酶的磷酸化调节酵母的中心代谢

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Regulation of yeast central metabolism by enzyme phosphorylation

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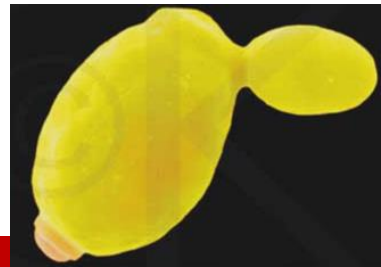
一.简介

Saccharomyces cerevisiae　酿酒酵母

蛋白磷酸化：一种常见的翻译后修饰，调控细胞代谢、渗透应力、生长、分裂等。

磷酸化的作用：激活或者抑制目标蛋白

1.改变蛋白构象
2.打开或封闭底物催化中心
3.决定物质转运
4.把蛋白和其他蛋白复合物结合在一起
5.标记要被降解的蛋白

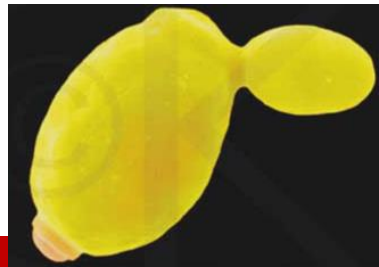


二.研究焦点

1.The majority of functional phosphoenyzmes are still unknown.

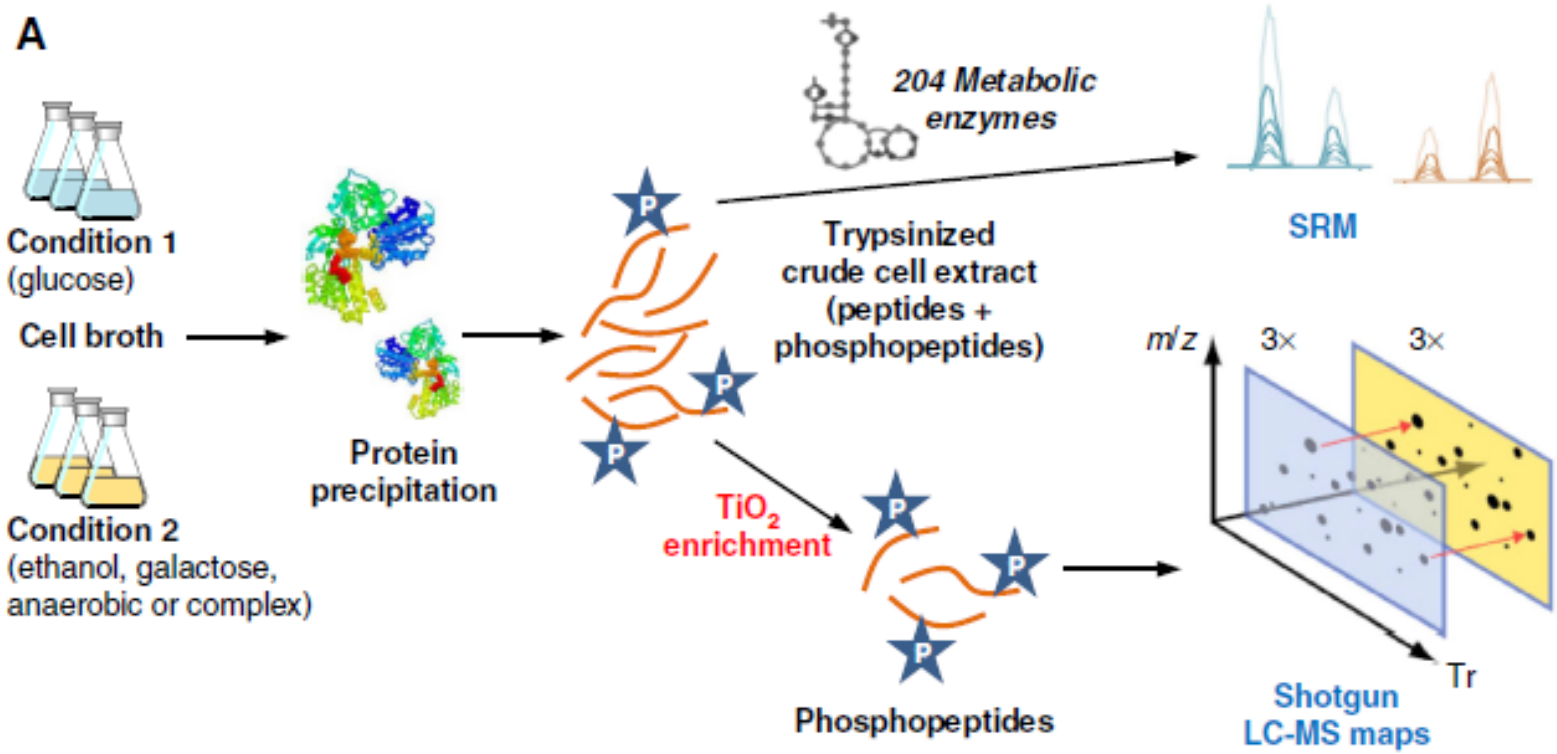
2. Previous studies focused on the qualitative effect of protein phosphorylation without considering the impact of phosphorylation on the operation of the metabolic network as whole.

