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Research review paper

Discovering novel hydrolases from hot environments

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工程技术 1区
IF:11.05

从炎热的环境中发现新型水解酶

目录

CATALOG

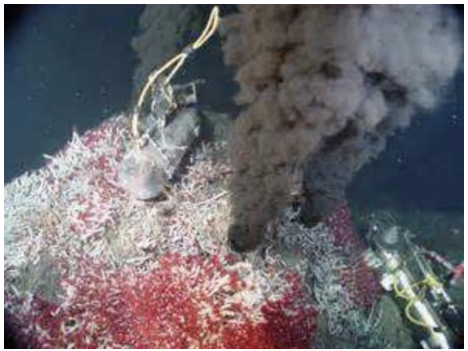
Introduction

Thermophiles
(嗜热菌)
growing optimally at
50℃ or higher

extreme thermophiles
(极端嗜热菌)
growing optimally at
65–79℃

Hyperthermophiles
(超嗜热菌)
growing optimally at
above 80℃


hot springs

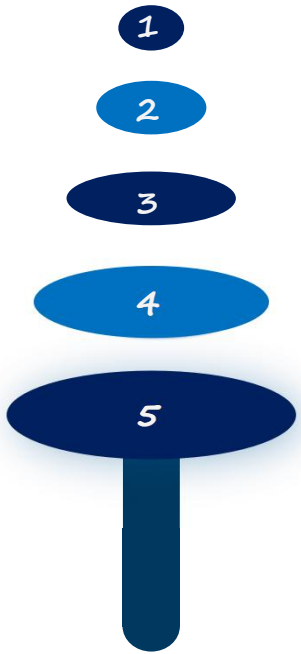

deep-sea hydrothermal vents


compost

高温！ + 极端的pH！ + 缺氧！ ⟹ 筛选新型生物催化剂

热稳定酶的好处

- 01 易于纯化
- 02 更好的稳定性
- 03 更高的生产率
- 04 降低污染风险
- 05 提高传质速率



挑战

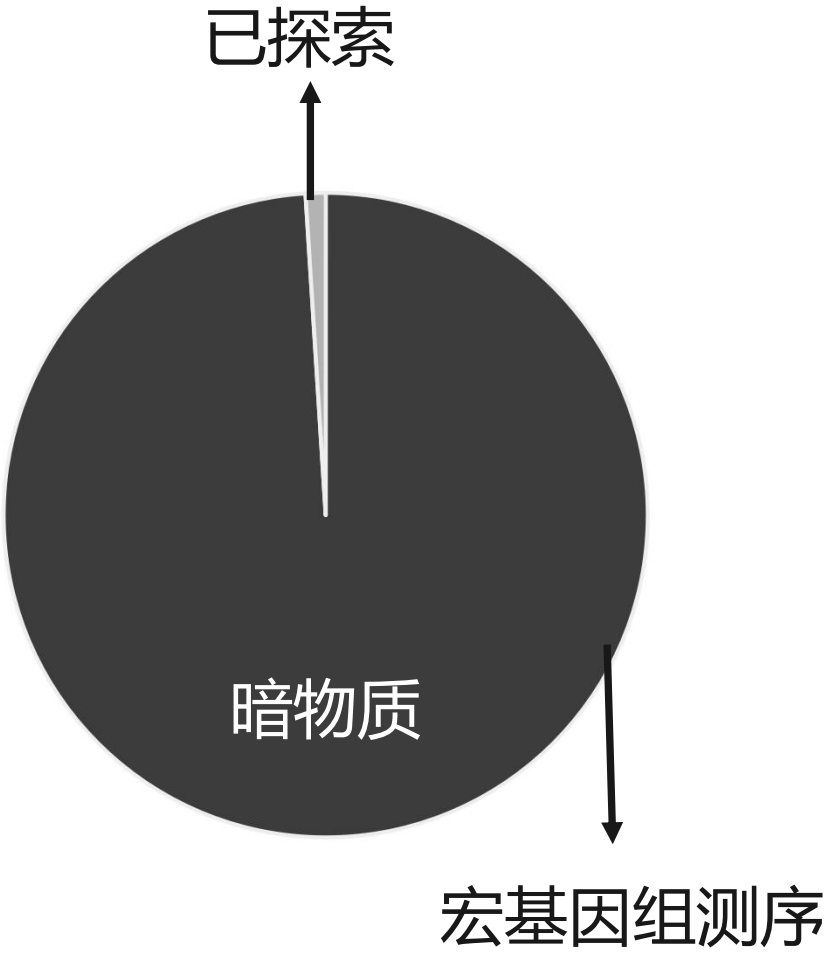
- 微生物取样
- 基因测序
- 生物信息学
- 基因表达
- 水解酶的表达生产

解决方法

01 取样及“原位”富集

02 宏基因组筛选方法

03 生物信息学和高通量筛选技术



EU contract #265933

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HotZyme

HotZyme (GA: 265933) is a project supported by the 7th framework programme for research and technological development (FP7) of the European Union.

The research project is a large scale integrating project collaborative project - on the systematic screening for novel hydrolases from hot environments.

Coordinator

University of Copenhagen	Xu Peng

European Commission

Scientific Officer	Angel Landabaso
Financial Officer	Dalibor Vojta

目的：从炎热环境中发现新型热稳定水解酶。
靶向酶：糖苷酶，酯酶，内酯酶，环氧化物水解酶和蛋白酶。

HotZyme项目于2011年启动，并于2015年完成。在此期间，HotZyme团队从全球温泉环境中收集了数百个样品，其中基于一系列工业利聚合物进行了数千种富集培养。最终，该团队获得了几十种能够降解聚合物底物的分离物。

利用新一代测序技术，该团队对15个温泉宏基因组和几个基因组和转录组进行了测序。从宏基因组文库中，该团队使用计算机模拟方法和功能筛选方法鉴定了数百种潜在的新水解酶，其中十多种被选择用于详细的生物化学和结构分析

