读书报告

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ARTICLE

Novel hydrogenases from deep-sea hydrothermal vent metagenomes identified by a recently developed activity-based screen

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基于活性筛选鉴定来自深海热液喷发物宏基因组的新型氢化酶

目 录

Contents

01 Introduction

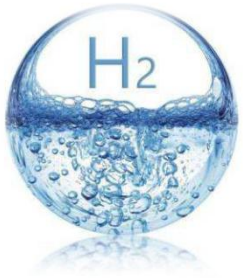
02 Materials and methods

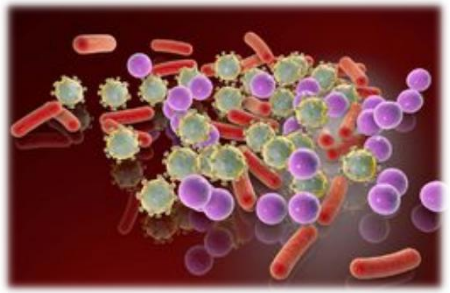
03 Results and discussion

04 Conclusion

01

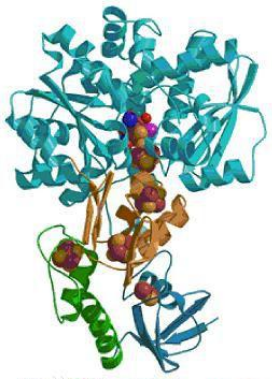
Introduction





Hydrogen is one of the most common elements on Earth.

In Bacteria, Archaea, and lower eukaryotes, hydrogen plays a central role for metabolic processes.



The enzymes converting molecular hydrogen into protons and electrons are the hydrogenases.

$H_2 \leftrightarrow 2H^+ + 2e^-$ 氢化酶

Types of hydrogenases (according to catalytic center):

(i) [NiFe]-hydrogenases (associated with hydrogen sensing and consumption)

(ii) [FeFe]-hydrogenases (the socalled hydrogen-evolving hydrogenases)

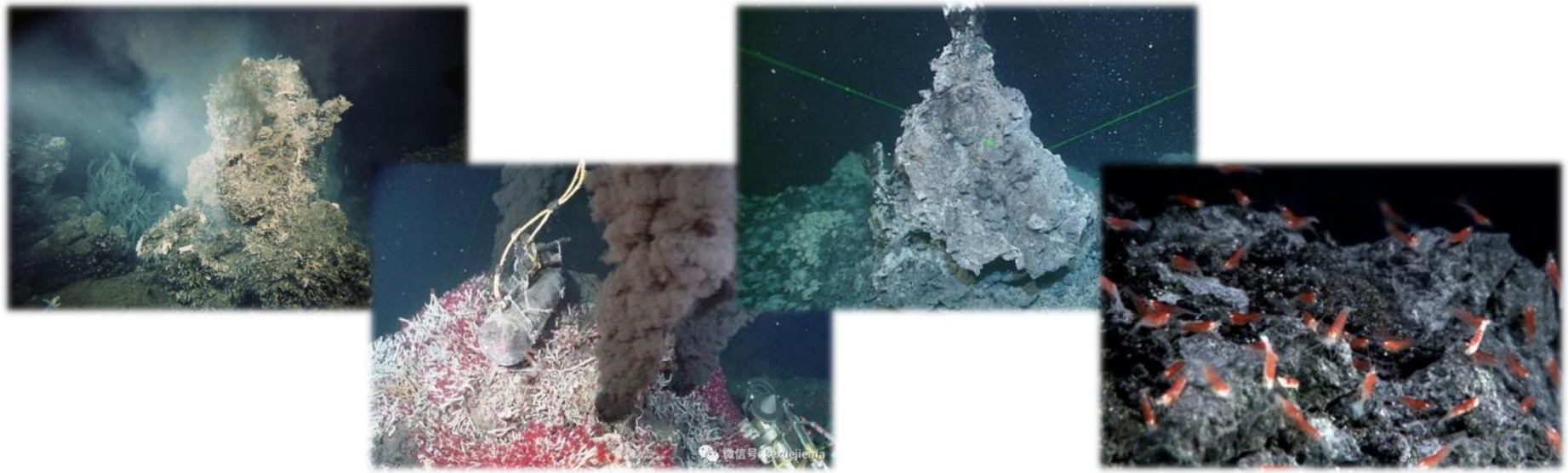
(iii) [Fe]-hydrogenases (involved in methanogenesis)

enrichment and isolation of hydrogen-oxidizing microorganisms or metagenomic sequencing

disadvantage: the majority of microorganisms are currently not culturable (>99%);

sequence-based analyses cannot aid in discovering new hydrogenases.

activity-based screen for seeking H_2-uptake enzymes from metagenomes



In hydrothermal vent systems （热液喷口）hydrogen can be highly enriched in the emitted fluids because of serpentinization processes (rock water interactions) or magma degassing.

