ERA CoBioTech (ERA-Net Cofund on Biotechnologies)

ACHEMA2018

Kick-off session: "Biotechnology for a sustainable bioeconomy"

Title: Cyanophycin in Tobacco

Project name: Tobacco as sustainable source of the natural biopolymer cyanophycin as co-product to oil and protein

Name: Jeroen Hugenholtz

WAGENINGEN
UNIVERSITY & RESEARCH

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant 722361

Frankfurt am Main, 14.06.2018

Project partners

Your Logo

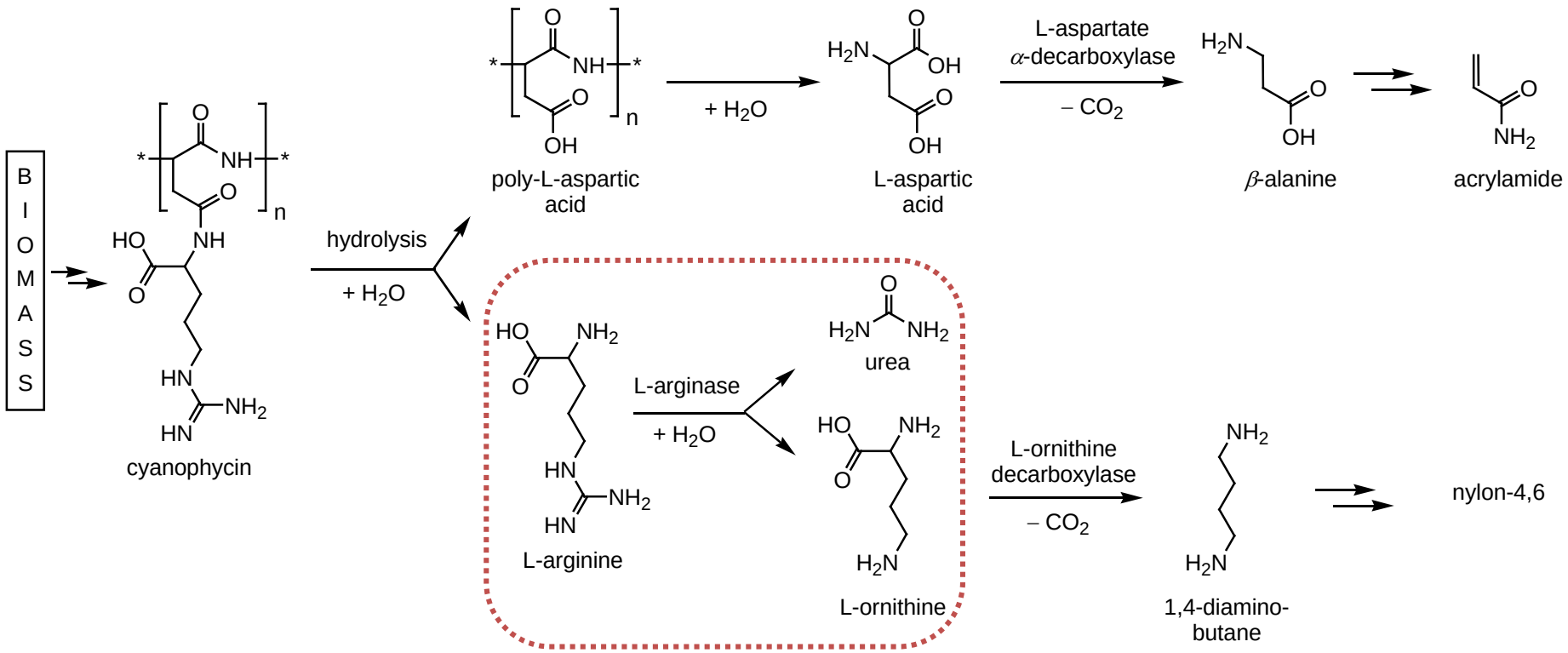
- Instituto de Agrobiotechnologi de Rosario (INDEAR, Argentina) – Coordinator
- IDROEDIL (IDR, Italy)
- University of Rostock, (UR, Germany)
- Leuphana University Luneburg (UL, Germany)
- Wageningen University and Research (WUR, The Netherlands)