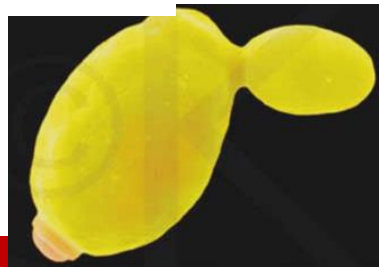
3.We quantitatively measured the phosphoproteome of S. cerevisiae during growth under five environmental conditions.



三.实验方法

We quantitatively measured the phosphoproteome of S. cerevisiae during growth under five environmental conditions.



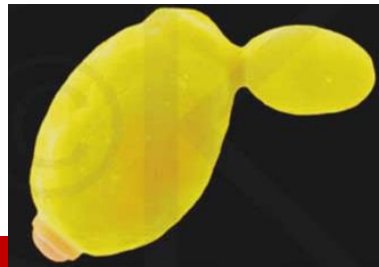


三.实验结果

1. Phosphopeptide patterns

556 proteins with at least one phosphorylation site.

Of the 681 enzymes contained in the reaction network of yeast metabolism, 70 with at least one phosphorylation site, 10 of which do not have a phosphorylation site yet listed in PhosphoPep.



2.Degree of phosphorylation

B

Proteins measured by SRM:

$$\text{Total protein fold-change} = \frac{6}{10}$$

Phosphopeptides measured by Shotgun:

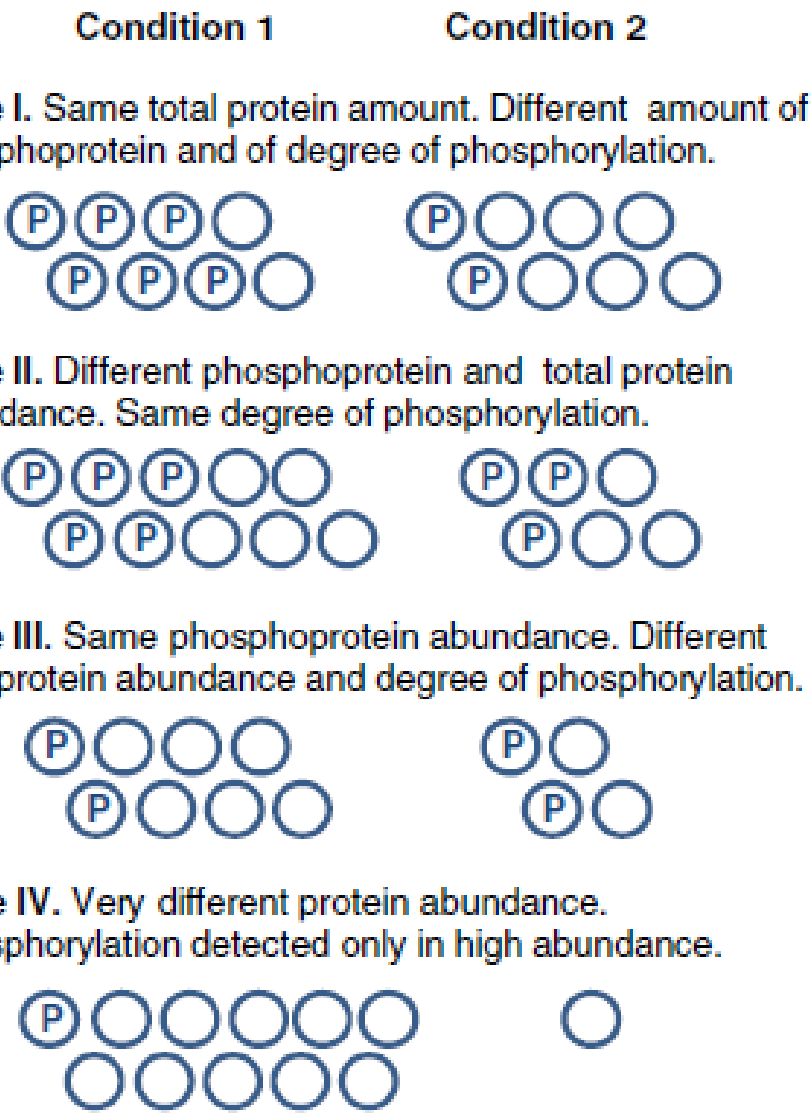
$$\text{Phosphorylated protein fold-change} = \frac{4}{5}$$

