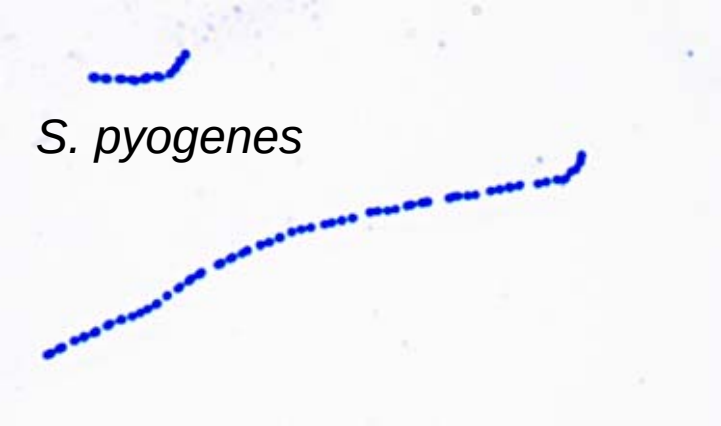
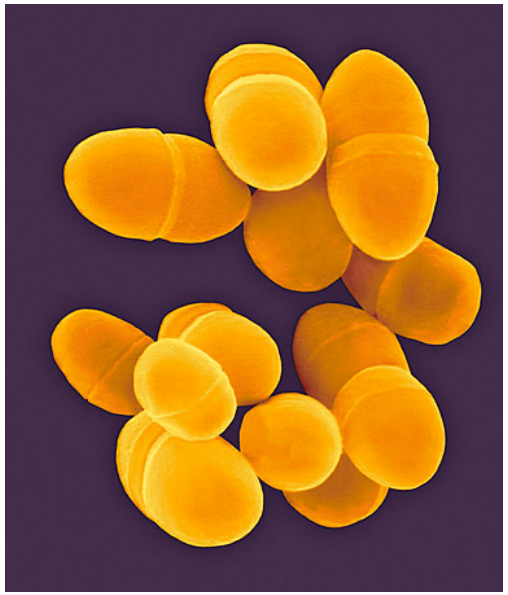
SYSMO-LAB 2

Progress-meeting Copenhagen Nov 22nd 2012

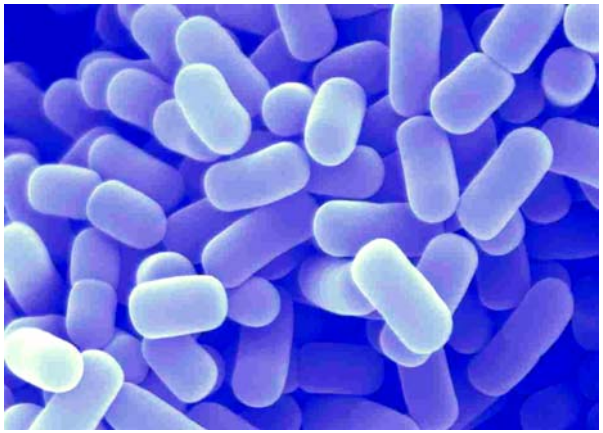
Hügenholtz-lab / University of Amsterdam
Koen van Grinsven



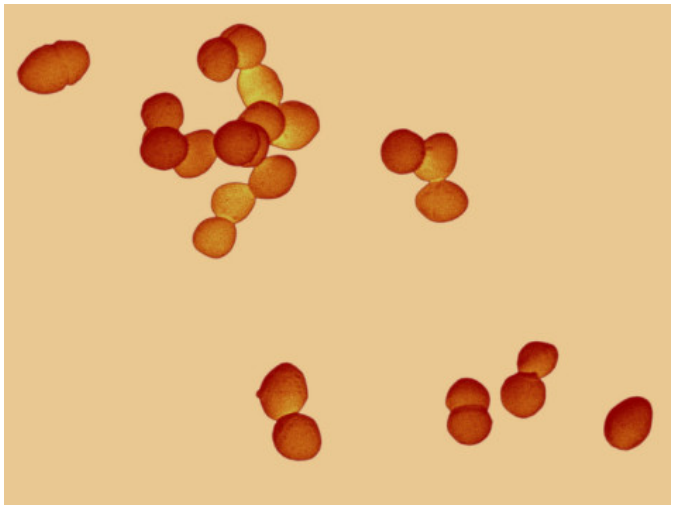
L. lactis



L. plantarum



E. faecalis



Experimental data last months

Genome scale metabolic models

Amino acid auxotrophies:

- *S. pyogenes*
- *E. faecalis*
- *L. plantarum* (4 strains)
- *L. lactis*

Kinetic model(s) for glycolysis

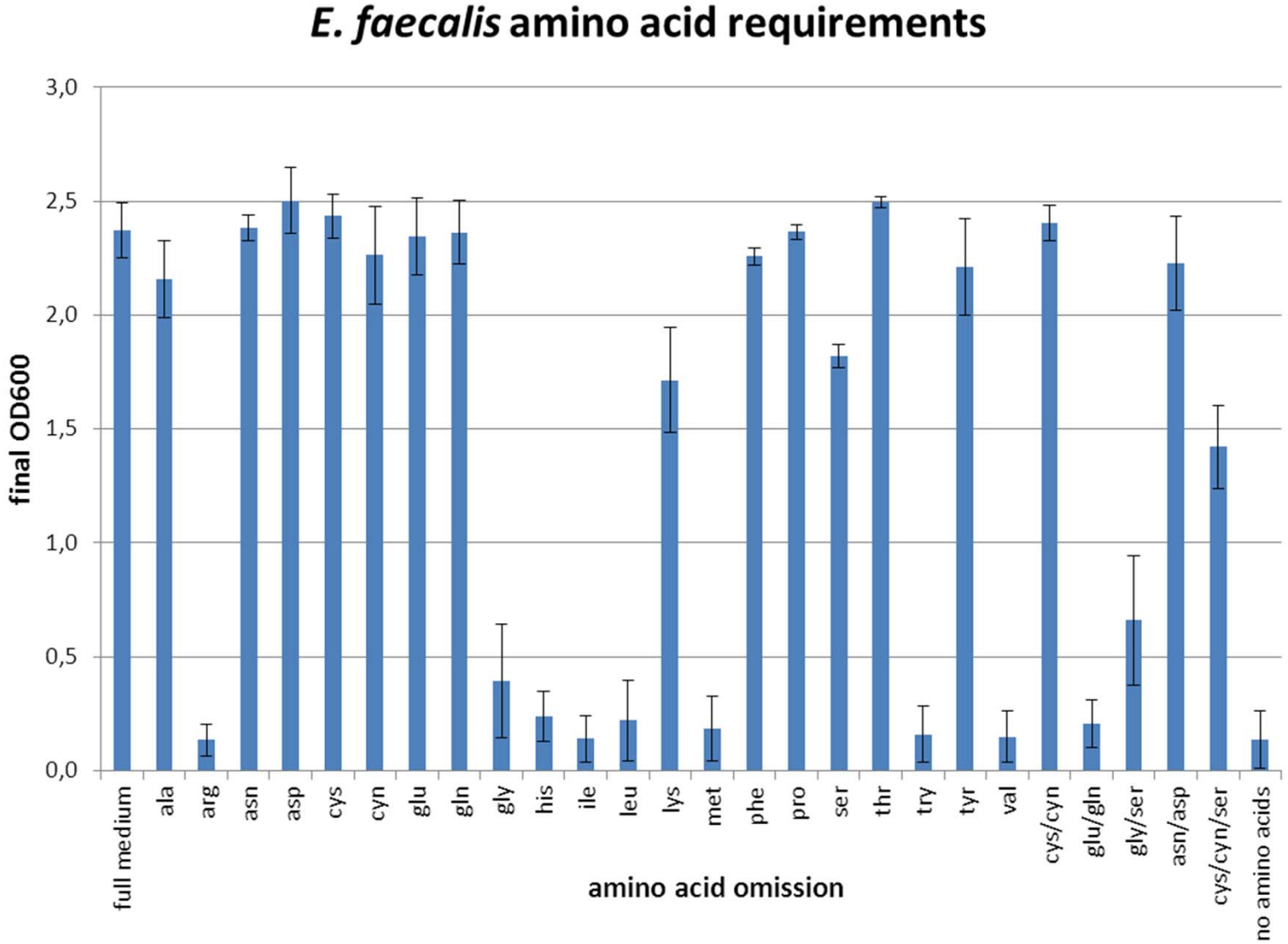
Vmaxes whole glycolysis @ different growthrates *E. faecalis*

Vmaxes glycolysis different *L. plantarum* strains

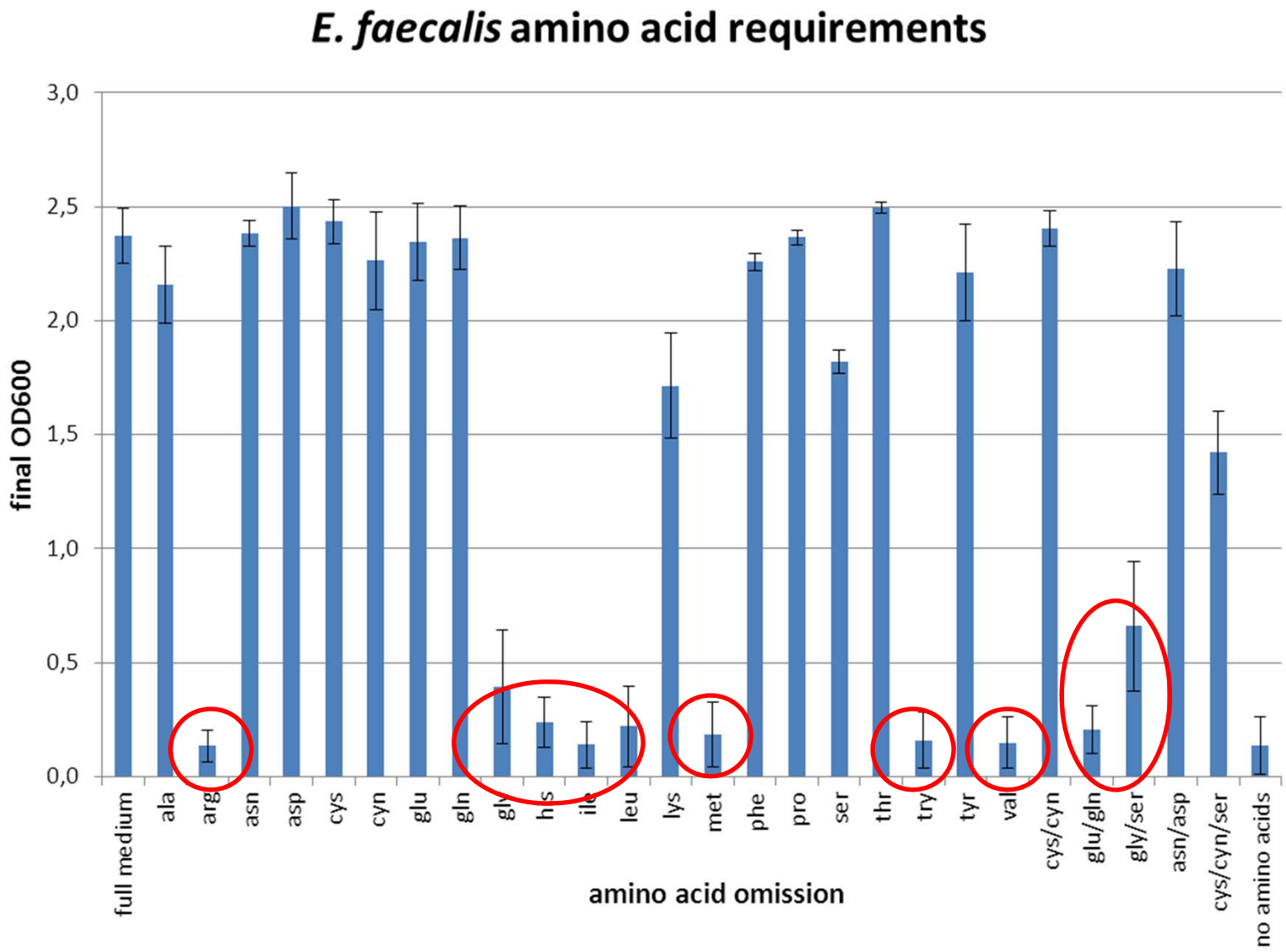
Vmaxes GAPDH/PYK/LDH *S. pyogenes* (Antje Sieg)

