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# Towards Sustainable Nylon-6: Fermentative Production of 6-Aminocaproic Acid

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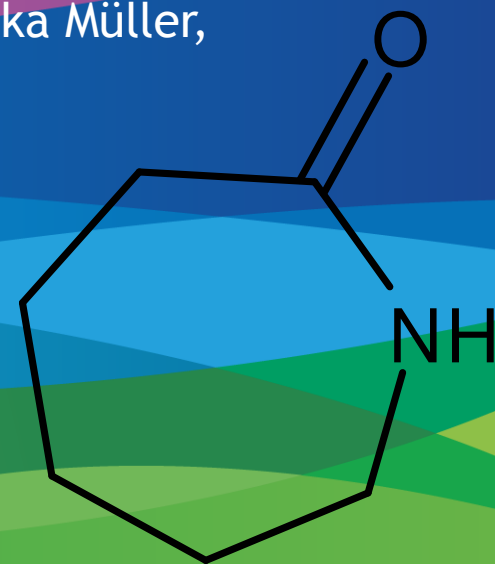
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# Towards sustainable Nylon-6: Fermentative production of 6-aminocaproic acid

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June 7, 2012

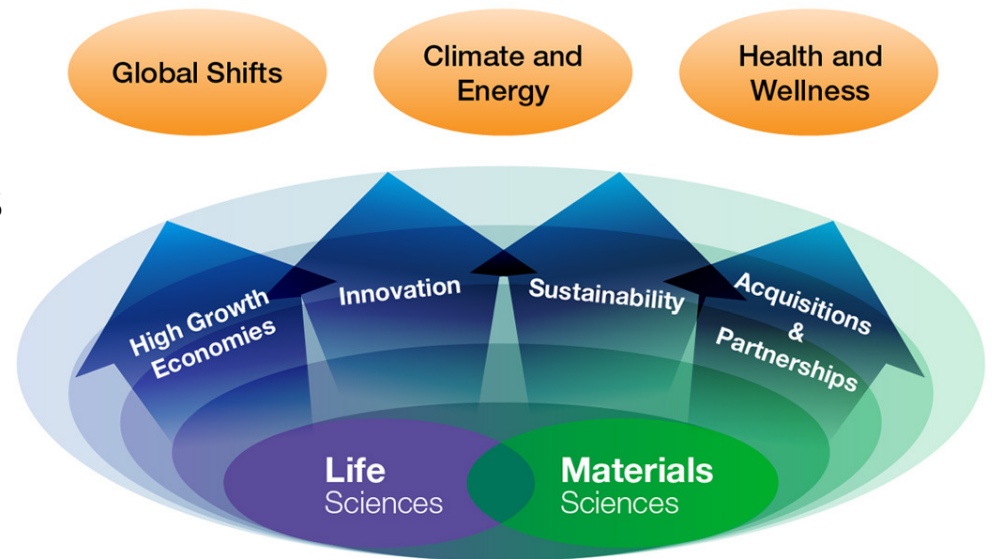


# Overview

- DSM Company Overview
- Caprolactam: What is it?
- Route Scouting
- Enzyme Discovery
- Strain Development
- Acknowledgements

# DSM, the Life Sciences and Materials Sciences company

- Some 22, 000 employees
- Net sales 2011: € 9.0 billion
- 4 drivers, directed to global trends
- Strong, solid balance sheet
- Leader in Dow Jones Sustainability Index 2011

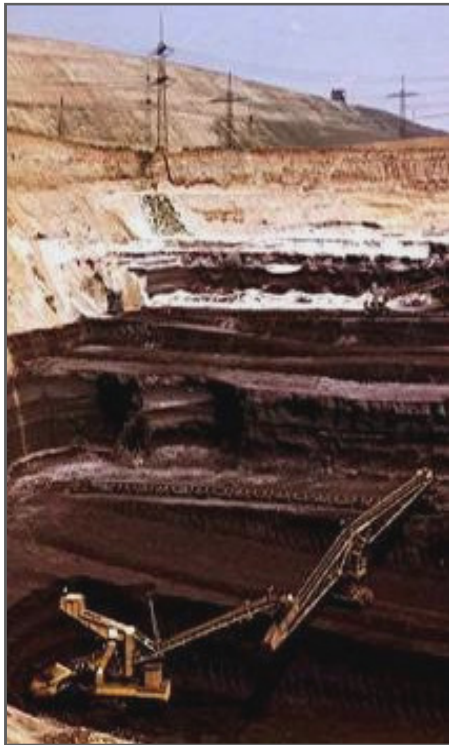


# DSM sales offices and production sites



# Where did it start... and what are we heading for?

Need for new and sustainable energy and material resources



Coal



Oil



Biomass

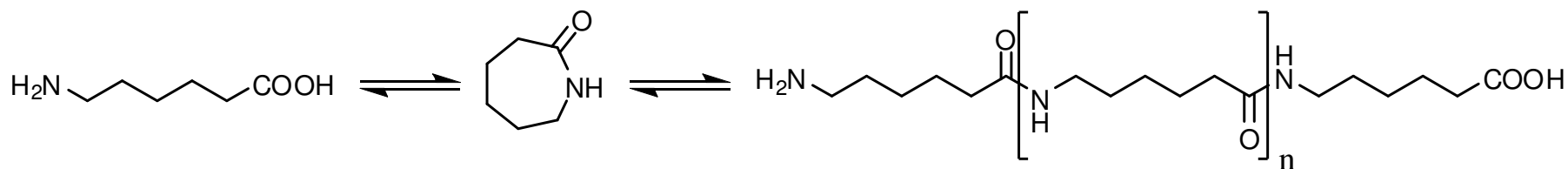
First generation **Renewable**

**Renewable**

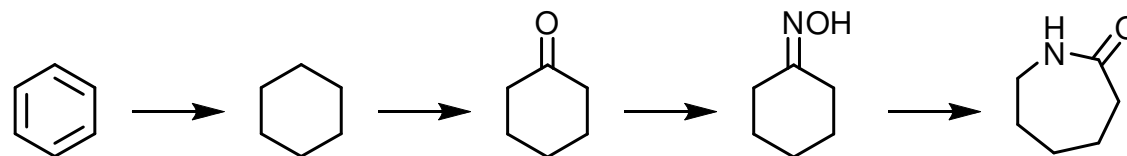


# Caprolactam

- Lactam of 6-amino-caproic acid
- Monomer for production of nylon 6



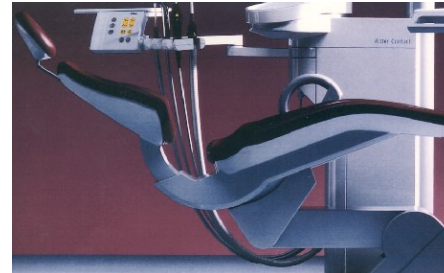
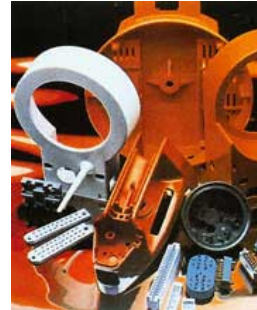
- Currently part of Benzene value chain