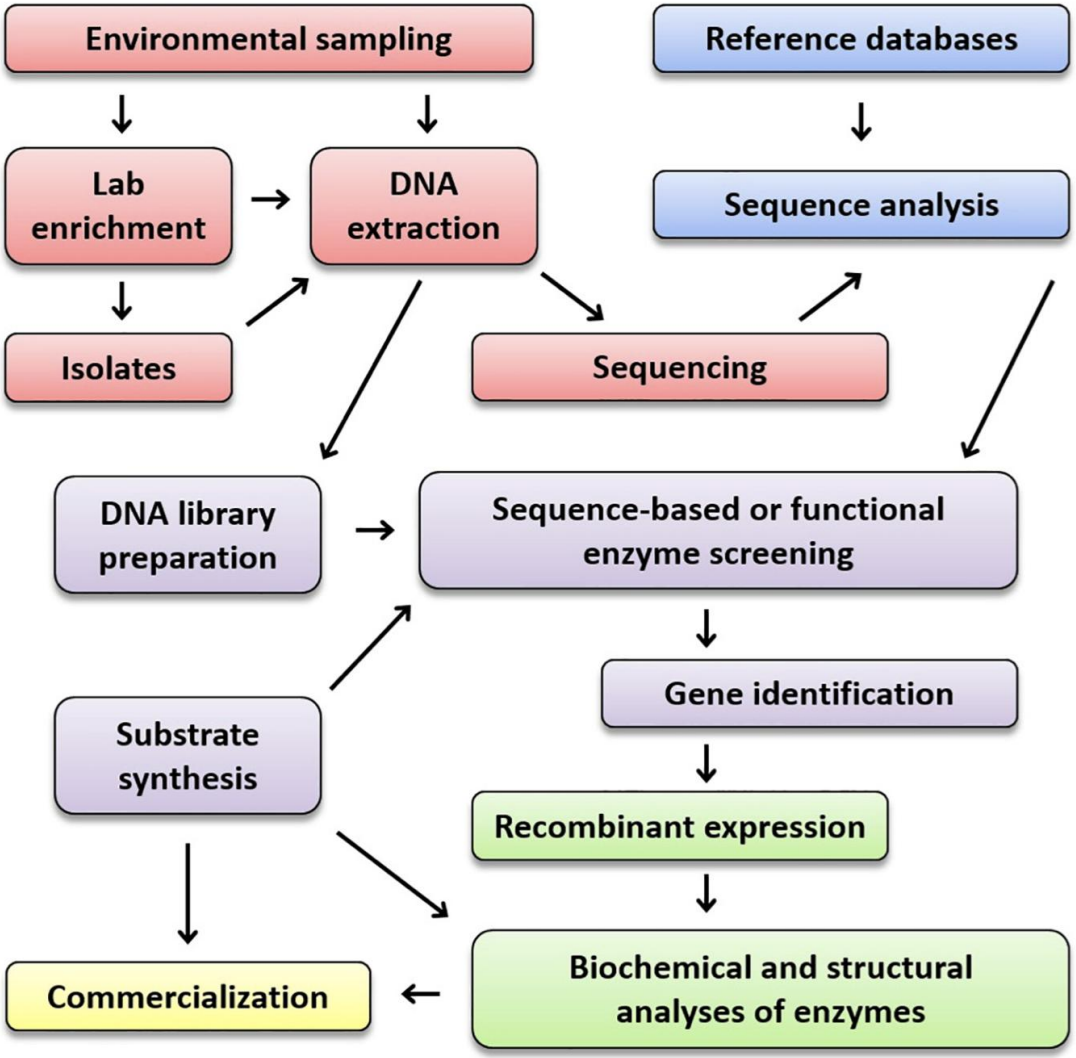


Fig. 1. Schematic workflow with exploration of the biodiversity of hot environments using different mining approaches (red boxes), involving the development of novel bioinformatic tools and platforms (blue boxes) and different screening methodologies (light velvet boxes). In the end, enzymes were subjected to more detailed functional and structural characterization (green boxes) and highly promising enzymes were patented for future potential commercialisation (yellow box). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

该项目是使用不同的采样方法探索热环境的生物多样性，包括开发新的生物信息学工具和平台以及不同的筛选方法。最后，对酶进行了更详细的功能和结构表征，并且将具有商业潜力的酶申请了专利，以用于未来潜在的商业化。

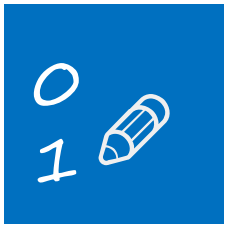
02
PART

Mining the environment

√ 原位富集具有水解活性的嗜热微生物

√ 高通量富集和分离具有水解活性的新型嗜热微生物

对世界各地发现的各种高温自然环境进行采样有两个主要目标



从这些环境中的微生物和病毒中获取总DNA样本，以便创建**功能性表达文库**，并进行详细的生物信息学分析以寻找选定的热稳定酶

创建微生物及其病毒的**富集培养物**，用于直接筛选选定的酶活性

采样地点

Fig. 2. Selected sample sites for mining natural thermal environments: A) Kunashir in situ, Dr. E. Taranov is gratefully acknowledged for providing Fig. 2A, B–D) Kunashir, E) Kamchatka Sun spring (Solnechniy), Russia, F—H) Yellowstone National Park, USA: (F) high temperature sulfur chloride hot spring, (G) acidic hot spring and (H) sulfuric hot spring.

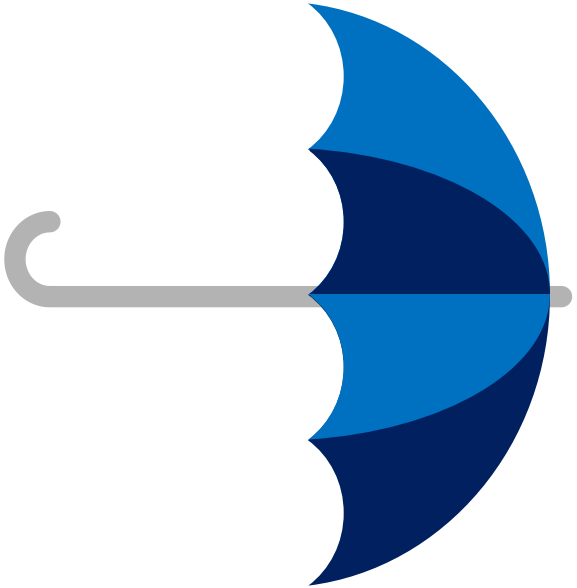
从世界各地的自然热环境中获得了300多个样本：冰岛，意大利，中国，黄石国家公园（美国），堪察加半岛，千岛湖，贝加尔湖地区（俄罗斯）和深层次生物圈（西西伯利亚，挪威海，巨魔，巴伦支海）

温度范围：40至151℃　　**样品类型**：水，沉积物和微生物过滤膜

pH范围：2.0至10.5　　**处理方式**：DNA提取以及富集和分离嗜热微生物

样品挑战

不应该阻止研究人员从热环境中进行采样



- 较低的细胞密度（低至102个细胞/ ml）
- 温泉环境的地球化学特性（例如低pH和高金属含量）
- 高温环境中收集和提取足够数量和质量的生物分子

原位富集具有水解活性的嗜热微生物

富集物：纤维素（微晶纤维素（MCC），羧甲基纤维素（CMC），玉米和竹子叶），木聚糖，淀粉，α和β-角蛋白，黄原胶，聚酯和聚乙烯醇作为底物

使用Illumina平台测序的总16S rRNA基因片段来研究在原代原位富集中发展的**微生物群落的组成**。 显示不溶性底物的可见降解和/或含有嗜热微生物的新系统发育基团的那些用于进一步表征和分离工作

高通量富集和分离具有水解活性的新型嗜热微生物

目的:是找到能够在各种大分子底物上生长的嗜热生物，从而找到降解主要底物的热稳定性水解酶

在**实验室**中用含有不同工业上感兴趣的聚合物底物的培养基培养数百种粗环境样品和**原位富集物**

底物:纤维素，木聚糖，木质素，淀粉，几丁质，竹叶，α-和β-角蛋白，黄原胶，地衣多糖，琼脂糖，聚对苯二甲酸乙二醇酯和聚乙烯醇

高温黄原胶降解酶对于深海油应用是有意义的。
富含降解纤维素或木质素组分的酶对于在纸浆和造纸工业中受到关注。

Table 1
Isolated pure bacterial and archaeal strains growing on polymeric substrates.

