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Fermentation and enzymology as tool for functional whey ingredients/products

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Sweet cheese whey
(components in g L-1)

Water
(938-943)

pH 5.9-6.6

Total Solids
(57-62)

Carbohydrates (47)
-Lactose (47)

Milk Fat (0.5)
- Triglycerides (0.25)
- Phopholipids (0.15)
- Diglycerids (0.05)
- Free Fatty Acids (0.05)

Proteins (6.2)
- *b*-Lactoglobulin (3.0)
- *a*-Lactalbumin (1.2)
- Immunoglobulin-G (0.7)
- Proteose Peptone (0.6)
- Serum Albumin (0.4)
- Other Protein (0.3)
  # Immunoglobulin-A (0.05)
  # Lactoferrin (0.045)
  # Lactoperoxidase (0.025)
  # Lysozyme (0.002)

Minerals (4.5)
- Potassium (1.5)
- Chloride (1.1)
- Phosphorous (0.7)
- Calcium (0.6)
- Sodium (0.5)
- Magnesium (0.1)
- Trace Elements (0.03)
  # Zinc (0.0015)
  # Iron (0.0006)
  # Iodine (0.0005)
  # Copper (0.0002)

NPN (0.132)
- Urea (0.08)
- Free Amino Acids (0.025)
- Cholin (0.015)
- Orotic Acid (0.012)

Vitamins (0.075)
- Vitamin B5 (0.004)
- Vitamin B2 (0.0015)
- Vitamin C (0.0015)
- Vitamin B6 (0.0005)

Fermentation using whey

Comparison: typical chemically defined medium

component	amount/L
glucose	10 g
NH_4Cl	1 g
K_2HPO_4	0.5 g
$MgSO_4 \cdot 7\ H_2O$	0.2 g
$FeSO_4 \cdot 7\ H_2O$	0.01 g
$CaCl_2 \cdot 2\ H_2O$	0.01 g
NaCl	0.5 g
trace element solution	1 ml

„molecular formula“ of a bacterial cell :

$C_1\ H_{1.6}\ O_{0.5}\ N_{0.15}\ P_{0.01}\ S_{0.001}$

(elemental analysis)

Whey is an undefined natural complex medium

pasteurization or sterilization (?)

The quality and/or quantity of the **carbon/energy** source and the **nitrogen source** is an issue

- *b*-galactosidase activity
- additional nitrogen source(s)

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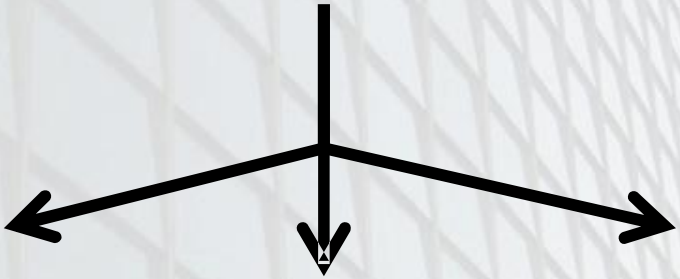
WP as fermentation medium

Processed whey concentrates (deproteinized)



sophorose lipid

Single cell protein (SCP)

- yeasts (GRAS)
  *K. marxianus**
  $Y_{X/S}$ 0.48-0.52
- feed
- (food)

Single cell oil (SCO)

- yeasts, fungi
  Cryptococcus curvatus
- triglycerides (27 g L^{-1})
  - è N-limitation
  - è unsaturated FA
    (ca. 50% oleic acid)
  - è possible use: biodiesel

Biosurfactants (SL)

- yeasts
  Candida boidinii
- biotensid (420 g L-1)
  - è on cell debris from
    C. curvatus

