



Proteome-wide systems analysis of a cellulosic biofuel-producing microbe

一种生物燃料微生物的蛋白质组分析

报告人：何长寿

组员：裴志华

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background



含量丰富

难以降解

利用率低



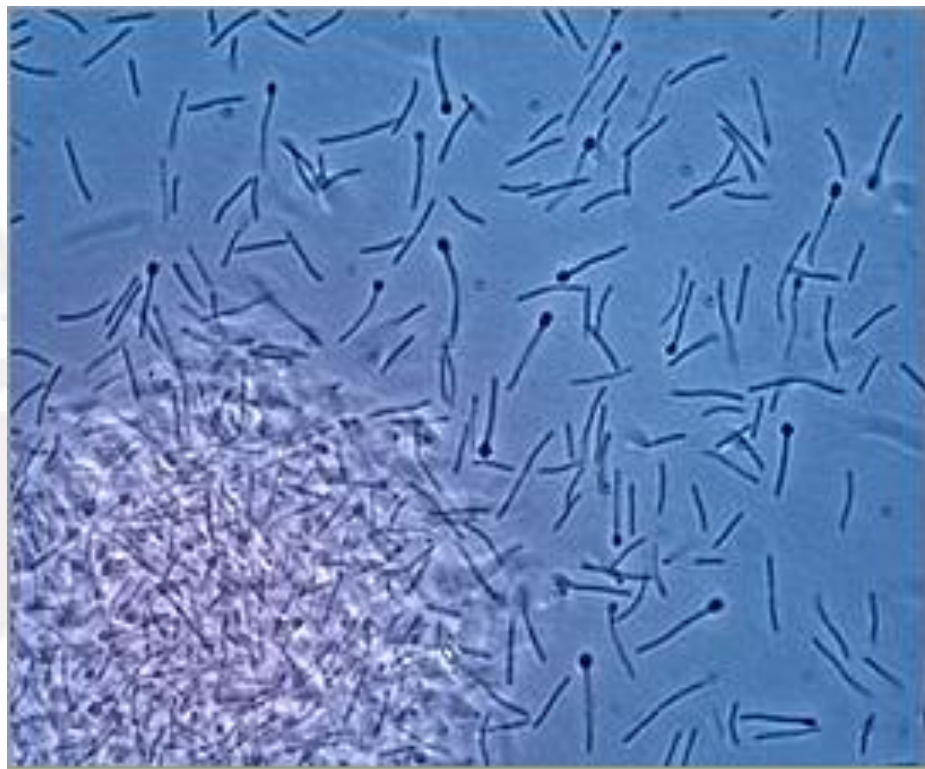
生物质资源

background



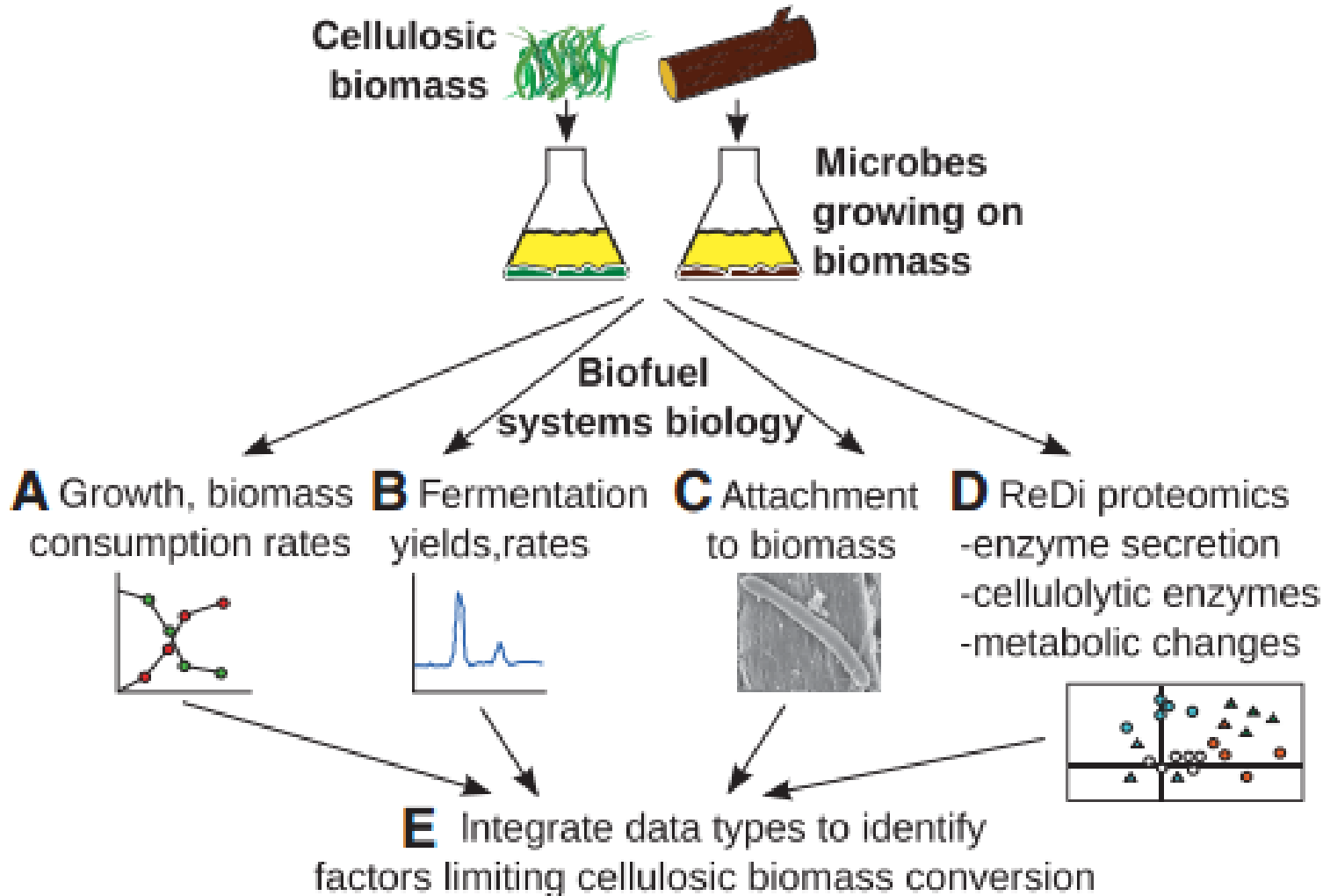
- 杆状, 专性厌氧, 革兰氏阳性菌。
- 能够利用纤维素, 多聚半乳糖醛酸, 和其它常见于植物的生物质发酵生产乙醇, 乙酸, 二氧化碳和氢气