- Does not occur in nature



# Caprolactam for Nylon-6 Applications





# Caprolactam Market Info

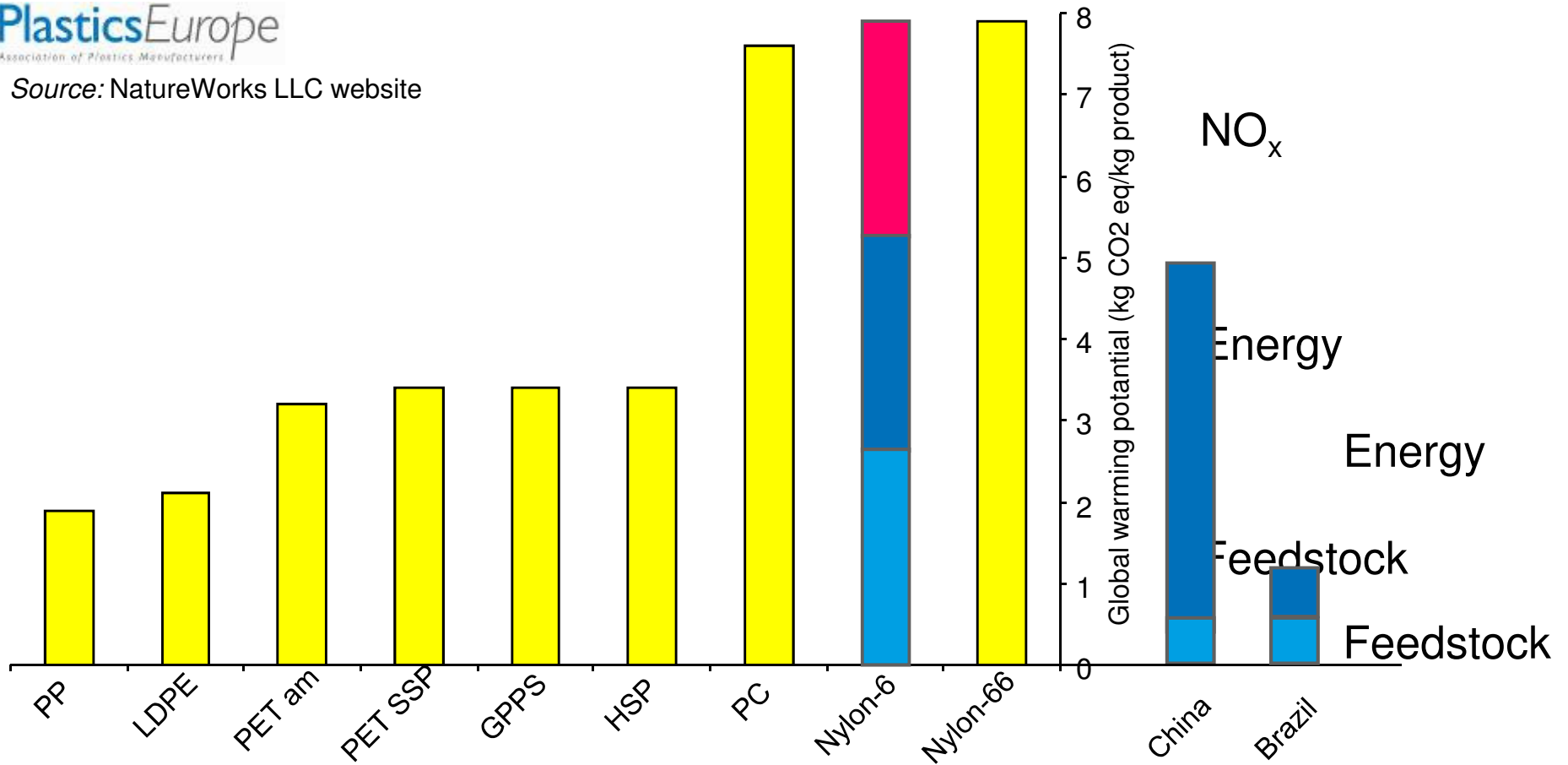
- World caprolactam market size: 4 Mtpa
- DSM largest merchant seller, 2nd largest producer
- Only global producer (NL, US, CN)
- DSM technology leader, 40% of Caprolactam is made using DSM technology



# How “green” is caprolactam / nylon-6?

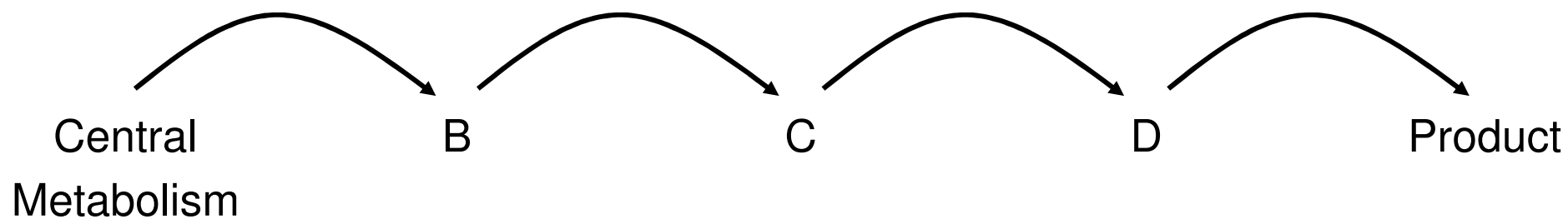
PlasticsEurope  
Association of Plastics Manufacturers

Source: NatureWorks LLC website



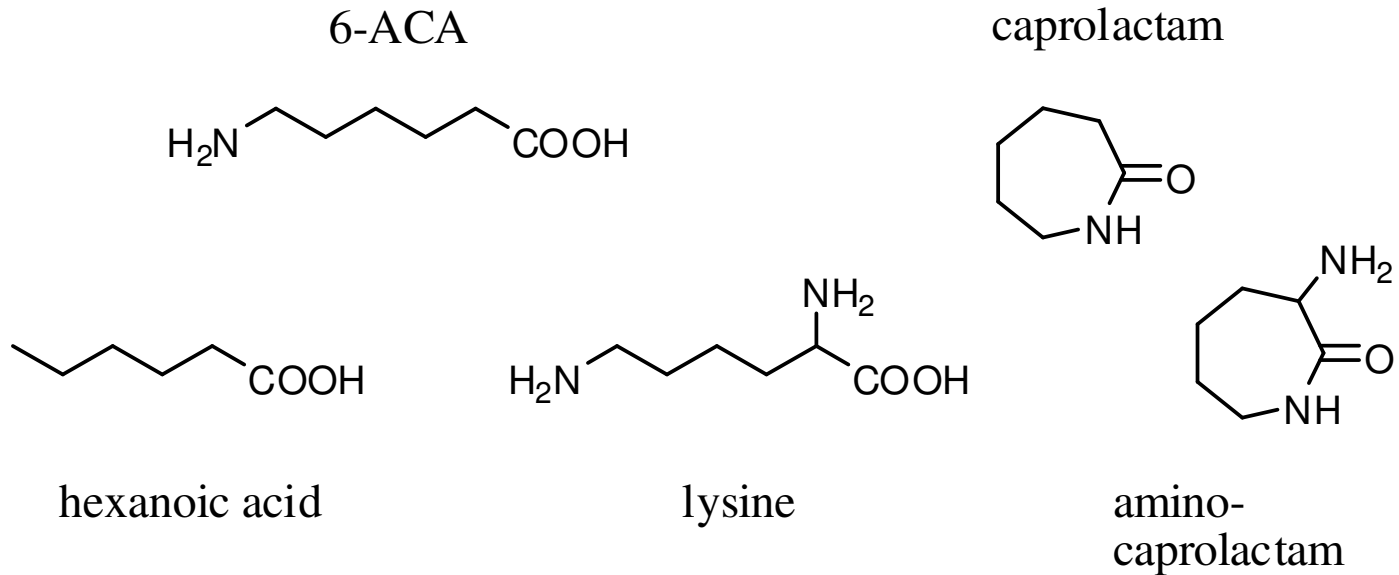
- Production of Caprolactam by fermentation has significant potential to improve sustainability of the nylon-6 value chain
- DSM has embarked on a project to develop a fermentation process that is based on sustainable raw materials