Name	Origin	Substrate, (concentration g/l)	T (°C)	pH	Closest relative at the time of isolation and 16S rDNA identity (b. – Bacteria, a. – Archaea)
7T	Iceland	Xylan, (10)	55	7.0	b. *Thermoanaerobacterium aciditolerans* 99%
7Tnr.1	Iceland	Xanthan, (0.5)	55	7.0	b. *Thermomicrobium roseum* 91%
7Tnr1 A	Iceland	Xanthan, (0.5)	55	7.0	b. *Geobacillus vulcani*
7Tnr.2	Iceland	Xanthan, (0.5)	55	7.0	b. *Cohnella laevinbosi/thermotolerans* 97%
6Tnr.2	Iceland	Xanthan, (0.5)	85	7	b. *Thermus aquaticus* 96%
6Tnr.3	Iceland	Xanthan, (0.5)	85	7	b. *Thermus aquaticus/thermophilus* 95%
8Tnr.3	Iceland	Xanthan, (0.5)	85	7.0	b. *Thermus antranikianii* 99%
2T 5 2 and Is2-7*6.2	Iceland	Xanthan, (0.5)	55	7.0	b. *Meiothermus silvanus* 99%
7T nr2,1 7T nr4,1 7T nr4,2	Iceland	Starch, (5)	55	7.0	b. *Meiothermus silvanus* 98%
2319x	Russia, Kunashir	Xylan, (10)	85	6.0	a. *Thermococcus alcaliphilus/aegaeus* 99%
2319cl	Russia, Kunashir	Sodium carboxy-methyl-cellulose, (2)	75	7.0	b. *Caldicellulosiruptor owensenis/hydrothermalis* 98%
8-7 nr.1	China	Xylan, (10)	78	7.0	b. *Dictyoglomus* sp. 99%
8-7 nr.2	China	Xylan, (10)	78	7.0	b. *Fervidobacterium islandicum* 98%
2410	Russia, Kamchatka	Polyvinyl-Alcohol, (5)	80	6.0	a. *Sulfolobus islandicus* 99%
DG#1 3,2	Denmark	Xanthan, (0.5)	55	7.0	b. *Paenibacillus ginsengihumi* 92%
DG#1 4,1	Denmark	Xanthan, (0.5)	55	7.0	b. *Cohnella laeviribosi* 97%
Is3-14,2	Iceland	Xanthan, (0.5)	55	7.0	b. *Thermus igniterrae* 98%
Is3-24,1 Is3-24,6 Is3-24,4	Iceland	Xanthan, (0.5)	55	5.0	b. *Alicyclobacillus sendaiensis* 98%
Is3-23,3 Is3-23,4	Iceland	Xanthan, (0.5)	70	5.0	b. *Alicyclobacillus acidocaldarius* 99%
DG#1 1,1	Denmark	Xylan, (10)	55	7.0	b. *Brevibacillus thermoruber* 99%
DG#1 2,1 DG#1 2,2	Denmark	Xylan, (10)	70	7.0	b. *Geobacillus vulcani* 98%
DG#1 3,2	Denmark	Xylan, (10)	55	7.0	b. *Brevibacillus thermoruber* 98%
DG#1 3,2	Denmark	Xylan, (10)	70	7.0	b. *Geobacillus thermoglucosidasius* 99%
Is3-23,2	Iceland	Xylan, (10)	70	5.0	b. *Alicyclobacillus sendaiensis* 98%
Is3-23,4	Iceland	Xylan, (10)	70	5.0	b. *Alicyclobacillus sendaiensis* 99%
Is3-24,4 Is3-24,7	Iceland	Xylan, (10)	55	5.0	b. *Alicyclobacillus sendaiensis* 99%
Is3-24,5	Iceland	Xylan, (10)	55	5.0	b. *Alicyclobacillus sendaiensis* 98%
Is3-21,2	Iceland	Xylan, (10)	55	7.0	b. *Brevibacillus thermoruber* 99%
Is3-21,4.1	Iceland	Xylan, (10)	55	7.0	b. *Geobacillus* sp. 99%
Is3-21,4.3	Iceland	Xylan, (10)	55	7.0	b. *Geobacillus thermoleovorans* 99%
Is3-23,1	Iceland	Xylan, (10)	70	5.0	b. *Geobacillus thermoleovorans* 99%
Is2-8	Iceland	Xylan, (10)	70	6.0	b. *Geobacillus kaustophilus* 97%
Is3-21,3	Iceland	Polyvinyl-alcohol, (0.5)	55	7.0	b. *Thermus brockianus* 98%
Is2-7*	Iceland	Polyvinyl-alcohol, (0.5)	55	6.0	b. *Thermus brockianus* 99%
It-5	Italy	Polyvinyl-alcohol, (0.5)	78	7.0	a. *Staphylothermus hellenicus* 96%
It-5.1	Iceland	Gelrite, (5)	78	5.0	a. *Sulfolobus shibatae* 99%
7T	Iceland	Polyethylene terephthalate, (2)	70	7.0	b. *Rhizobium leguminosarum* 91%
BPi-2ag	Russia, Baikal Rift	Agarose, (5)	54	7.3	b. *Caloramator australicus* 97%
BPi-4-40	Russia, Baikal Rift	Xylan, (2)	40	7.3	b. *Paenibacillus lautus* 99%
2842	Russia, Baikal Rift	Xanthan, (0.5)	47	7.5	b. *Phycisphaera mikurensis* 80%
2918	Russia, Kamchatka	Xanthan, (0.5)	54	6.0	b. *Phycisphaera mikurensis* 80%
Rift-s3	Guaymas Basin	Microcrystalline cellulose (10)	65	6.5	b. *Thermosipho atlanticus* 96%

虽然建立了超过一千种富集物，但其中有10%在三次转移中幸存下来。只有这些所谓的稳定富集培养物随后用于DNA提取，用于16S rRNA测序和分离纯菌株。

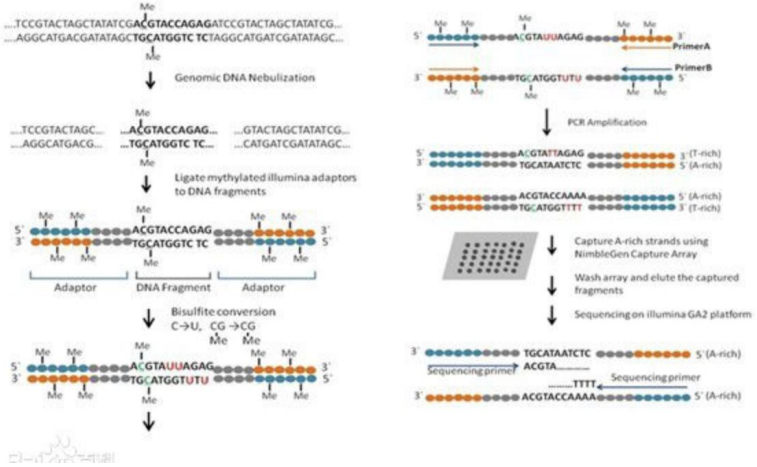
尽管大多数分离株显示出与已知物种的高16S rRNA序列同一性，但是一些是非常新颖的并且一些被发表为新的分类群

Sequencing and bioinformatics

在早期环境DNA测序中，Sanger测序是首选方法，类似于对个体基因组进行测序。

这些分析通常依赖于16S rRNA基因的部分序列，因为大多数NGS平台的读取长度相对较短。
为此，设计用于扩增16S rRNA可变区的引物
V4-V8、V3-V4

用过的。在过去的几年中，通过这种方法分析了大量的极端温度环境，特别是温泉，由于这种方法，已经生产并沉积了大量的16S rRNA序列。在公共数据库中，如核糖体数据库项目、或SILVA数据库



第二代DNA测序技术
(Next-generation sequencing)

TABLE 1 | Examples of hot springs studied using the amplification of the variable regions of 16S rRNA.

Hot spring	Type of sample	pH	Temperature (°C)	Sequencer	Region amplified	References
Siloam, Limpopo, South Africa		9.5	63	Roche 454 GS FLX	V4–V7	Tekere et al., 2011
Lake Bogoria, Kenya					V3–V4	Dadheech et al., 2013
Arzakan and Jermuk, Armenia					V4–V8	Hedlund et al., 2013
Bacon Manito Geothermal Field, Philippines					V4–V8	Huang et al., 2013
Furnas Valley, Saõ Miguel, Azores	Water, sediment and microbial	2.5–8	51–92	Roche 454 GS FLX	V2–V3	Sahm et al., 2013
Yunnan province and Tibet, China					V4	Song et al., 2013
Zavarzin, Uzon Caldera, Kamchatka, Russia					V3	Rozanov et al., 2014
Sungai Klah, Malaysia	Water and sediment	8	75–85	Illumina MiSeq	V3–V4	Chan et al., 2015
Jakrem, Meghalaya, India	Microbial mat	–	–	Illumina	V3	Panda et al., 2015
Odisha, Deulajhari, India	Sediment	7.14–7.83	43–55	Illumina GAIIX	V3–V4	Singh and Subudhi, 2016