The *C. phytofermentans* genome encodes 161 CAZy.



Clostridium phytofermentans

idea



Materials and methods



Growth and HPLC

Electron microscopy

Quantitative proteomics

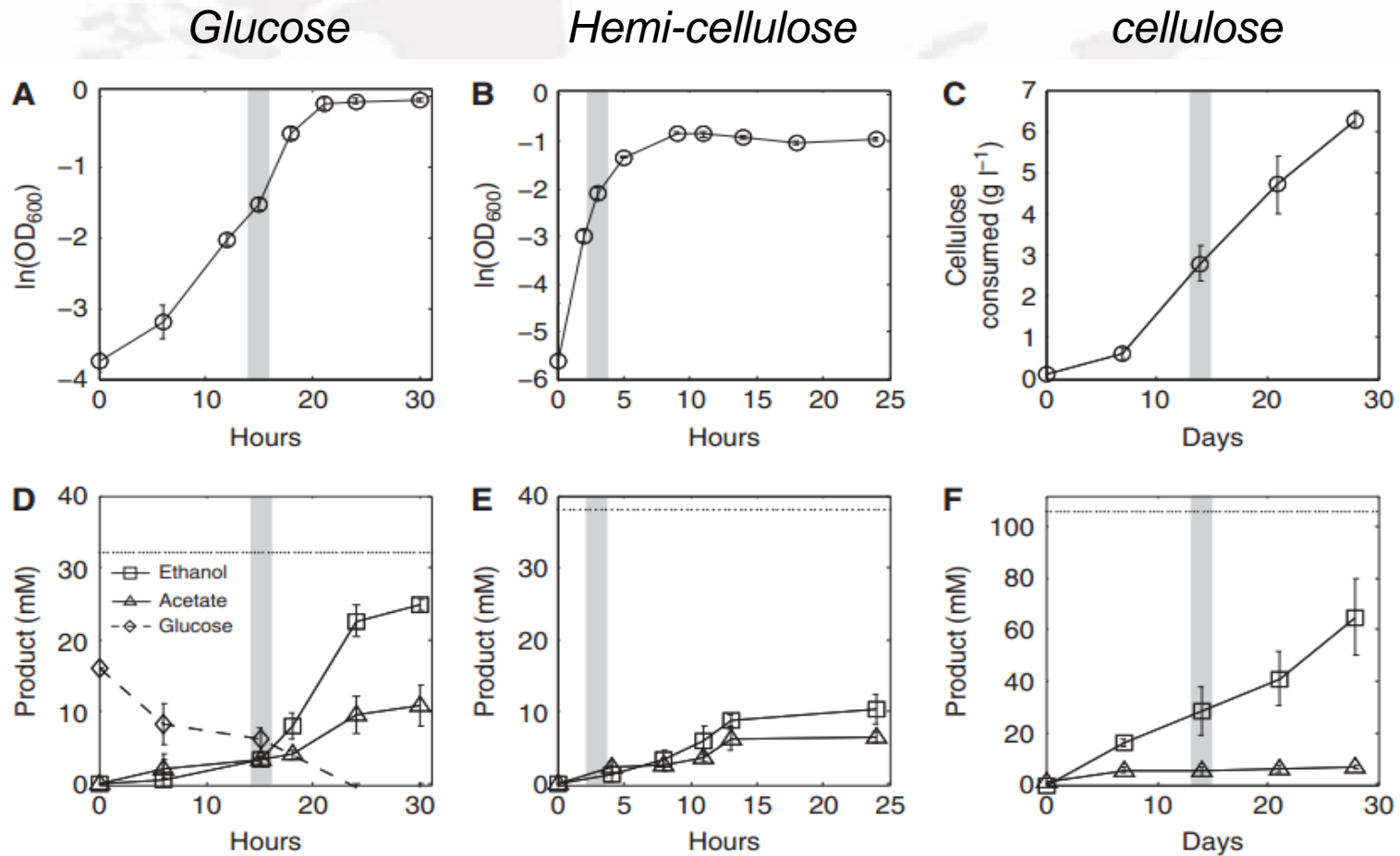
DNS reduced sugar assay

qRT-PCR

result



1、Growth and fermentation, cell adhesion



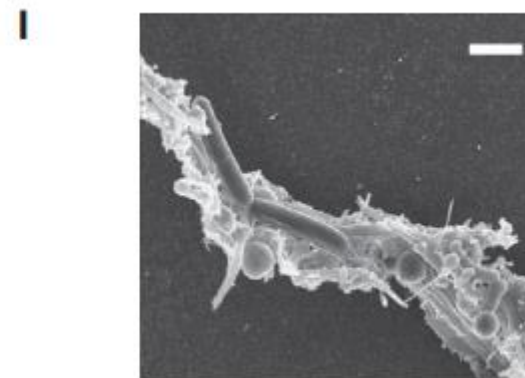
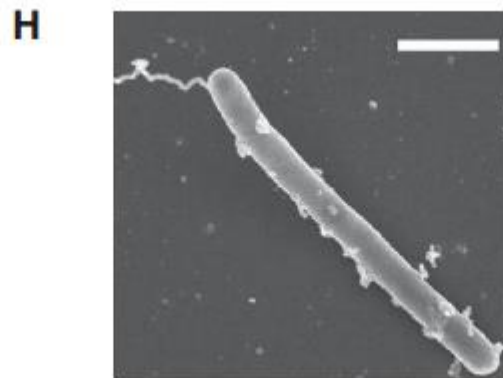
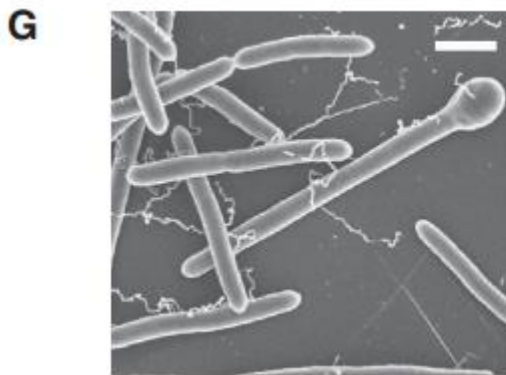