# Route Scouting



# Identification of Biochemical Route

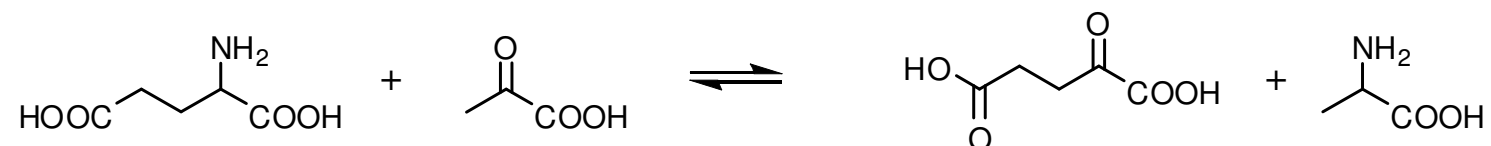
- Identify close natural metabolites



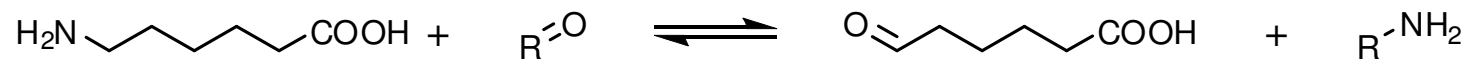
- Microbial metabolites contain too much or too little N

## Solving the N-problem

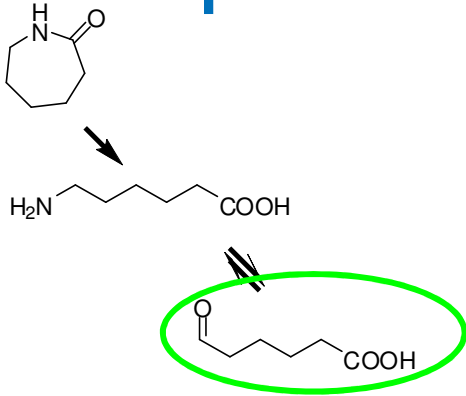
- Transamination is most common way to add/ remove N



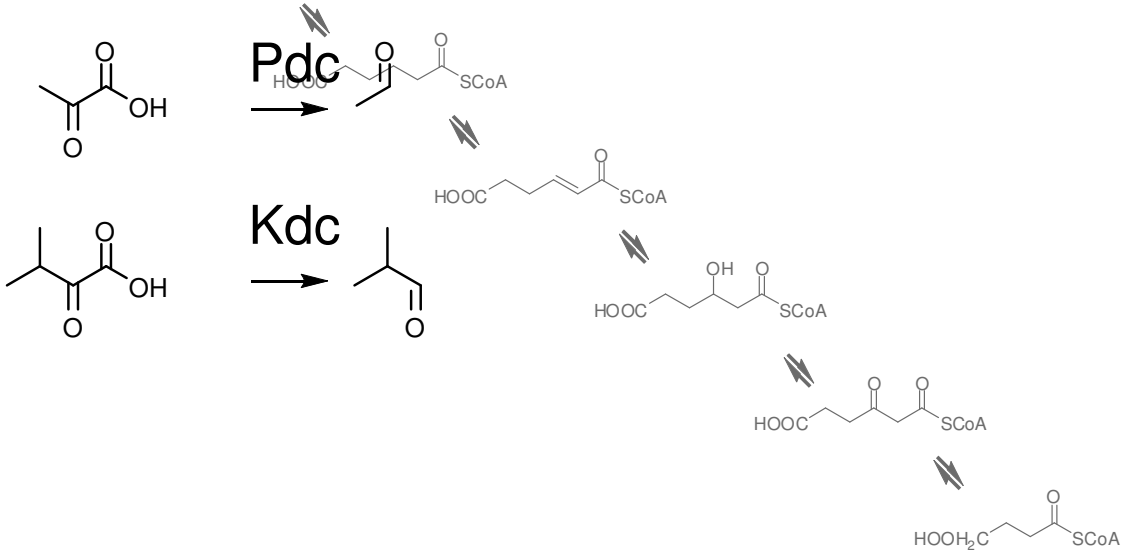
- Transamination of 6-ACA is known from caprolactam degradation