Here microbial hydrogen oxidation can be vital for providing energy to fuel autotrophic carbon fixation.

Steep thermal (4 °C to several 100 s °C) and chemical (oxic to anoxic) gradients.
A broad repertoire of hydrogen-oxidizing microorganisms producing enzymes with distinct biochemical properties can be expected.

Generally, a high diversity among membrane-bound H_2-uptake [NiFe]-hydrogenases can be observed in hydrothermal fluids , but it has remained unresolved whether these hydrogenases are indeed functional.

activity-based screen to seek H_2-uptake enzymes from fosmid metagenomic libraries

Shewanella oneidensis **MR-1 (*S. oneidensis* ΔhyaB)** **[NiFe]- hydrogenase deletion mutant**

an H_2-uptake active metagenomic fosmid clone

Restore the original phenotype. It makes use of *S. oneidensis* MR-1's ability to couple hydrogen oxidation (catalyzed by the [NiFe]-hydrogenase HyaA/HyaB) with the reduction of Fe(III)citrate to Fe(II)citrate.

The Fe(III) reduction reaction results in a color change (from yellow to colorless) of FW medium.

Activity-Based Screening of Metagenomic Libraries for Hydrogenase Enzymes

来自 Springer | ♡ 喜欢 0　阅读量：1

作者： Nicole Adam , Mirjam Perner

摘要： Abstract Here we outline how to identify hydrogenase enzymes from metagenomic libraries through an activity-based screening approach. A metagenomic fosmid library is constructed in E. coli and the fosmids are transferred into a hydrogenase deletion mutant of Shewanella oneidensis (ΔhyaB) via triparental mating. If a fosmid exhibits hydrogen uptake activity, S. oneidensis' phenotype is restored and hydrogenase activity is indicated by a color change of the medium from yellow to colorless. This new method enables screening of 48 metagenomic fosmid clones in parallel.

收起

关键词：Metagenome Function-based screen Hydrogenase Hydrogen uptake

DOI： 10.1007/978-1-4939-6691-2_17

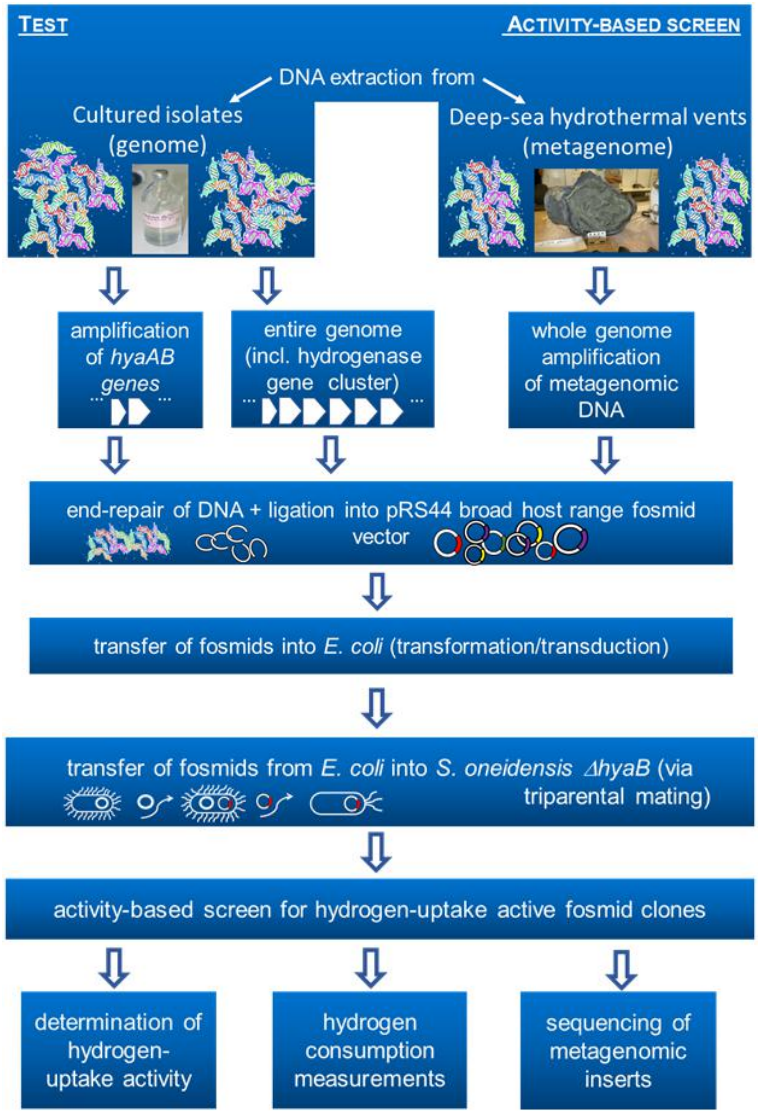
Triparental mating: 三亲交配法是将中间质粒转入受体菌的过程，此过程需要三种细菌，即含有中质粒的大肠杆菌供体菌，含有游动质粒pRK2013的大肠杆菌“协助”菌(helper)和受体菌。当这三种菌混合时，协助质粒pRK2013游动进入大肠杆菌内，提供游动(mol)和转移(tra)功能，把供体的中间质粒转移进受体菌内。

