment cultures. **a** Enrichment cultures supplemented with 0.10 % yeast extract, **b** enrichment cultures supplemented with 0.01 % (w/v) yeast extract. [a]Numbers indicate the enrichment conditions used (e.g., 53_1.4_01 indicates the enrichment culture incubated at 53°C, pH 1.4, and in the pres- ence of 0.10 % yeast extract)

Acidianus brierleyi (**AB**)

Metallosphaera hakonensis (**MH**)

M. sedula (**MS**)

Sulfolobus shibatae (**SS**)

Sulfurisphaera ohwakuensis (**SO**)

Thermogymnomonas acidicola (**TGA**)

Thermoplasma acidophilum (**TPA**)

Uncultured *Sulfolobales* I (**US-I**)

Uncultured *Sulfolobales* II (**US-II**)

Uncultured *Sulfolobus* I (**S-I**).

Table 2 Representative clones, phylotypes, and closest species (closest environmental clones) derived from each enrichment culture (archaea)

Source	Representative clone (clonal frequency %)	Phylotype	Closest species, accession no. (Closest environmental clones, accession no)	Sequence similarity (%)
53_1.4_01[a]	53_14_01arc_07 (21)[b]	*Thermogymnomonas acidicola*	*Thermogymnomonas acidicola*, AB269873	99.9
	53_14_01arc_12 (79)	*Thermoplasma acidophilum*	*Thermoplasma acidophilum*, AL139299	99.7
68_1.4_01	68_14_01arc_42 (22)	*Acidianus brierleyi*	*Acidianus brierleyi*, D26489	99.4
	68_14_01arc_51 (9)	*Metallosphaera hakonensis*	*Metallosphaera hakonensis*, D86414	100.0
	68_14_01arc_21 (2)	*Metallosphaera sedula*	*Metallosphaera sedula*, CP000682	99.4
	68_14_01arc_53 (44)	Uncultured Sulfolobales I	*Stygiolobus azoricus*, X90480	86.4
			(Thermal soil clone, AF391996)	97.2
	68_14_01arc_43 (22)	Uncultured Sulfolobales II	*Sulfolobus metallicus*, D85519	86.8
			(Bacterial crushed coral clone, KC190231)	88.4
68_4.0_01	68_4_01arc_12 (100)	*Sulfolobus shibatae*	*Sulfolobus shibatae*, M32504	99.5
68_6.0_01	68_6_01arc_01 (100)	*Sulfolobus shibatae*	*Sulfolobus shibatae*, M32504	99.3
83_1.4_01	83_14_01arc_09 (100)	*Sulfurisphaera ohwakuensis*	*Sulfurisphaera ohwakuensis*, D85507	100.0
83_4.0_01	83_4_01arc_11 (100)	*Sulfurisphaera ohwakuensis*	*Sulfurisphaera ohwakuensis*, D85507	100.0
83_6.0_01	83_6_01arc_05 (100)	*Sulfurisphaera ohwakuensis*	*Sulfurisphaera ohwakuensis*, D85507	99.9
68_1.4_001	68_14_001arc_22 (39)	*Metallosphaera hakonensis*	*Metallosphaera hakonensis*, D86414	100.0
	68_14_001arc_37 (3)	*Metallosphaera sedula*	*Metallosphaera sedula*, CP000682	99.9
	68_14_001arc_17 (58)	*Thermogymnomonas acidicola*	*Thermogymnomonas acidicola*, AB269873	100.0
68_4.0_001	68_4_001arc_01 (100)	*Sulfolobus shibatae*	*Sulfolobus shibatae*, M32504	99.6
68_6.0_001	68_6_001arc_06 (100)	*Sulfolobus shibatae*	*Sulfolobus shibatae*, M32504	99.3
83_1.4_001	83_14_001arc_08 (100)	*Sulfurisphaera ohwakuensis*	*Sulfurisphaera ohwakuensis*, D85507	100.0
83_4.0_001	83_4_001arc_38 (16)	*Sulfolobus shibatae*	*Sulfolobus shibatae*, M32504	99.6
	83_4_001arc_29 (73)	*Sulfurisphaera ohwakuensis*	*Sulfurisphaera ohwakuensis*, D85507	100.0
	83_4_001arc_36 (11)	Uncultured *Sulfolobus* I	*Sulfolobus shibatae*, D32504	95.9
			(Icelandic hot spring clone, DQ441483)	99.3
83_6.0_001	83_6_001arc_08 (3)	*Sulfolobus shibatae*	*Sulfolobus shibatae*, M32504	99.2
	83_6_001arc_03 (5)	*Sulfurisphaera ohwakuensis*	*Sulfurisphaera ohwakuensis*, D85507	100.0
	83_6_001arc_01 (92)	Uncultured *Sulfolobus* I	*Sulfolobus shibatae*, D32504	95.9
			(Icelandic hot spring clone, DQ441483)	99.3