# Caprolactam Degradation as Inspiration: AKP-route



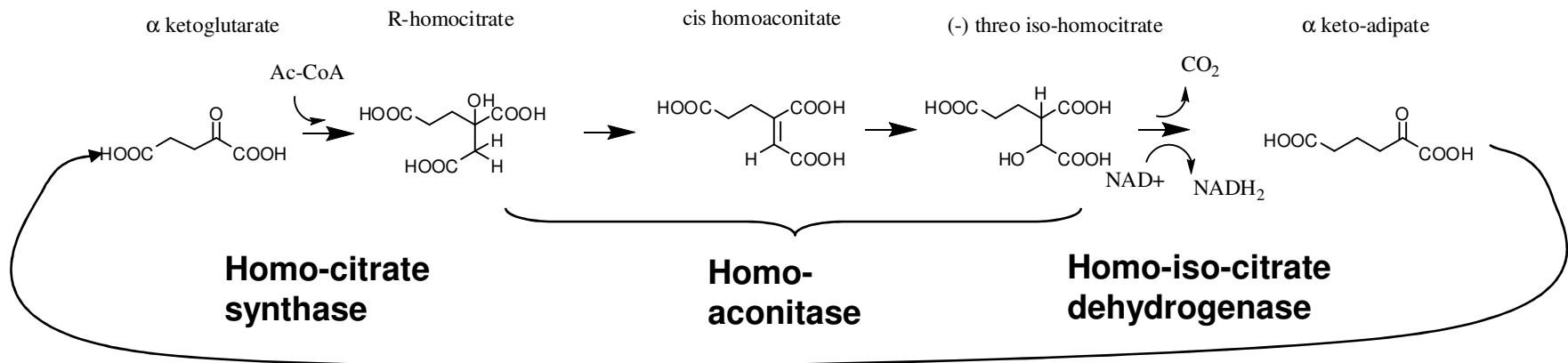
Decarboxylase





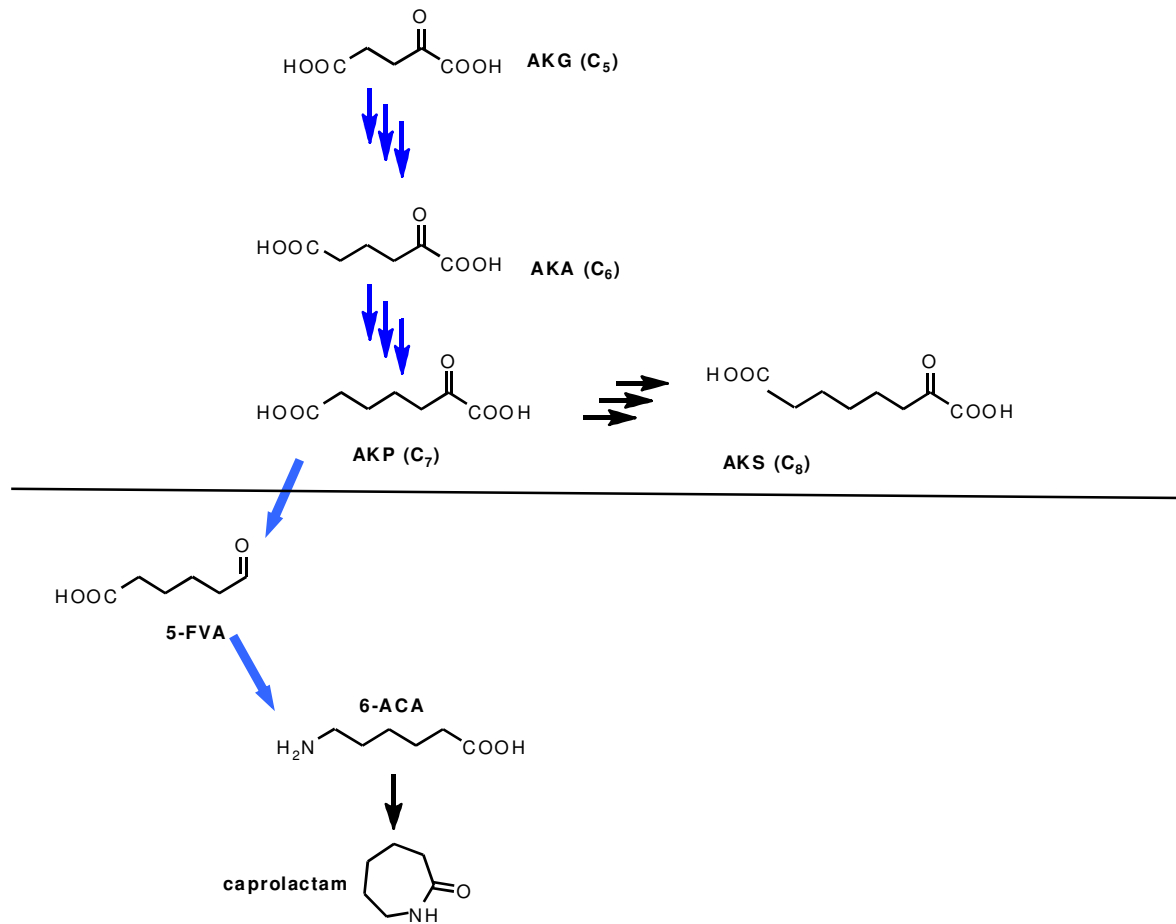
# How to make $\alpha$ -keto pimelate (AKP)

- AKP only present in methanogens
- Intermediate in  $C_1$ -elongation from  $\alpha$ -keto glutarate to  $\alpha$ -keto suberate in the pathway for coenzymeB biosynthesis

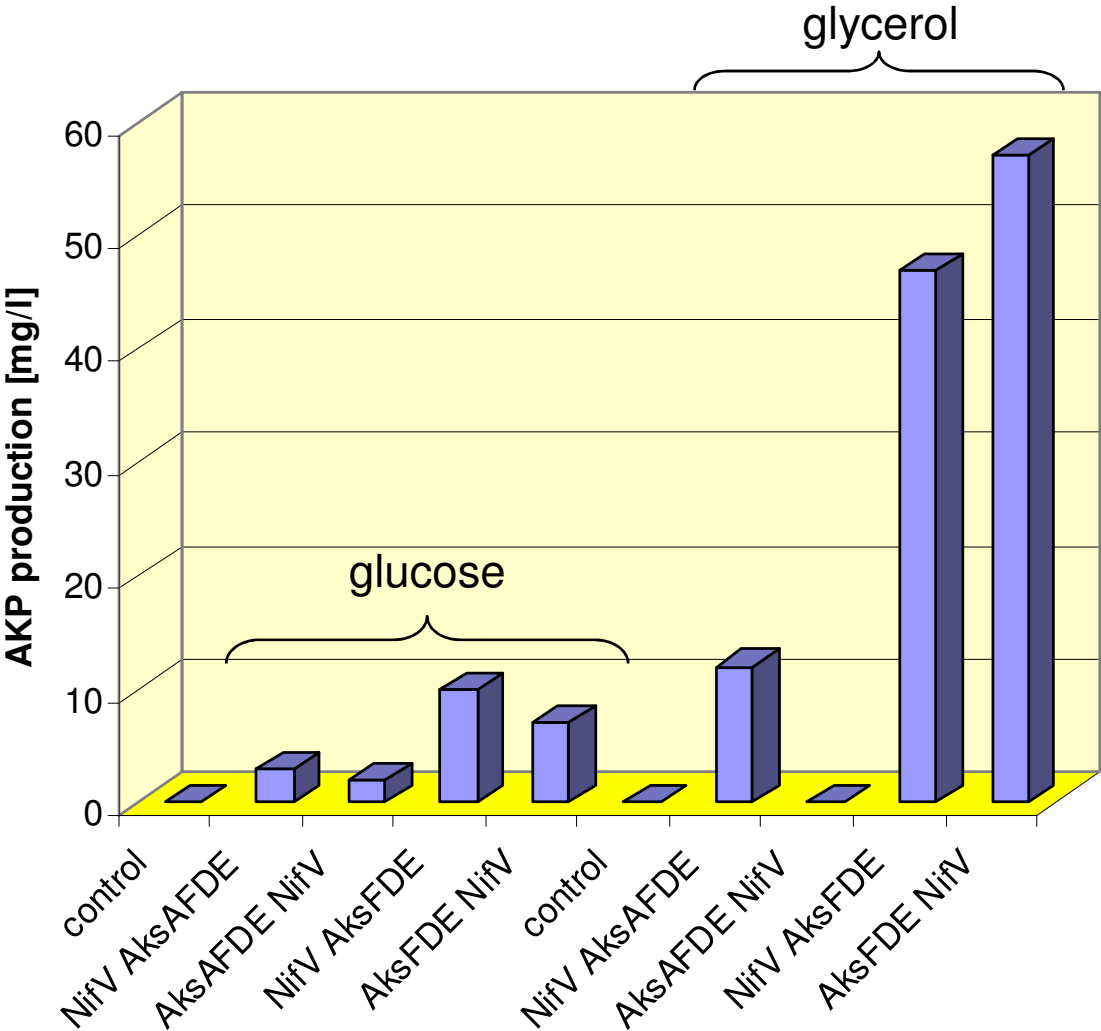


- Characterized in *Methanocaldococcus jannashii*
- Proceeds in 3 cycles from AKG via AKA and AKP to AKS
- N-fixing bacteria have homocitrate synthase specific for AKG and AKA (e.g. NifV from *Azotobacter vinelandii*)