Syldatk, C. (2006) *Lecture Stuttgart*
*Schultz et al. (2006) *AMB* 69, 515

Lactic acid bacteria and whey

Constituent[b]	Final concn (mM)[a]	
	MPL_{ox}[c]	MPL (amt/liter)
Lactose	58.4	58.4 (20 g)
Sodium thioglycolate*	4.38	4.38 (0.5 g)
Tween 80* (% vol/vol)	0.1	0.1 (1 g)
$K_2HPO_4 \cdot 3H_2O$*	7	
Na_2HPO_4		2.11 (0.3 g)
$NaH_2PO_4 \cdot H_2O$		5.8 (0.8 g)
Potassium acetate*	15	75 (7.35 g)
Ammonium citrate*	10	
Orotic acid*	3.2	
Pyruvic acid*	0.9	
Formic acid*	0.2	
Oleic acid*	0.033	
NaCl	10	
$CaCl_2$*	5	2.5 (0.27 g)
$MgSO_4 \cdot 7H_2O$*	0.8[d]	0.8 (0.2 g)
$MnSO_4 \cdot H_2O$	0.12[e]	0.12 (0.02 g)
$FeSO_4 \cdot 7H_2O$ (μM)	70[f]	0.23 (0.066 mg)
Micronutrients* (μM)		
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.003[g]	0.003 (0.0037 mg)
$CoCl_2 \cdot 6H_2O$	0.03[g]	0.03 (0.007 mg)
H_3Bo_3	0.4[g]	0.4 (0.025 mg)
$CuSO_4$	0.01[g]	0.01 (0.0016 mg)
$ZnSO_4 \cdot 7H_2O$	0.01[g]	0.01 (0.0029 mg)
Bases*		
Adenine	0.37[c]	0.37 (0.05 g)
Guanine	0.33[c]	0.33 (0.05 g)
Xanthine	0.32	0.32 (0.05 g)
Uracil	0.45[c]	0.45 (0.05 g)
2′-Deoxyguanosine*	0.19[c]	
Hypoxanthine*	0.37	
Amino acids		
L-Alanine	2.24[d]	1.12[f] (0.1 g)
L-Arginine	0.29[d]	1.84[f] (0.32 g)
L-Asparagine	1.9[d]	2.27 (0.3 g)
L-Aspartate	7.5	3.75[f] (0.5 g)
L-Cysteine · HCl	2.28[d]	1.6 (0.28 g)
Glycine	2.66[d]	2.13[f] (0.16 g)
L-Glutamate	5.9[d]	2.25[f] (0.38 g)
L-Glutamine	2[f]	2 (0.3 g)
L-Histidine	3.22[d]	1.3[f] (0.2 g)
L-Isoleucine	3.8[d]	2.75[f] (0.36 g)
L-Leucine	1.52[d]	4.57[f] (0.6 g)
L-Lysine · HCl	0.27[d]	2.4[f] (0.44 g)
L-Methionine	3.35[d]	0.8[f] (0.12 g)
L-Phenylalanine	1.21[d]	2.06[f] (0.34 g)
L-Proline	4.7	8[f] (0.92 g)
L-Serine	1.9[d]	3.42[f] (0.36 g)
L-Threonine	0.42[d]	2.52[f] (0.3 g)
L-Tryptophan	1[d]	0.5[f] (0.1 g)
L-Tyrosine	1.1[d]	0.66[f] (0.12 g)
L-Valine	4.27[d]	4[f] (0.48 g)
L-Cystine	0.42[d]	
Vitamins (μM)		
B_{12} (cobalamin)*	7.4[h]	7.4 (0.01 mg)
Riboflavin*	2.66[c]	0.53 (0.2 mg)
Calcium pantothenate*	2[c]	0.2 (0.1 mg)
Niacin*	14[d]	1.4 (0.17 mg)
Folic acid*	0.72[d]	0.072 (0.032 mg)
Pyridoxal*	2.45[c]	0.245 (0.05 mg)
Pyridoxamine*	0.83	
p-Aminobenzoate*	2.92[d]	
Biotin*	0.04[c]	
Thiamine*	3[c]	
L-Ascorbic acid* (mM)	2.8	
Spermine · $6H_2O$*	2.5	