[a] Numbers indicate the enrichment conditions used (e.g., 53_1.4_01 indicates the enrichment culture incubated at 53 °C, pH 1.4, and in the presence of 0.10 % yeast extract)

[b] Clonal frequency (%) of the clones affiliated with same phylotype as the representative clone in each enrichment culture is indicated in the parenthesis

Microbial communities of enrichment cultures

Table 3 Number of clones, phylotypes, and homologous coverage of enrichment cultures (archaea)

Source	Number of clones	Number of phylotypes	Homologous coverage
53_1.4_01[a]	24	2	0.92
68_1.4_01	45	5	0.89
68_4.0_01	14	1	0.93
68_6.0_01	13	1	0.92
83_1.4_01	14	1	0.93
83_4.0_01	14	1	0.93
83_6.0_01	14	1	0.93
68_1.4_001	36	3	0.92
68_4.0_001	12	1	0.92
68_6.0_001	13	1	0.92
83_1.4_001	14	1	0.93
83_4.0_001	37	3	0.92
83_6.0_001	38	3	0.92
Total	288	10	0.97

[a] Numbers indicate the enrichment conditions used (e.g., 53_1.4_01 indicates the enrichment culture incubated at 53 °C, pH 1.4, and in the presence of 0.10 % yeast extract)

Isolation of novel archaea detected in the enrichment cultures

68_1.4_0.1
83_6.0_0.01

(US-I)

(US-II)

(S-I).

68_1.4_0.1

107倍稀释液（10ml）
培养21天
将浑浊培养物再次稀释1010倍

⇨ HS-1

83_6.0_0.01

培养13天
平板纯化三次

⇨ HS-3

HS-1

1438bp 16S rRNA
S. shibatae (88.6 %)
S. acidocaldarius (88.3 %)
S. solfataricus (88.3 %)

HS-3

1440bp 16S rRNA
S. shibatae (96.2 %)
S. solfataricus (96.1 %)
S. ohwakuensis (92.9 %)

