HERBASPIRILLUM SEROPEDICAE: STUDY OF POLYHYDROXYALKANOATES PRODUCTION

Molecular Microbiology Laboratory-Institute of Biological Research "Clemente Estable" (IIBCE)

Montevideo, Uruguay

FAPESP
Workshop for Sustainable Production of Biopolymers and other Bio-based Products
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Molecular Microbiology Laboratory-IIBCE

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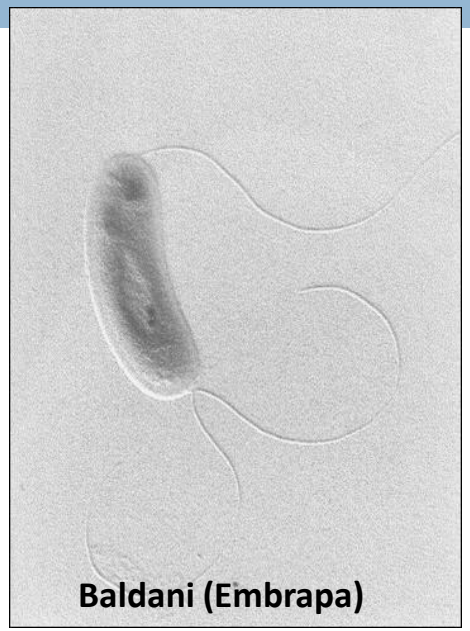
Project Focus: PHA production by *Herbaspirillum seropedicae* Z69

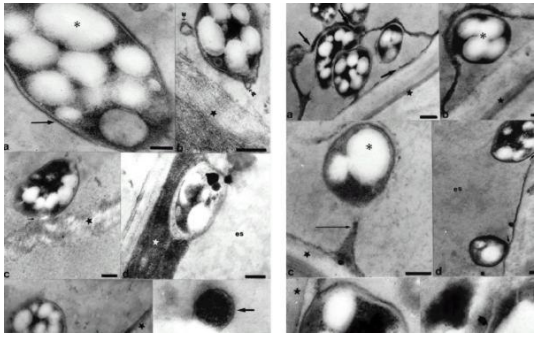
H. seropedicae: General background


Baldani (Embrapa)

- **Origin: Isolated in Brazil by the group of Prof. J. Döbereiner.**

- **Beta-Proteobacterium isolated from rice, corn or wheat root systems first described in 1986: Baldani JI et al. (1986) "Characterization of *Herbaspirillum seropedicae* gen. nov. sp. nov. a root-associated nitrogen-fixing bacterium." Int. J. Syst. Bacteriol.**

- **Strickly aerobic organism, flagellar motility, diazotroph. It can grow microaerophilically.**


James et al.. 2002

- **Soil bacterium and endophyte, colonizes roots (rice, sugar cane, maize, coffe, etc).**

- **Plant growth-promoting and non-pathogenic.**

H. seropedicae: General background

- Public access to the genome of strain Z78 (Univ Paraná, Curitiba- Brasil) (Genopar Project, responsible: F. Pedrosa).
- Carbon metabolism: we determined enzymatic activities for ED and TCA. It was not possible to determine the presence of Phosphofrutokinase (EMP) and Phosphogluconate dehydrogenase (Pentose Phosphate).
- Curitiba's group studied N and arabinose metabolism.
- No special growth requirements (optimal growth temp. 34º C).