- Total project budget: M€ 1.95

- Project start: 1-7-2018

Solaris Tobacco; lots of flowers/seeds, small leaves

N-chemicals from cyanophycin

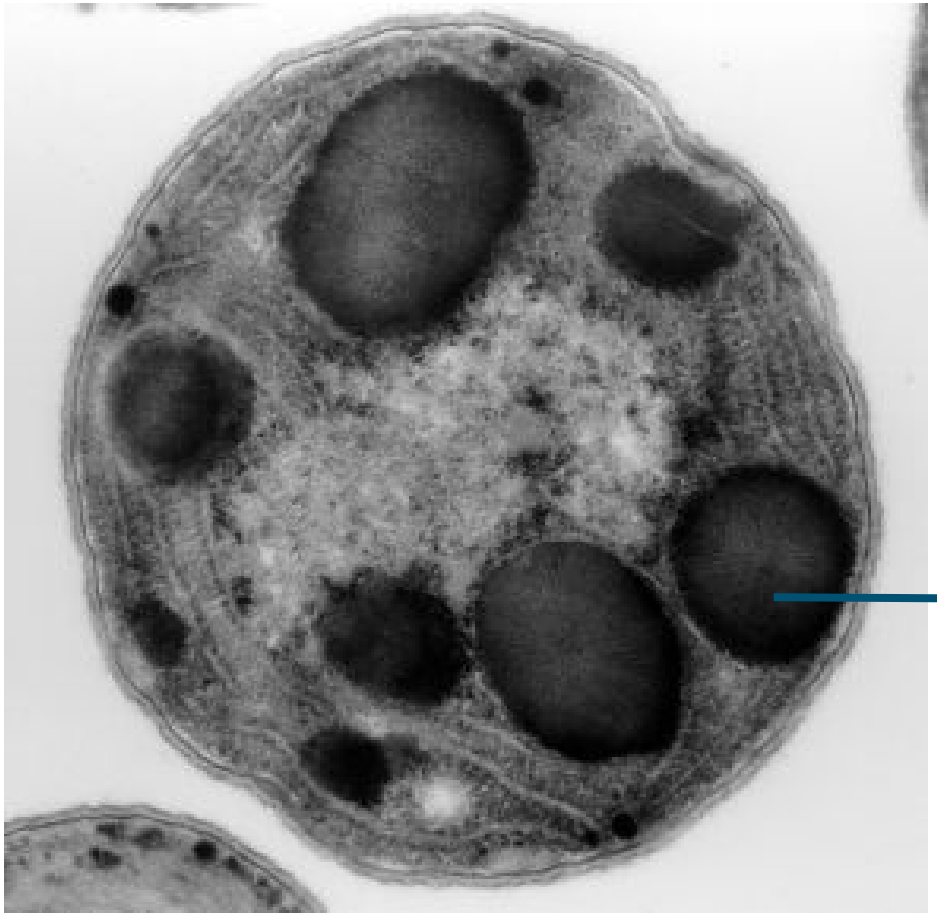


Focus:

- cyanophycin hydrolysis
- α-decarboxylation of L-aspartic acid
- L-arginine hydrolysis
- L-ornithine decarboxylation

Konst et al
Steunenberg et al

Ethanol and Cyanophycin building blocks from yeast: two products for the price of one