Comparison pH sensitivity/robustness 4 LAB-species

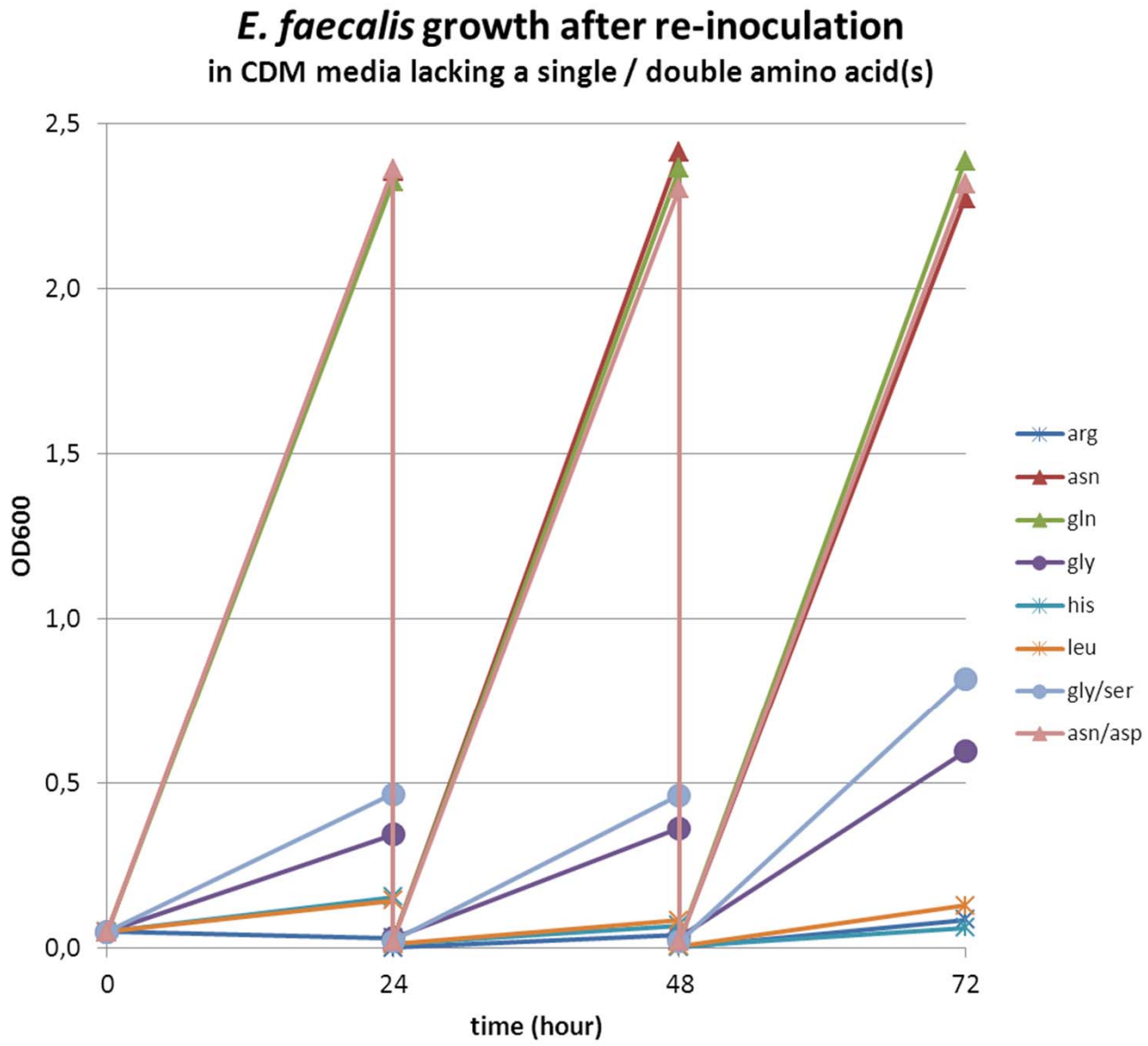
Amino acid auxotrophies; defined media lacking 1 or a combination of amino acids, monitor growth after inoculation with PBS-washed cells



Growth or no growth?



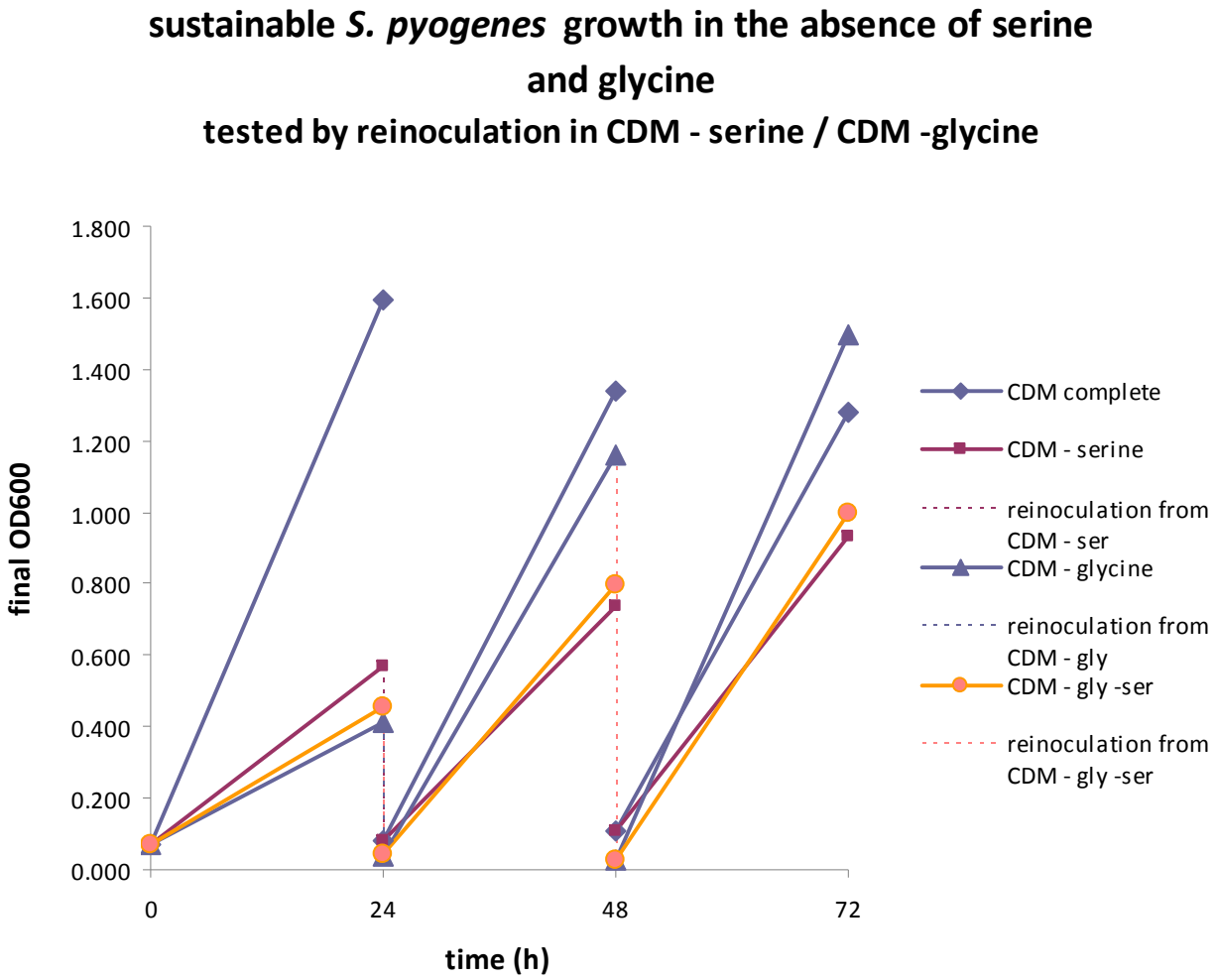
Successive inoculation in the same medium



Re-inoculation of pyogenes after full growth in CDM does not work !

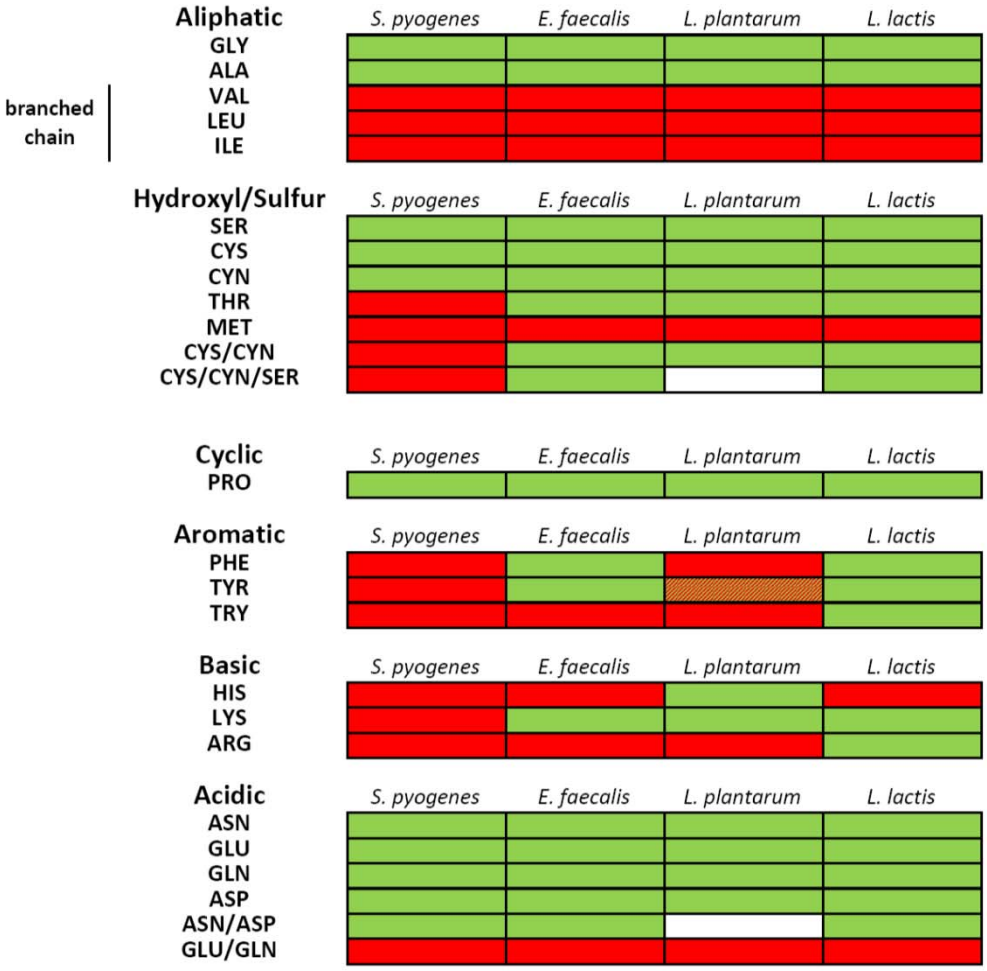
- Pyogenes acidifies CDM to +/- pH 5, but looses all viability
- However,