02

Materials and methods

（i）测试从系统发育不同的氢氧化细菌分离物中成功H_2–吸收活性酶的能力；

（9株变形菌门的不同菌株）



氢吸收活性判定　氢消耗测量

（ii）应用筛选于宏基因组fosmid文库用于寻找重组H_2–吸收活性酶。

Cultivation of tested strains and DNA extraction

Cloning of [NiFe]-hydrogenase genes

Sampling of hydrothermal environments and isolation of metagenomic DNA

Construction of (meta)genomic fosmid libraries

Fosmid libraries were created using genomic DNA from *E. coli* K-12, *S. oneidensis* MR-1, *P. leiognathi* L1, *W. succinogenes* DSM1740, *S. denitrificans* DSM1251, *R. capsulatus* SB1003, *D. vulgaris* Hildenborough, and *T. denitrificans* AB7 as well as metagenomic DNA from the three vent environments. Repeated attempts to construct a genomic library for *A. aeolicus* failed.

PCR-based identification of [NiFe]-hydrogenase genes in (meta)genomic fosmid libraries

Function-based screen for H_2-uptake active enzymes

Preparation of crude cell extracts and partial purification of recombinant H_2-uptake active enzymes

H_2-uptake enzyme activity assay

Hydrogen consumption measurements

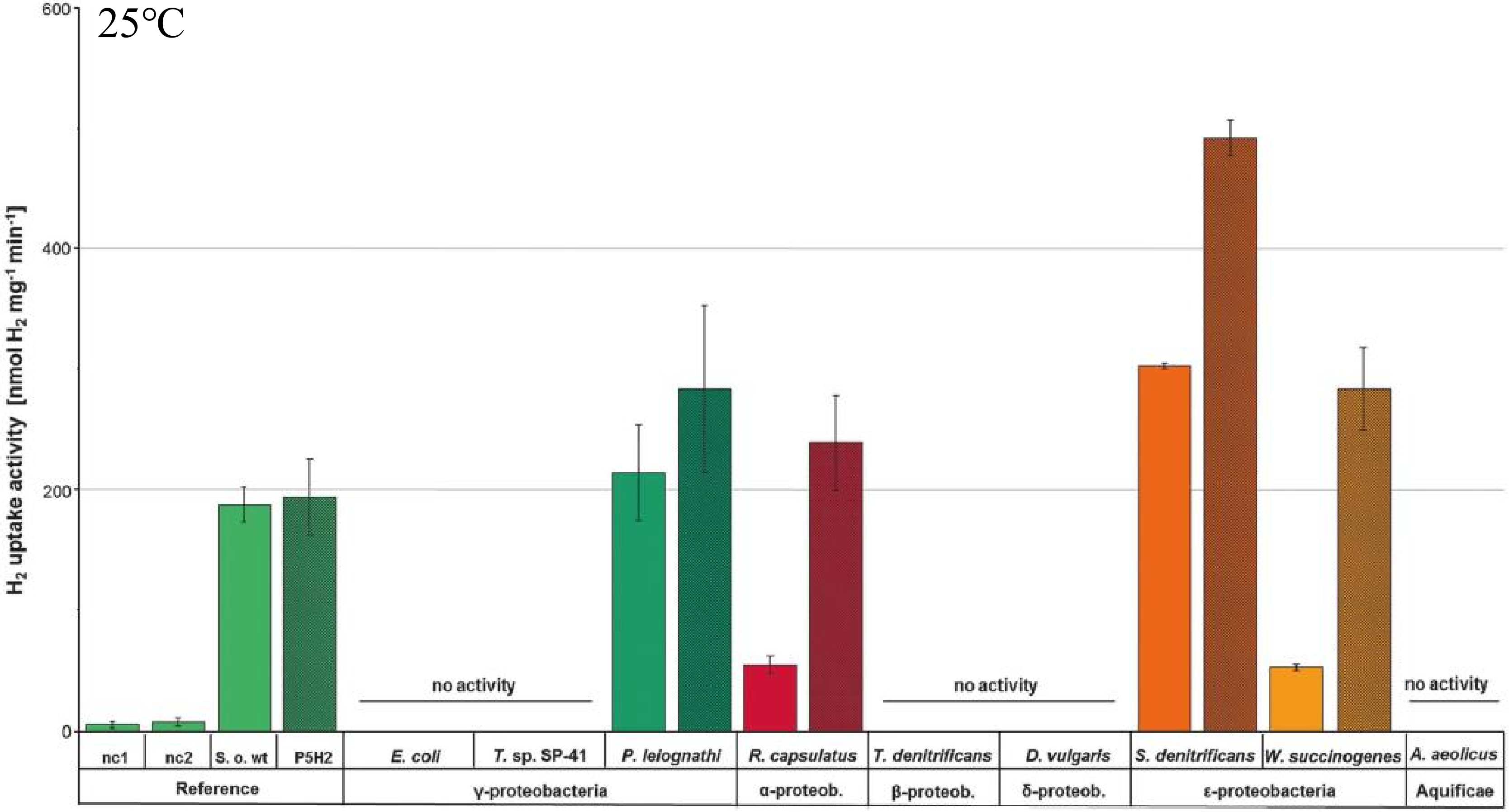
Sequencing and sequence analysis of the metagenomic fragments