Calculated degree of phosphorylation:

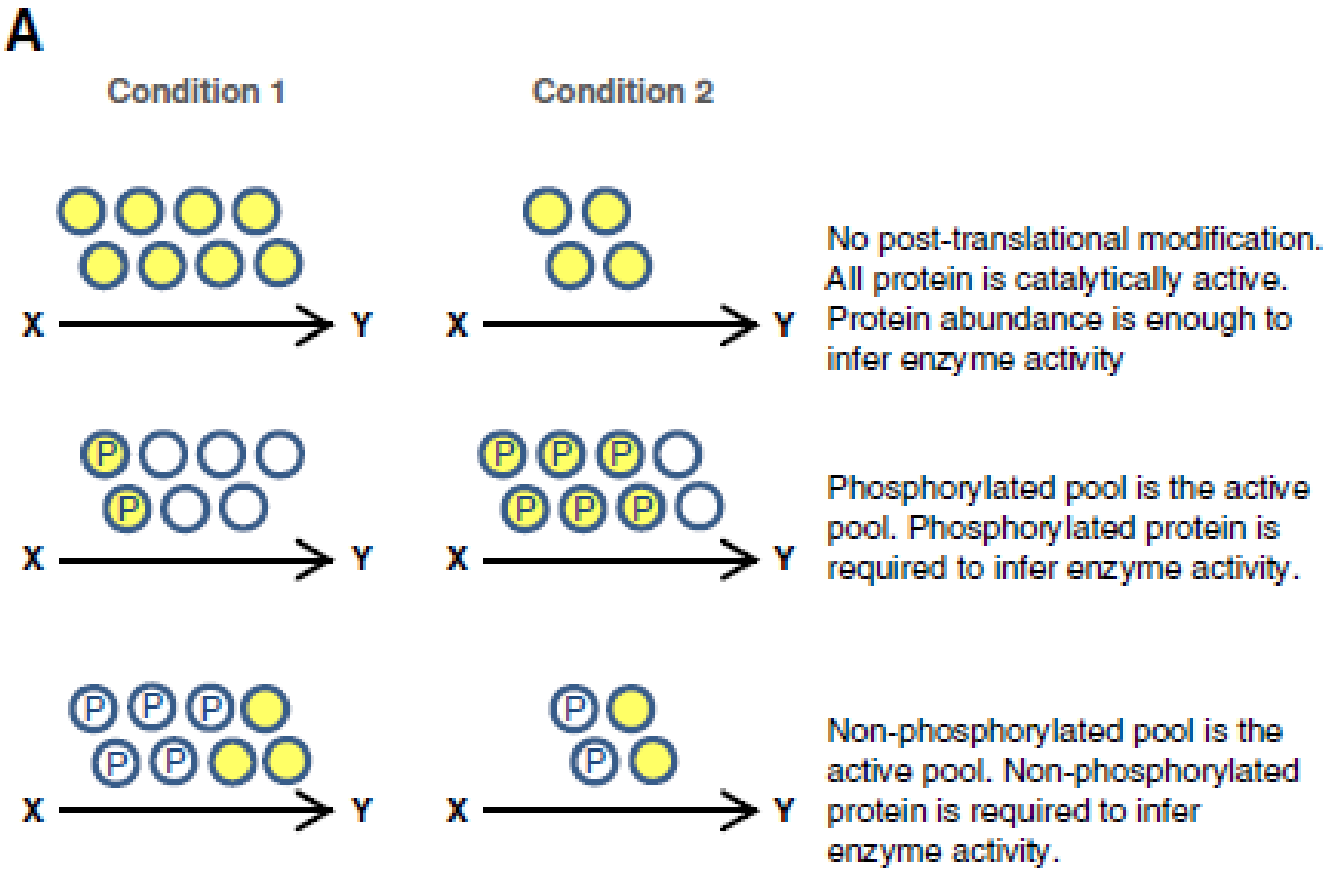
$$\text{Fold-change in degree of phosphorylation} = \frac{4/6}{5/10} =$$