- lactic acid bacteria need a huge number of supplements (auxotrophic)
- typical complex medium: **MRS** (peptone, meat extract, yeast extract, glucose, salts, minerals)
- **production of *L. casei*** using supplemented and diluted, partly deproteinated whey[a] (from powder)
  - è supplements: ammonium salts (2.5 g L^{-1}), yeast extract (0.25 g L^{-1})
  - è biomass yield to lactose: $Y_{X/S}$ =0.165 kg kg^{-1}

by-products: **lactic acid** and **EPS**

Chervaux et al. (2000) *AEM* 66. *Vol. 12*. 5306

[a]Mondragon-Parada et al. (2006) *ABB* 134, 223

Lactic acid production in whey

- production of pure L(+)- or D(-)-lactic acid*
  - 80.000 t anno^{-1}, 90% by LAB fermentation
  - polylactic acid: biodegradable polymers
  - synthon in chemistry
  - *food*: preservative, flavour agent, acidity adjuster

Lactic acid isomers produced by common dairy lactobacilli

Name of bacteria	D(−) Lactic acid	L(+) Lactic acid	DL (±) mixture
L. acidophilus	No	No	Yes
L. delbrueckii subsp. *lactis*	Yes	No	No
L. delbrueckii subsp. *bulgaricus*	Yes	No	No
L. helveticus	No	No	Yes
L. casei	No	Yes	No
L. paracasei subsp. *tolerans*	No	Yes	No
L. paracasei subsp. *paracasei*	No	Yes[a]	No
L. rhamnosus	No	Yes	No
L. plantarum	No	No	Yes
Lc. lactis	No	Yes	No
S. thermophilus	No	Yes	No
Leuconostoc sp.	Yes	No	No

[a] Some strains produce D(−) and L(+) lactic acid (*Source*: Curry an Crow, 2003a).

+ yeast extract

pH 5.5, 37°C, 4 g L^{-1} h^{-1}

continuous production using immobilised cells (entrapment by gels)
--> **productivity 12 g L^{-1} h^{-1}**

*Review see: Panesar et al. 2007, *Food Chem.* 105, 1

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Catalysis on lactose

Lactose
(Gal-Glu)

enzymatic hydrolysis

Lactose-free milk, whey products
- Increased digestibility, sweetness, solubility

trans galactosylation

Galactosyl oligo-saccharides (GOS)
- Increased solubility
- Prebiotic (baby foods)
- Laxative