H. seropedicae: PHAs producer

- **Classic pathway for PHB synthesis.**
- **Acumulates PHB and copolymer P(3HB-*co*-3HV) with different carbon sources.**
- **Acumulates up to 60 % cell dry weigth of PHB when consumes glucose as sole carbon source (under optimized conditions).**

Carbon Source	Polymer		
	Z67	Z69	Z78
Glucose	PHB	PHB	PHB
Xylose	PHB	PHB	PHB
Mannitol	PHB	PHB	PHB
Galactose	PHB	PHB	PHB
Arabinose	nd*	PHB	nd
Succinate	PHB	PHB	PHB
Nonanoic acid	P(3HB-co-3HV)	P(3HB-co-3HV)	P(3HB-co-3HV)
Propionic acid	nd	P(3HB-co-3HV)	nd

*nd.: Not determinated

H. seropedicae: PHAs producer

Use of low cost carbon sources

PHB production in batch cultures using carbohydrates present in hemicellulose hydrolyzate

Carbon source	Xt (g/l)	Xr (g/l)	PHB (g/l)	PHB (%)
Glucose 30 g/l	12.5	5.3	7.2	58
Xylose 30 g/L	7.6	3.8	3.8	50
Xylose 24 g/l –glucose 4.5 g/l -arabinose 1.5 g/l	8.9	3.9	5.0	56

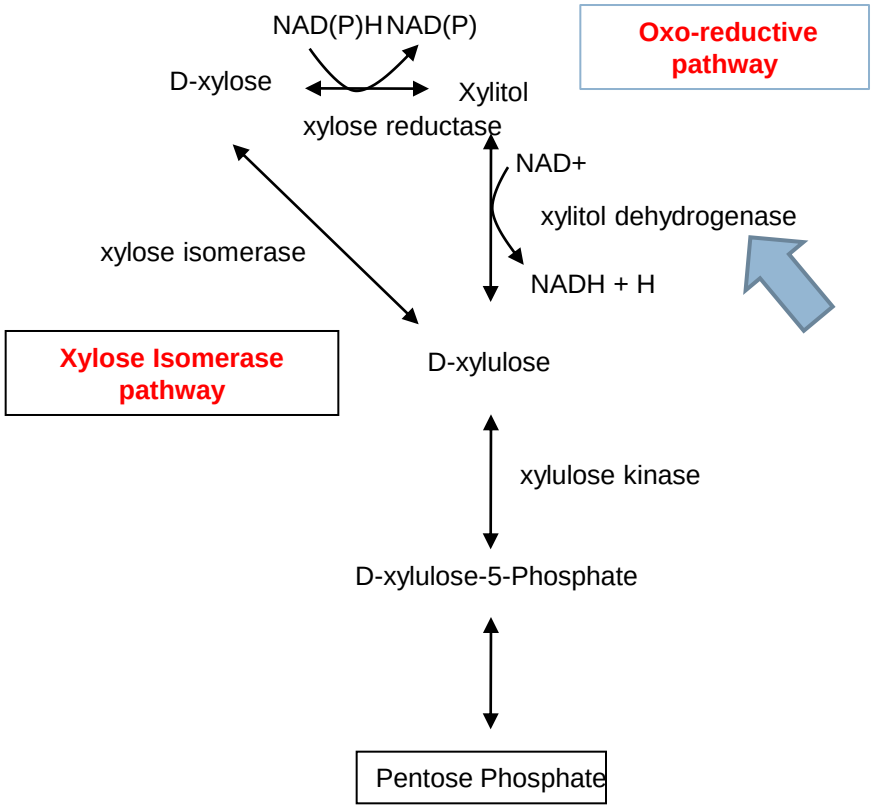
We studied the accumulation of PHB in batch cultures and have obtained a yield of 50% cell dry weight