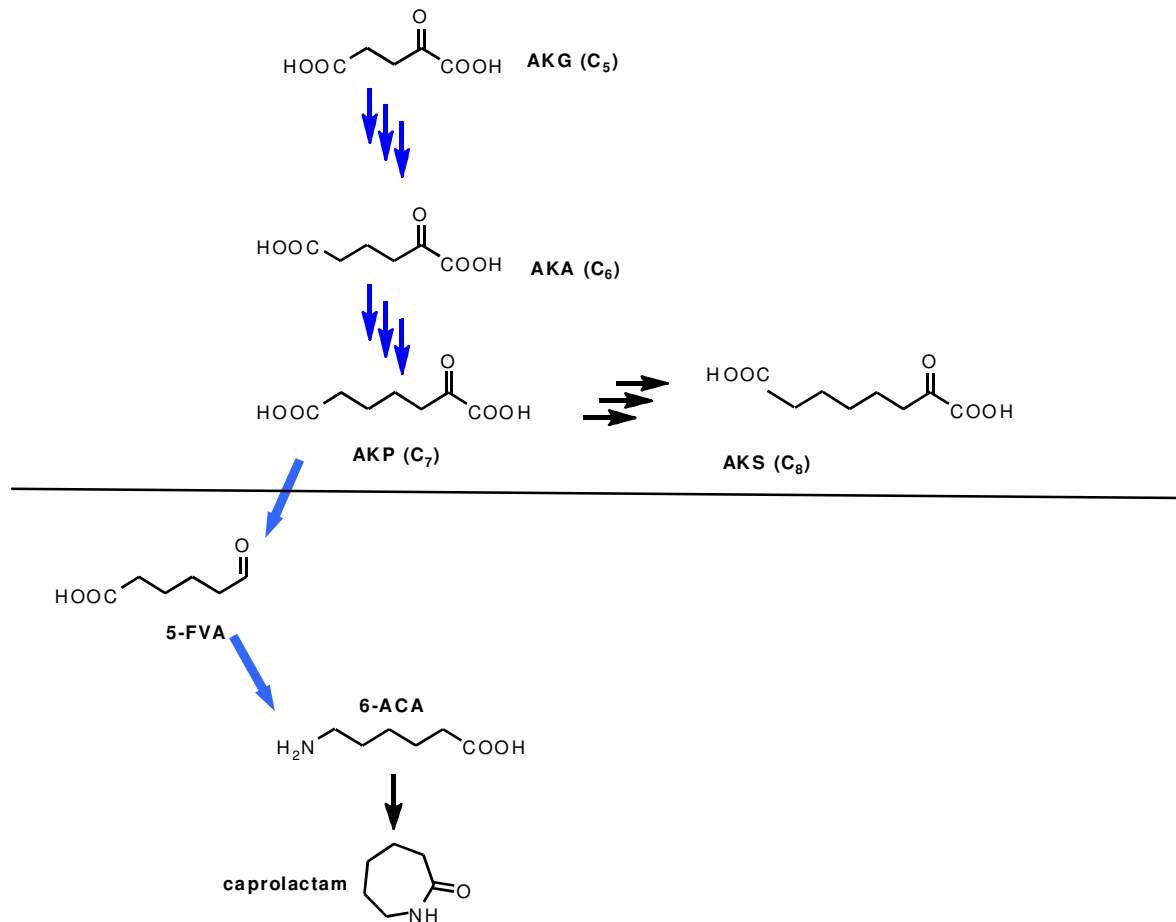
# the “AKP” - pathway



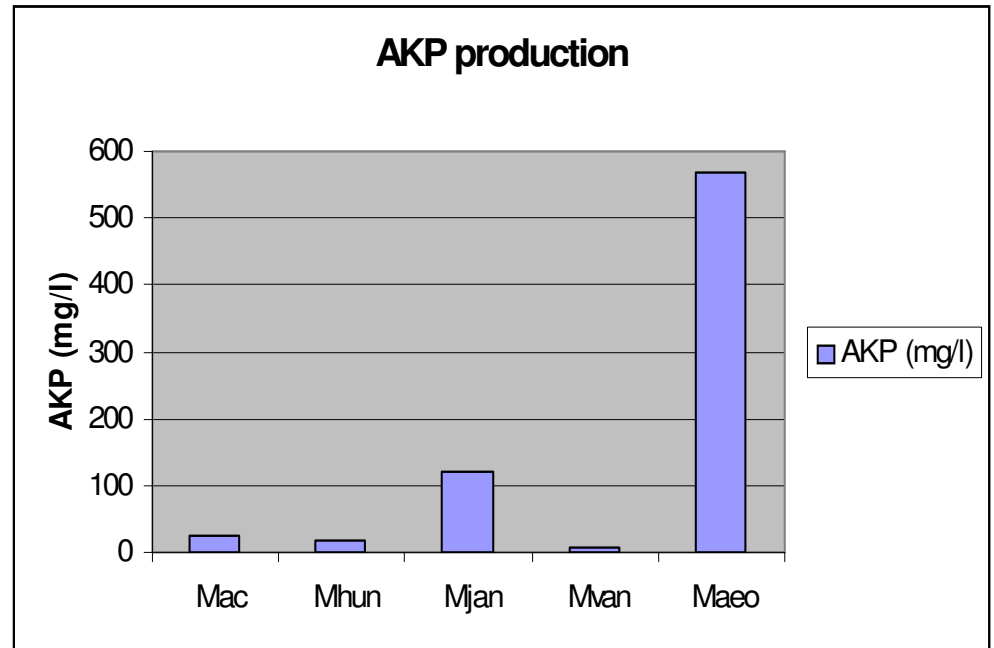
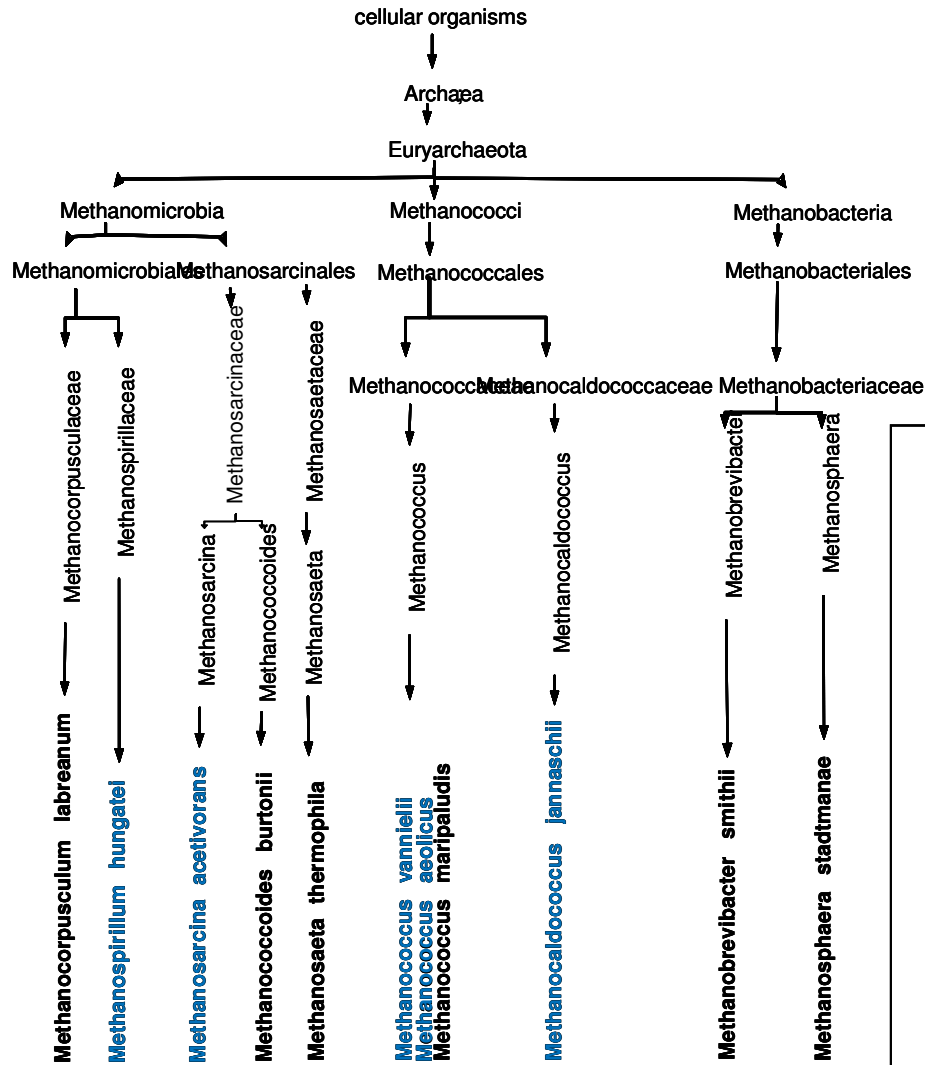
# AKP production in *E. coli*