Cyanophycin mainly in cyanobacteria as nitrogen and energy reserve material

= Asp + Arg

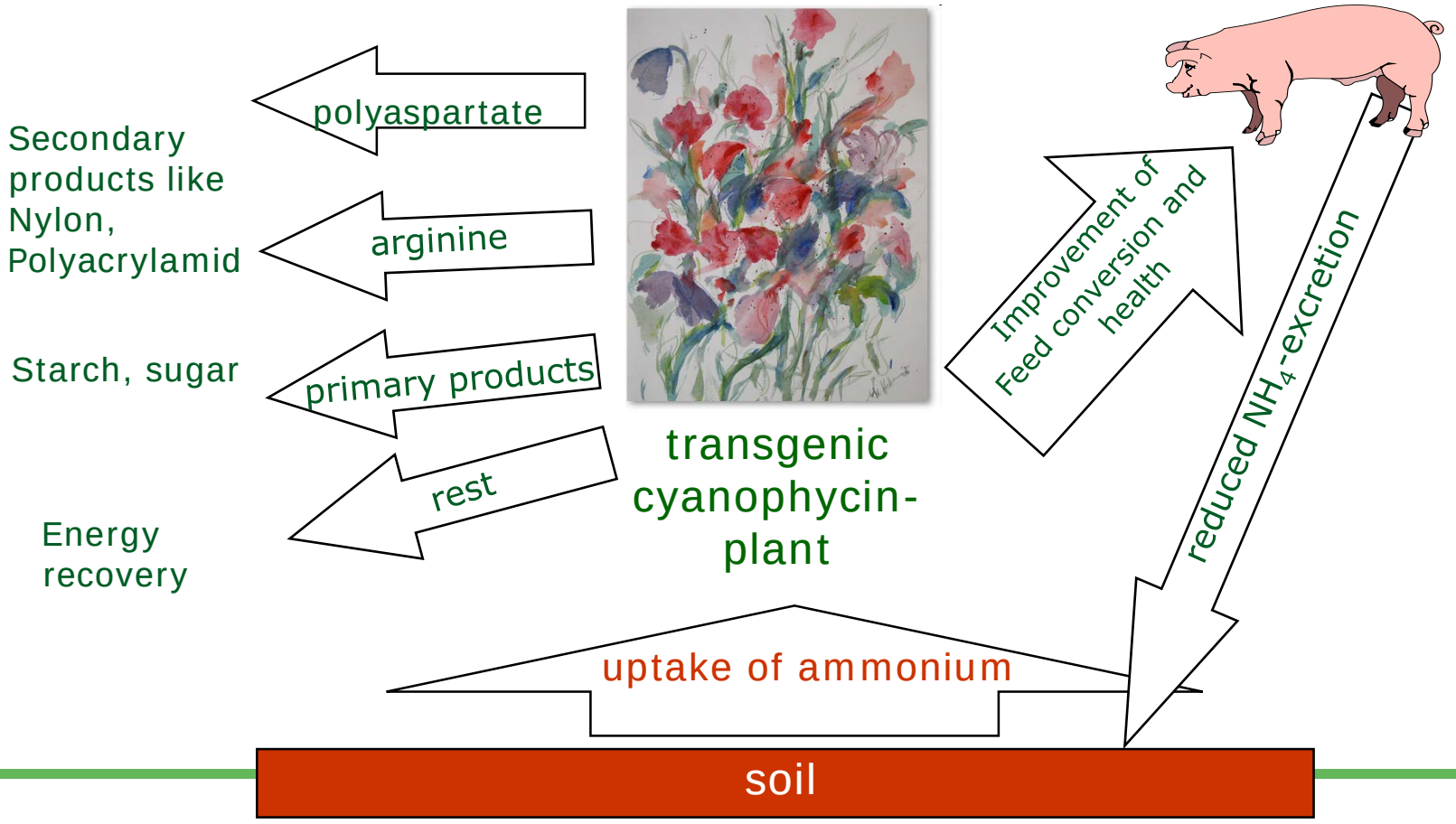
Granule
35% (wt/wt) and slow growth

Steinle et al

Introduction

- Project objectives (problem to be solved)
  - Cyanophycin production by tobacco (seeds)
  - Tobacco as nitrogen source (Asp, Arg) for feed
  - Production of novel biobased polymers
  - Production of biobased nylon
- Scientific approach and project topic area
  - Inserting cyanophycin-production in Solaris Tobacco
  - Oil- and Cyanophycin-extraction from Solaris
  - Comparison cyanophycin production in Tobacco and microorganisms
  - Life-cycle and economic assessment of cyanophycin production by Tobacco
  - Conversion of cyanophycin in (novel) biopolymers (nylon)