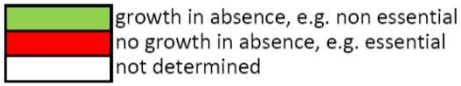
  (Non)Essentiality of several amino acids could be confirmed by reinoculation for media supporting reduced growth



Summary amino acid auxotrophies 4 LAB species

n.b. all 4 plantarum strains have the exact same auxotrophies





L. lactis agrees with literature: P. Ruhdal Jensen, K. Hammer A
L. plantarum: severely reduced, but sustainable growth in abs
L. plantarum agrees with literature: Teusink et al. AEM 2005

Vmaxes glycolytic enzymes at different growthrates (kinetic models)

AEM
Journals.ASM.org

Standardized Assay Medium To Measure *Lactococcus lactis* Enzyme Activities while Mimicking Intracellular Conditions

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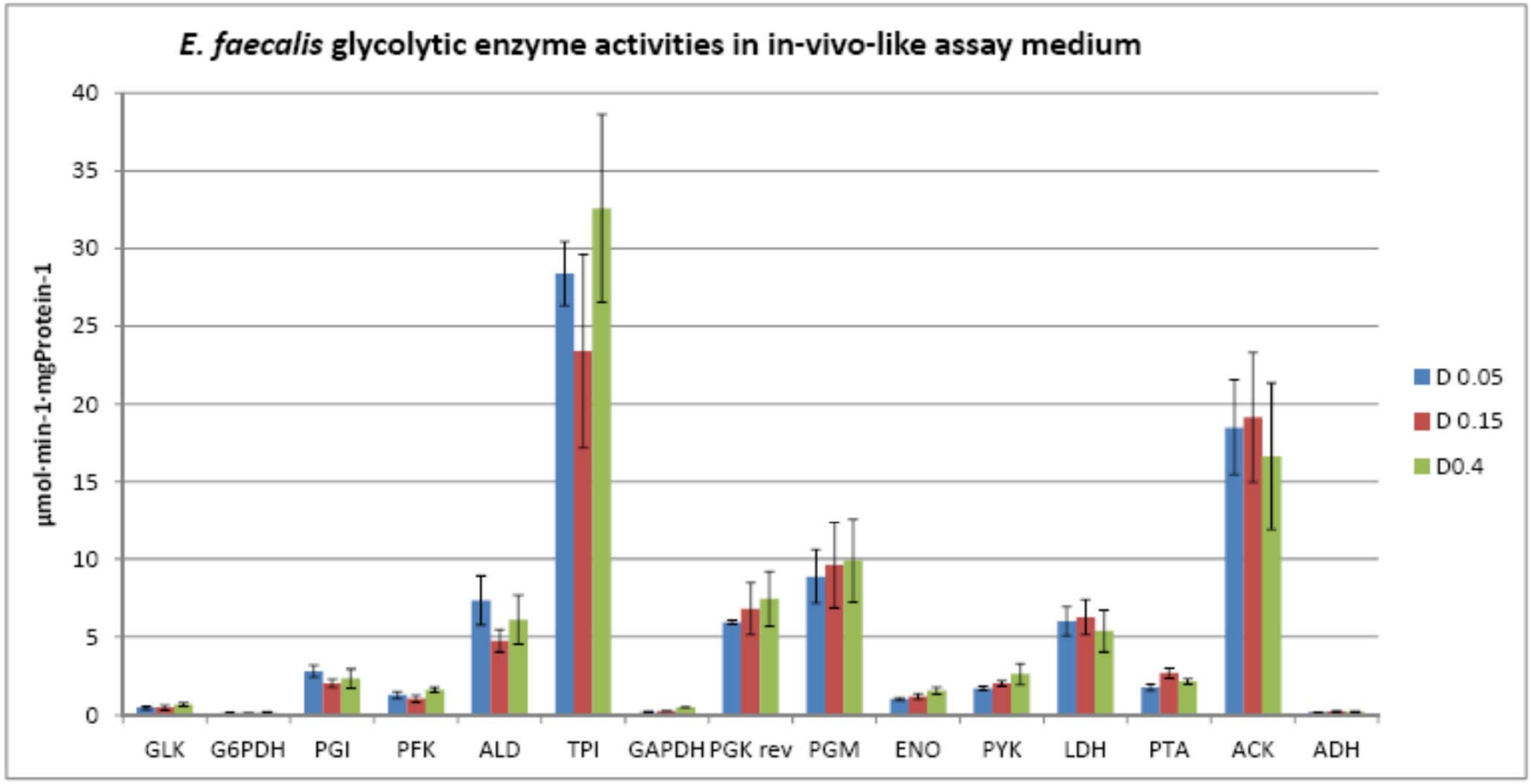
Centre for Integrative Bioinformatics, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands[a]; Laboratory of Microbiology, Wageningen University and Research Centre, Wageningen, The Netherlands[b]; and Kluyver Centre for Genomics of Industrial Fermentation/NCSB, Delft, The Netherlands[c]

TABLE 2 Composition of reagent mixtures for all enzyme assays[a]

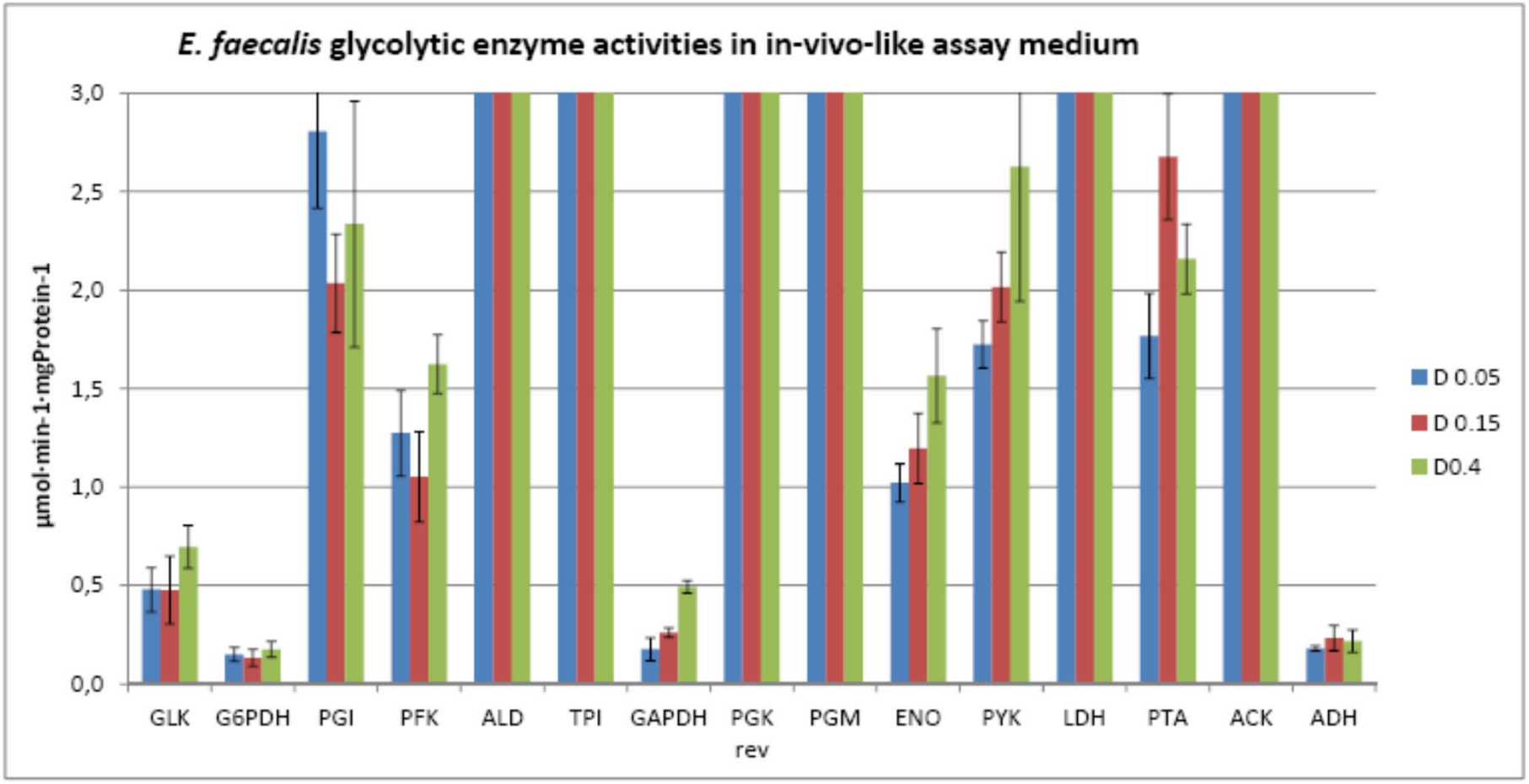
Enzyme	Reaction mixture components
GLK[b] (EC 2.7.1.2)	4 mM $MgSO_4$, 2 mM ATP, 0.4 mM $NADP^+$, 5 U · ml^{-1} G6PDH and initiator: 10 mM glucose
G6PDH[b] (EC 1.1.1.49)	2 mM $MgSO_4$, 0.4 mM $NADP^+$ and initiator: 10 mM glucose-6P
PGI (EC 5.3.1.9; physiological direction)	7 mM $MgSO_4$, 5 mM ATP, 0.3 mM NADH, 1 U · ml^{-1} PFK, 1 U · ml^{-1} ALD, 2 U · ml^{-1} G3PD, 5 U · ml^{-1} TPI, and initiator: 10 mM glucose-6P
PGI (nonphysiological direction)	2 mM $MgSO_4$, 0.4 mM $NADP^+$, 1.75 U · ml^{-1} G6PDH and initiator: 20 mM fructose-6P
PFK (EC 2.7.1.11)	7 mM $MgSO_4$, 5 mM ATP, 0.3 mM NADH, 1 U · ml^{-1} ALD, 2 U · ml^{-1} G3PD, 5 U · ml^{-1} TPI, and initiator: 20 mM fructose-6P
ALD (EC 4.1.2.13)	2 mM $MgSO_4$, 0.3 mM NADH, 2 U · ml^{-1} G3PD, 5 U · ml^{-1} TPI, and initiator: 30 mM fructose-1,6BP
TPI (EC 5.1.3.1)	2 mM $MgSO_4$, 0.3 mM NADH, 2 U · ml^{-1} G3PD, and initiator: 6 mM glyceraldehyde-3P
GAPDH (EC 1.2.1.12)	5 mM $MgSO_4$, 5 mM cysteine-HCl, 50 mM potassium phosphate, 3 mM ADP, 14.5 U · ml^{-1} PGK, 5 mM NAD+ and initiator: 10 mM glyceraldehyde-3P
PGK (EC 2.7.2.3; nonphysiological direction)	7 mM $MgSO_4$, 5 mM ATP, 0.3 mM NADH, 8 U · ml^{-1} GAPDH, and initiator: 10 mM 3-PGA
PGM (EC 5.4.2.1)	5 mM $MgSO_4$, 3 mM ADP, 0.1 mM 2,3BPG, 0.3 mM NADH, 2 U · ml^{-1} ENO, 5 U · ml^{-1} PYK, 10 U · ml^{-1} LDH, and initiator: 5 mM 3P-glycerate
ENO (EC 4.2.1.11; PEP absorbance[c])	5 mM $MgSO_4$, 3 mM ADP, 0.3 mM NADH, 5 U · ml^{-1} PYK, 10 U · ml^{-1} LDH, and initiator: 5 mM 2P-glycerate; 2 mM $MgSO_4$, and initiator: 5 mM 2P-glycerate
PYK (EC 2.7.1.40)	5 mM $MgSO_4$, 3 mM ADP, 5 mM fructose-1,6BP, 0.3 mM NADH, 10 U · ml^{-1} LDH, and initiator: 6 mM PEP
LDH (EC 1.1.1.27)	2 mM $MgSO_4$, 3 mM fructose-1,6BP, 0.3 mM NADH, and initiator: 6 mM PYR
ACK (EC 2.7.2.1)	5 mM $MgSO_4$, 3 mM ADP, 2 mM glucose, 0.4 mM $NADP^+$, 8.5 U · ml^{-1} hexokinase, 12.7 U · ml^{-1} G6PDH, and initiator: 5 mM acetyl-P
PTA (EC 2.3.1.8; PTA control)	2 mM $MgSO_4$, 0.08 mM DTNB, and initiator: 0.4 mM acetyl-coenzyme A. 2 mM $MgSO_4$, 0.008 mM DTNB, 2 mM acetyl-P, and initiator: 0.4 mM acetyl-CoA
ADH (EC 1.1.1.1)	2 mM $MgSO_4$, 0.3 NADH, and initiator: 20 mM acetaldehyde
ALDH[d] (EC 1.2.1.10; nonphysiological direction)	2 mM $MgSO_4$, 0.1 mM CoA, 1 mM DTT, 0.5 mM NAD^+, and initiator: 40 mM acetaldehyde