2、cell adhesion

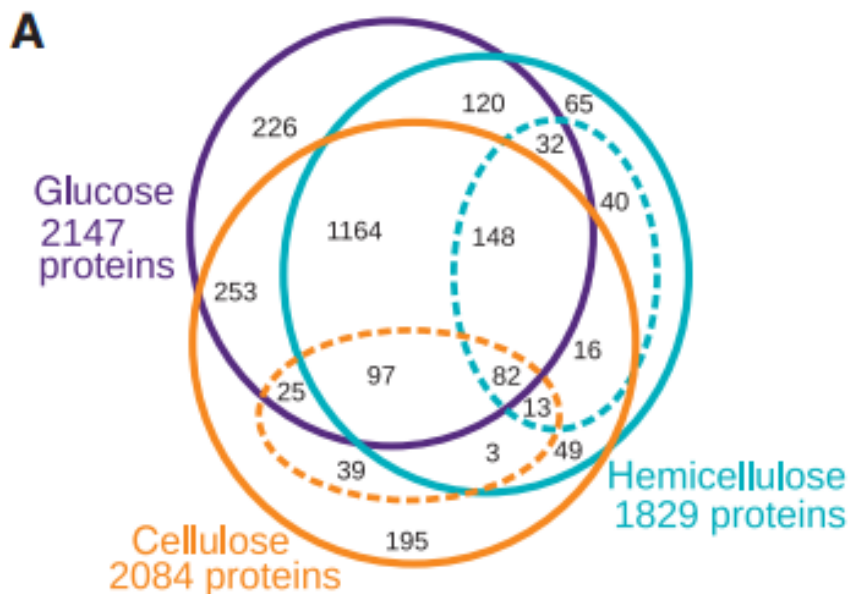
Glucose

Hemi-cellulose

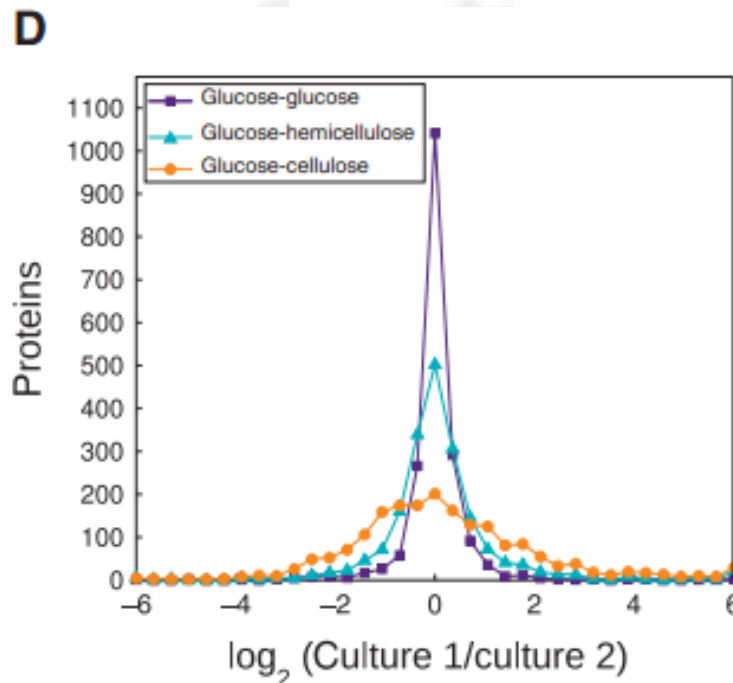
cellulose



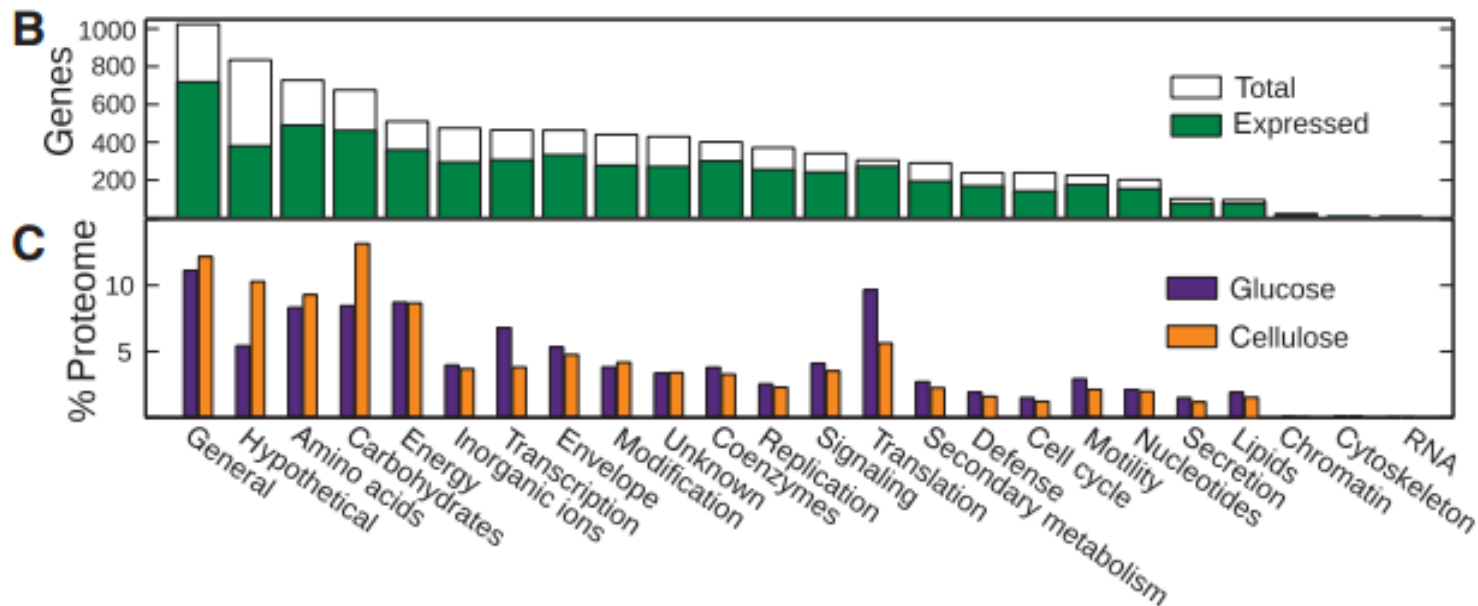
3、Protein identification and quantification by mass spectrometry