Possibility of usage of cyanophycin from plants



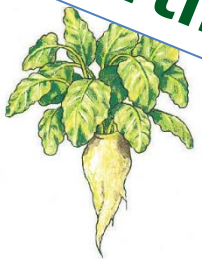
Potential candidates to produce cyanophycin

potato

as by product of starch

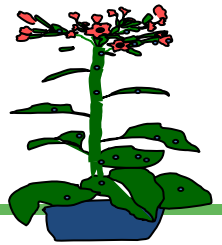
Leftover: usage as feed, energy source or fertilizer

sugar beet



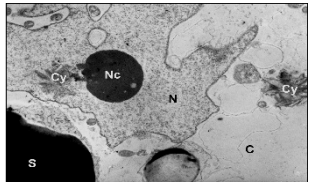
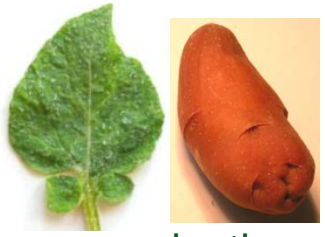
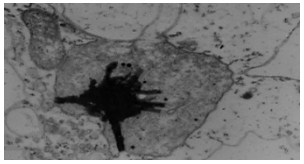
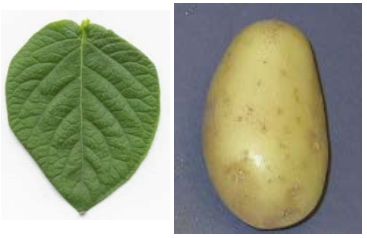
f sugar

tobacco

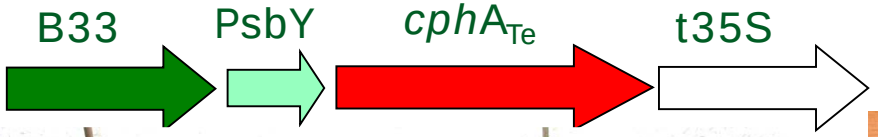


as by product of nicotin

Cyanophycin accumulation in potato

construct	Localization in plant	max. cyanophycin content in % dw	phenotype
p35S *cph*A t35S **p35S-*cph*A**	cytoplasm 	Leaf: 0.2 tuber: 0.2	 no germination Neumann et al., 2005)
p35S TP *cph*A t35S **pFNR-*cph*A**	cytoplasm 	Leaf: 0.2 tuber: 0.01	
pPsbY-*cph*A	plastids of leaf and tuber 	Leaf: 3.7 tuber: 0.9	

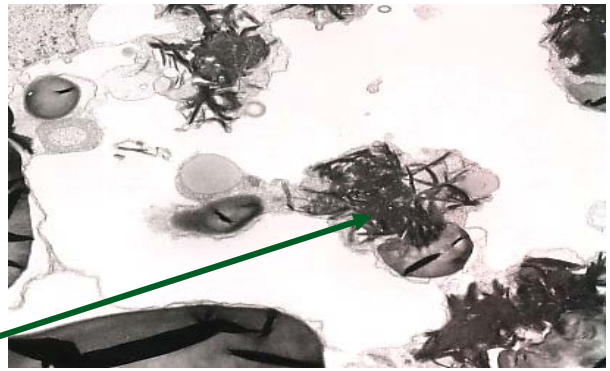
Increase in cyanophycin production by tuber specific expression of the synthetase



nic event 7

nic event 7

max. 7.5% in dw



cyanophycin exclusively in amyloplasts

(Hühns et al., accepted)