缺点

这种基于PCR的方法由于引物的限制，嵌合体等因素以及可能存在于样本中的抑制因素而存在偏差
成本高以及缺乏足够的测序深度

16S rRNA序列的分析可能导致分类学的错误识别
因为密切相关的物种可能含有几乎相同的16S rRNA基因

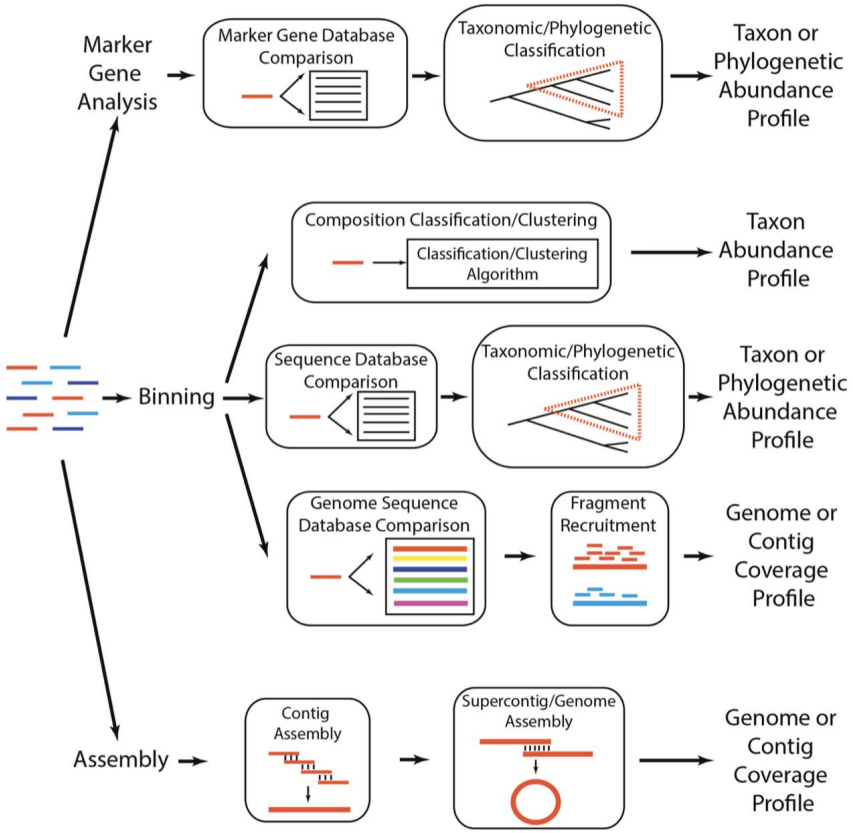


FIGURE 2 | Analytical strategies to determine which taxa are present in a metagenome. A metagenome (colored lines, left) can be subject to three general analytical strategies that ultimately produce a profile of the taxa, phylogenetic lineages, or genomes present in the community. *Marker gene analyses* involve comparing each read to a reference database of taxonomically or phylogenetically informative sequences (i.e., marker genes), using a classification algorithm to determine if the read is a homolog of a marker gene, and annotating classified reads based on their similarity across marker gene sequences. There are several methods for *binning* metagenomes, including (1) compositional binning, which uses sequence composition to classify or cluster metagenomic reads into taxonomic groups, (2) similarity binning, which classifies a read into a taxonomic or phylogenetic group based on its similarity to previously identified genes or proteins, and (3) fragment recruitment, wherein reads are aligned to nearly identical genome sequences to produce metagenomic coverage estimates of the genome. Finally, sequences can be subject to *assembly*, wherein reads that share nearly identical sequence at their ends are merged to create contigs, which can subsequently be assembled into supercontigs or complete genomes.

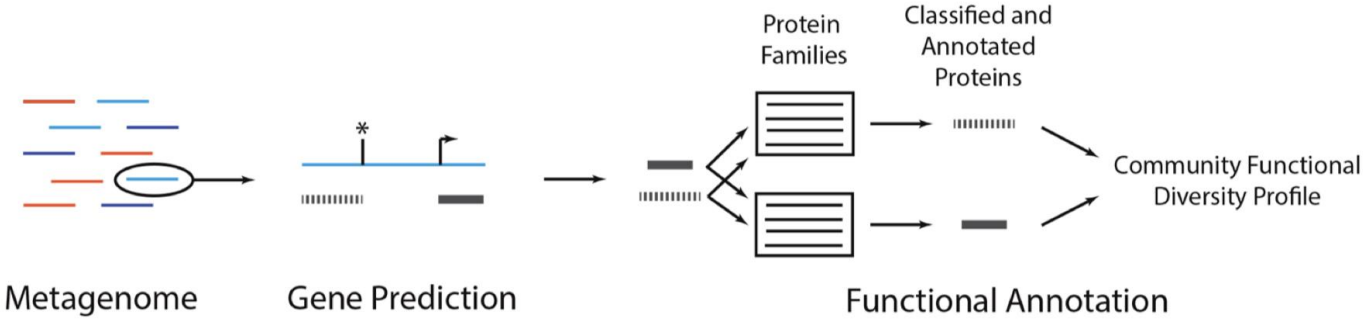
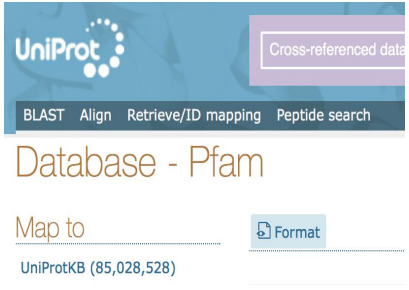
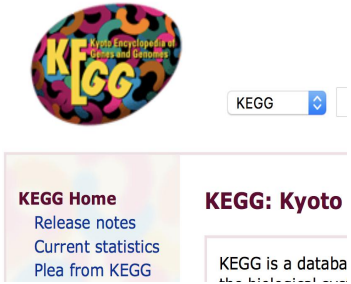


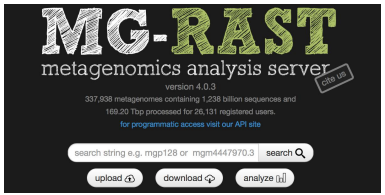
FIGURE 3 | A metagenomic functional annotation workflow. A metagenome (colored lines, left) can be annotated by subjecting each reads to gene prediction and functional annotation. In *gene prediction*, various algorithms can be used to identify subsequences in a metagenomic read (blue line) that may encode proteins (gray bars). In some situations, coding sequences may start (arrow) or stop (asterisk) upstream or downstream the length of the read, resulting in partial gene predictions. Each predicted protein can then be subject to *functional annotation*, wherein it is compared to a database of protein families. Predicted peptides that are classified as homologs of the family are annotated with the family's function. Conducting this analysis across all reads results in a community functional diversity profile. As discussed in the main text, there are alternative annotation strategies and variations on this general procedure.

功能注释数据库





基因预测、数据库搜索、分类注释



IMG/M

SUPER-FOCUS

Gene expression and screening
基因表达和筛选

√用于功能筛选的重组文库

√功能性体外筛选表达文库

√用全细胞生物传感器进行功能筛选

选择合适的表达系统与适当的筛选宿主是非常重要的。 在HotZyme项目中，质粒文库在载体pUC18。
选择**大肠杆菌系统**的主要原因是它符合HotZyme项目的标准

- 由于携带该基因的相关基因组被不编码这种酶的基因组稀释，特定酶基因的命中率下降，因此需要具有非常高转化率的重组宿主。。

- 大肠杆菌已被证明是高度接受外来的SD翻译起始点

为了克隆到pUC18载体中，用*Bsp*143I和*Hin*1II对DNA进行部分酶切，凝胶提取> 2kb的片段并与*Bam*HI / *Sph*I酶切的pUC18连接。 将连接的DNA转化到感受态的E.cloni 细胞中

对于大肠杆菌表达文库的成功功能筛选需要考虑几个因素：

- 需要合适的方法来检测所需的酶活性，例如通过使用显色透明圈。
- 底物必须可被表达的酶接近，因为底物被细胞吸收，酶被分泌或通过细胞裂解释放，尽管可能发生高表达的细胞内酶释放到细胞外空间。
- 为了获得良好的信噪比，筛选宿主不应含有对筛选底物的显着内源活性。