[a] Detailed methodology can be accessed in Document S3 in the supplemental material. Except where indicated, all enzyme reagent mixtures were adapted from reference 9. G3PD, glycerol-3P dehydrogenase.

Vmaxes *E. faecalis* (ferm. Margrete Solheim) (kinetic model Nadine Veith)

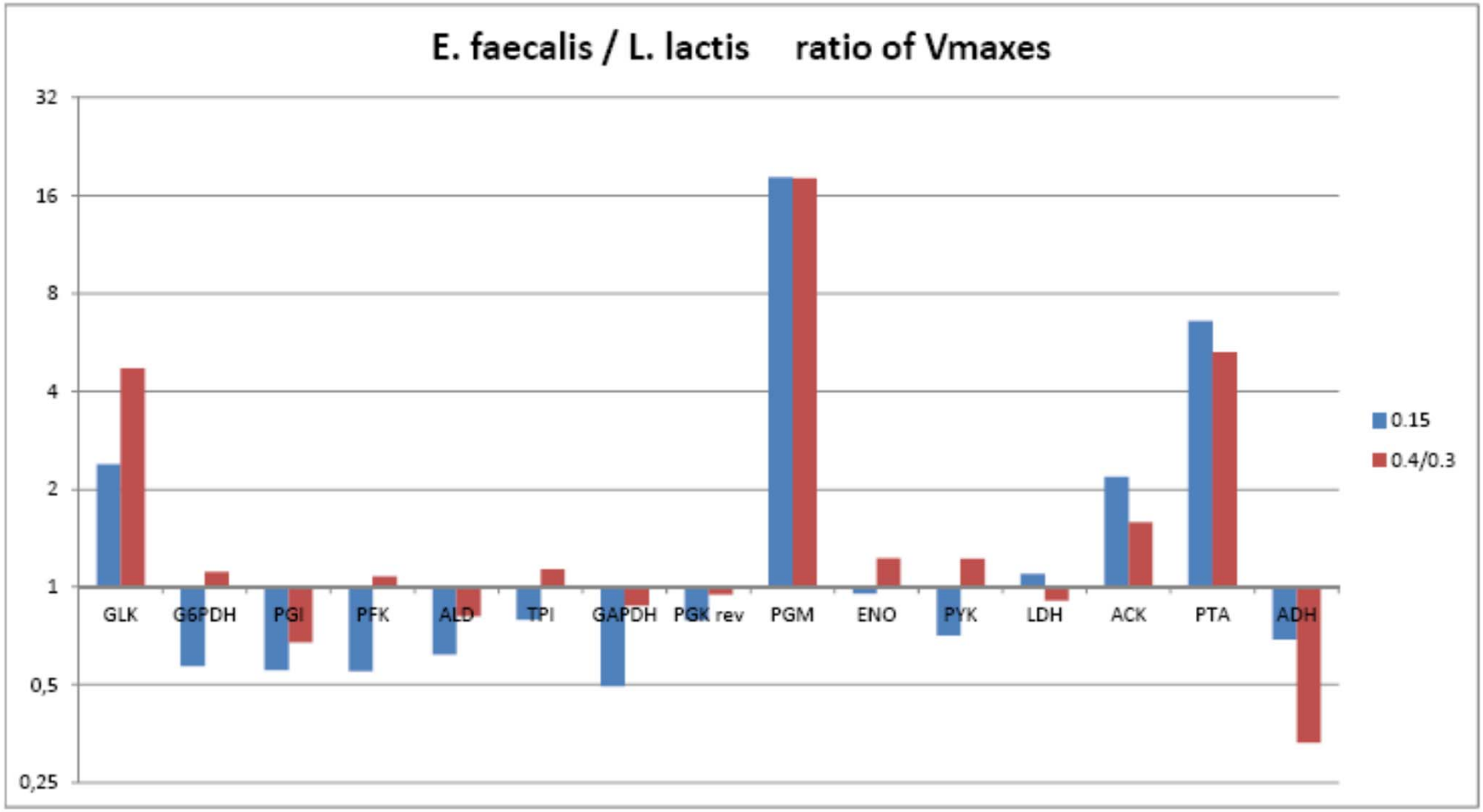


Vmaxes E. faecalis (ferm. Margrete Solheim)
Kinetic model (Nadine Veith)



Appears to be growthrate-dependent expression of GAPDH / ENO / PYK
(different from L. lactis)

Interspecies comparisons Vmaxes (data L. lactis Anisha Goel)

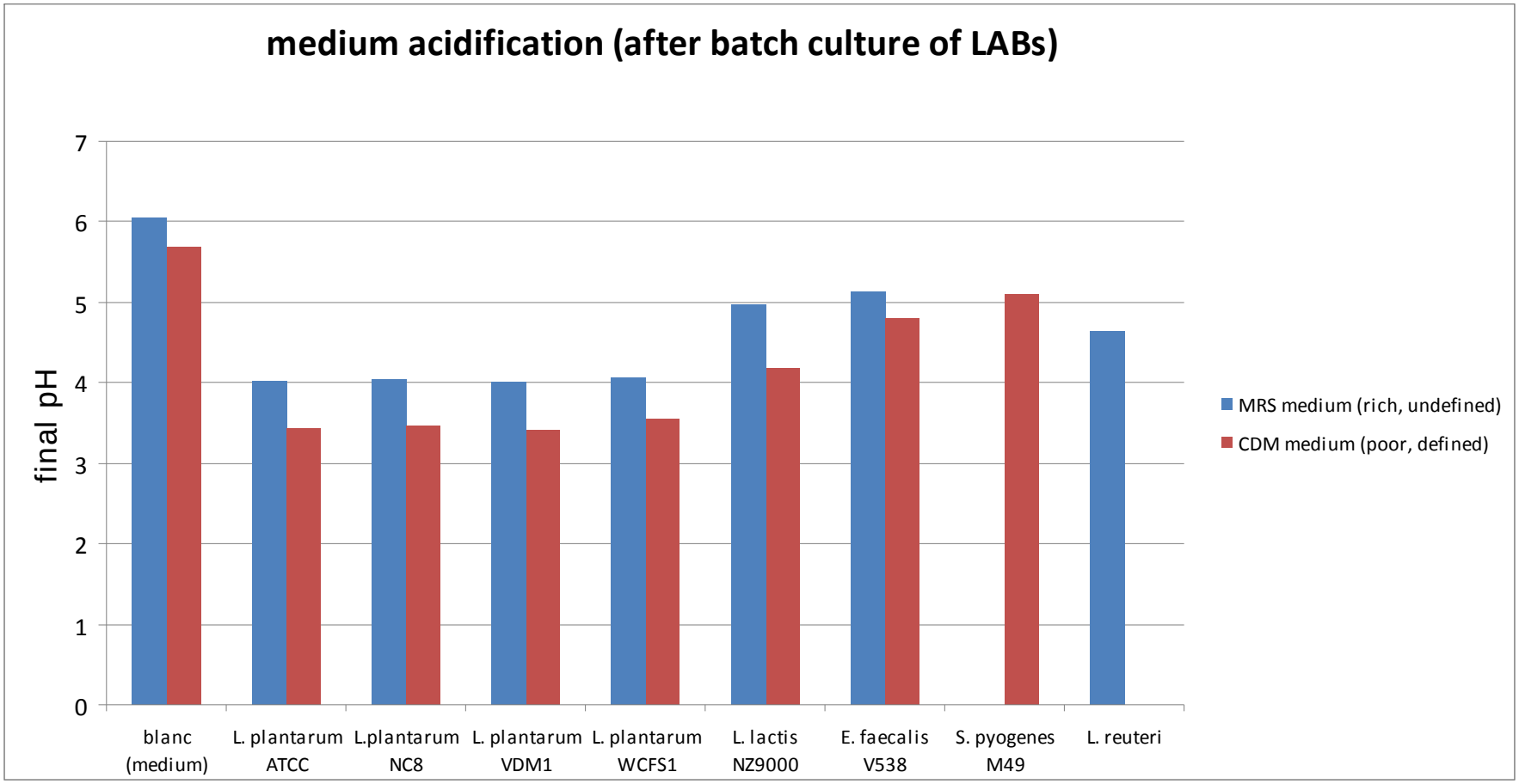


Extend this data to all 4 LAB species

Vmaxes L. plantarum (ferm. Anette McLeod) Kinetic model (Domenico Bellomo)