Data availability

03

Results and discussion

A novel activity-based screen for recovering H_2- uptake active enzymes from metagenomes



S. oneidensis ΔhyaB complemented with the large (*S. denitrificans*) or large and small subunit structural genes of the [NiFe] hydrogenases of *E. coli*, *Thiomicrospira* sp., *P. leiognathi*, *R. capsulatus*, *T. denitrificans*, *D. vulgaris*, *W. succinogenes*, and *A. aeolicus*.

negative controls ... containing the hydrogenase gene ... **nc2**-*S. oneidensis* ... cluster (84G4II)

Due to the highly specific maturation apparatus of hydrogenases, the heterologous expression of functional recombinant hydrogenases often fails.

For each tested isolate we expressed the hyaAB genes (in case of *S. denitrificans* only hyaB was used) only or the entire hydrogenase gene cluster to elucidate whether (i) functional hydrogenases could be produced in our host and (ii) genes from the hydrogenase gene cluster were needed for expressing a functional structural hydrogenase.

Our complementation experiments demonstrated that the heterologous expression of different proteobacterial [NiFe]-hydrogenases in a hyaB deletion mutant of *S. oneidensis* is possible and can produce H_2-uptake active enzymes.

Seeking H_2-uptake active enzymes from hydrothermal vent metagenomes

Sisters Peak chimney（烟囱）

hydrothermal fluids emanating from a crater（火山口） at Nibelungen

low-temperature fluids from the Lilliput field along the southern Mid-Atlantic Ridge

Each library consisted of 4800 fosmid clones. Of the 14400 screened clones, 4 clones exhibited H_2-uptake activity, which equals a hit rate of 1:3600 clones.