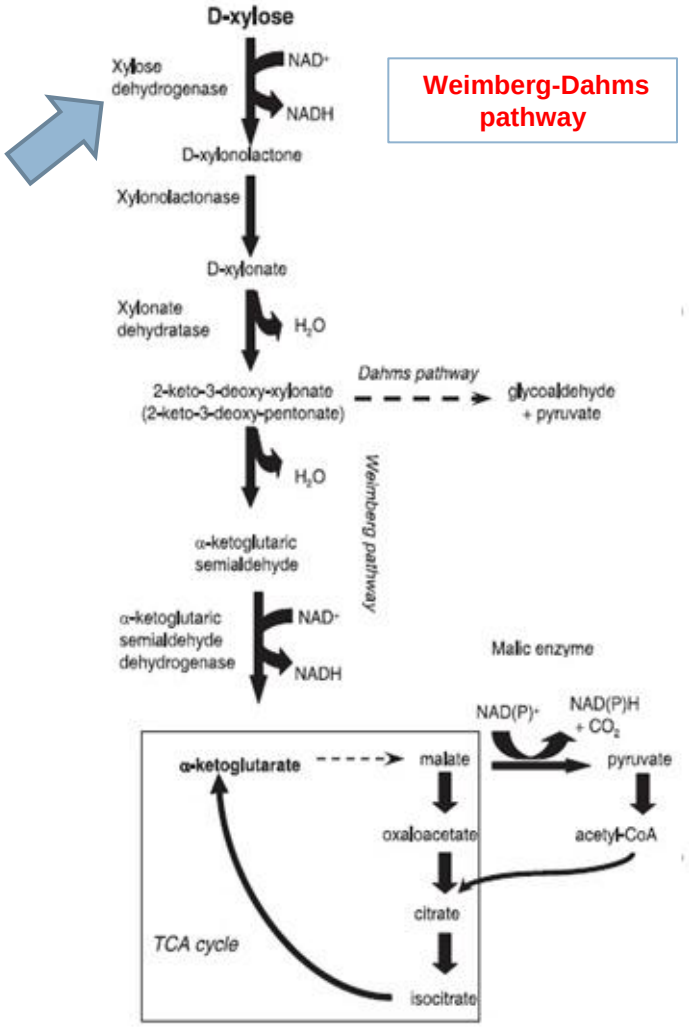
OBJECTIVE

Characterization of xylose metabolism and optimization of PHB production when xylose is used as the carbon source

Xylose metabolism

Pathways described in different microorganisms





Stephems et al. 2007

Biochemical strategy:

Determination of enzymatic activities

Enzyme	Specific activity (nmol/min.mg prot.)			
	Extracts Glucose 30g/l	Extracts Xylose 10 g/l	Extracts Xylose 30g/l	Extracts Xylitol 10g/l
Xylose deshydrogenase	15.7	29.2	30.4	0
Xylitol deshydrogenase	0	0	0	46.5

These results suggest the presence of the Dahms-Weimberg pathway for xylose degradation.

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L-Arabinose Metabolism in *Herbaspirillum seropedicae*