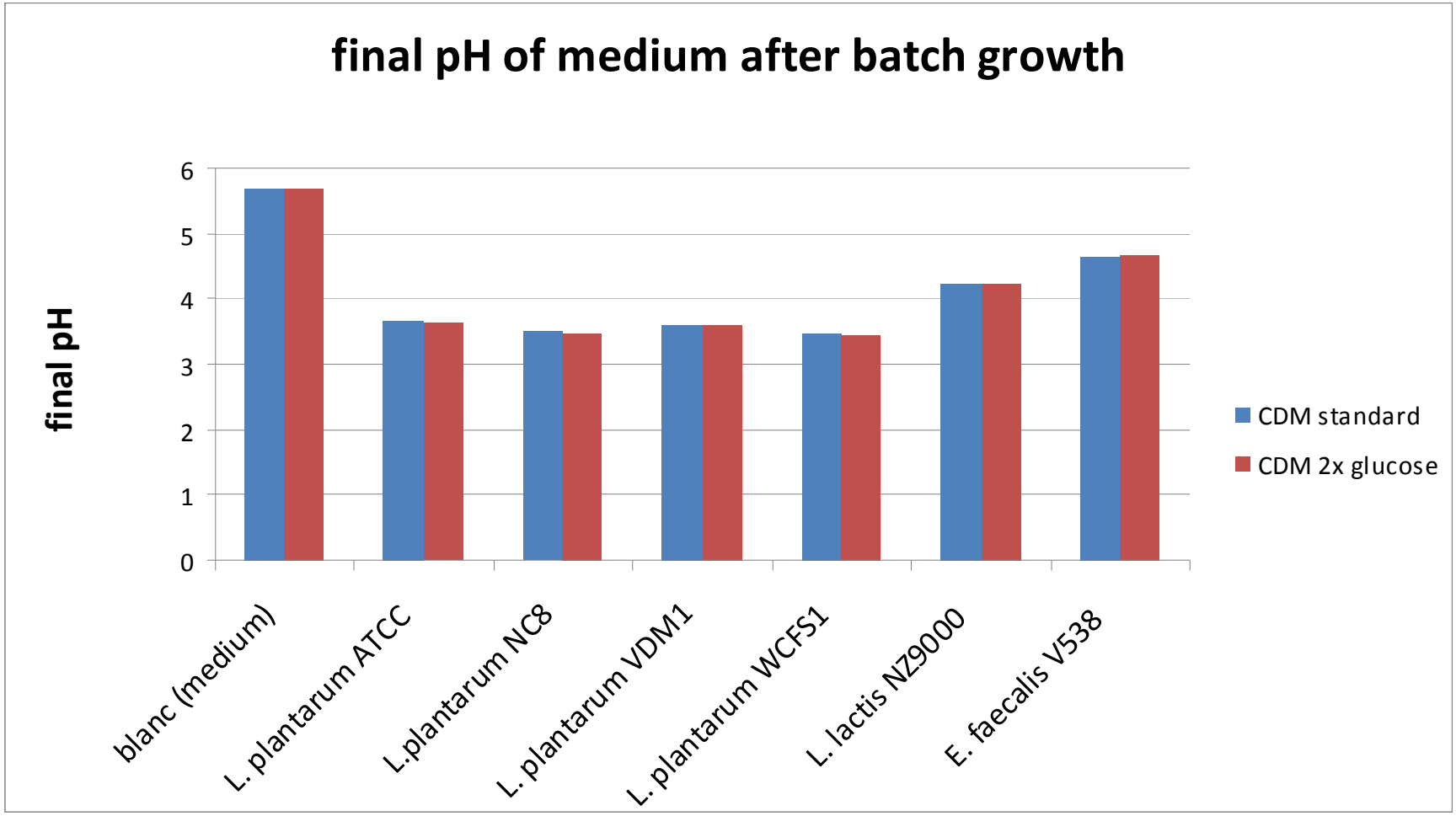
- Measured for WCFS1 (D=0.05 en D=0.4)
- Problem: samples stayed in the freezer for one year and have been defrosted once
- From measured values WCFS-strain 6 enzymes (GLK, G6PDH, PGI, TPI, PGM, LDH) seem ok, the activity for the rest is too low

Comparison pH sensitivity/robustness 4 LAB-species



Different species acidify medium to a different pH, pH-limited batch growth (?)

Batch culture is not limited by glucose;
growth stops at same pH in the presence of 2x glucose



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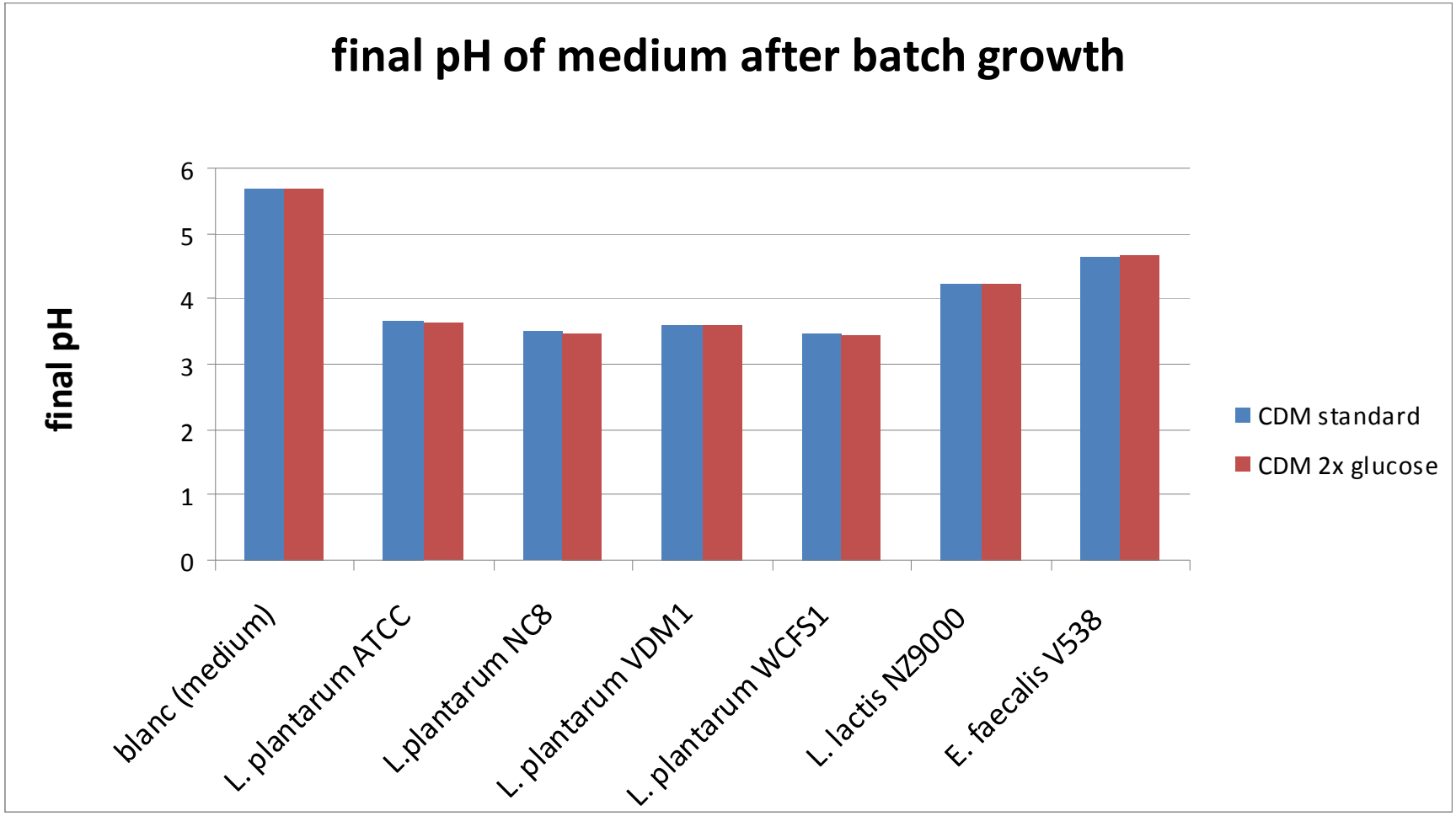

Does more acidification also mean more growth ???

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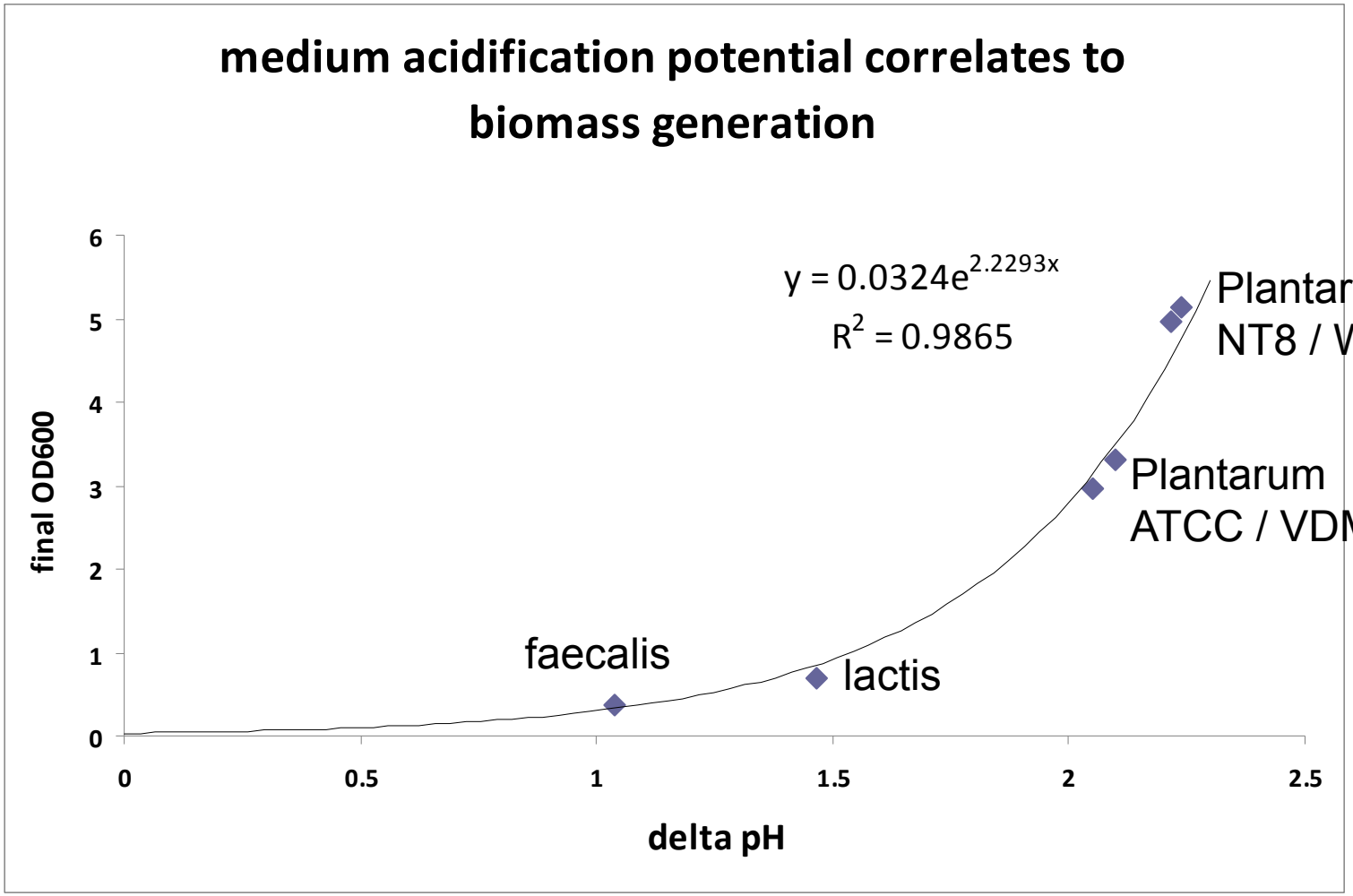


The benefit of still growing at low pH is clear, why don't lactis and faecalis do the same?