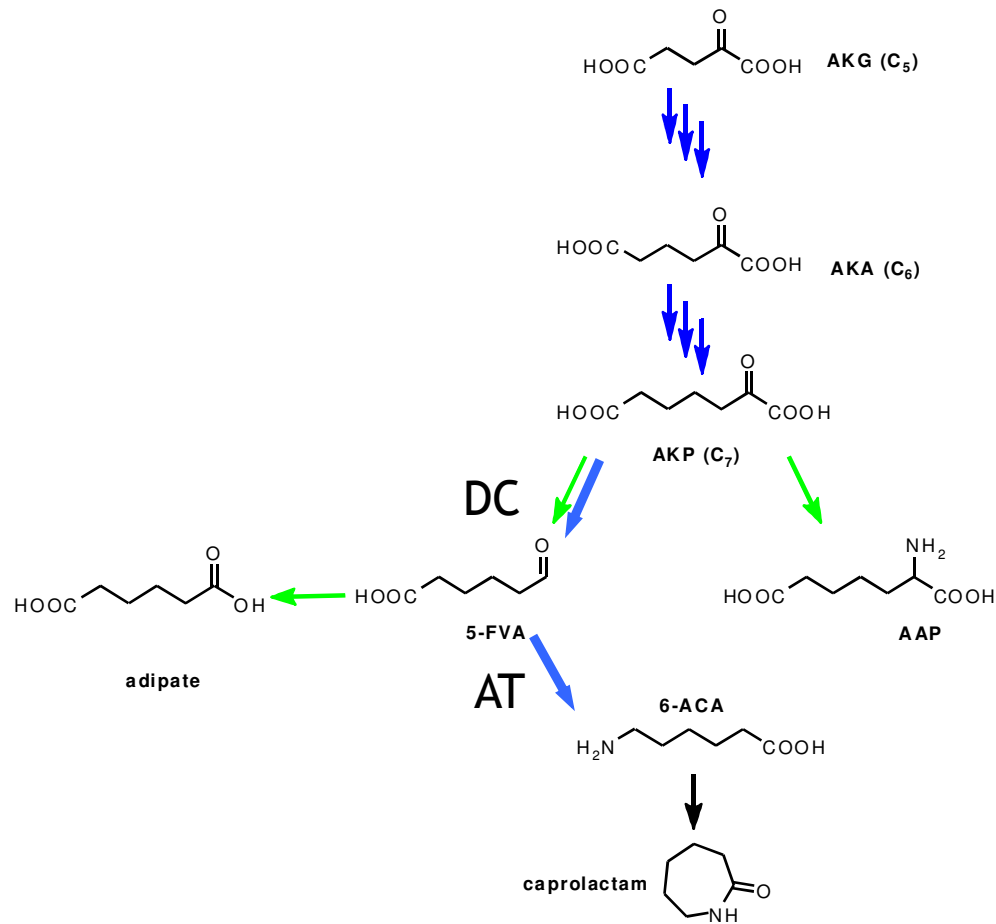
# the “AKP” - pathway





# Aks genes from various archaee bacteria

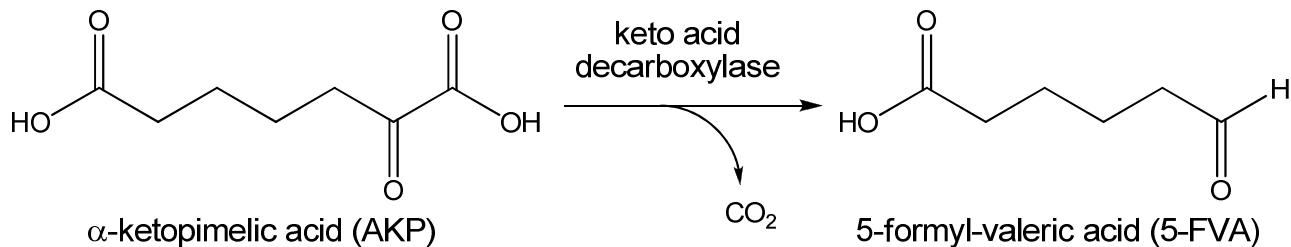


# the “AKP” - pathway



 Introduced genes  
 Endogenous *E. coli* enzymes

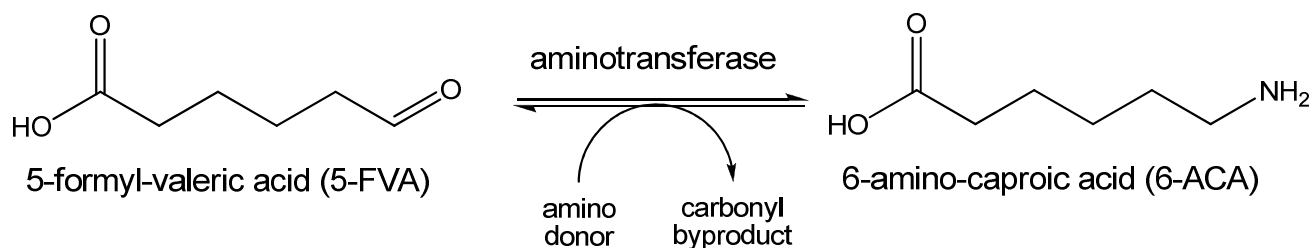
# Identification of AKP Decarboxylases



- Identification of suitable decarboxylases in databases
- Cloned and tested candidates in AKP decarboxylation

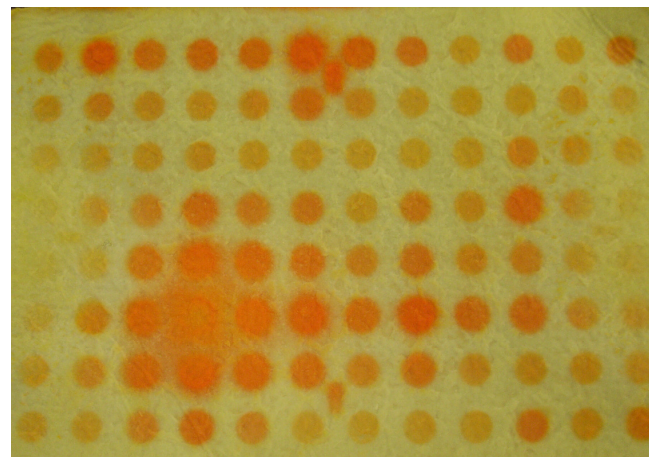
Biocatalyst	5-FVA concentration [mg/l]		
	3 h	18 h	48 h
LysA ( <i>E. coli</i> )	150	590	720
PDC 1472A ( <i>S. cerevisiae</i> )	2000	2000	1600
KdcA ( <i>L. lactis</i> )	3300	2300	2200
KivD ( <i>L. lactis</i> )	820	1400	1500
control	n.d.	n.d.	n.d.

# Identification of 5-FVA aminotransferase



- Screening proprietary DSM aminotransferase platform
- Primary screening with sensitive colour assay identified numerous hits
- Confirmation of hits with LC/MS

Biocatalyst	6-ACA [mg/l]
<i>Vibrio fluvialis</i>	43
<i>Pseudomonas aeruginosa</i>	25
<i>Bacillus weihenstephanensis</i>	24
<i>Bacillus subtilis</i>	6
<i>Pseudomonas aeruginosa</i>	21
Vector control	0.6
Negative control	n.d.