Microbial communities of enrichment cultures

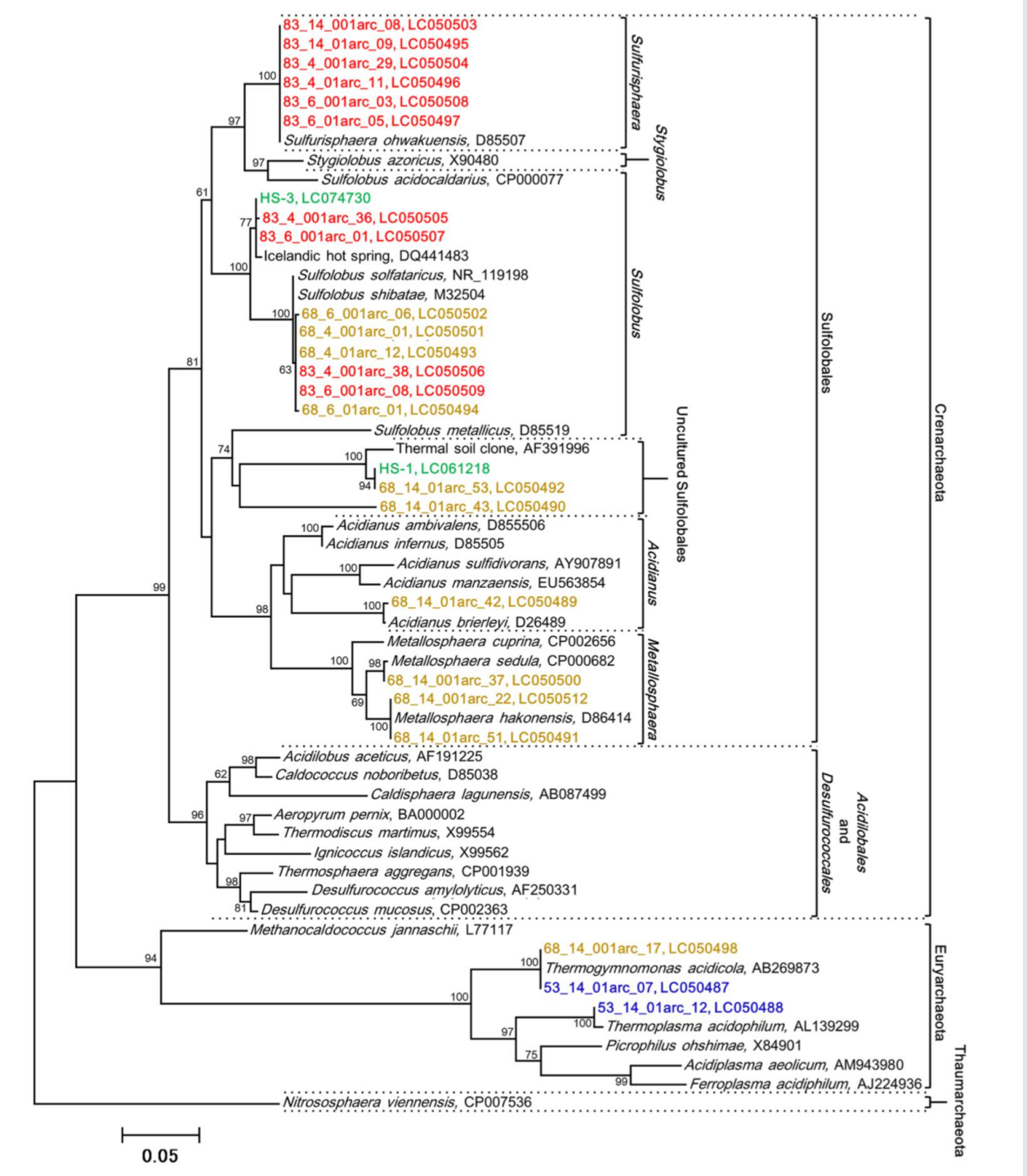


Fig.2 Neighbor-joining tree of representative clones and novel strains obtained in this study (archaea). Bootstrap values (n = 1000) greater than 50 % are indicated at the nodes. The scale bar indi cate the representative clones and novel strains obtained in this study. Each color indicates clones from enrichment cultures conducted at 53 °C (blue), 68 °C (yellow), 83 °C

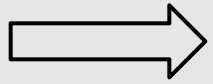
4 Discussion

Discussion

这些结果表明，ENFE策略可以使用现有的培养条件恢复以前未培养的微生物。

使用苛刻的条件可能使我们有机会分离出少量新型微生物，因为这些条件很少用于分离。

53_4.0_01 未检测到古细菌
53_1.4_0016S rRNA基因，但
53_4.0_001 检测到细菌克隆



因此，如果分离目标是古细菌，并且孵化温度相对较低（约50°C），则应考虑防止富集培养中细菌繁殖的措施，包括添加抗生素如氨苄西林。

Discussion

培养环境样品中微生物的一个障碍，无法预测合适的生长条件。在这项研究中，我们使用了广泛的丰富培养方式，而不是试图重现自然环境。

我们假设，在四次亚培养之后，如果在浓缩物中检测到以前未培养的微生物，它们应该能够在相同条件下增殖。基于这一假设，试图从含有以前未培养的微生物的富集物中分离以前未培养的微生物

Discussion

据了解，一些古菌和细菌不能单独分离，并且需要与特定物种共同培养才能生长。在某些情况下，这些需求可以通过将来自另一物种的废培养基或细胞提取物添加到分离培养基中来实现

尽管创新的分离方法在分离先前未培养的微生物方面发挥了战略性作用，但ENFE策略显示出使用常规培养基和技术表征和分离这些微生物的潜力。

谢谢聆听！

Thank you for listening