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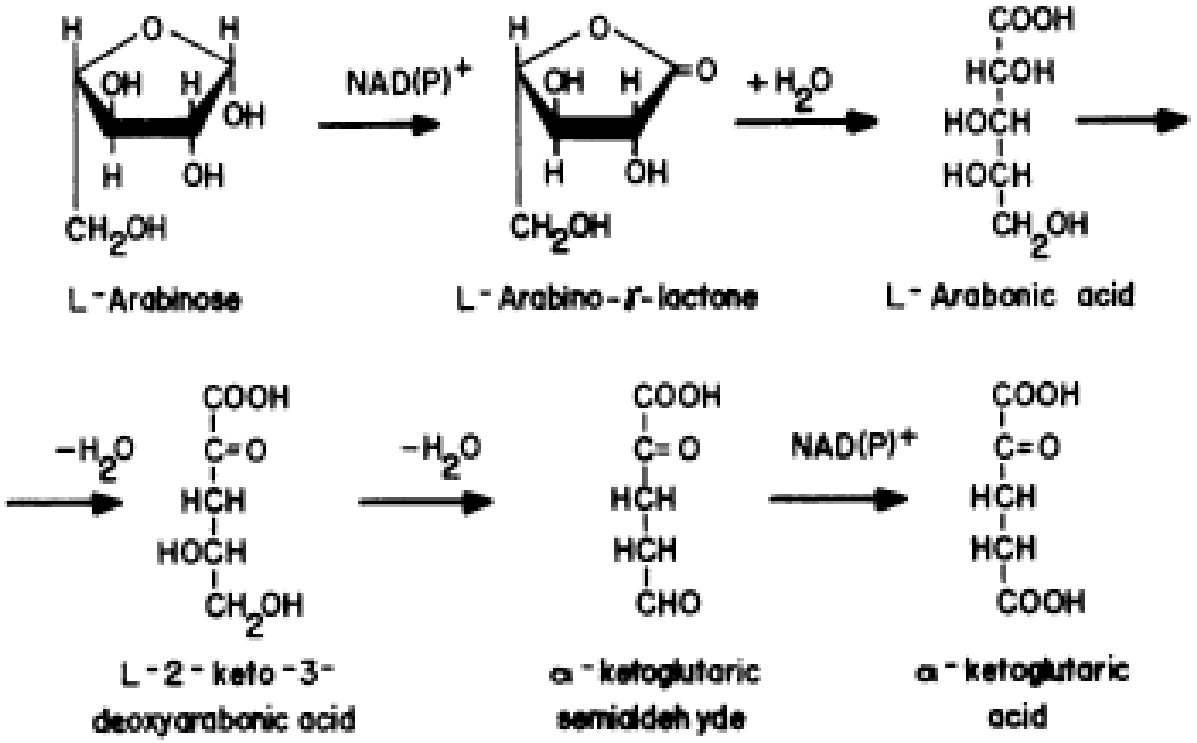


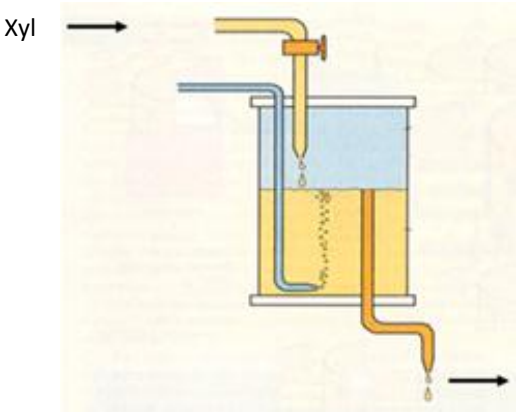
FIG. 2. Pathway of L-arabinose metabolism in *H. seropedicae*.

Metabolic strategy:

Metabolic pathway analysis using elementary modes (EM)

The EM represent metabolic pathway involved in conversion of the substrate into the products

Continuous culture experiment



D (h-1)	Xt (g/l)	Xr (g/l)	PHB (g/l)	PHB (%)	Xyl initial (g/l)	Xyl residual (g/l)	Xyl consumed (g/l)	NH_4Cl initial (mM)	NH_4Cl residual (mM)	$Y_{Xr/xil}$ (g/g)	$Y_{PHB/xil}$ (g/g)
0.021					7.11			7.8			
	0.96										
	1.35	0.47	0.88	65		0	7.11		0	0.066	0.124
	1.50	0.53	0.97	65		0	7.11		0	0.074	0.136
	1.47	0.47	1.00	68		0	7.11		0	0.066	0.140
	1.57	0.64	0.93	69		0	7.11		0	0.090	0.131

Metatool

Input File

Metabolic network: ED, Weimberg-Dahms, anaplerotic pathway (Gluconeogenesis and Phosphate Pentose), aerobics respiration

Constraints

67 % cell dry weigth PHB acumulation

XT= Xr+PHB

Output File:

1: ATP = ADP
2: 20241.4 XYLext + 301819 ADP + 103446 O = 301819 ATP + 55999.8 CO2 + XT
3: XYLext + 29 ADP + 10 O = 29 ATP + 5 CO2
4: 34046.6 XYLext + 715976 ADP + 241498 O = 715976 ATP + 125026 CO2 + XT
5: 20241.4 XYLext + 274208 ADP + 103446 O = 274208 ATP + 55999.8 CO2 + XT