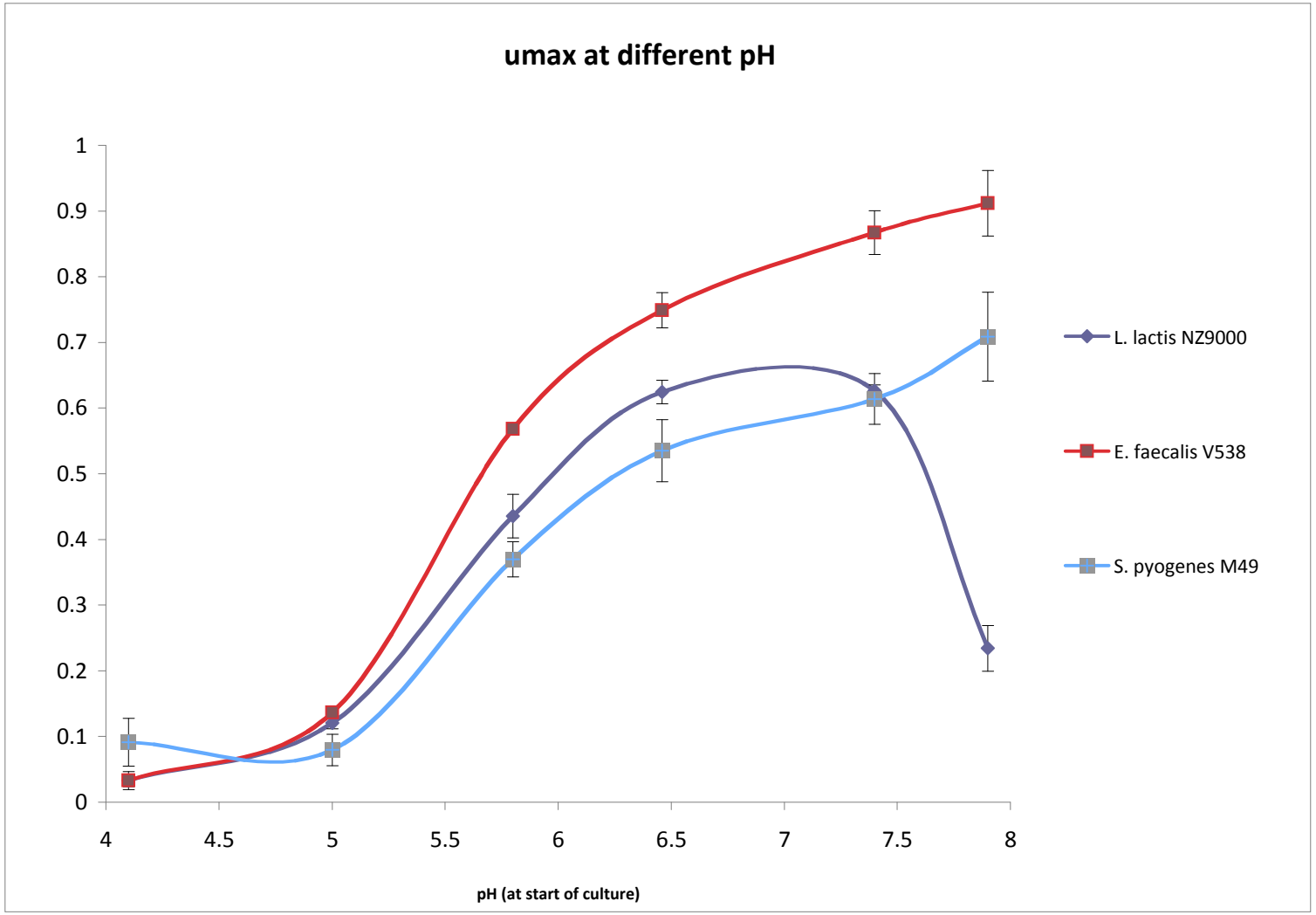
Maximum growthrates at different pHs

measured in modified CDM, containing McIlvaine-Buffer

prepare CDM without citrate and $K_xH_xPO_4$, 1.1 x concentrated addition per liter:

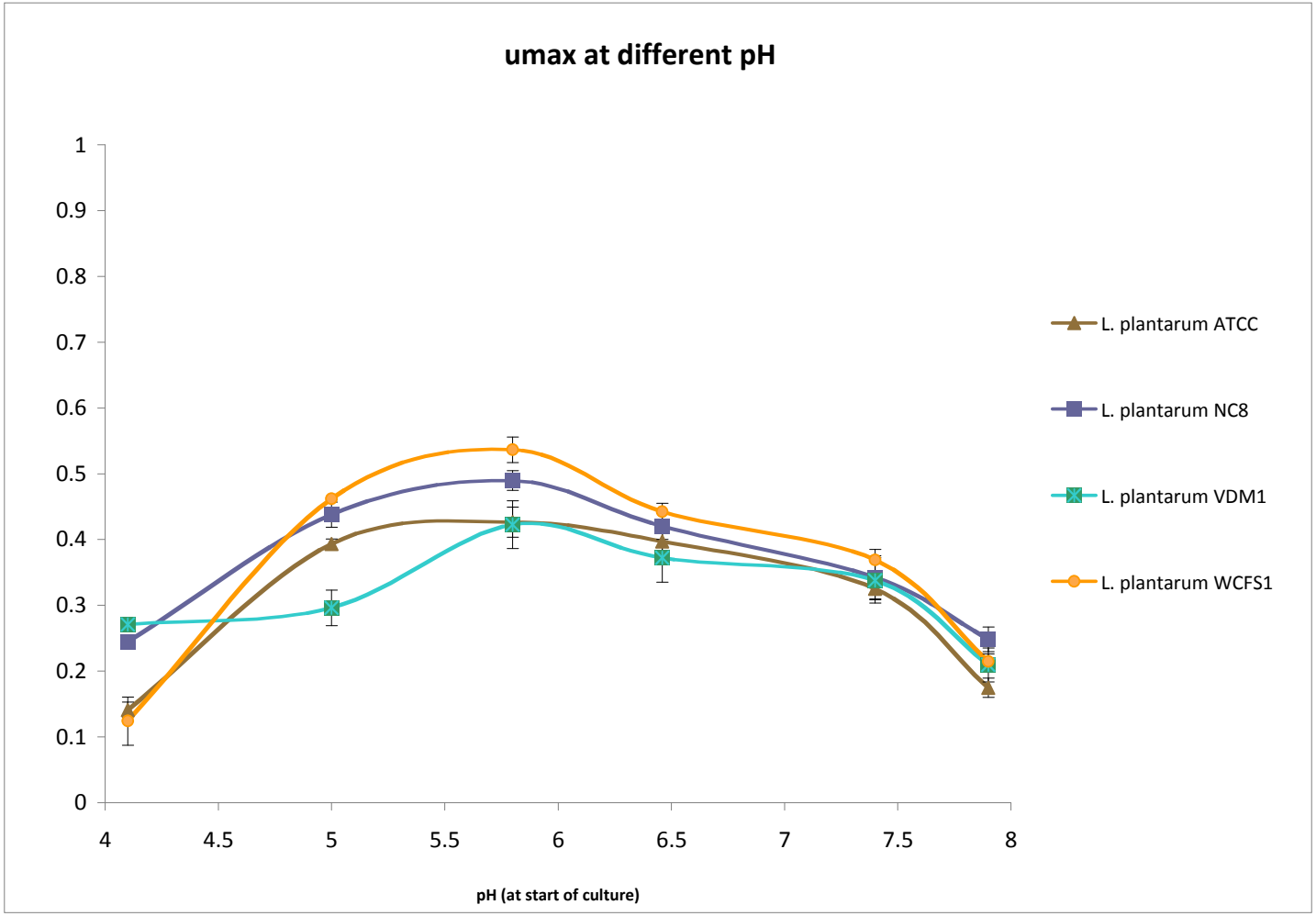
1 M K_2HPO_4 (ml)	**0.5 M Citric Acid (ml)**	**pH...**
20.55	79.45	3.0
38.55	61.45	4.0
51.50	48.50	5.0
63.15	36.85	6.0
82.35	17.65	7.0
97.25	2.75	8.0

Maximum growthrates at different pHs



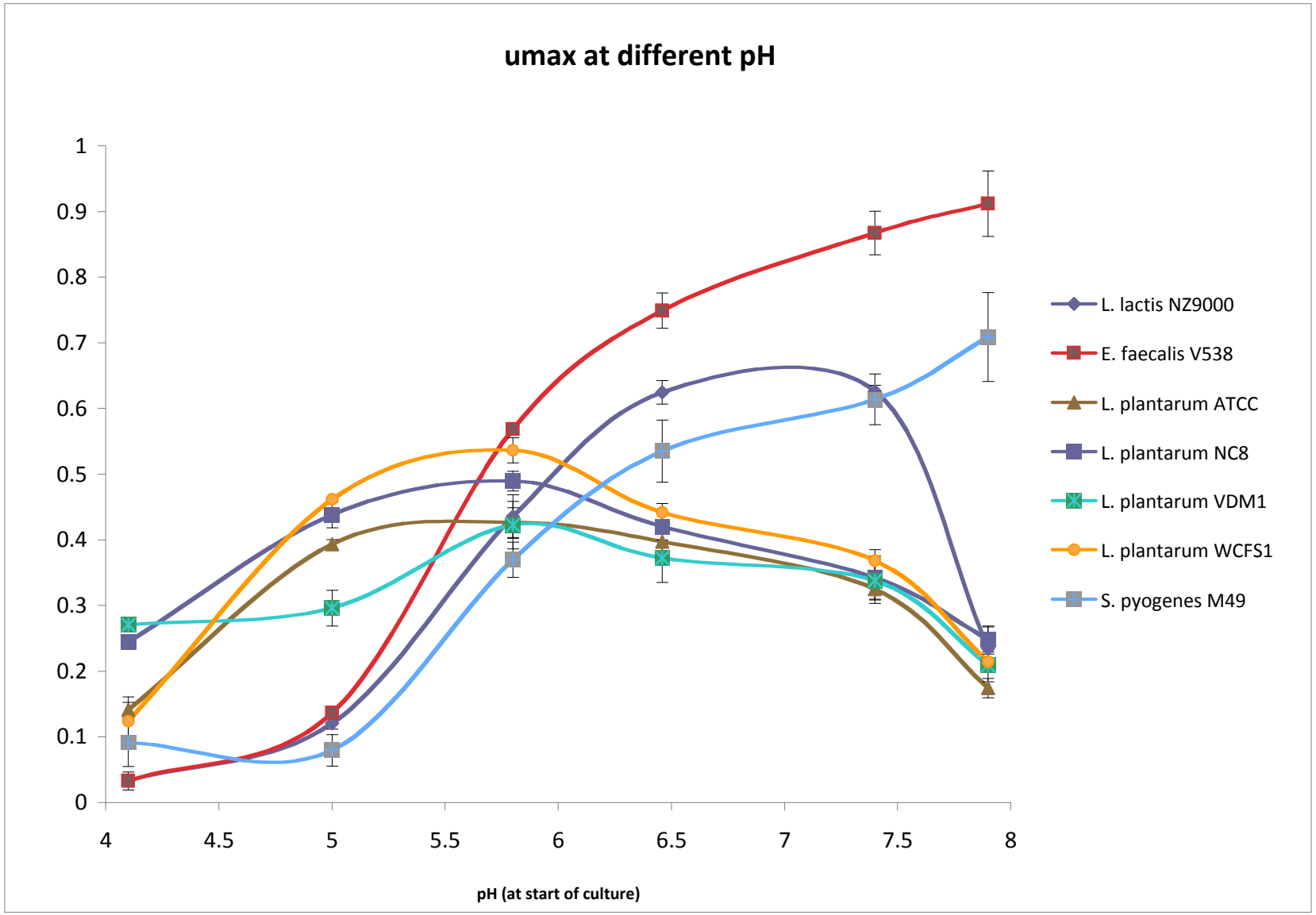
Lactis / faecalis / pyogenes, clear preference for neutral pH