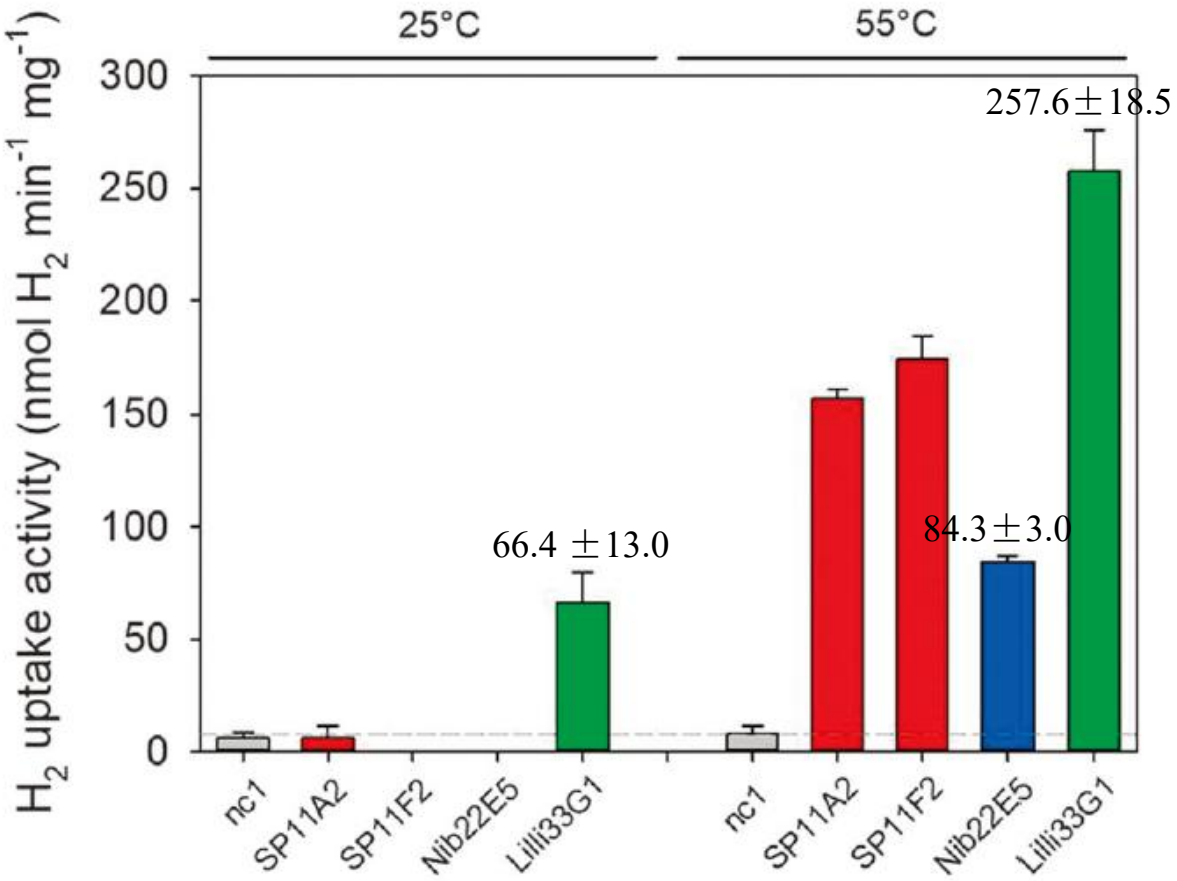
H_2-uptake active enzymes from hydrothermal vent metagenomes

Supplementary Table S3: Environmental properties from the examined hydrothermal vent habitats.

	two clones **Sisters Peak**	one clone **Nibelungen**	one clone **Lilliput**
sample type	massive sulfide (rock)	fluid	diffuse fluid
fluid temperature	400°C	120°C	9°C
most abundant electron donor	H_2S	H_2	H_2S
H_2 concentration [µM]	1600	22	0.9

Properties are taken from Perner et al (2013) (Nibelungen and Lilliput), Perner et al (2014) (Sisters Peak) and references therein.