N° EM			Experimental	
	$Y_{Xr/xil}$ (g/g)	$Y_{PHB/xil}$ (g/g)	$Y_{Xr/xil}$ (g/g)	$Y_{PHB/xil}$ (g/g)
2	0.108	0.221	0.075	0.133
4	0.064	0.131		
5	0.108	0.221		

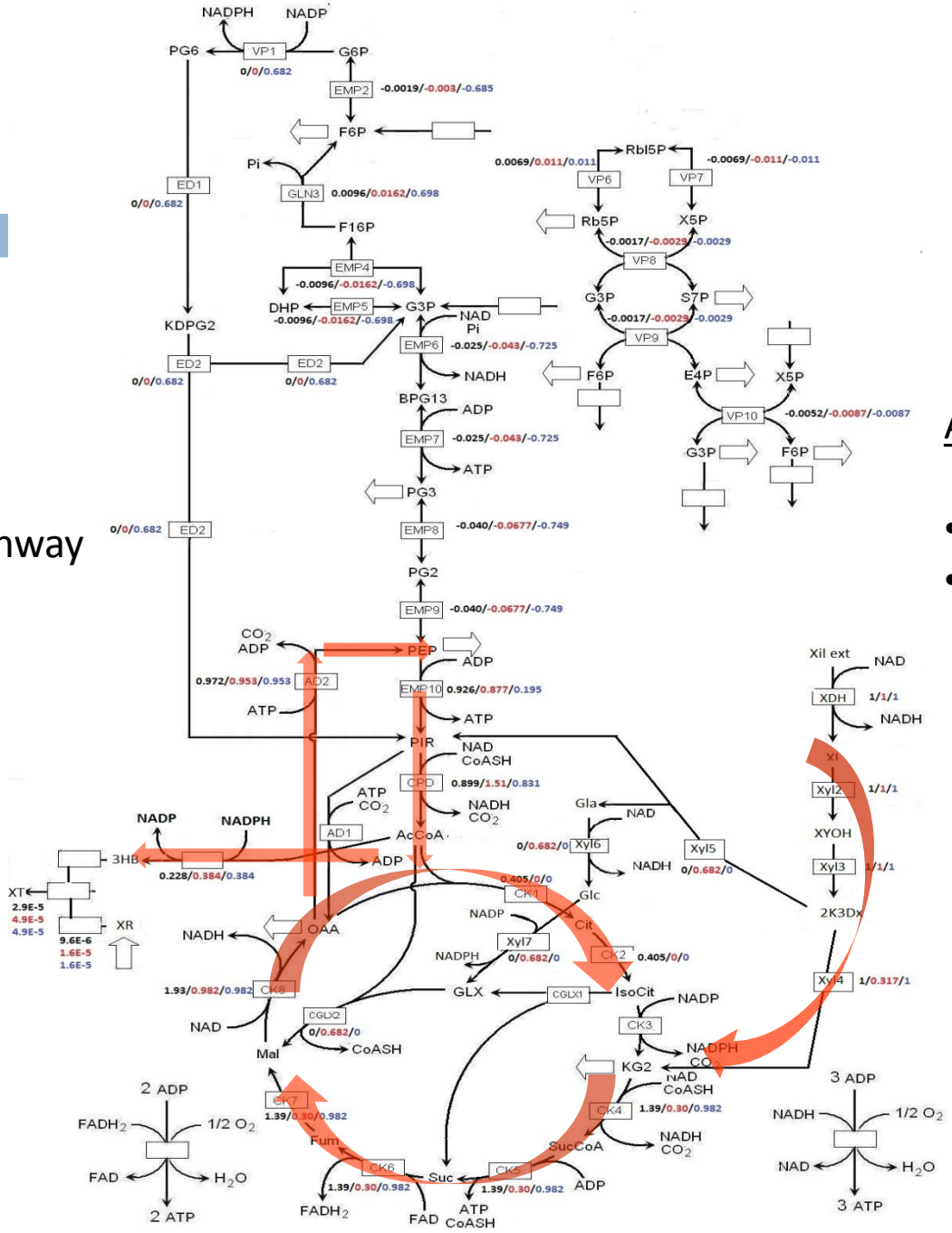
EM N°4

Catabolic pathways

- The Weimberg pathway
- TCA

Anabolic pathways

- Gluconeogenesis
- Pentoses Phosphate



Xylose metabolism