$$2567/3926=65\%$$

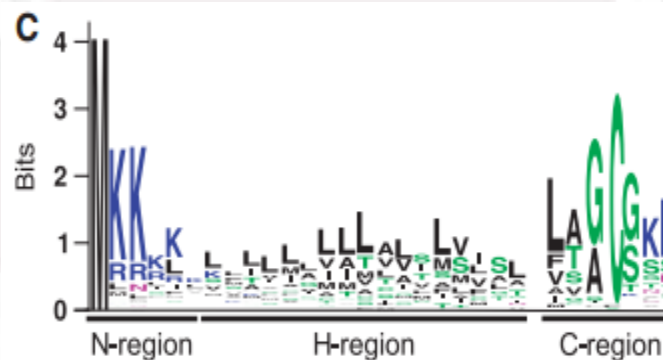
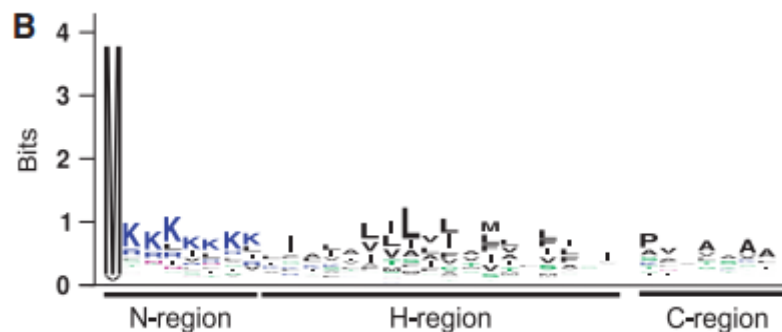
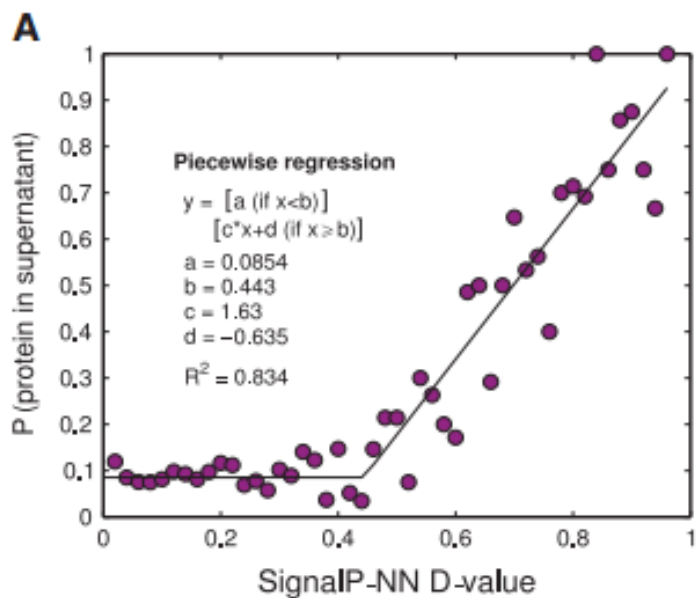


Relative protein expression in different cultures quantified by ReDi labeling



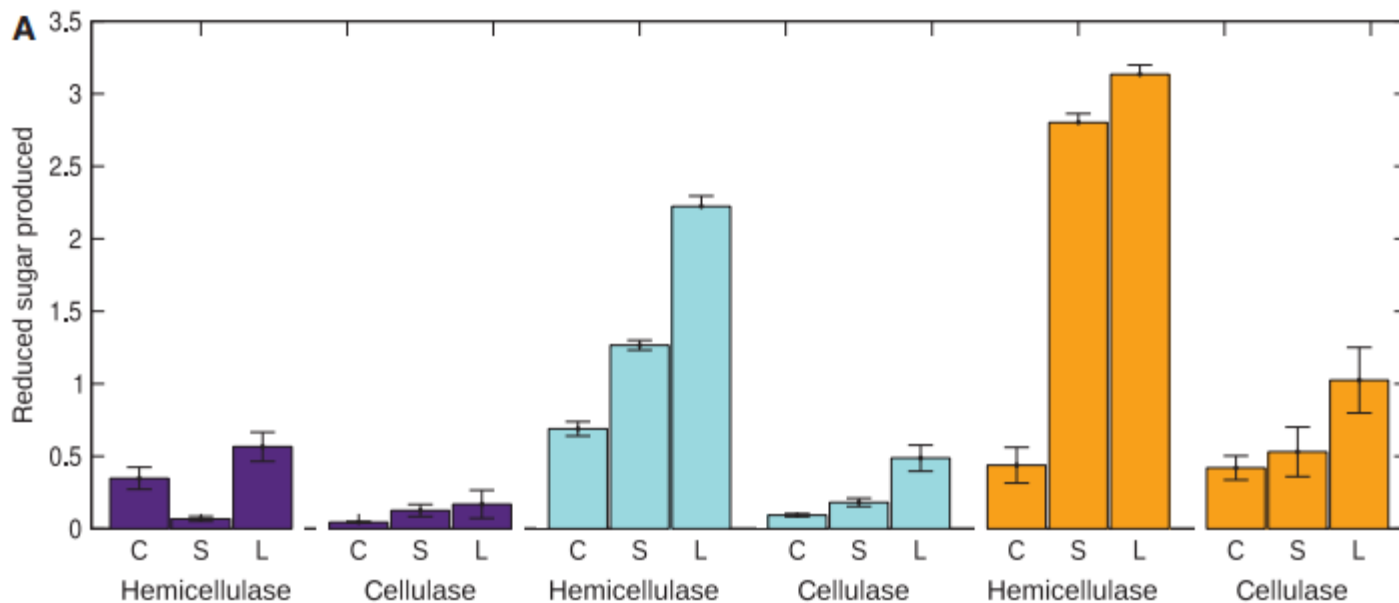
These Protein(65%) is conserved across Clusters of Orthologous Genes (COG) functional categories