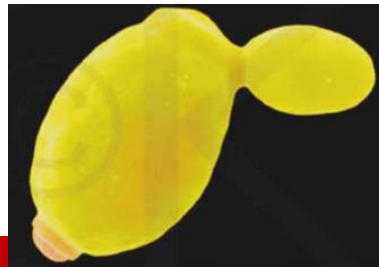
$$= \frac{\text{Phosphorylated protein fold-change}}{\text{Total protein fold-change}}$$

C

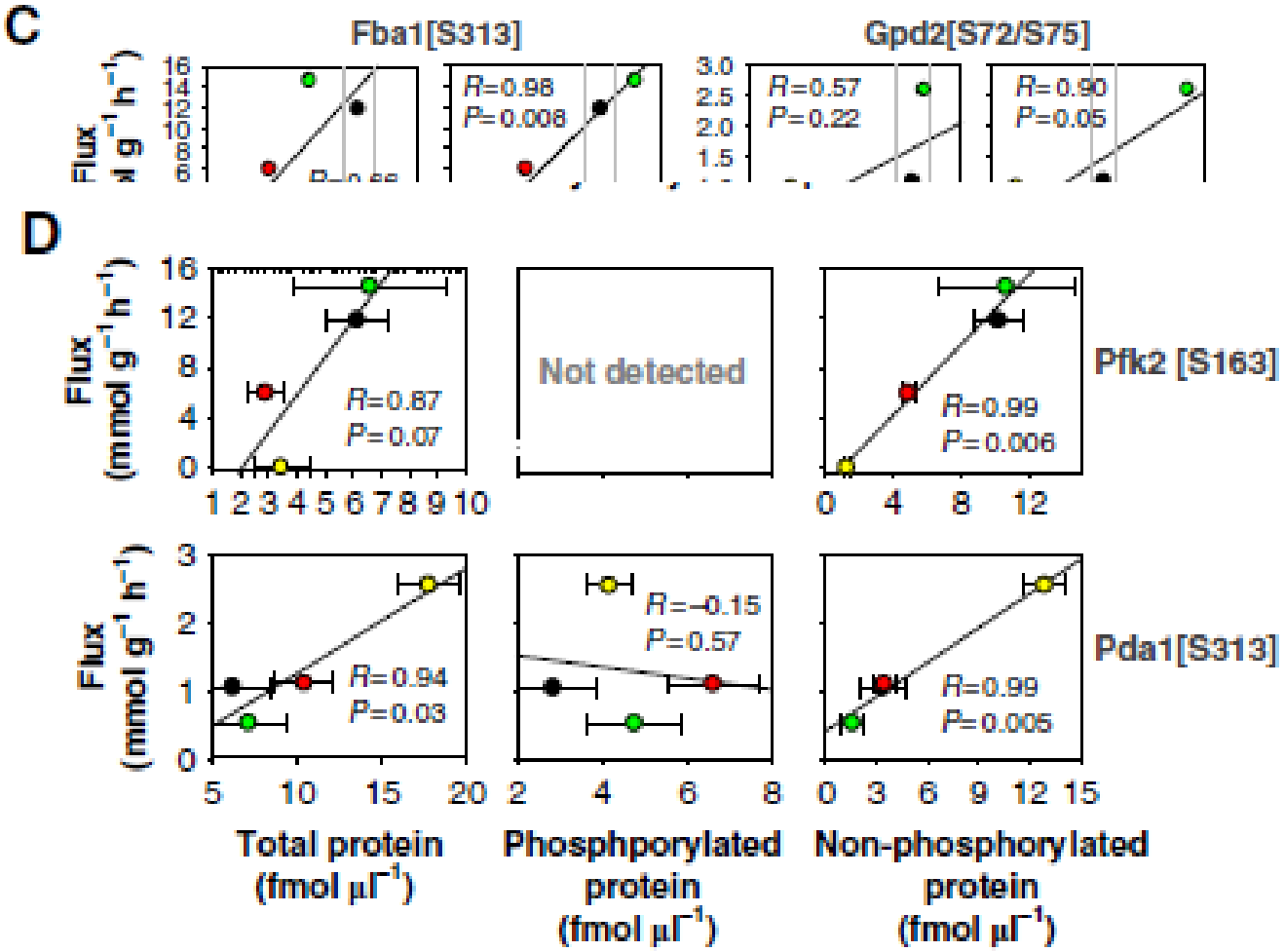


3.The impact of phosphorylation on enzyme activity

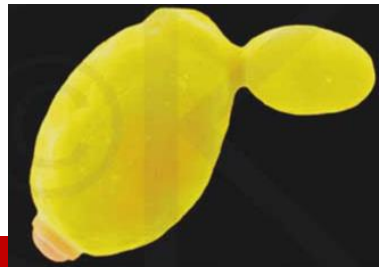




4. 在代谢网络中酶磷酸化的功能相关性



glucose (black)
galactose (red)
anaerobic (green)
ethanol (yellow)



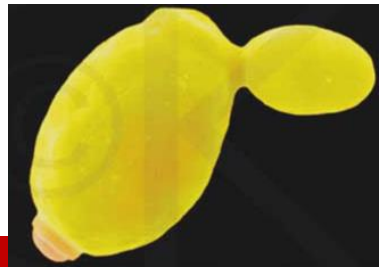
三.实验结果

5.Functional impact of phosphosite removal on metabolic activity.