Conclusion

- **These results strongly suggest that *H. seropedicae* uses the Weimberg pathway to metabolize xylose**

Optimization of PHB production

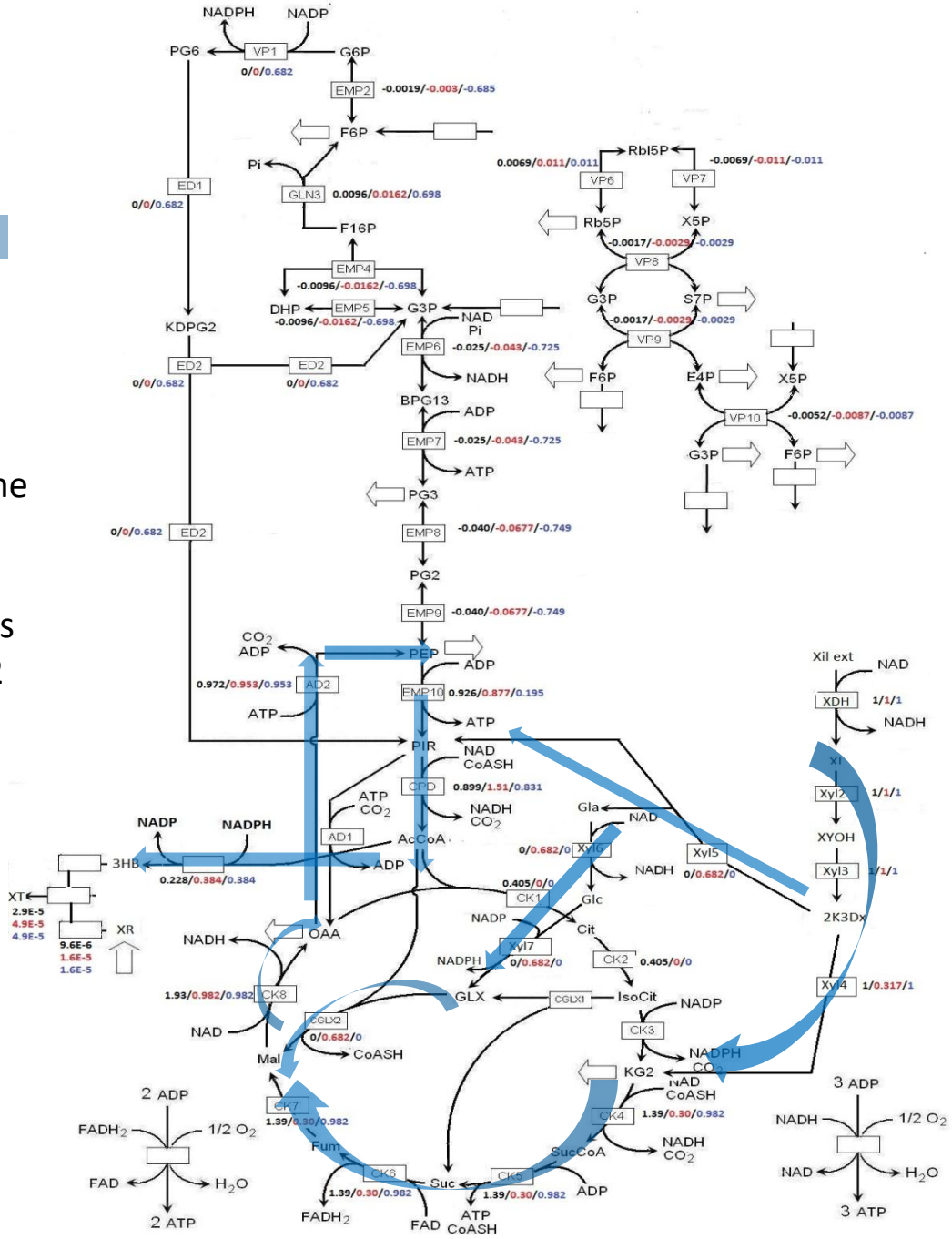
- EM N°4 does not have the highest yield transformation of carbon source into polymer compared with those calculated from EM N° 2 and N°5.