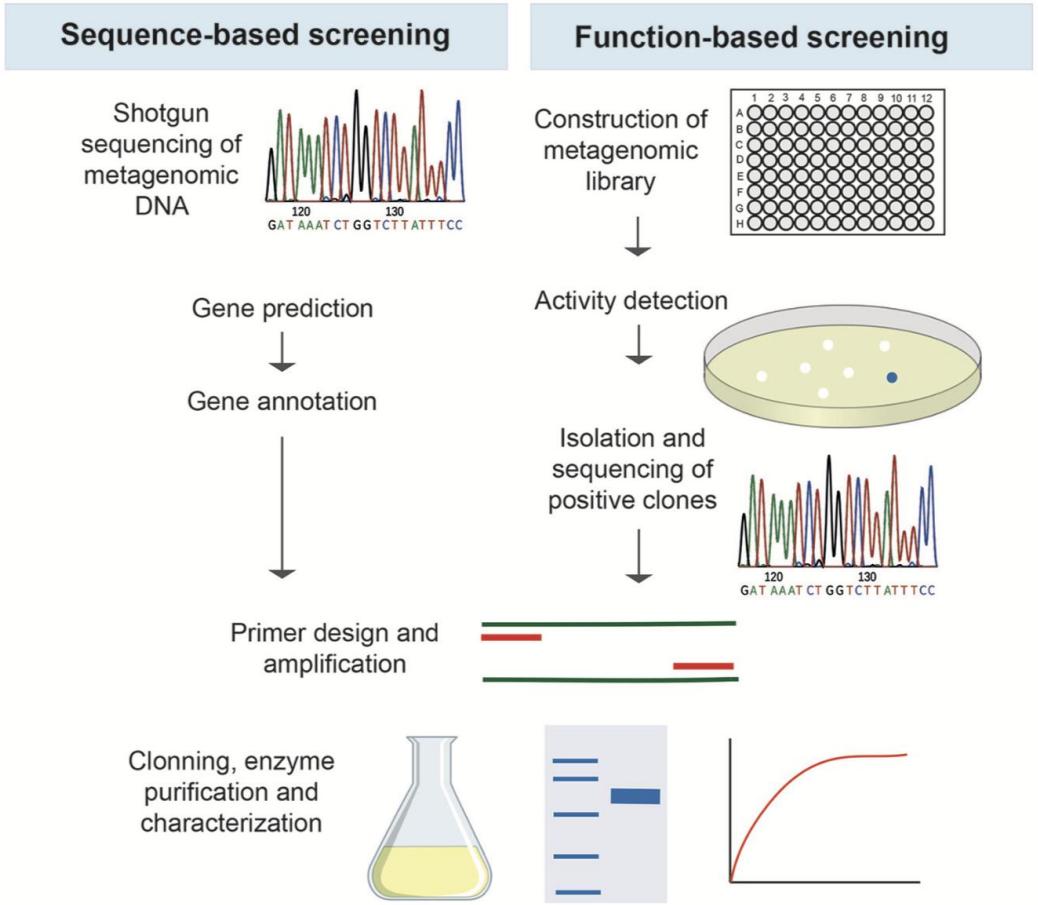


FIGURE 2 | The two major strategies used for screening metagenomes in search of new thermozymes.

该技术的优点是**不需要培养天然微生物或已知基因的先前序列信息**，因此代表了用于开采具有新特征的酶的有价值的方法

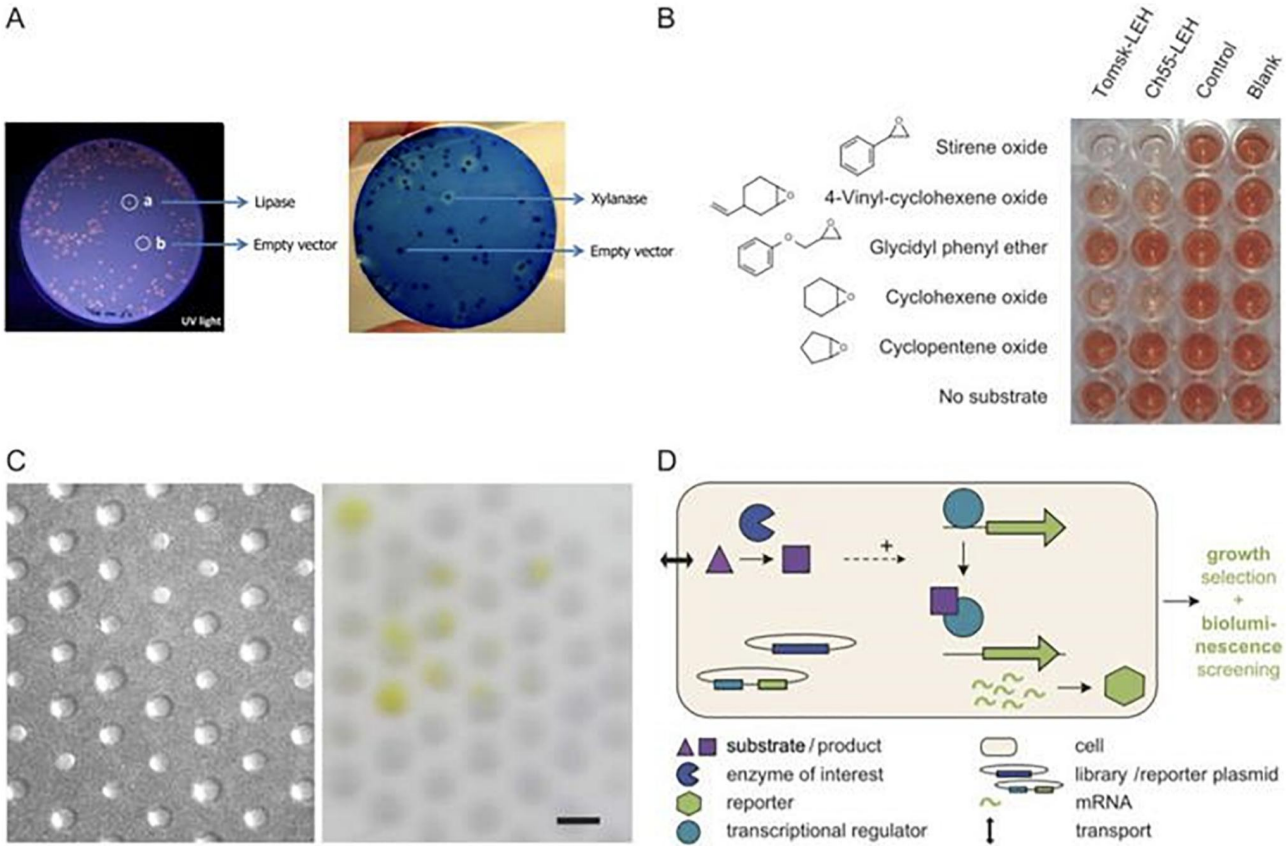


Fig. 3. Overview of the different functional screening strategies. (A) Agar plate based screening. (B) Microtiterplate-based screening. Detection and substrate profiling of epoxide hydrolase activity with the colorimetric adrenaline assay (Cedrone et al., 2005). (C) Microbial culture chip (MCC) based screening. Left: light microscope image of a small section of an MCC with 100 μm circular compartments, inoculated with a mixture of *E. coli* expressing an esterase or carrying an empty vector. Right: a similar area is shown after transfer of the microcolonies onto nitrocellulose and incubation on a filter paper soaked with assay buffer containing substrate and the pH indicator bromophenol blue. The scale bar is approximately 300 μm. (D) Whole-cell bioreporter-based screening. Adapted from van Rossum et al. (2013). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

05
PART

Functional and structural enzyme characterization
酶的功能和结构

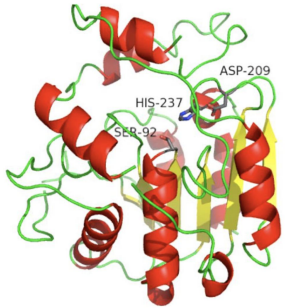


Fig. 6. The overall α/β structural fold of the *C. hydrogenoformans* lactonase enzyme with the catalytic triad characteristic of esterase enzymes shown in stick representation. Subtle differences in the aminoacids within the binding pockets determine the preference for lactone substrates.

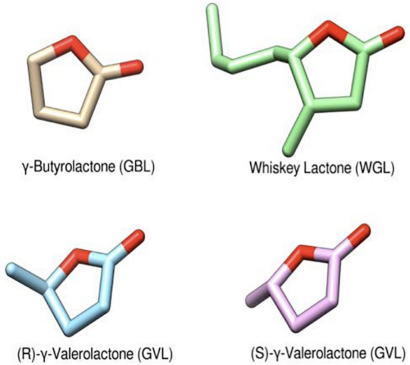


Fig. 7. The structure of the substrates used in the molecular modelling ligand docking studies of the *C. hydrogenoformans* lactonase enzyme.