Maximum growthrates at different pHs



Different strategy plantarum species, lower u-max, but broader range

Maximum growthrates at different pHs



Trade-off growthrate and pH robustness

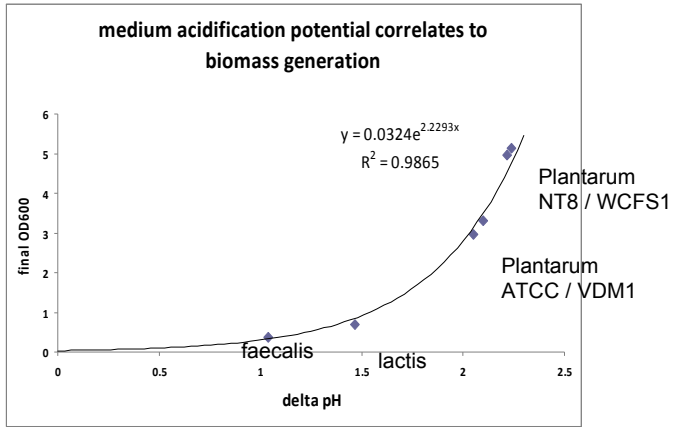
- Hypothesis; at low extracellular pH LABs spent a lot of ATP to pump out protons in order to keep their cytosolic pH neutral. plantarum evolved to grow in low pH. Cytosolic pH of plantarum (partly) acidifies along with extracellular pH; less energy spent on pumping out protons

Exps:

- Determine cytosolic pH
- Determine pH sensitivities glycolytic enzymes

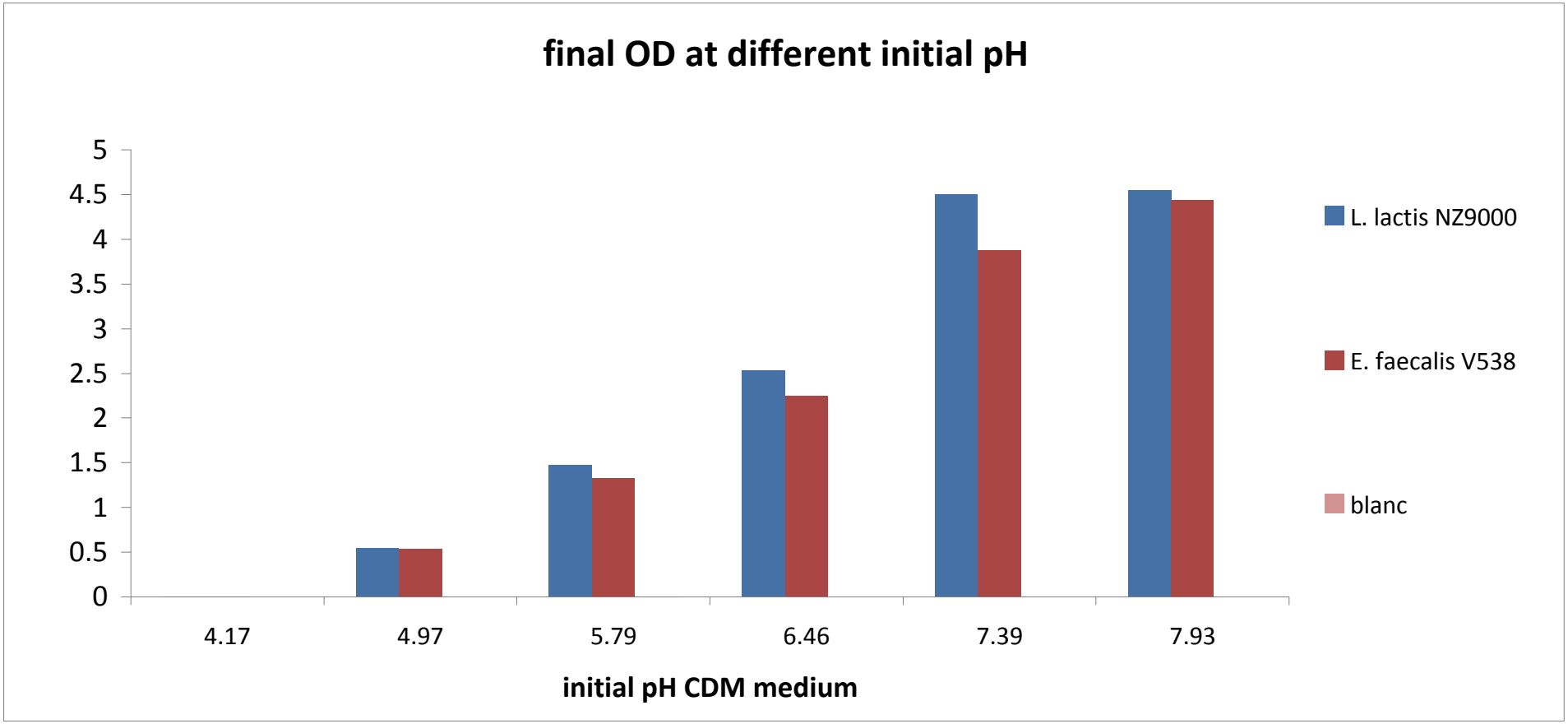
  Does plantarum have enzymes that are less pH sensitive (but with lower specific activity/kCat, resulting in a lower growthrate at neutral pH?)

Previously shown; acidification correlates with growth

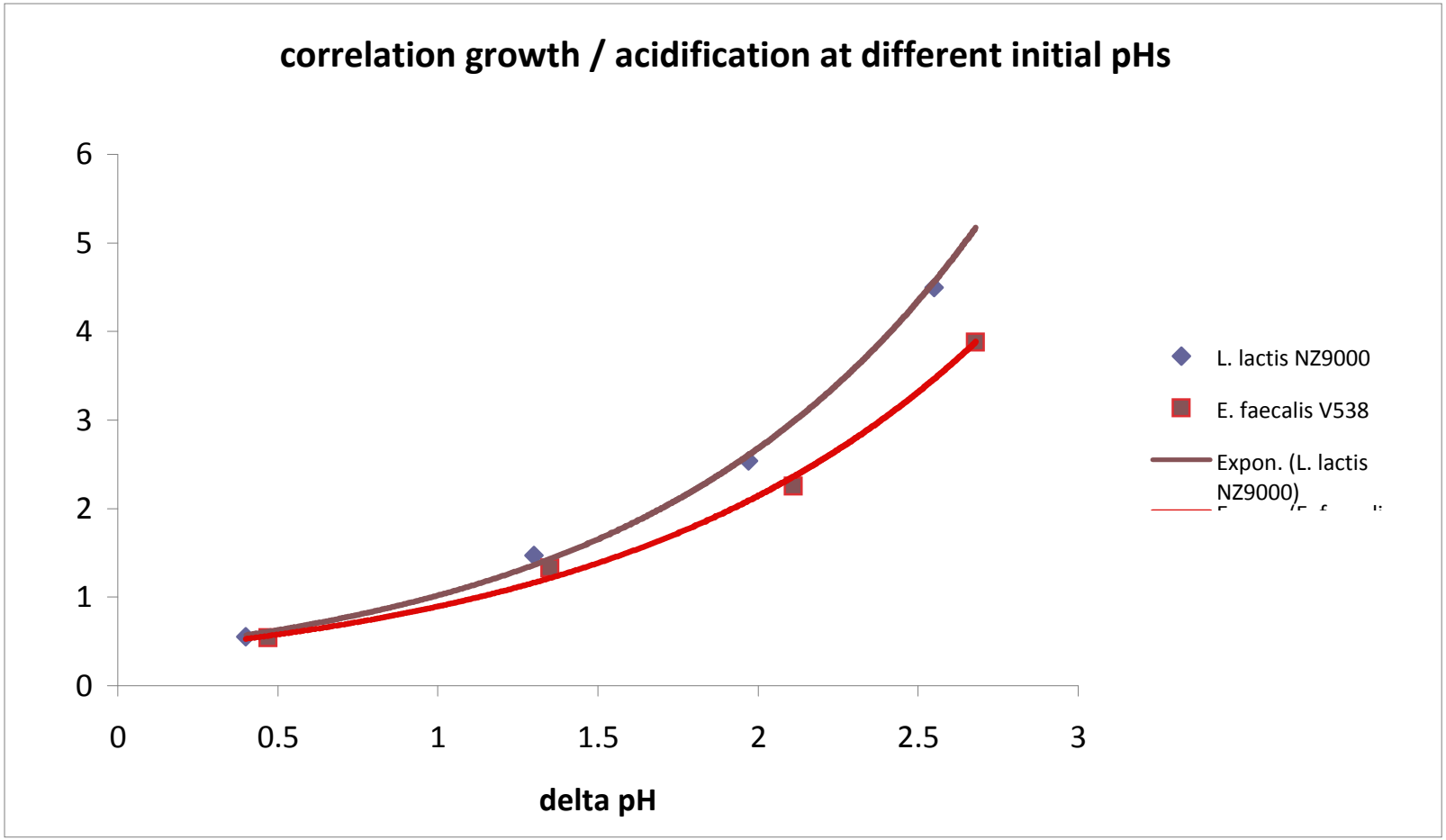


- Grown 4 LABs at different initial pH
- Determined u-max (previously shown 96 well-plate)
- Measured final pH after full growth (test tube)
- Measured final OD after full growth (test tube)
- Plot ΔOD/ΔpH for each species