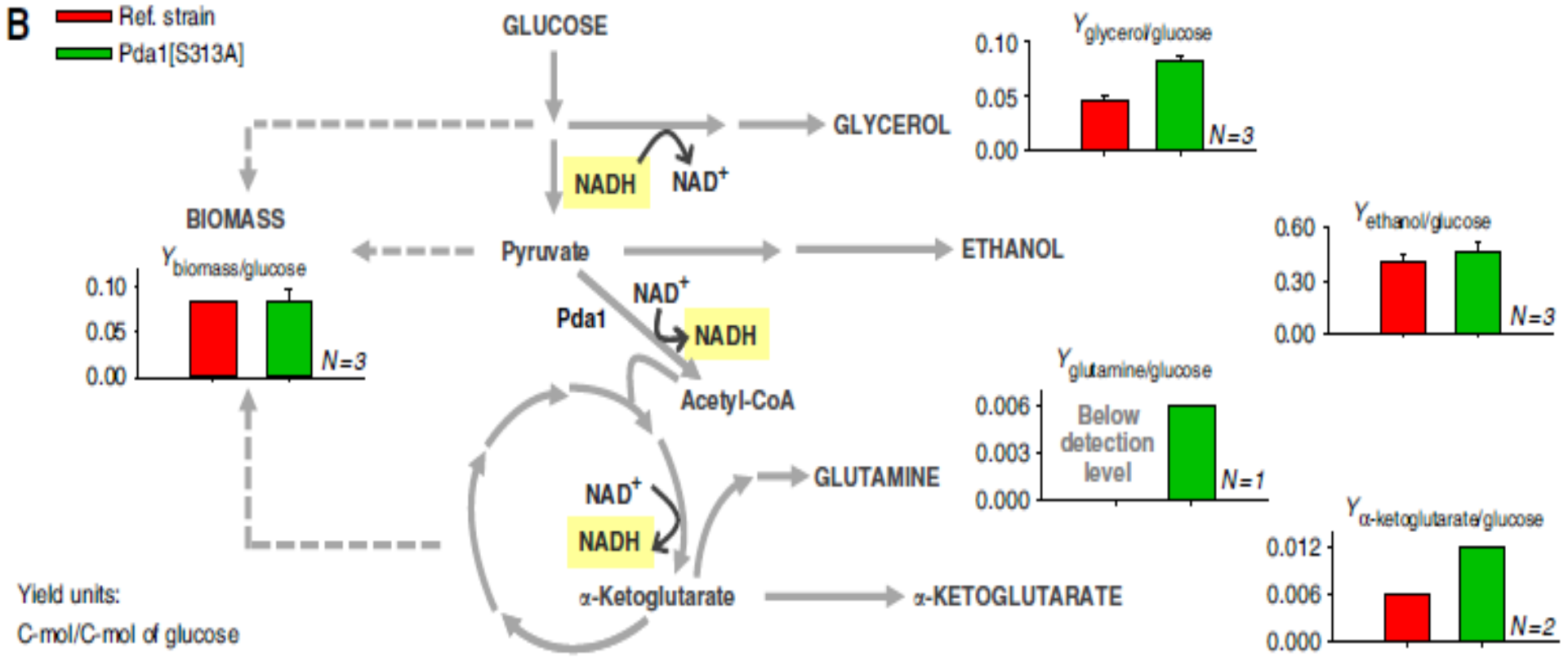
A

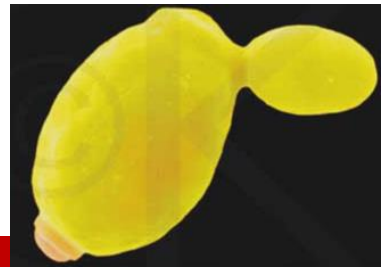
Enzyme	Phosphopeptide	Phosphosite	Fold-change (Eth/Glc)	Constructed site mutations
Gpd1 (YDL022W)	RSS[P]SSVS[P]LK	S23,S27	–6.2	Gpd1[S23A] Gpd1[S24A] Gpd1[S25A] Gpd1[S27A] Gpd1[S24/27A] Gpd1[S23/24/25A] Gpd1[S23/24/25/27A]
	SSS[P]SVS[P]LK	S24,S27	–2.0	
	SSS[P]SVS[P]LKAAEKPFK	S24,S27	–6.2	
	SSS[P]SVSLK	S24	–3.3	
	SSSS[P]VSLK	S25	–1.2	
Pda1 (YER178W)	YGGHS[P]MSDPGTTYR	S313	–3.6	Pda1[S313A]
Pfk2 (YMR205C)	NAVSTKPTPPPAPEASAES[P]GLSSK	S163	19.9	Pfk2[S163A] Pfk2[S167A] Pfk2[S171A]
	NAVSTKPTPPPAPEASAESGLSS[P]KVHS[P]YTDLAYR	S167,S171	1.8	
	NAVSTKPTPPPAPEASAES[P]GLSS[P]KVHS[P]YTDLAYR	S163,S167,S171	Only ethanol	