Experimental release of two independent clones of PsbY-*cph*A 2006-2012

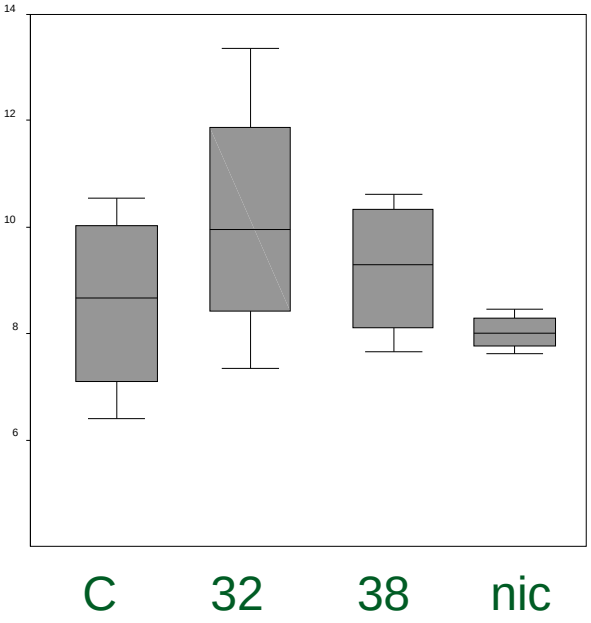
July

August

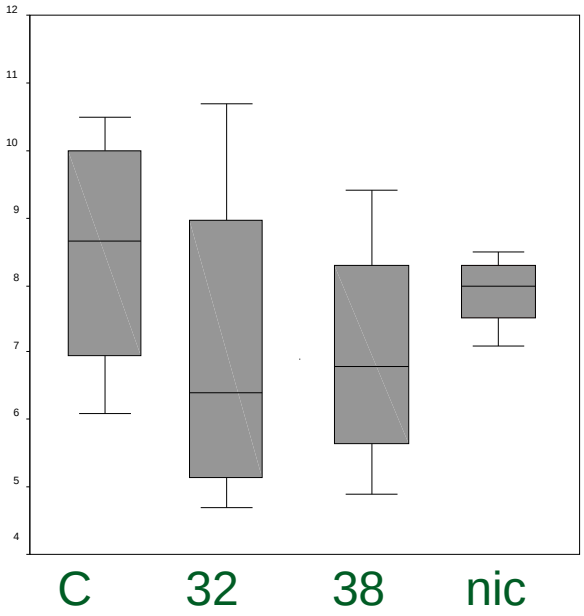
September

October

Influence cyanophycin production on protein content



Protein content with cyanophycin



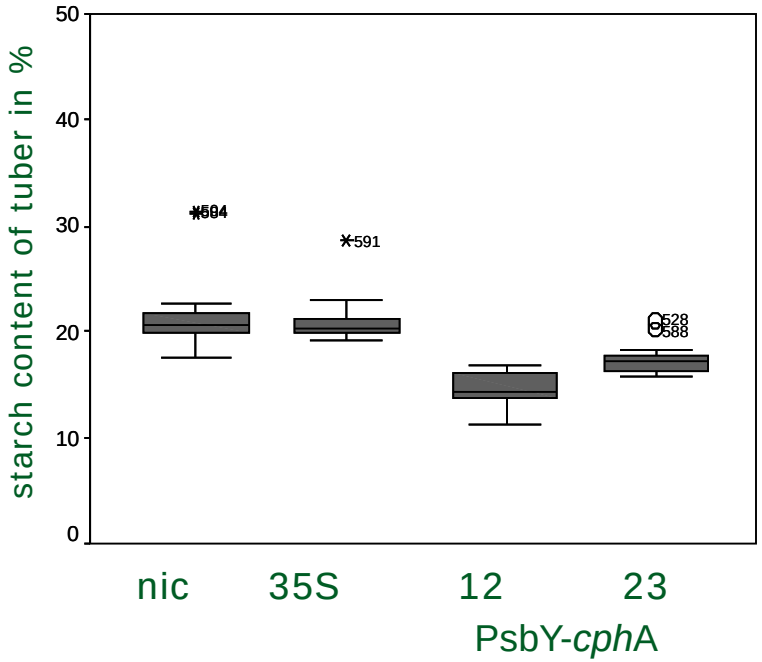
Protein content without cyanophycin

Total protein content including cyanophycin increased in transgenic tubers, while total protein content measured without cyanophycin was slightly reduced

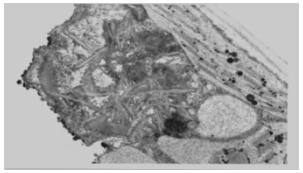
(Hühns et al., accepted)

Influence cyanophycin accumulation on starch content

From field grown tubers



Cyanophycin accumulation in tobacco Petit Havanna SRI

construct	Localization in plant	max. cyanophycin content in % dw	phenotype
p35S *cph*A t35S **p35S-*cph*A**	cytoplasm 	1.0 (no progeny)	
p35S TP *cph*A t35S **pRieske-*cph*A**	cytoplasm	0.2	
pFNR-*cph*A	cytoplasm	1.0	
pCP24-*cph*A	cytoplasm	1.3	
pPsbY-*cph*A	plastids 	**1.7**, in progeny up to **6.8 %**	