N° EM			Experimental	
	$Y_{Xr/xil}$ (g/g)	$Y_{PHB/xil}$ (g/g)	$Y_{Xr/xil}$ (g/g)	$Y_{PHB/xil}$ (g/g)
2	0.108	0.221	0.075	0.133
4	0.064	0.131		
5	0.108	0.221		

EM N°2

It involves the Weimberg and Dahms pathways and part of the Glioxylate cycle

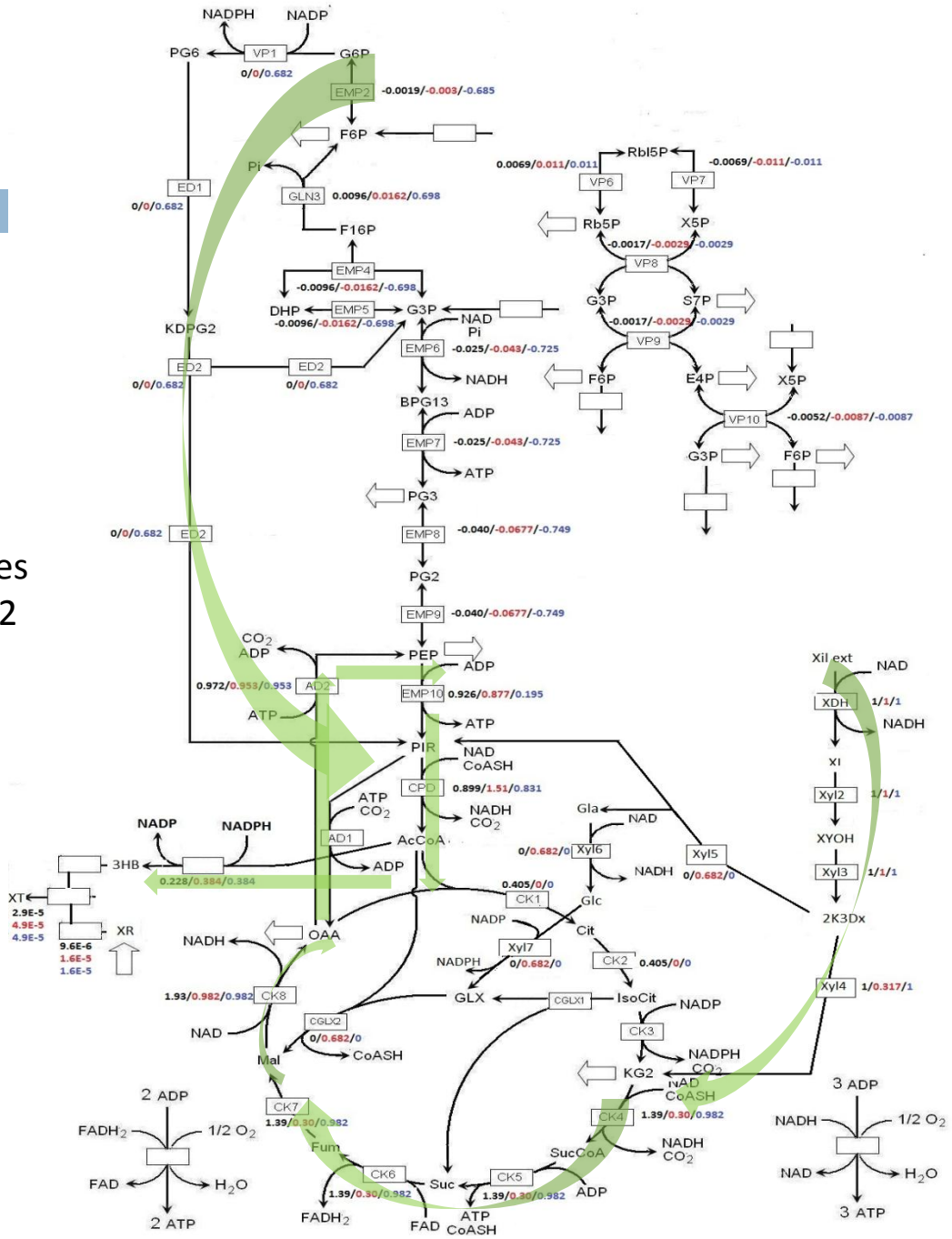
It does not involve the enzymes CK1 (citrate synthase) and CK2 (aconitase) of the TCA