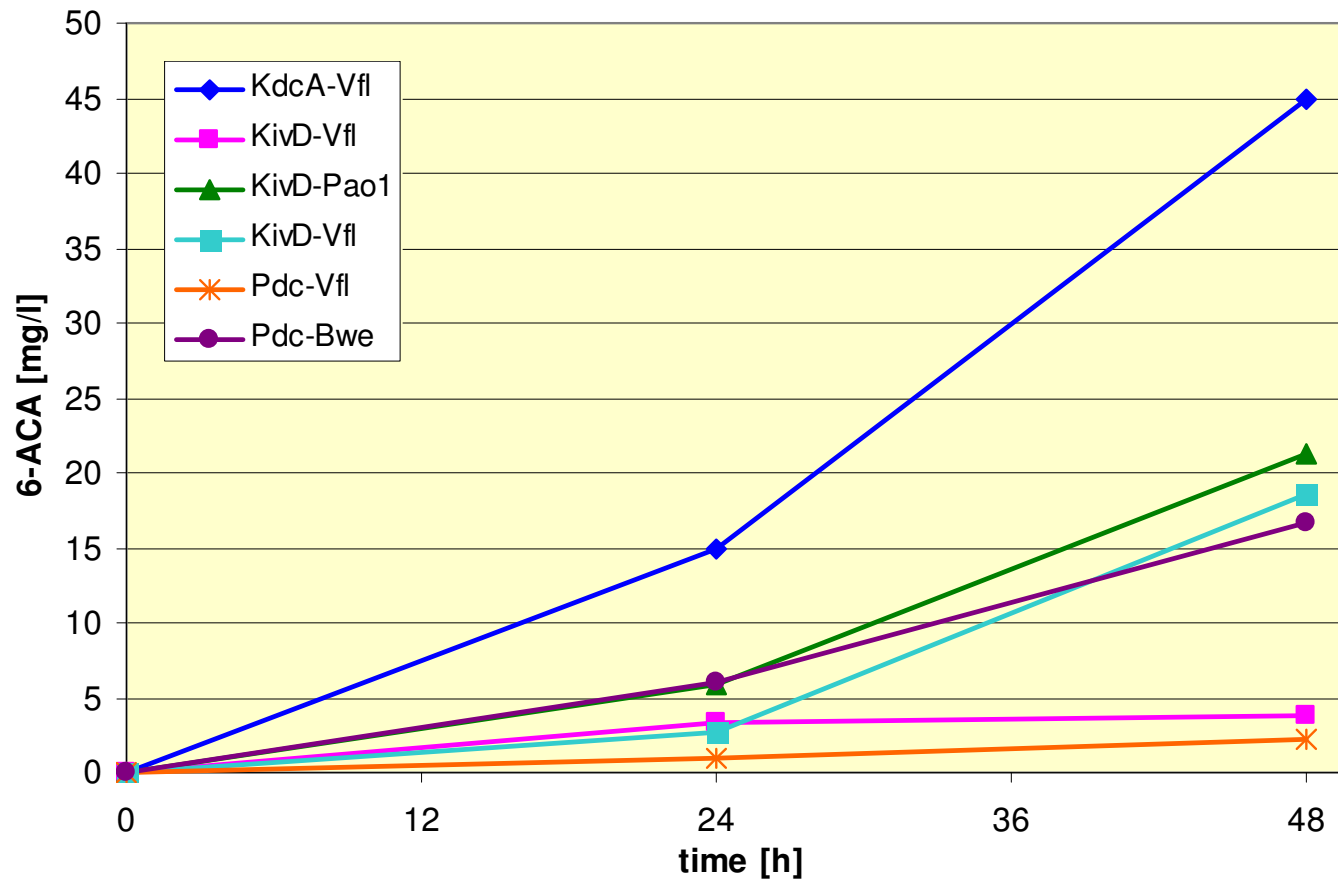


colorimetric assay



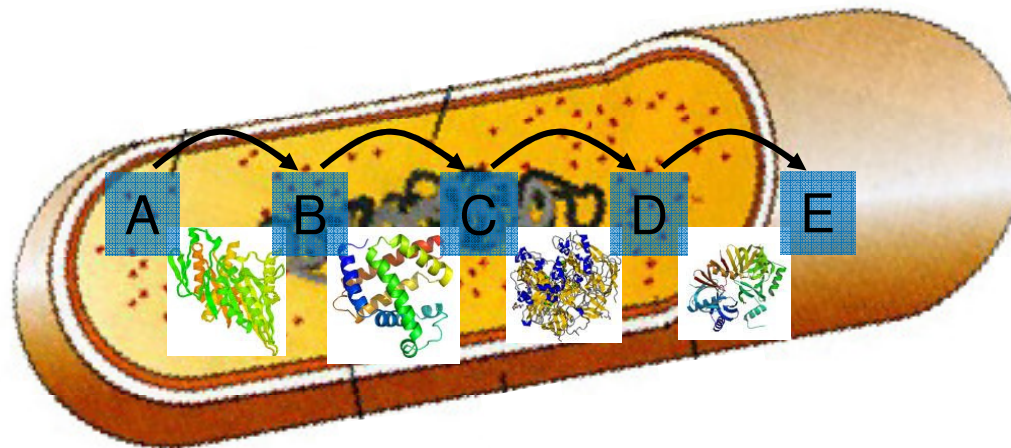
# DC and AT are functional *in-vivo*

- Several combinations tested in AKP-feeding experiments

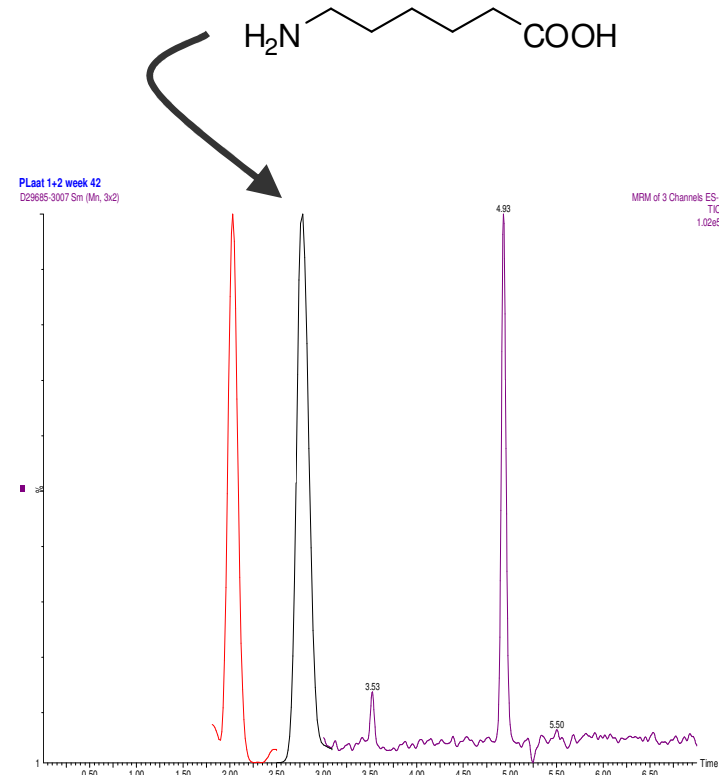
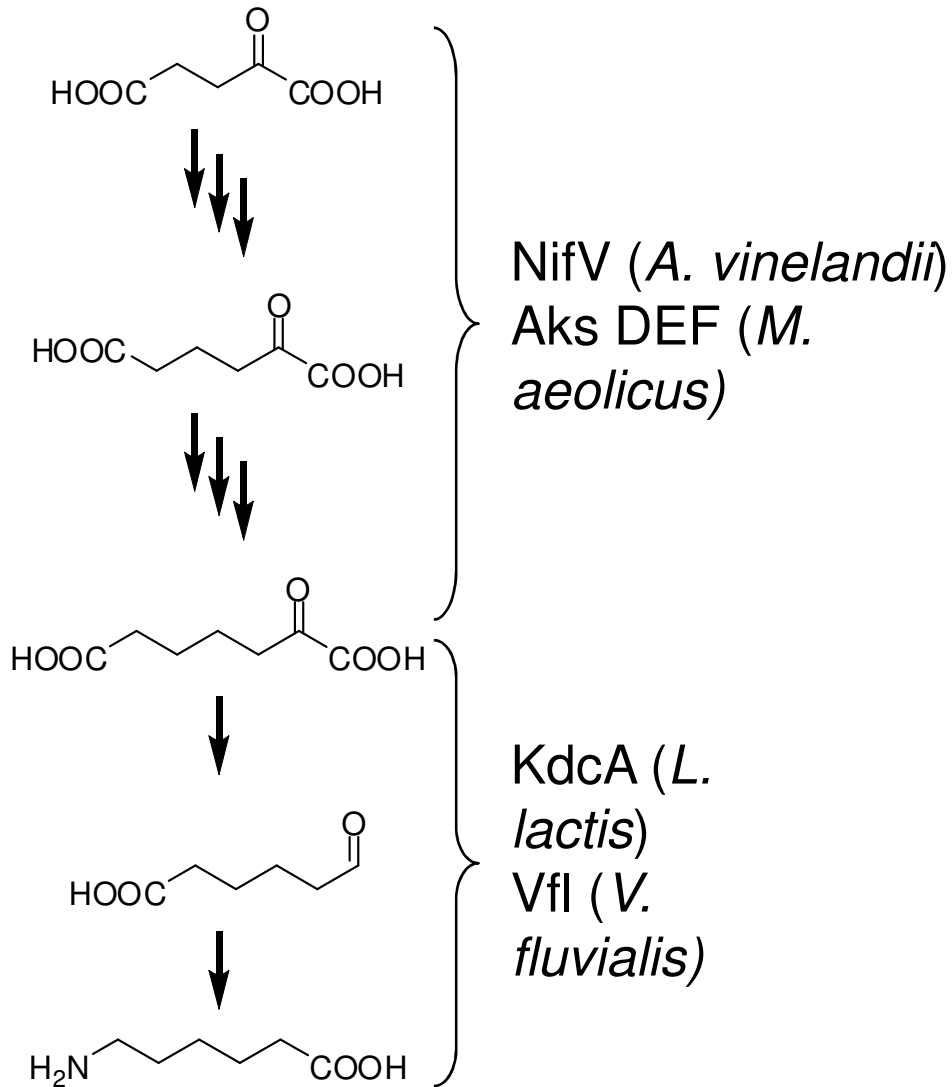