H_2-uptake active enzymes from hydrothermal vent metagenomes



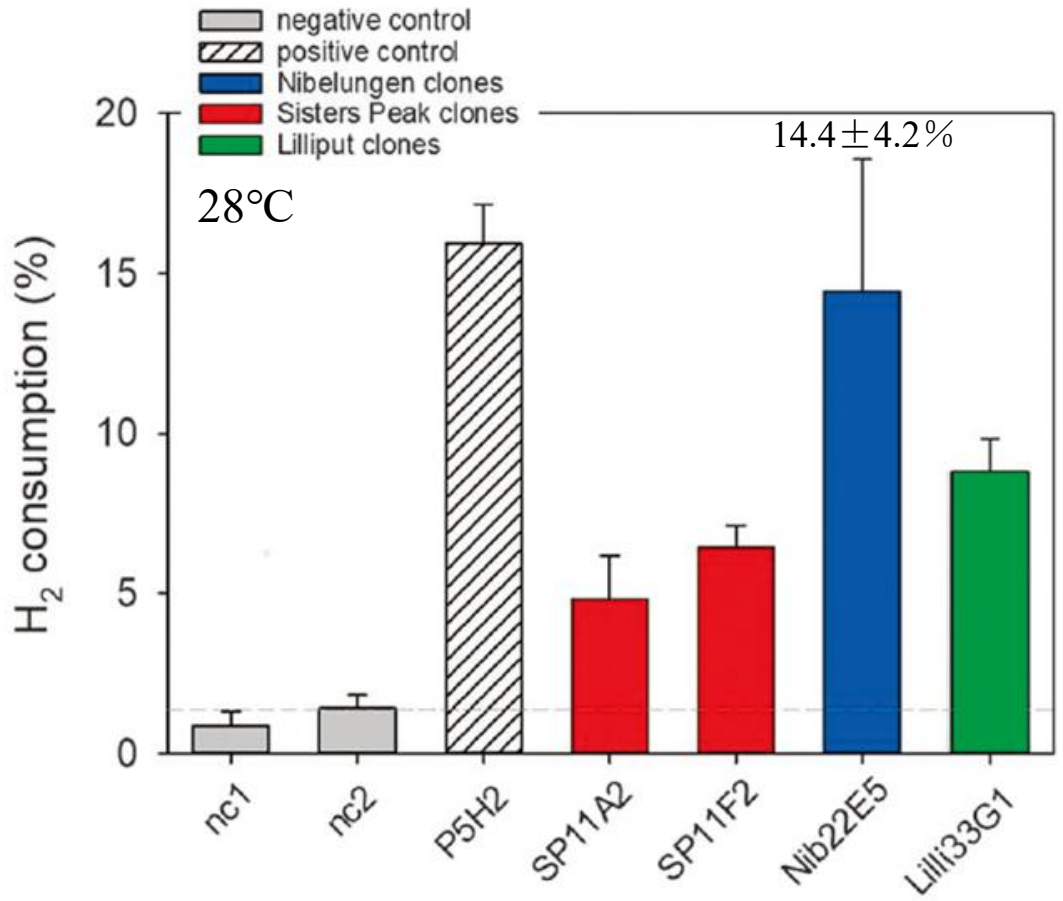
partially purified protein

may be related to the temperatures of the habitats

previously report:

H_2-uptake activities determined from recombinant [NiFe]-hydrogenases displayed a relatively wide range of activities, i.e. 3.75 nmol H_2/min/mg of total protein, 150 and 444 nmol H_2/min/mg of partially purified proteins.

H_2-uptake active enzymes from hydrothermal vent metagenomes



a 80:20 H_2:CO_2 gas mixture after 3 weeks

previously report:

The *Epsilonproteobacterium Sulfurimonas denitrificans*, for example, consumes 51 and 26% hydrogen of the 80:20 H_2:CO_2 gas mix within 14 days of incubation without or with added thiosulfate(硫代硫酸盐), respectively.

Sequencing the metagenomic DNA fragments

Four H_2- uptake active *S. oneidensis* ΔhyaB fosmid clones, namely **SP11A2**, **SP11F2**, **Nib22E5**, and **Lilli33G**1 (the latter was also detected by sequence-based approach).

BLASTP

SP11A2: 49–68% AA similarity

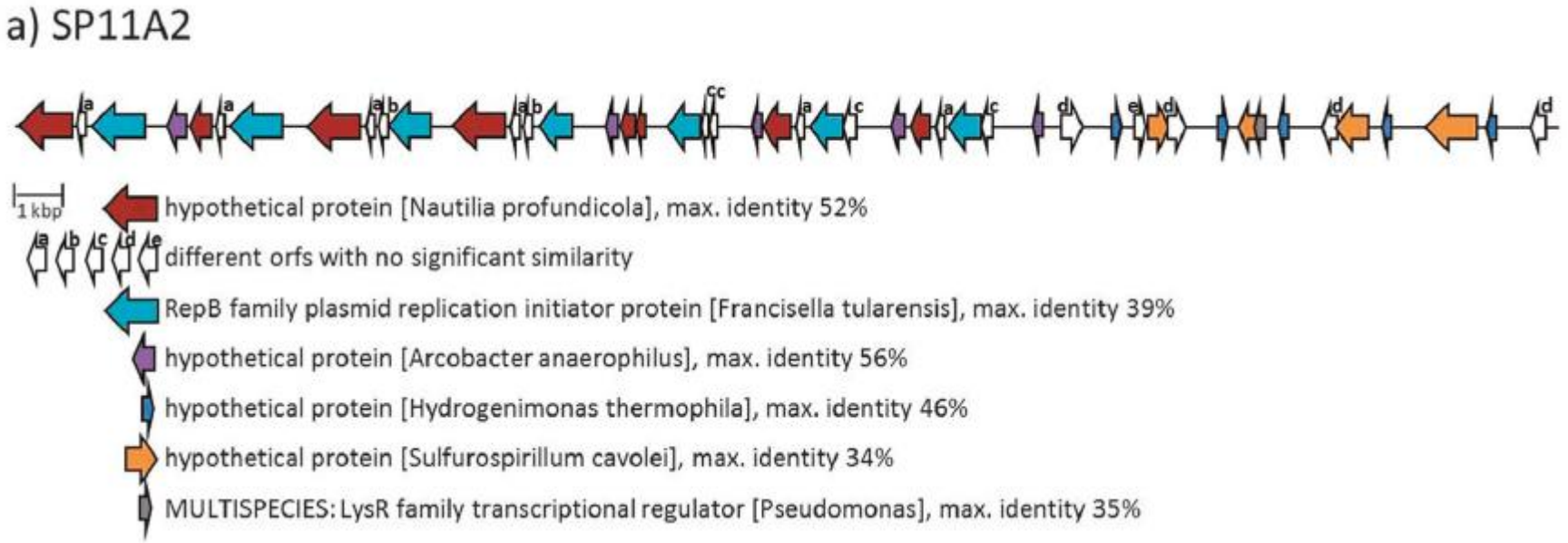
SP11F2: no significant hit or 27–38% AA similarity