(Neumann et al., 2005; Hühns et al., 2008)

Cyanophycin production in commercial tobacco varieties

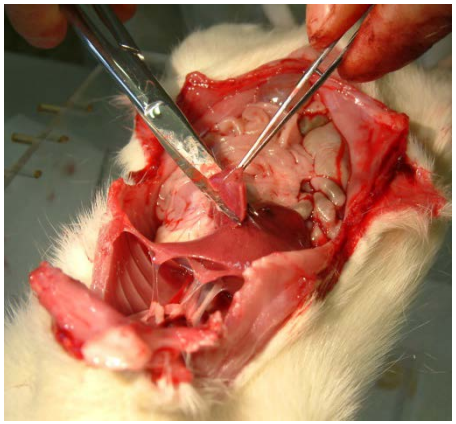
SRI

(6,8% dw)

Badischer
Geudertheimer

?

Toxic potential

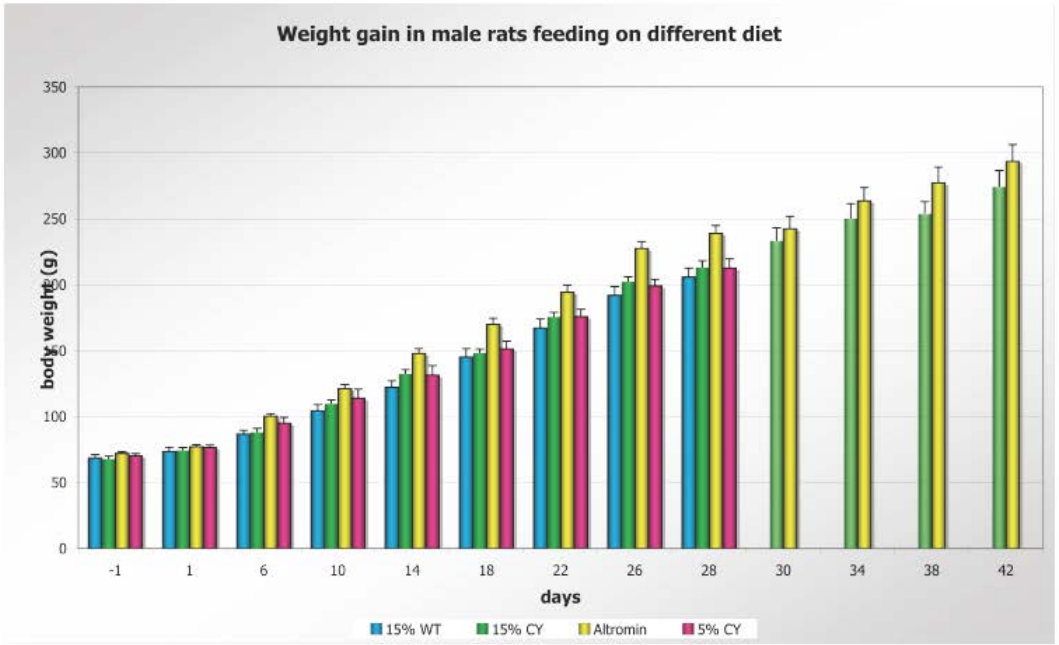


Primacyt GmbH

Bioserv GmbH

1. Feeding for 26 and 90 days with 15% freeze dryed potatoes in the diet:

   no negative effect on health

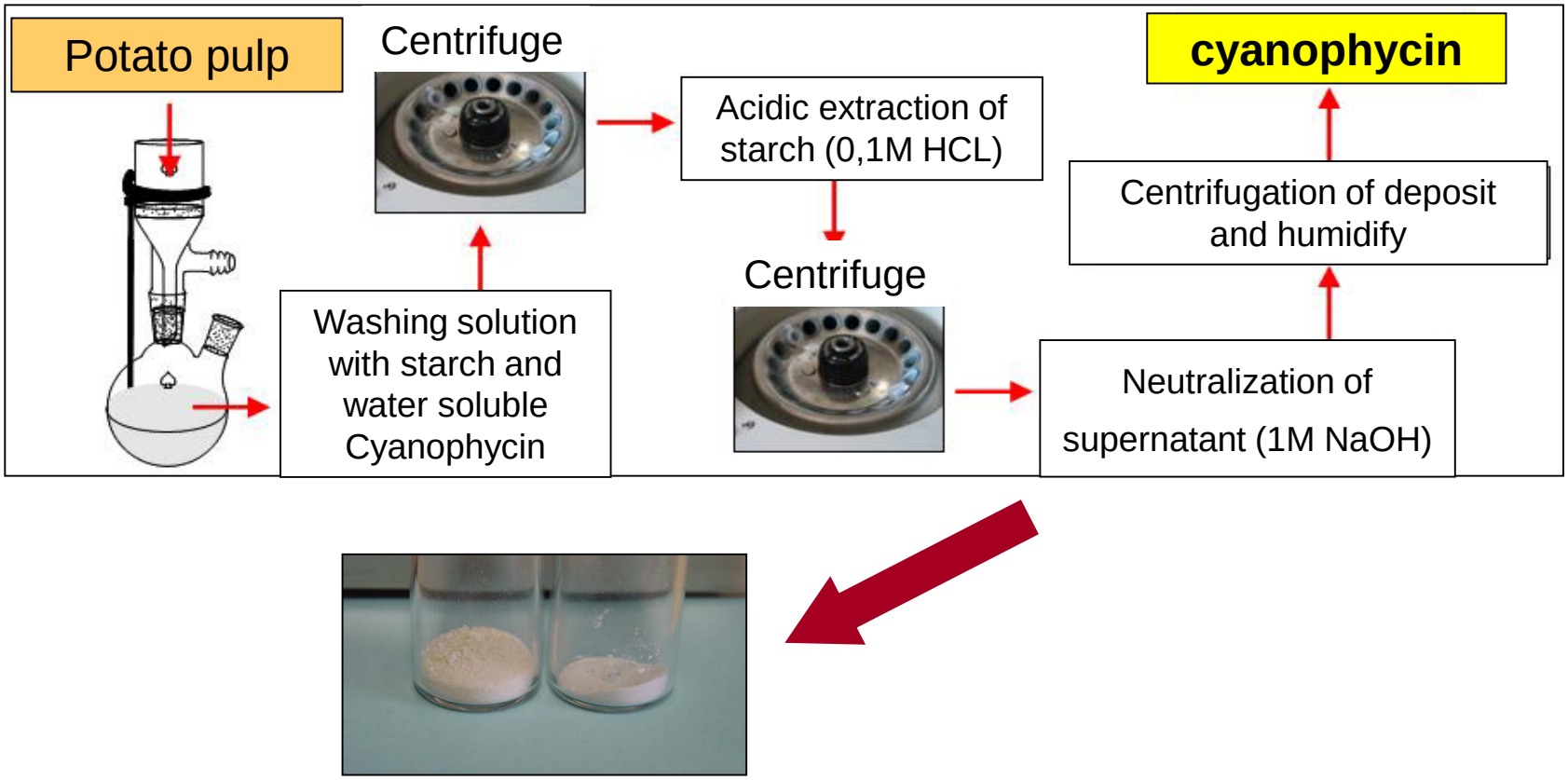


Bioserv AG

Allergeniv potential

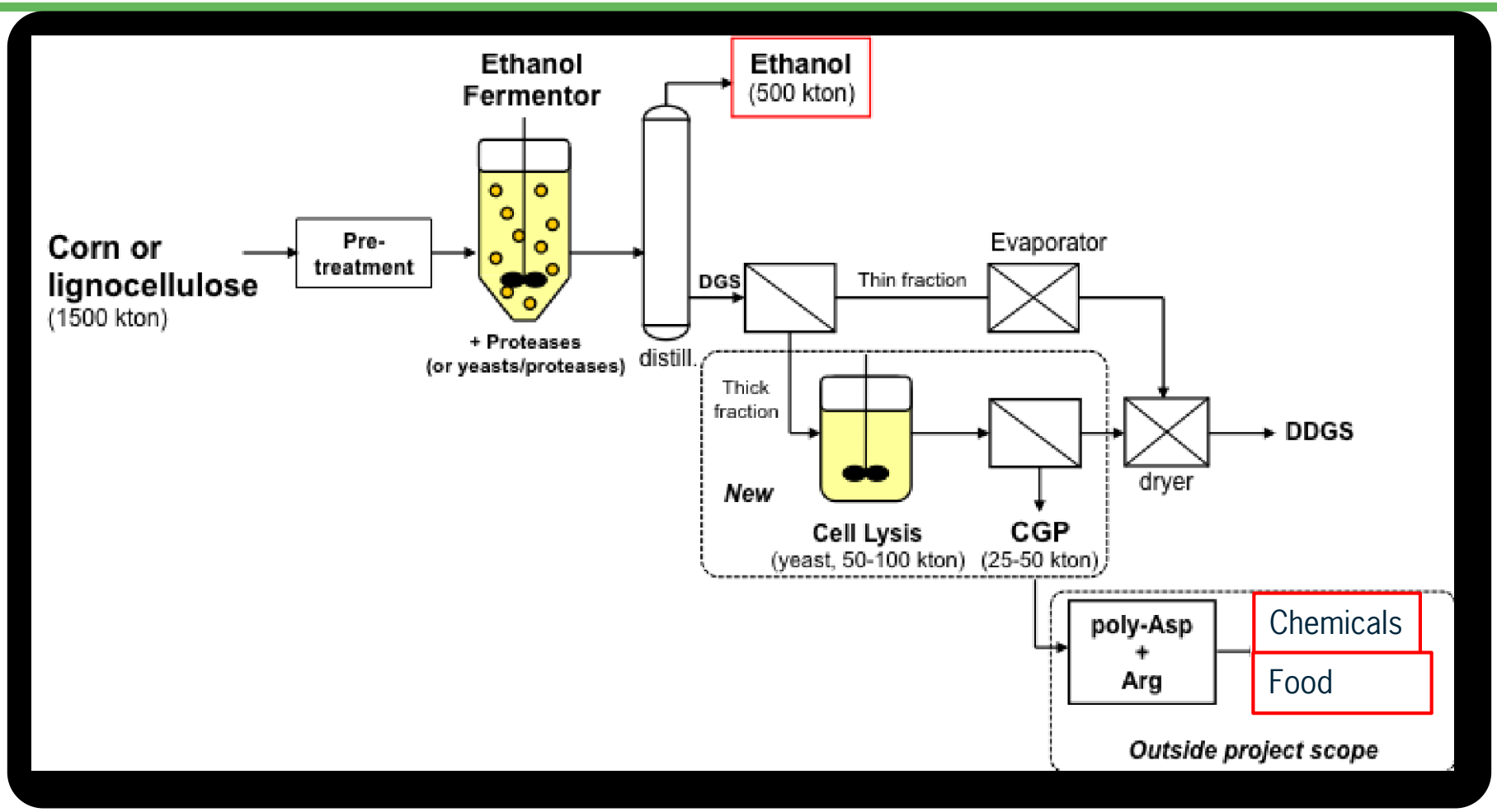
Test	Species	Duration	Result
Irritation	Rabbit	1 week	No changes
Subacute Toxicity	NMRI-mice	4 weeks	No changes
Chronical Toxicity	NMRI-mice	365 days	No changes
Delayed allergy (Type IV)	Guiney pig	33 days	No changes
Immediate allergy (oral application)	Brown Norway rats	45 days	No changes
Immediate allergy (systemic application)	Balb/c mice	45 days	No changes

Isolation of cyanophycin from plants



90% pure cyanophycin from plants

Cyanophycin: Hardly additional capital costs



Conclusion

Cyanophycin is promising as nitrogen (Arg, Asp) source for the feed industry

Cyanophycin is a promising source for biomaterials (nylon, poly-Asp

Cyanophycin can be produced in potato and tobacco

Cyanophycin can be produced in plants up to more than 9% dw

Production in leaves is higher but organ-directed synthesis is possible

Very high cyanophycin content in potato leads to:

- Slightly reduced yield
- Slightly reduced starch content
- No toxic and allergenic effects

Cyanophycin can be isolated in a relatively easy process parallel to starch up to 80 % purity.

Large-scale isolation machinery (Grassa) is available

Contact details

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