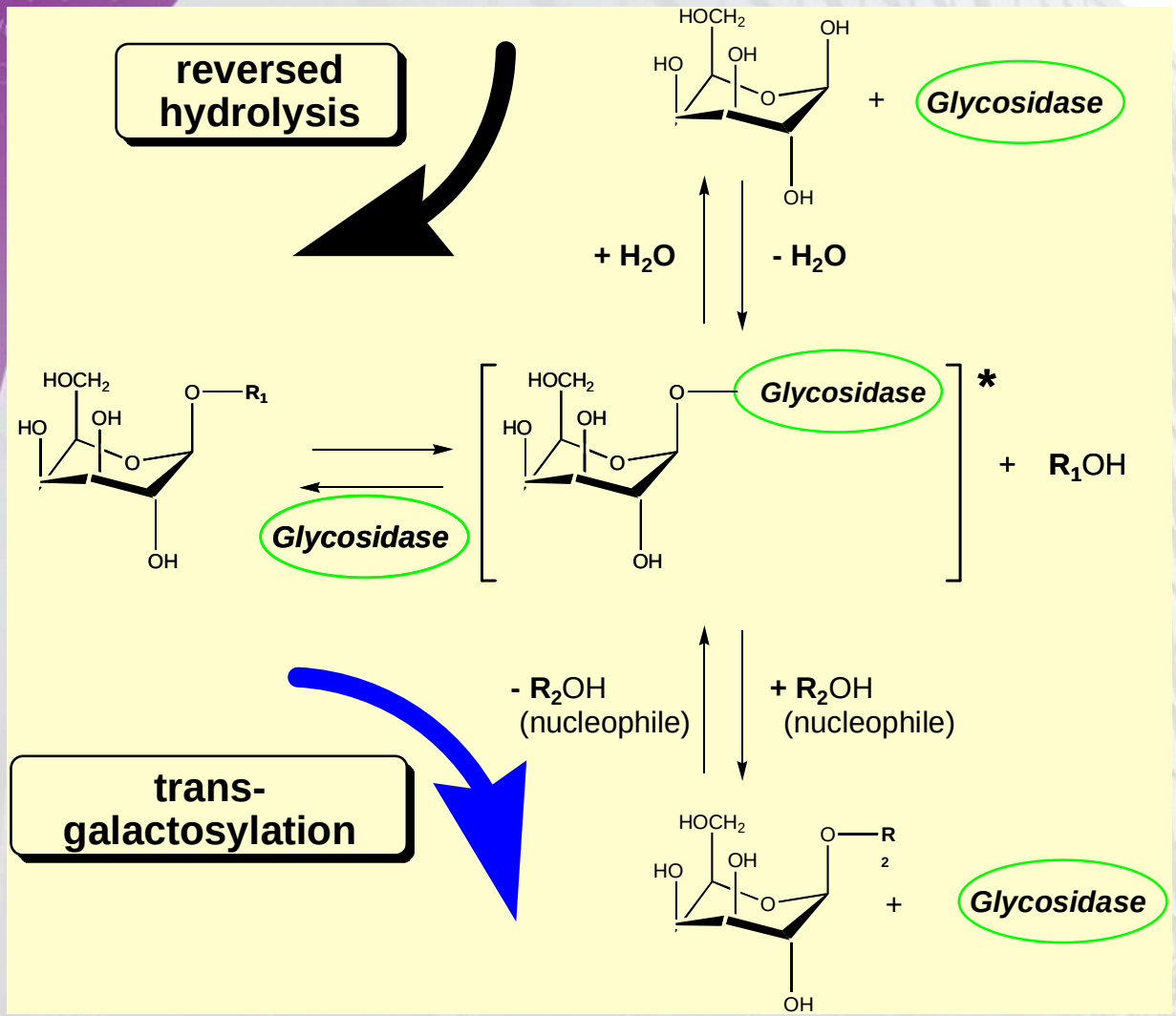
friesland foods
Vivinal® GOS

enzymatic catalysis ?

Lactulose (Gal-Fru)
- Most popular laxative
- 10-times value of lactose
- Prebiotic (FOSHU list)
- Baby foods as bifidogenic facto
- Increased sweetness, solubility

Harju, M. (2001) *Int. J. Dairy Technol.* 54

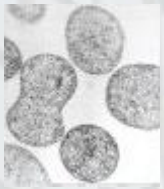
Synthesis using a hydrolase?

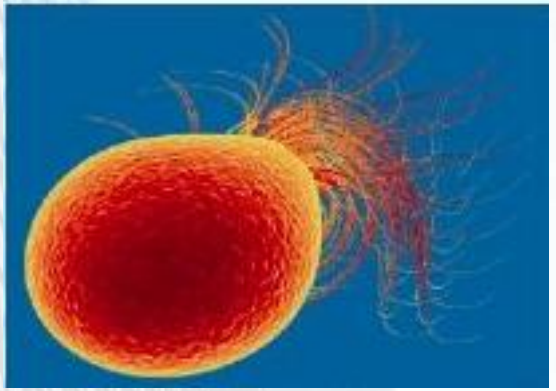


- reversed hydrolysis (thermodynamically controlled)
  - a_w-value
  - concentration donor/acceptor
- transgalactosylation (kinetically controlled)
  - nucleophilic character
  - concentration of acceptors

Unusual biocatalyst

geothermal heated
marine sediments
(Stetter, Uni Regensburg)