EM N°5

It involves the Weimberg, ED pathways.

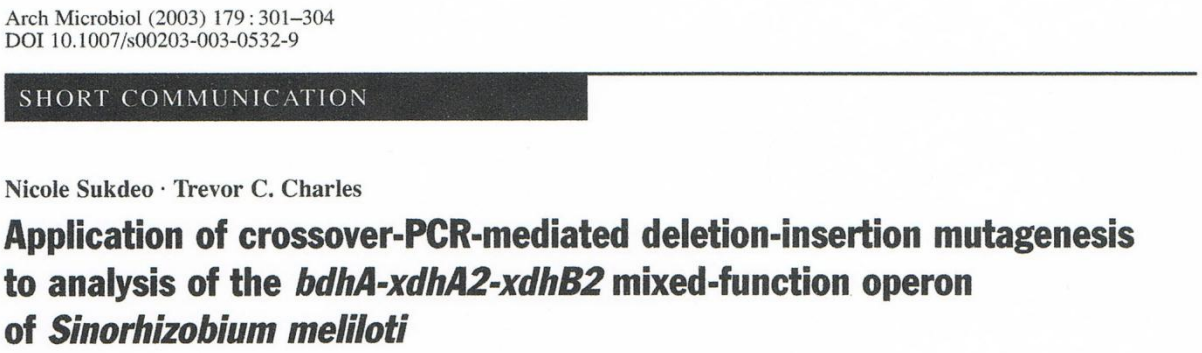
It does not involve the enzymes CK1 (citrate synthase) and CK2 (aconitase) of the TCA



Citrate synthase: key enzyme as a target for mutagenesis

In *H. seropedicae,* this enzyme is encoding by *prp*C gene

Site directed mutagenesis in *prp*C gene using the method described by Sukdeo and Charles



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SHORT COMMUNICATION

Nicole Sukdeo · Trevor C. Charles

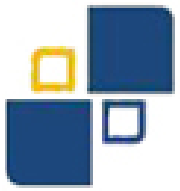
Application of crossover-PCR-mediated deletion-insertion mutagenesis to analysis of the *bdhA-xdhA2-xdhB2* mixed-function operon of *Sinorhizobium meliloti*

- We designed the primers and performed the PCR.
- We obtained a fragment of the expected size cloned and sequenced it to verify that the sequence is correct.

To be continued….

- Once obtained *H. seropedicae prpC* mutant, we will analyze metabolic pathways and it performance.

- Production assays using hemicellulose hydrolizate as carbon source.

Montevideo

Thank you!