4、 The C. phytofermentans secretion



Consensus sequences of (B) type I and (C) type II lipoprotein N-terminal signal peptides for proteins found in the supernatant of cellulose cultures

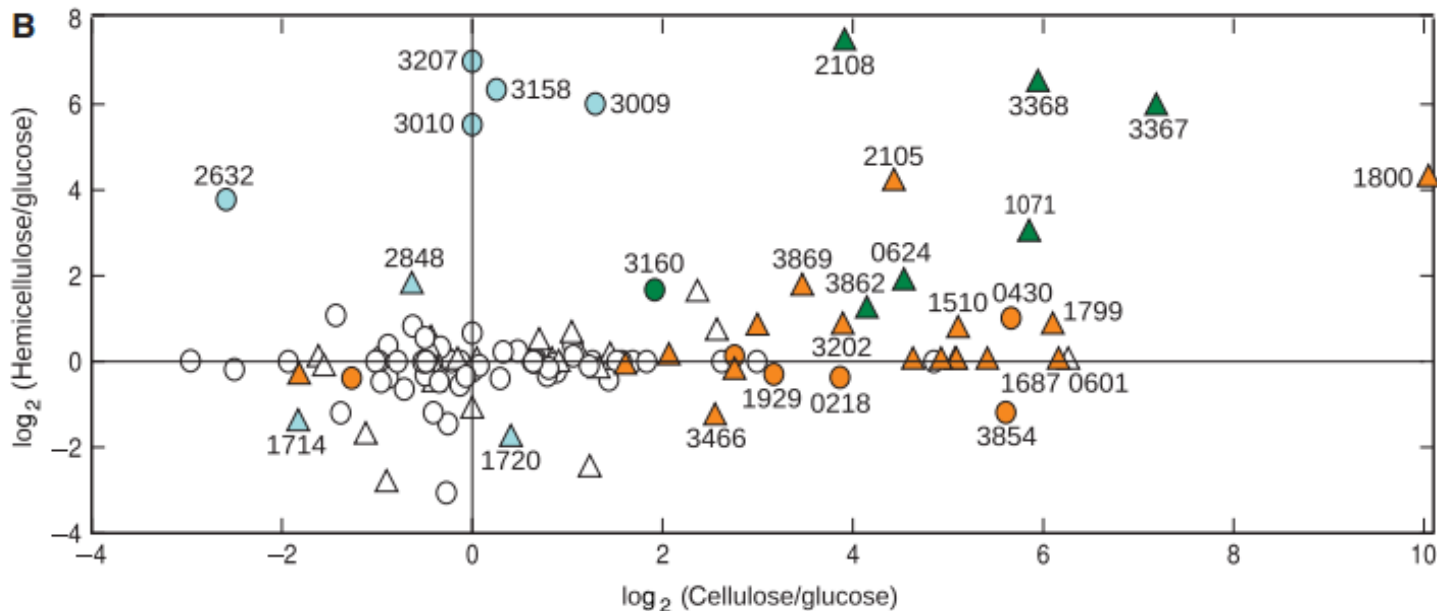
5、 Carbohydrate-active enzyme (CAZy) expression and activities in glucose, hemi-cellulose, and cellulose cultures.