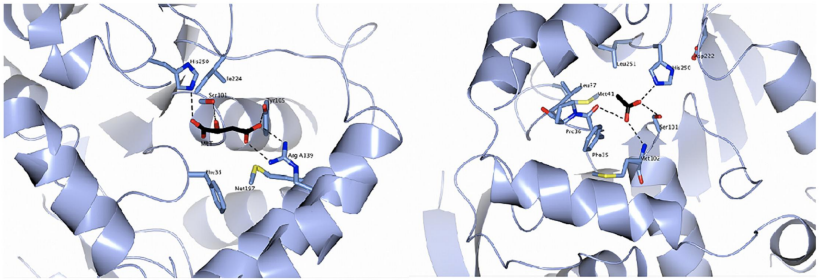


Fig. 9. Structure of carboxyl esterase TtEst (PDB: 4UHC) from *T. terrifontis*. A) The alcohol binding site of TtEst showing the protein backbone in cartoon mode. The important amino acid residues and the bound ligand are shown in stick mode. The dashed represent hydrogen bonds and ionic interactions. B) The carboxyl binding site which restricts the size of the substrate acyl group that can bind is shown. The bound acetate group and the important amino acid side chains are shown as stick models and the protein backbone in cartoon mode. The hydrogen bonds are shown as dashed lines.

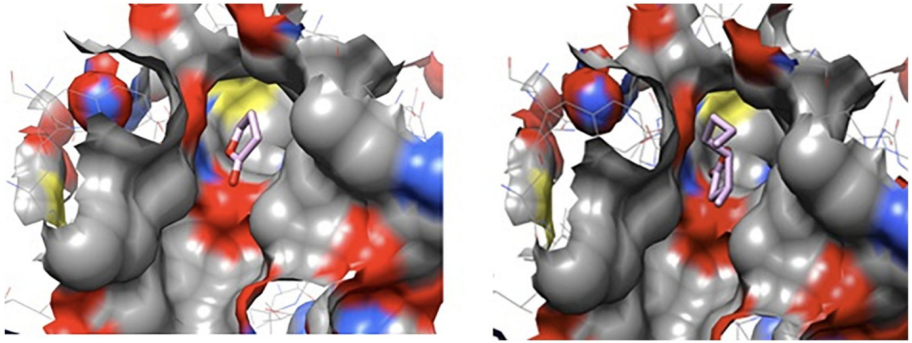


Fig. 8. Docking of substrates into the active site of the C. hydrogenoformans lactonase A) the GBL docked into the active site of the lactonase enzyme. B) a view of the (3S,4S)-WGL docked into the active site of the lactonase enzyme. The methyl group of the WGL prevents this substrate binding in the active conformation for catalysis and explains the experi- mental results showing no activity as on this sub- strate.

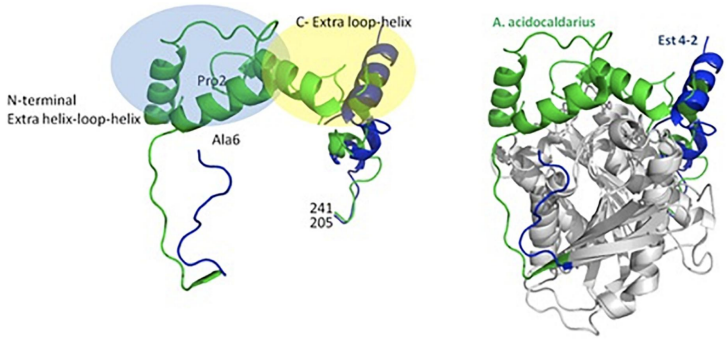


Fig. 10. A comparison of the carboxyl esterase TtEst2 (PDB: 5AO9) of *T. terrifontis* (blue) with the *A. acidocaldarius* esterase (PDB: 1EVQ) (green) highlighting the differences in the cap domain at the N-terminus and towards the C-terminal end of the enzyme. The lack of a cap domain in the TtEst2 enzyme means that this esterase has a very exposed active site compared to related esterases.The conserved core domain is shown in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

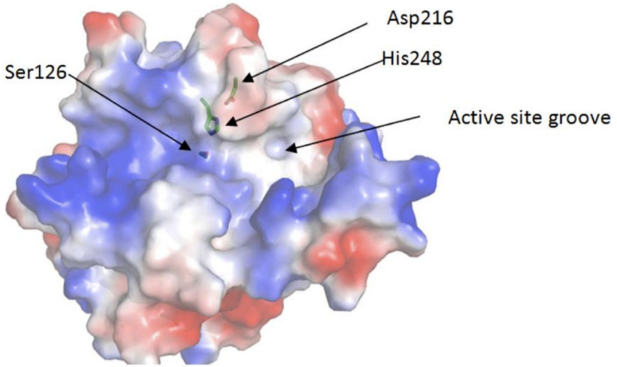


Fig. 11. An electrostatic surface representation of the carboxyl esterase TtEst2 of *T. terrifontis* with the esterase catalytic triad highlighted as stick models. This highlights the exposed active site groove running along the surface of the enzyme.

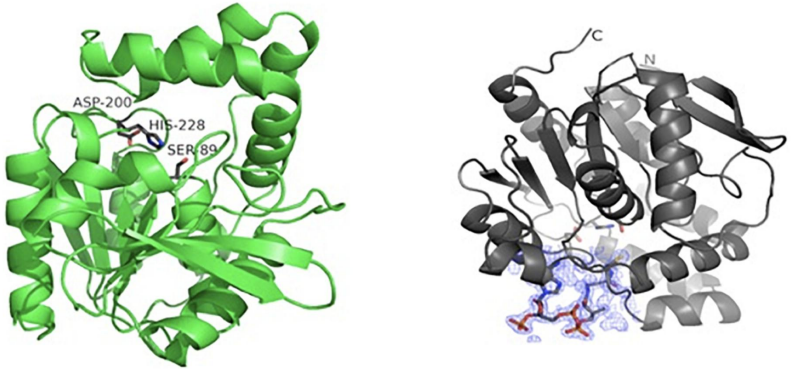


Fig. 12. The α/β hydrolase fold of the *A. fulgidus* Est-2 with the esterase catalytic triad shown as stick models (PDB code 5FRD). This enzyme has a coenzyme A molecule tightly bound at a unique position which is different from that seen for the thioesterases. The $2F_o$-F_c electron density map contoured at 1.2σ shows the presence of coenzyme A clearly in the protein structure bound close to the active site. The CoA molecule and active site residues are shown as stick models.

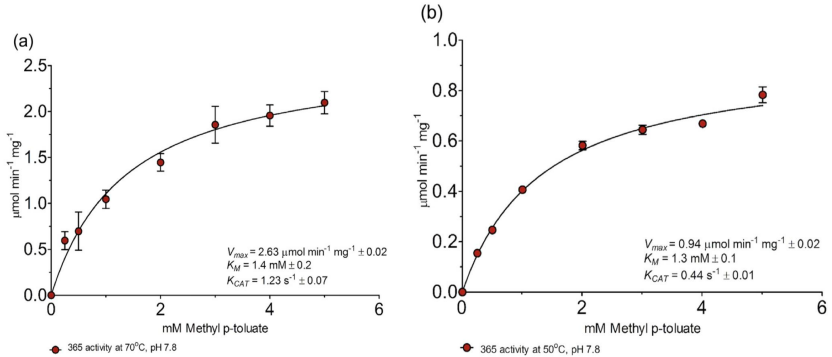


Fig. 13. A figure showing the kinetics of the reaction of the A. fulgidus Est-2 at 50 and 70 °C with the industrially interesting substrate, methyl p-toluate.