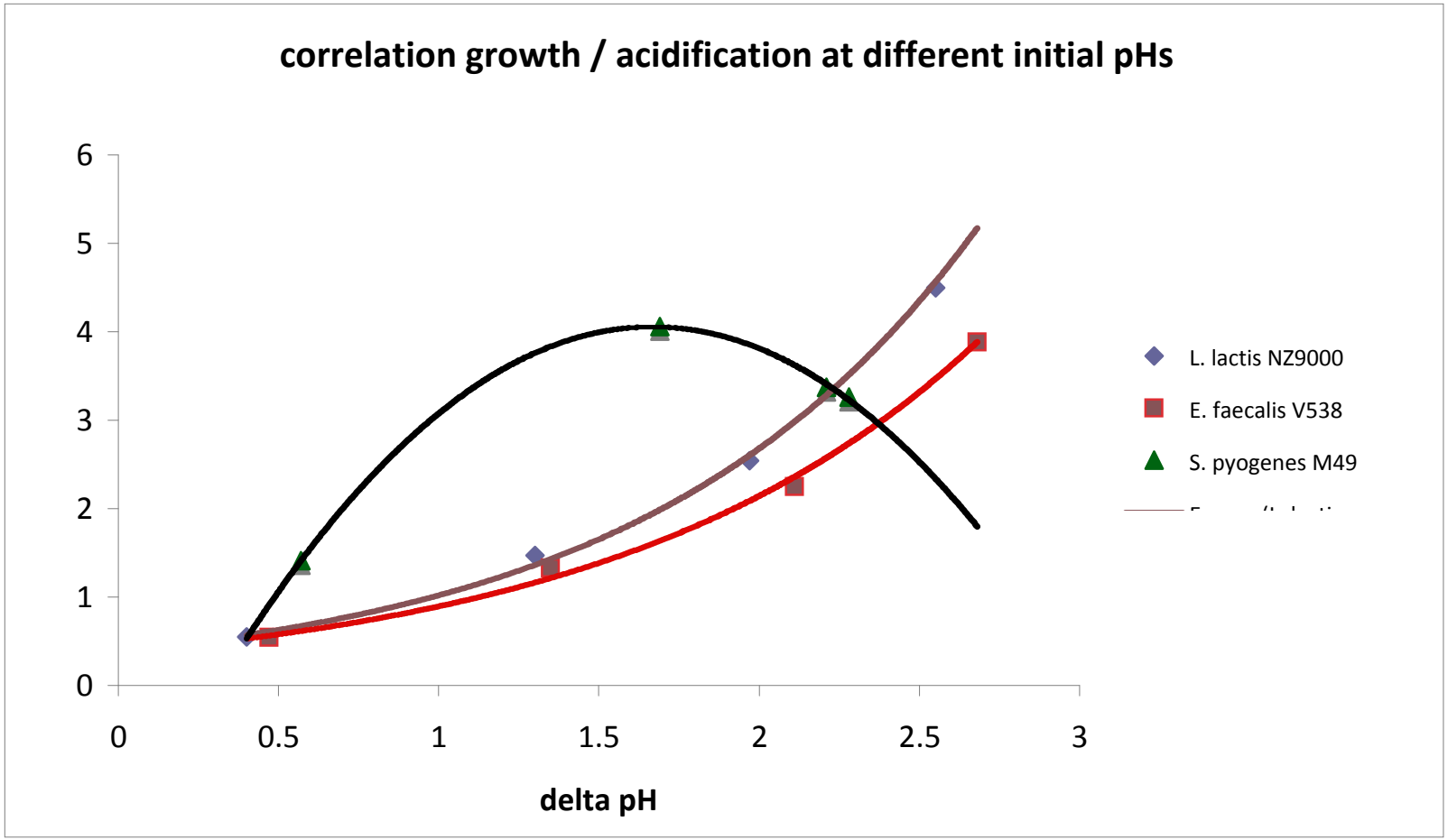
Lactis and faecalis; growth correlates exponentially with acidification of medium



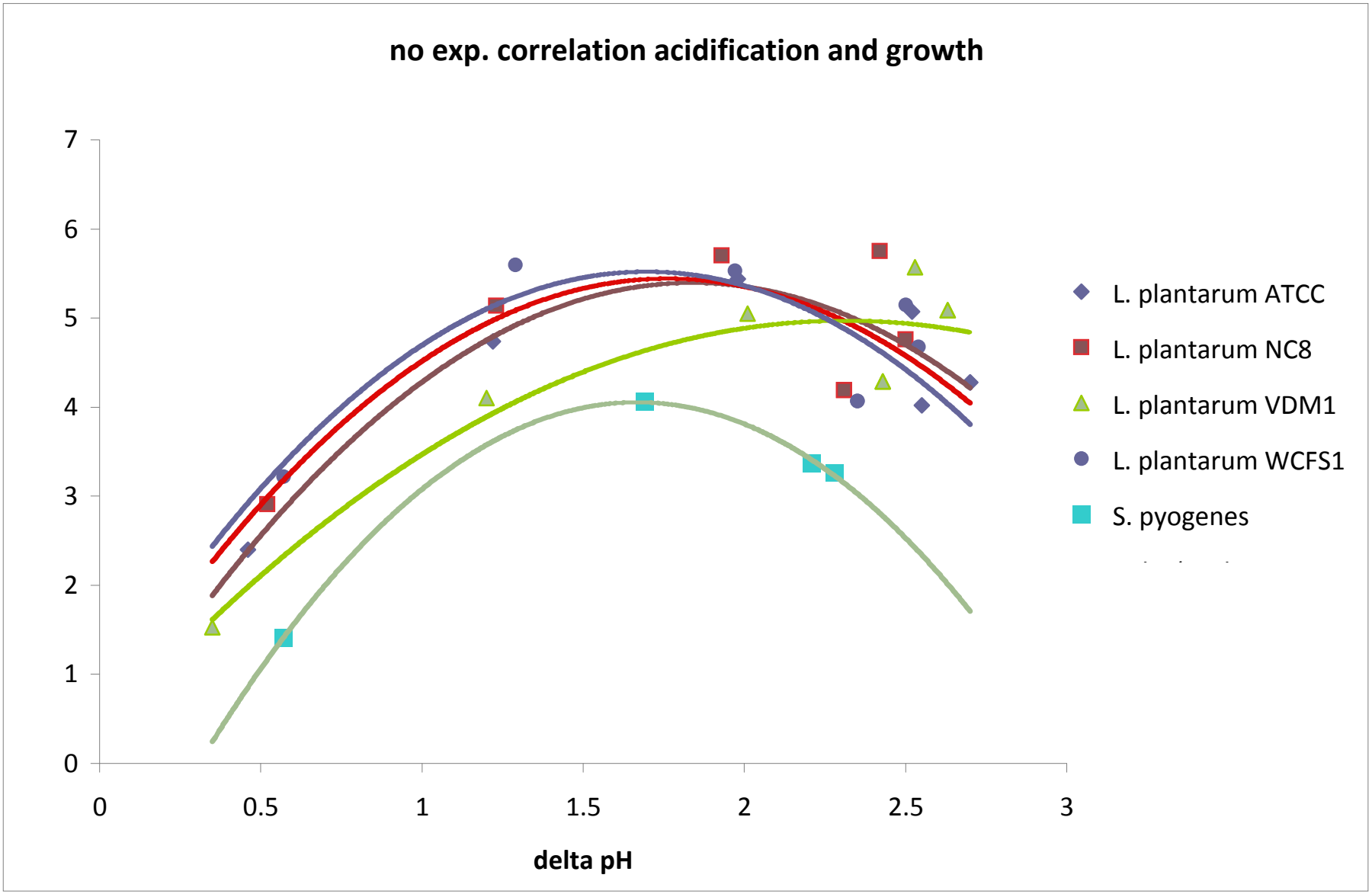
Lactis and faecalis; growth correlates exponentially with acidification of medium (lactis grows has a higher growth/acidification ratio as faecalis)



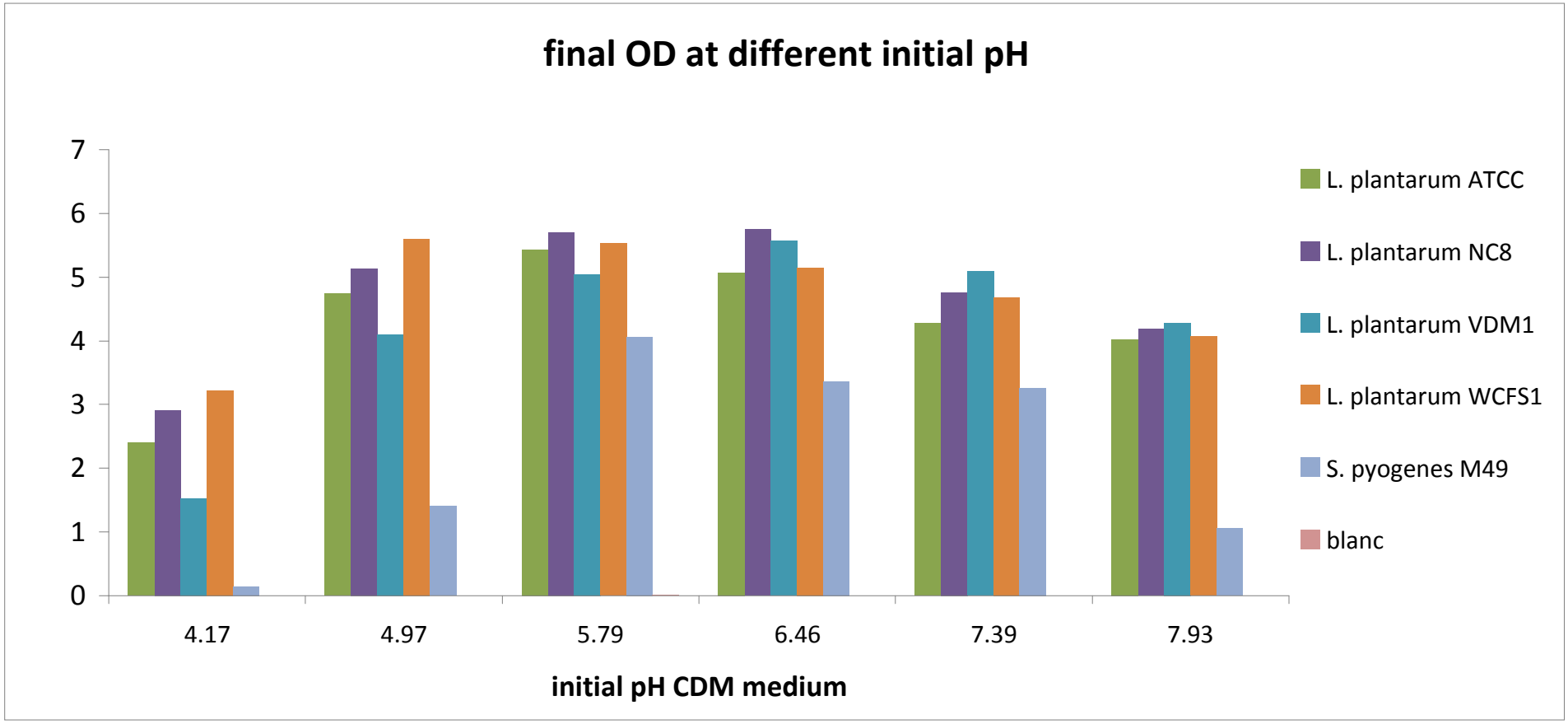
Lactis and faecalis; growth correlates exponentially with acidification of medium
For pyogenes this dependency is different !!!



For pyogenes this dependency is different !!!
plantarum shows similar behaviour ….



Plantarum and pyogenes reach a higher final OD when initial pH is lower



- Plantarum/pyogenes ; How do cells grow to higher/equal ODs eventhough acidification is less?

- Does external pH regulate a metabolic switch in plantarum (and pyogenes) ?
- Do cells reach the highest OD when initial pH is close to their cytolic pH ?

Future;

4 LAB species

- Measure end products after inoculation at different initial pHs
- Intracellular pH
- pH sensitivity glycolytic enzymes