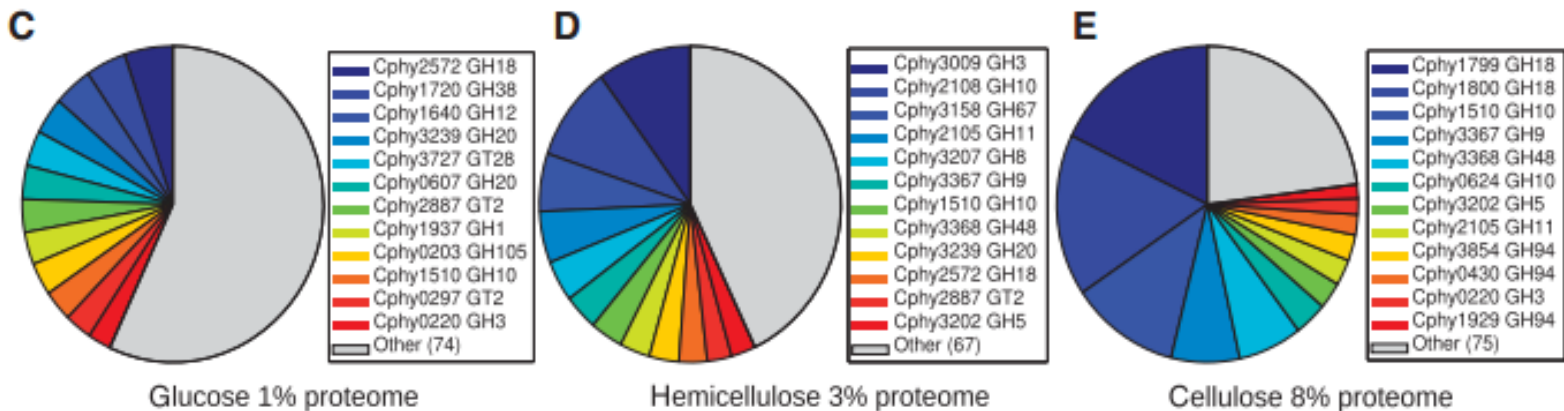
glucose

hemi-cellulose

cellulose

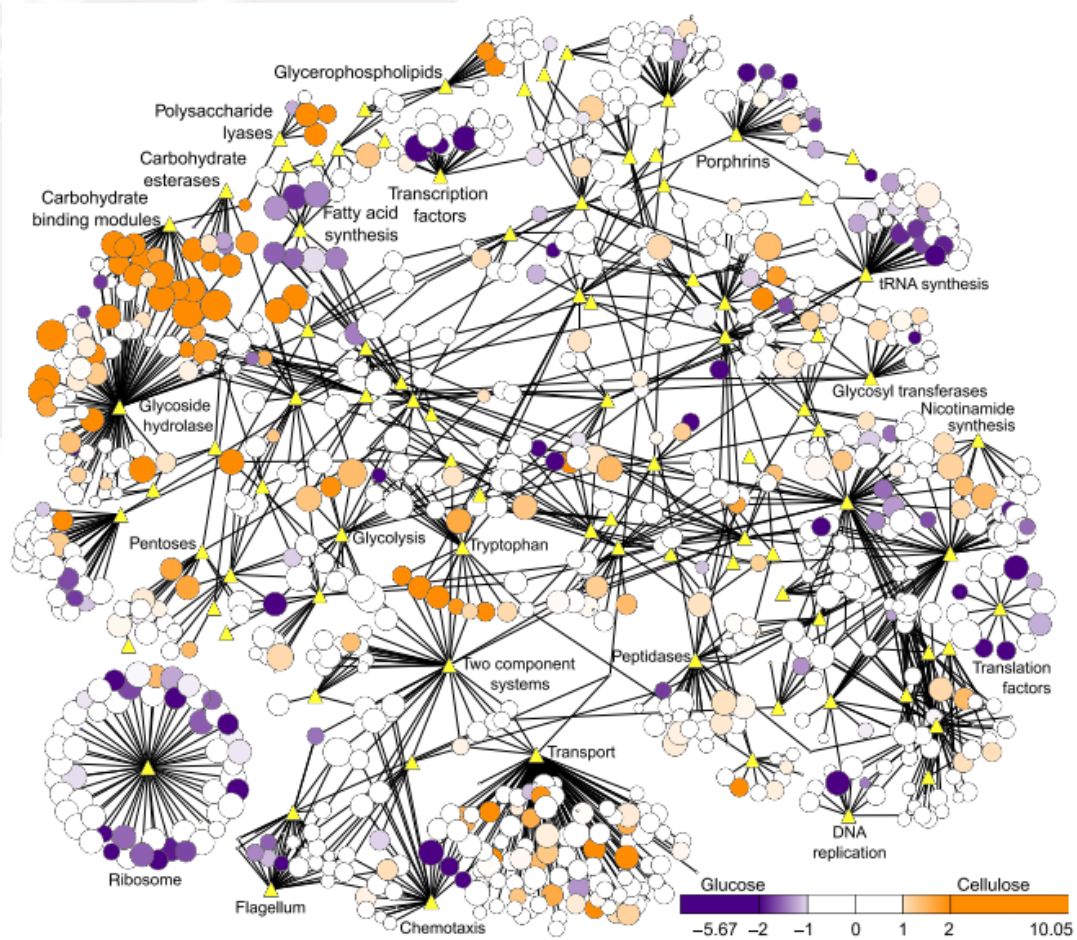


CAZy expression changes on hemi-cellulose and cellulose versus glucose showing differentially expressed proteins on hemi-cellulose (天蓝色), cellulose (橙色), or both (绿色). Symbols show cellular proteins(圆) and supernatant proteins (三角形)

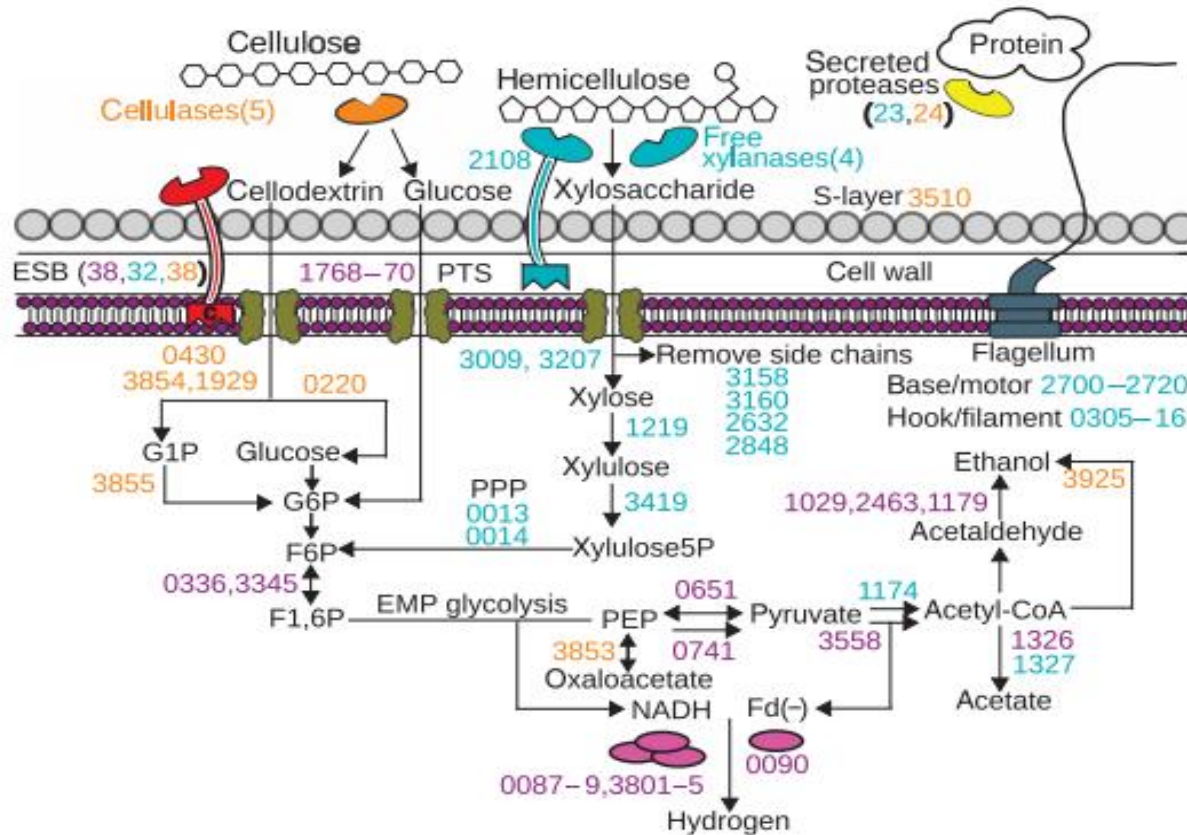


Shifts in the relative abundances of CAZy proteins in glucose (C) hemi-cellulose (D), and cellulose (E) treatments by Absolute Protein EXpression (APEX) show acclimation to different carbon sources

6、 Carbon metabolism



Discussion



Model of the key secreted and intracellular proteins for the degradation and fermentation of plant biomass.

summary



本实验分析了 *C. phytofermentans* 在不同碳源培养基中菌株生长、碳源消耗、以及蛋白质组学质谱定量和功能分析；

探索碳水化合物水解酶的胞外分泌机制和以及全体酶活；

根据不同不同碳源培养基中该菌蛋白表达量的变化构建了碳代谢的网络模型；

最后依据整体数据和前几步的结果构建出一个主要外分泌酶和胞内酶（都是 CAZy）对生物质降解过程的机制模型。



Thank You!