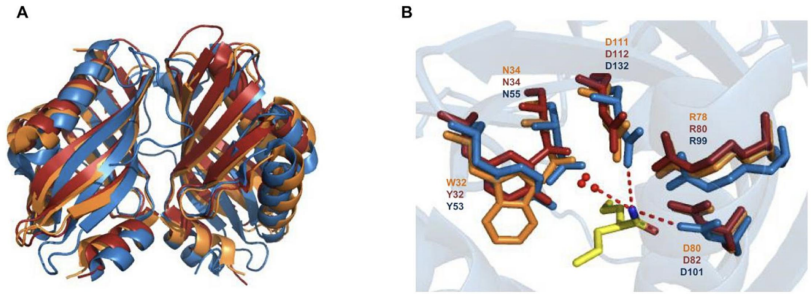


Fig. 14. Structure comparison of the thermostable limonene-1,2-epoxide hydrolases with the LEH of *R. erythropolis*, A) Overall structure alignment of Tomsk-LEH (PDB: 5AIF, *orange*), CH55-LEH (PDB: 5AIH, *red*), and *R. erythropolis* LEH (*Re*-LEH, PDB: 1NU3, *blue*). B) Key residue side chains in the active pocket (Tomsk-LEH, *orange*; CH55-LEH, *red*, and *Re*-LEH, *blue*). The inhibitor valpromide co-crystallized in the *Re*-LEH structure is coloured yellow (colour by element) which locates the potential position of substrate binding, while the catalytic water molecules are shown as red spheres. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

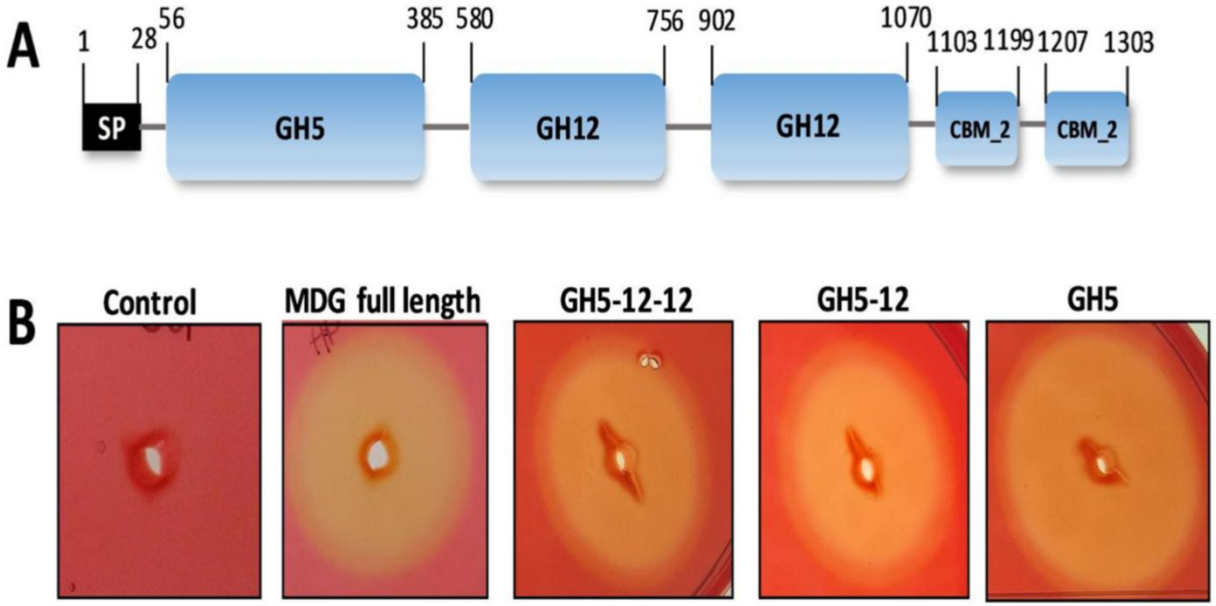
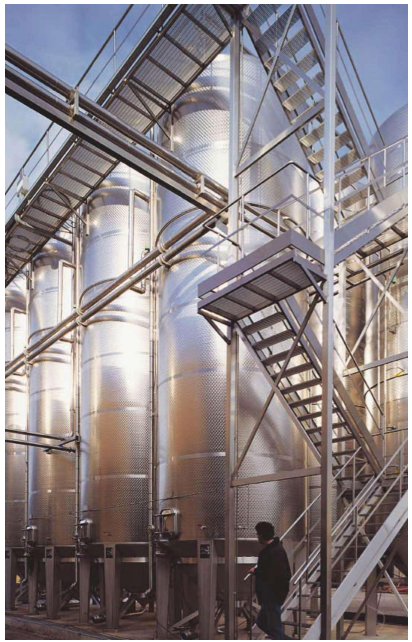


Fig. 18. Overview of the modular domain structure and cellulolytic activities of recombinant MDG and its truncated versions (modified from Gavrilov et al., 2016). Multidomain structure of the full length MDG (A), consisting of three glycoside hydrolase (GH) family domains and two family two carbohydrate binding modules (CBM2s). Domain order, N- to C-terminal direction: GH5-12-12-CBM2-2. (B) Hydrolytic activities of the full length MDG and truncated versions were analysed on CMC screening plates. Recombinant proteins were cloned without the signal peptide (SP) and the expression strain with empty plasmid was used as control. Plates were stained using 0.2% (w/v) Congo red and destained with 1 M NaCl, three times, 15 min at room temperature. For detailed discussion see text. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Industrial applications

大麦麦芽中的麦芽淀粉酶

从胃组织中分离出来蛋白酶用于干酪生产



微生物生产酶的优点

1、微生物种类多，品种齐全
2、微生物生长繁殖快，生长周期短，产量高；
3、培养方法简单，原料来源丰富，价格低廉，经济效益高，并可以通过控制培养条件来提高酶的产量；
4、微生物具有较强的适应性和应变能力。
5、可以进行大规模生产

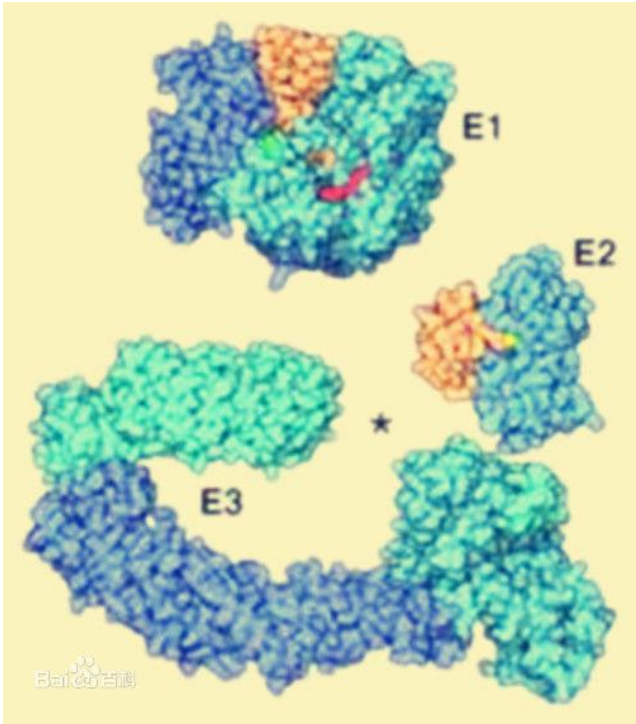
这些优势使得酶的生产量能够在现代工业过程中使用
工业酶市场估计每年约为145亿美元，预计增长将持续

绝大多数工业酶是水解酶。 它们作用于各种键，催化它们的裂解并将高分子量的化合物降解成较小的组分

售总额计算，洗涤剂酶是最大的市场之一。
Savinase是丝氨酸蛋白酶，几乎存在于所有洗衣洗涤剂配方中。

蛋白酶可有效去除衣物中富含蛋白质的污渍，如血渍。

除了蛋白酶或脂肪酶之类的酶，其主要作用是去除污渍
酶也可以作用于织物本身。 某些对无定形纤维素有活性的纤维素酶可以从棉织物表面除去纤维素纤维，同时使构成织物本身的结晶纤维素纤维保持完整



淀粉酶

两个主要领域：
新织物的退浆和**生物燃料的生产**

在第一种情况下，在编织之后，必须除去淀粉上浆剂，这是淀粉酶用于水解淀粉并有助于该过程的地方。

淀粉酶也用于淀粉的总水解以产生葡萄糖，其可以直接使用或发酵成乙醇，即所谓的第一代乙醇生产

从纤维素生物质生产葡萄糖需要一套酶。 这是因为木质纤维素作为植物细胞壁的重要组成部分，可以抵抗降解。
需要各种**内切和外切纤维素酶**来释放葡萄糖二糖纤维二糖，其通过葡糖苷酶进一步降解为葡萄糖

新型耐热水解酶的具体应用

Common features and specific applications of the novel thermostable hydrolases.