Nib22E5: no significant hit or 34–56% AA similarity

Lilli33G1: 99% similarity to the [NiFe]- hydrogenase from *W. succinogenes*

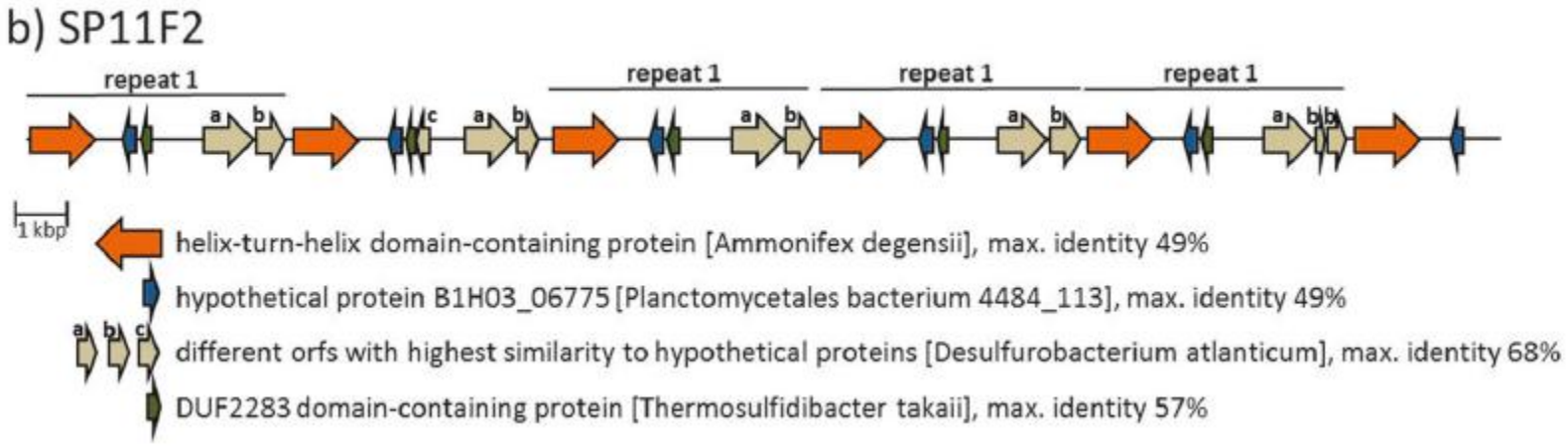
new enzymes

Sequencing the metagenomic DNA fragments



PacBio RII sequencing

Sequencing the metagenomic DNA fragments



PacBio RII sequencing

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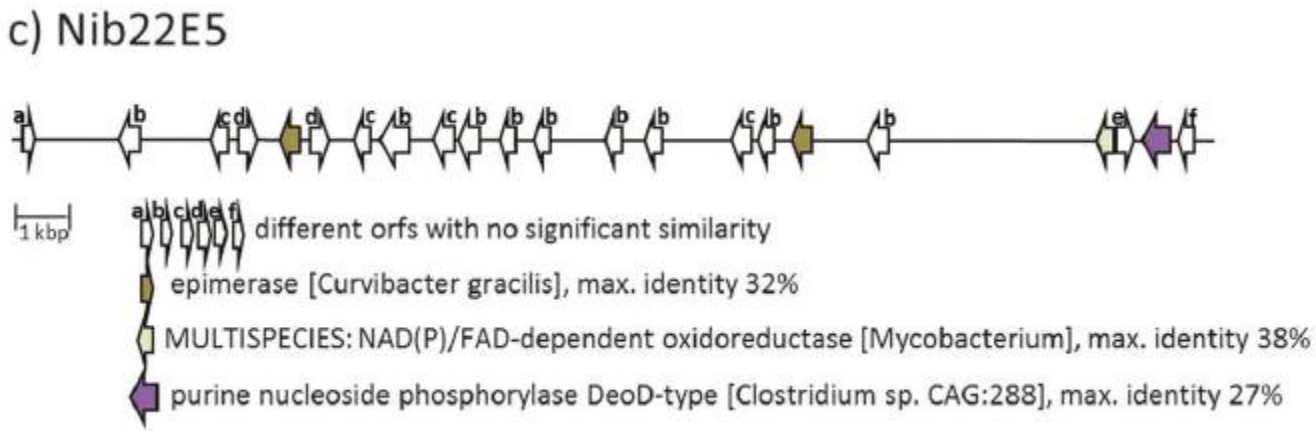