- Best combination (KdcA and Vfl) selected for further work

# Strain Development for 6-ACA production

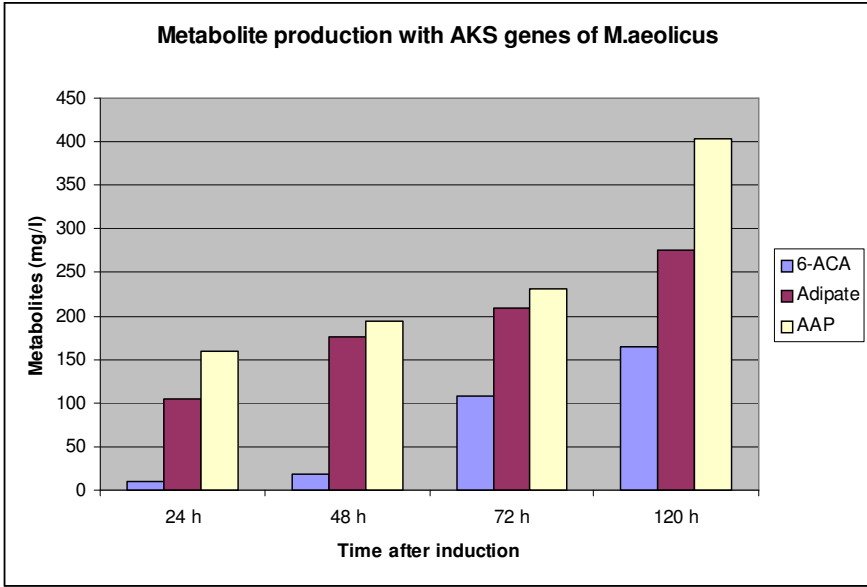
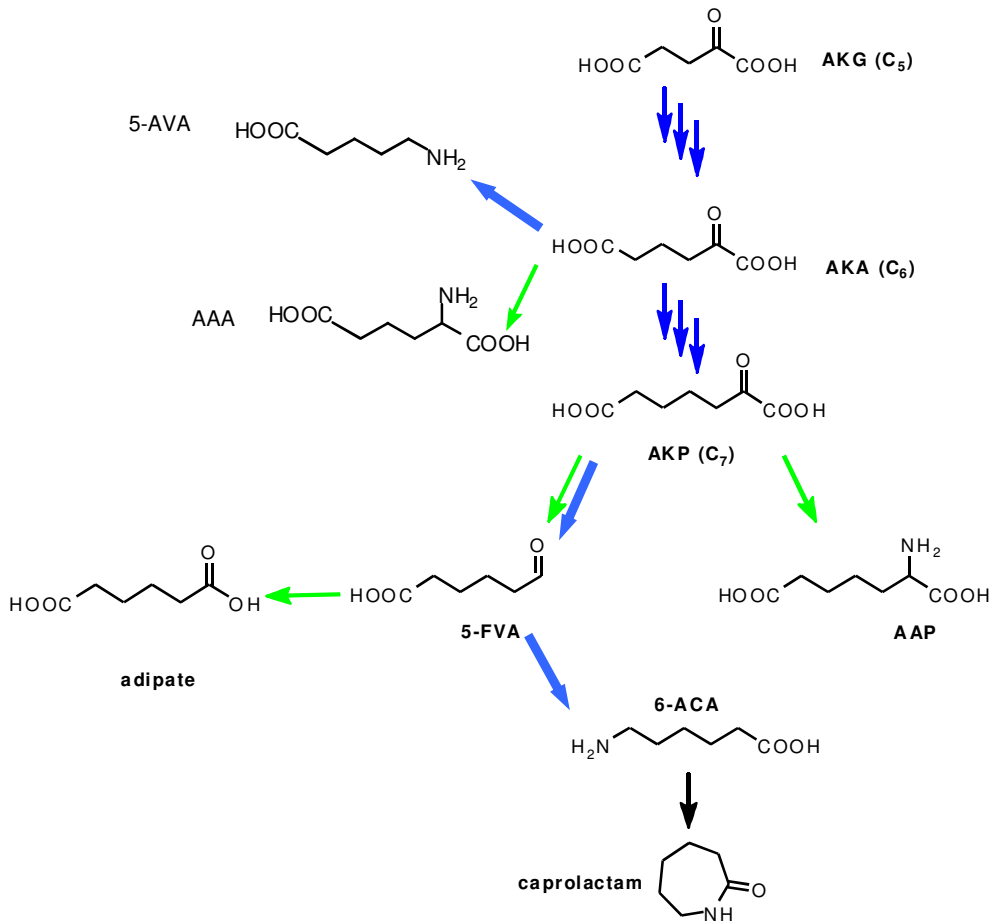


# Production of 6-ACA in *E. coli*



- Observed  $M+H^+$ : 132.09
- Identical to chemical standard

# Production of 6-ACA in *E. coli*



Introduced genes

Endogenous *E.coli* enzymes



# Summary

- Fermentative production can improve sustainability
  - Identified enzymes for production of AKP
  - Demonstrated first fermentative production of 6-ACA and 5-AVA in a microbial host
  - Fermentative production of Adipate, AAP aan AAA
- 
- Next to 6-ACA, 5-AVA was also produced from AKA indicating that this pathway can also be used for the production of compounds with another chain length, such as 7-amino suberate a building block for nylon 7 which is currently not produced at large scale, but is expected to have interesting properties.

# Outlook



Traditional CAP production Today



Traditional CAP production Future

# Acknowledgements

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- Michael Kuczynski

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- Marco Molling (Vereenigde)

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- Emile van de Sandt
- Marcel Wubbolts
- Roel Bovenberg
- John Perkins
- Herman Pel
- Oliver May
- Bernard Kaptein
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- Ronald Gebhard
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