Enzyme activities	Common features and specific applications
Lactonases	Synthesis of γ-hydroxycarboxylic acids and resolution of racemic lactones by selective hydrolysis of lactone rings
Esterases	Hydrolysis of small carboxylic esters and γ-lactams, resolution of racemates, high stability at elevated concentrations of organic solvents
Lipases	Hydrolysis of large carboxylic esters, resolution of racemates, stain removal by fat and lipid hydrolysis
Epoxide Hydrolases	Synthesis of epoxides and vicinal diols, resolution of mixtures of cis/trans-isomers by selective ring-opening of epoxides
Cellulases	Hydrolysis of cellulose in biomass conversion and textile treatment
Xylanases	Hydrolysis of xylan polysaccharides in pulp and paper applications and biomass conversion
Proteases	Stain removal by protein hydrolysis in detergent and laundry applications
Cutinases	Degradation of polyethylene terephthalate
Lignin degrading enzymes	Depolymerization of lignin in biomass conversion, pulp and paper applications
Glycosidase-Endoglucanase/ Mannosidase	Hydrolysis of β-glucan

纤维素酶：纤维素在生物质转化和纺织品处理中的水解作用

木聚糖酶：木聚糖多糖在纸浆和造纸中的水解及生物量转化

木质素降解酶：木质素在生物质转化、纸浆和造纸中的解聚作用

07
PART

Perspectives
观点

为了向更可持续的过程过渡，我们需要新型强大的生物催化剂，可以抵抗极端的pH和温度，溶剂和盐。 **极端微生物**是这种强力酶的天然储库。

过去几十年来各种“组学”技术的进步 - 例如基因组学，转录组学，蛋白质组学或代谢组学，使其应用于各种高温环境。

生成巨大的环境数据集，但是，为相关目标**挖掘**这些庞大而复杂的数据集仍然具有挑战性。最大的挑战是**基因注释**，尤其是大量没有明显功能的基因。

由于数据库中的错误，以及通过序列同一性来推断基因功能，注释可能不会给出实际的功能。因此，序列注释只是功能表征的第一步。

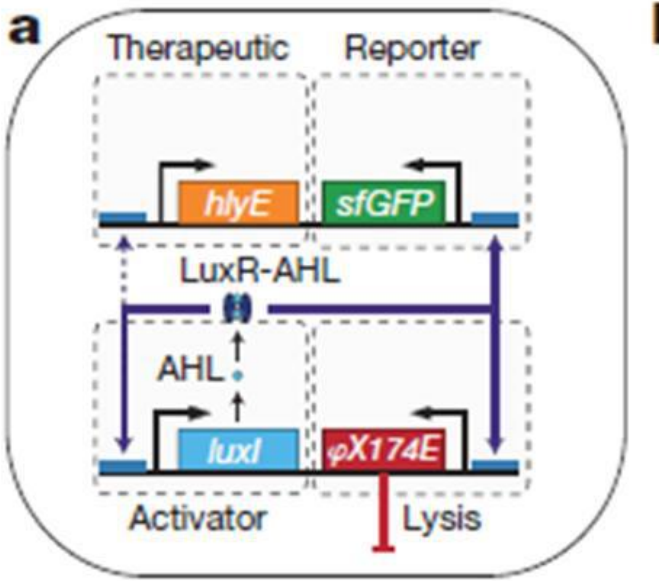
它需要多种方法才能取得成功。在未来，继续努力了解高温环境中微生物的生物化学过程，无疑将提高我们开发这些环境以获得有用产品的能力

对于功能筛选，可获得大量不同的筛选技术，但不幸的是它们尚不适用于每种酶。

每种筛选方法的挑战是：
酶的异源表达
酶的底物可用性

基于微流体的筛选和开发新的底物合成方法，扩大宿主的数量，可以帮助解决大多数这些挑战。

通过在适当的宿主有机体（如大肠杆菌）中引入非自然途径模块，可以开发出一种**合成生物学方法**，用于重要分子的体内生产



HotZyme项目的成功表明

仍然有很多水解酶被发现。特别是多学科方法，其中13个小组各自拥有不同的专业知识，在一个联盟中共同努力，使这个项目如此成功。

来自炎热环境的水解酶的广泛多样性，开发的新方法和工具为扩展已经完善的水解酶应用提供了巨大的宝贵财富，并将成为进一步探索未知新水解酶领域的宝贵资产

合作-多学科交叉合作

敬请各位老师同学批评指正

THANKS

D.O.I.	Title
10.1007/s00248-015-0576-9	Comparative Metagenomics of Eight Geographically Remote Terrestrial Hot Springs
10.1007/s13213-013-0704-z	Metagenomic analyses reveal phylogenetic diversity of carboxypeptidase gene sequences in activated sludge of a wastewater treatment plant in Shanghai, China
10.1128/JVI.01495-14	Unveiling Cell Surface and Type IV Secretion Proteins Responsible for Archaeal Rudivirus Entry
10.1016/j.jmb.2014.09.016	First Experimental Evidence for the Presence of a CRISPR Toxin in Sulfolobus
10.3389/fmicb.2012.00207	Diversity and subcellular distribution of archaeal secreted proteins
10.1099/ijs.0.063156-0	Thermosipho activus sp. nov., a thermophilic, anaerobic, hydrolytic bacterium isolated from a deep-sea sample
10.1134/S0026261714060034	Capacity of hyperthermophilic Crenarchaeota for decomposition of refractory proteins (α- and β-keratins)
10.1099/ijs.0.000009	Thermogutta terrifontis gen. nov., sp. nov. and Thermogutta hypogea sp. nov., thermophilic anaerobic representatives of the phylum Planctomycetes
10.1099/ijs.0.070151-0	Tepidisphaera mucosa gen. nov., sp. nov., a moderately thermophilic member of the class Phycisphaerae in the phylum Planctomycetes, and proposal of a new family, Tepidisphaeraceae fam. nov., and a new order, Tepidisphaerales ord. nov.
10.1111/febs.12281	Reporter-based screening and selection of enzymes
10.1111/febs.13326	Structural studies of a thermophilic esterase from a new Planctomycetes species, Thermogutta terrifontis
10.1111/febs.13328	Discovery and characterization of thermophilic limonene-1,2-epoxide hydrolases from hot spring metagenomic libraries
10.3389/fcell.2014.00070	Integrative workflows for metagenomic analysis	Efthymios Ladoukakis, Fragiskos N. Kolisis, Aristotelis A. Chatziioannou	19/11/2014	Frontiers in Cell and Developmental Biology Vol. 2	-
10.1016/j.nbt.2014.05.2042	ANASTASIA a versatile web platform for metagenomic analysis	Efthymios Ladoukakis, Eleftherios Pilalis, Aristotelis Chatziioannou, Fragiskos Kolisis	01/07/2014	New Biotechnology Vol. 31	S170
10.1016/j.jbiotec.2014.04.026	Characterization of a phosphotriesterase-like lactonase from the hyperthermoacidophilic crenarchaeon Vulcanisaeta moutnovskia	Verena Kallnik, Alina Bunescu, Christopher Sayer, Christopher Bräsen, Roland Wohlgemuth, Jennifer Littlechild, Bettina Siebers	01/11/2014	Journal of Biotechnology Vol. 190	11-17
	A Galaxy Workflow for the Functional Annotation of Metagenomic Samples	Pilalis E.	01/01/2012	Lecture Notes in Computer Science 7297/ 2012	247-253
	Economic transformation in Hungary and Poland	Directorate-General for Economic and Financial Affairs	01/01/2000	EEAG Report on the European Economy 43/1990	151-167
10.1002/cctc.201500608	Efficient epoxide hydrolase catalyzed resolutions of (+)- and (-)-cis/trans-limonene oxides	E. E. Ferrandi, C. Marchesi, C. Annovazzi, S. Riva, D. Monti, R. Wohlgemuth	31/08/2015	ChemCatChem, 7	3171-3178
10.3389/fmicb.2015.01294	The Structure of a Novel Thermophilic Esterase from the Planctomycetes Species, Thermogutta terrifontis Reveals an Open Active Site Due to a Minimal 'Cap' Domain	Christopher Sayer, Zalan Szabo, Michail N. Isupov, Colin Ingham and Jennifer A. Littlechild	23/11/2015	Frontiers in Microbiology	-
http://dx.doi.org/10.1101/031229	Fast and sensitive taxonomic classification	Peter Menzel, Kim Lee Ng, Anders	16/11/2015	BioRxiv. preprint.	-