phosphorylated serine residues ⟶ non-phosphorylatable alanine



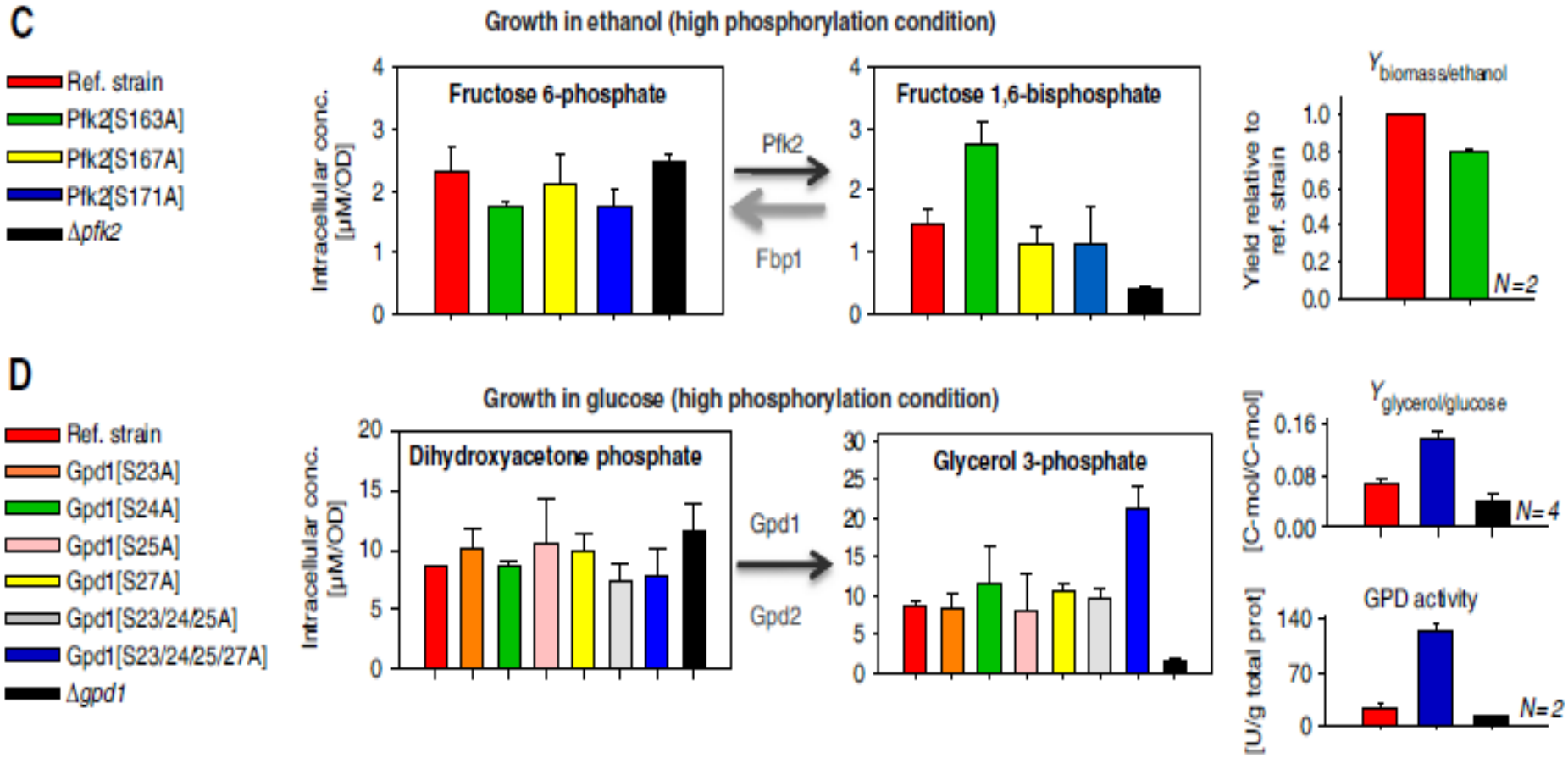
三.实验结果

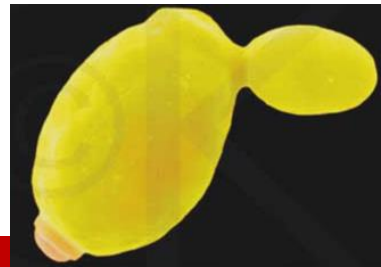

B
Ref. strain
Pda1[S313A]
GLUCOSE
GLYCEROL
NADH
NAD+
BIOMASS
Pyruvate
ETHANOL
NAD+
Pda1
NADH
Acetyl-CoA
GLUTAMINE
NAD+
NADH
α-Ketoglutarate
α-KETOGLUTARATE
Yield units:
C-mol/C-mol of glucose
Y biomass/glucose
0.10
0.05
0.00
N=3
Y glycerol/glucose
0.10
0.05
0.00
N=3
Y ethanol/glucose
0.60
0.30
0.00
N=3
Y glutamine/glucose
0.006
0.003
0.000
Below detection level
N=1
Y α-ketoglutarate/glucose
0.012
0.006
0.000
N=2



三.实验结果

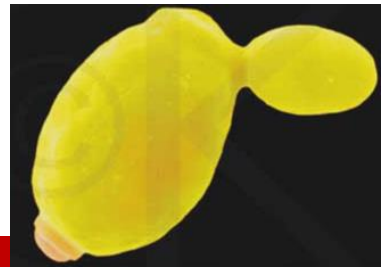
6.Identification of functional phosphorylation sites for Pfk2 and Gpd1





四.研究结论

1. 建立了一种在体内代谢网络水平上，研究酶的磷酸化的新方法。
2. 对酵母中磷酸化修饰的酶的功能进行了研究，也鉴定了一些新的与磷酸化修饰相关的酶。
3. 研究了酶的磷酸化对酶活的影响。
4. 对部分酶的磷酸化位点进行了突变，从而鉴定这些磷酸化位点的功能。



Thank You

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