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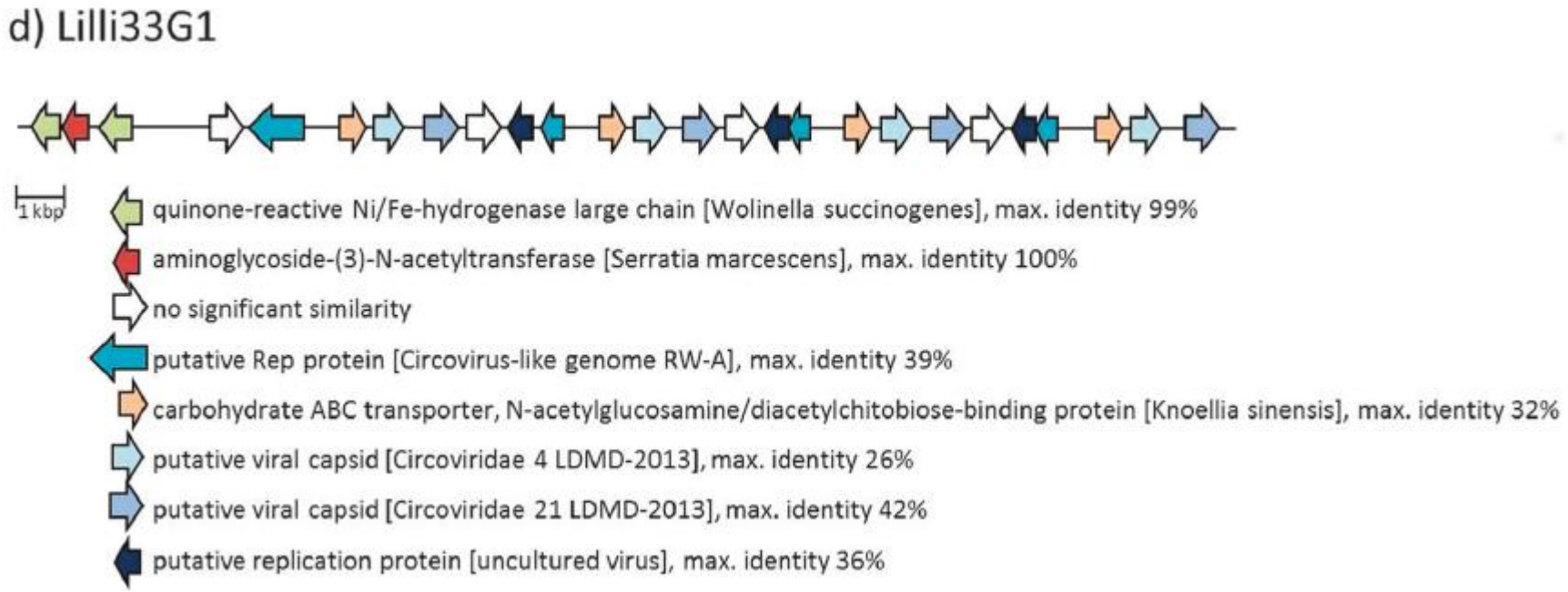
Sequencing the metagenomic DNA fragments



PacBio RII sequencing

04

Conclusion

hydrogen-converting organisms and their enzymes

limit our ability to culture the microorganisms

metagenomic and transcriptomic sequences is restricted by the genes and respective products

activity-based screens

not all bacterial and archaeal hydrogenases can be identified with the host–vector system used here.

currently the only way to identify and recover novel enzymes capable of hydrogen conversion from uncultured organisms.

[Fe]-hydrogenases - uncommon hydrogenase

（有机金属配体与铁中心配位，结构蛋白的组装相当简单并且只需较少组装和成熟蛋白）

[NiFe]- and [FeFe]- hydrogenases - typical hydrogenase

（缺乏铁 - 硫簇并且不含氧化还原活性过渡金属）

热液喷口中（稀有）金属可以容易地获得，可能在这样的系统中进化产生与常见和已知类型不同的氢化酶。

敬请各位老师同学批评指正！