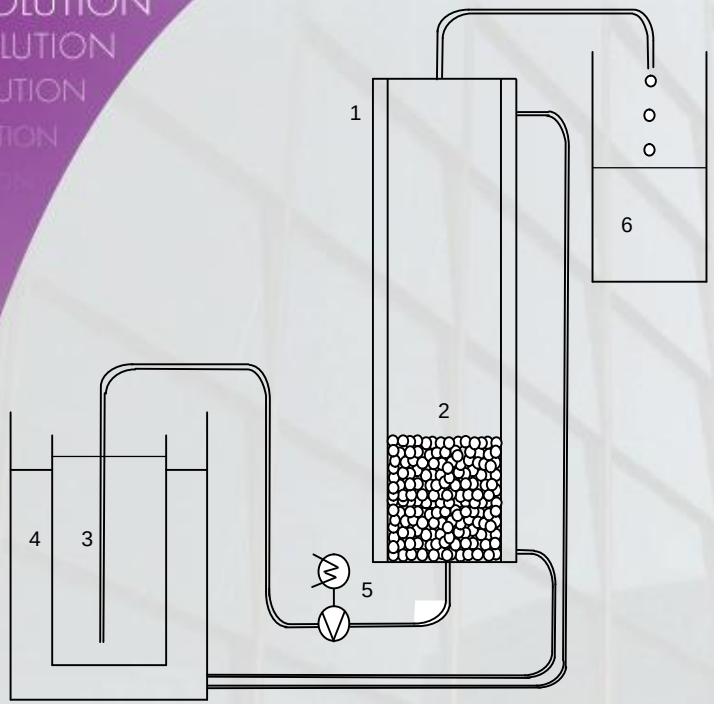




Quelle: ArchiMeDes

- *Pyrococcus furiosus* (extremophilic species of Archaea)
- enzyme CelB:
  b-glucosidase as well as b-galactosidase activity
- stability: $t_{1/2}$ (100°C) = 85h, $t_{1/2}$ (110°C) = 13h
- CelB produced in *E. coli* (1 mkat L^{-1} = 16.6 Mill. U L^{-1})

Expanded bed reactor system



Expanded Bed => $\frac{H_{total}}{H_{beads}}$

1: column, optimum temperature

2: immobilised enzyme (Cel B)

3: substrate solution (processed whey)

4: water bath

5: pump

6: product solution

- covalently immobilised Cel B
- whey UF permeate
- at 75°C

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Continuous lactulose synthesis

Whey-UF-permeate	sugar	H_{total}/H_{beads}			
		start t_0	1.3	1.6	2.0
diluted + 0.1 *M* Frc	**Lactose** [g/L]	45.61	5.93	8.20	11.02
	hydrolysis [%]		**87.0**	**82.0**	**75.8**
	Lactulose [g/L]		0.96	1.25	1.44
	yield [%]		**2.4**	**3.3**	**4.2**
+ 0.5 *M* Frc	Lactose [g/L] **hydrolysis [%]**	44.17	6.68 **84.9**	8.49 **80.8**	10.9[illegible] **75.2**
	Lactulose [g/L] **yield [%]**		5.66 **15.1**	6.47 **18.1**	6.83 **20.6**
concentr. + 0.1 *M* Frc	Lactose [g/L] **hydrolysis [%]**	109.66	34.11 **68.9**	41.56 **62.1**	48.0[illegible] **56.1**
	Lactulose [g/L] **yield [%]**		2.36 **3.1**	1.71 **2.5**	1.75 **2.8**
+ 0.5 *M* Frc	Lactose [g/L] **hydrolysis [%]**	105.13	20.81 **80.2**	33.65 **68.0**	39.61 **62.3**
	Lactulose [g/L] **yield [%]**		3.84 **4.6**	10.62 **14.9**	10.62 **16.2**

Selective protein hydrolysis

Swiss, Finland:
Evolus

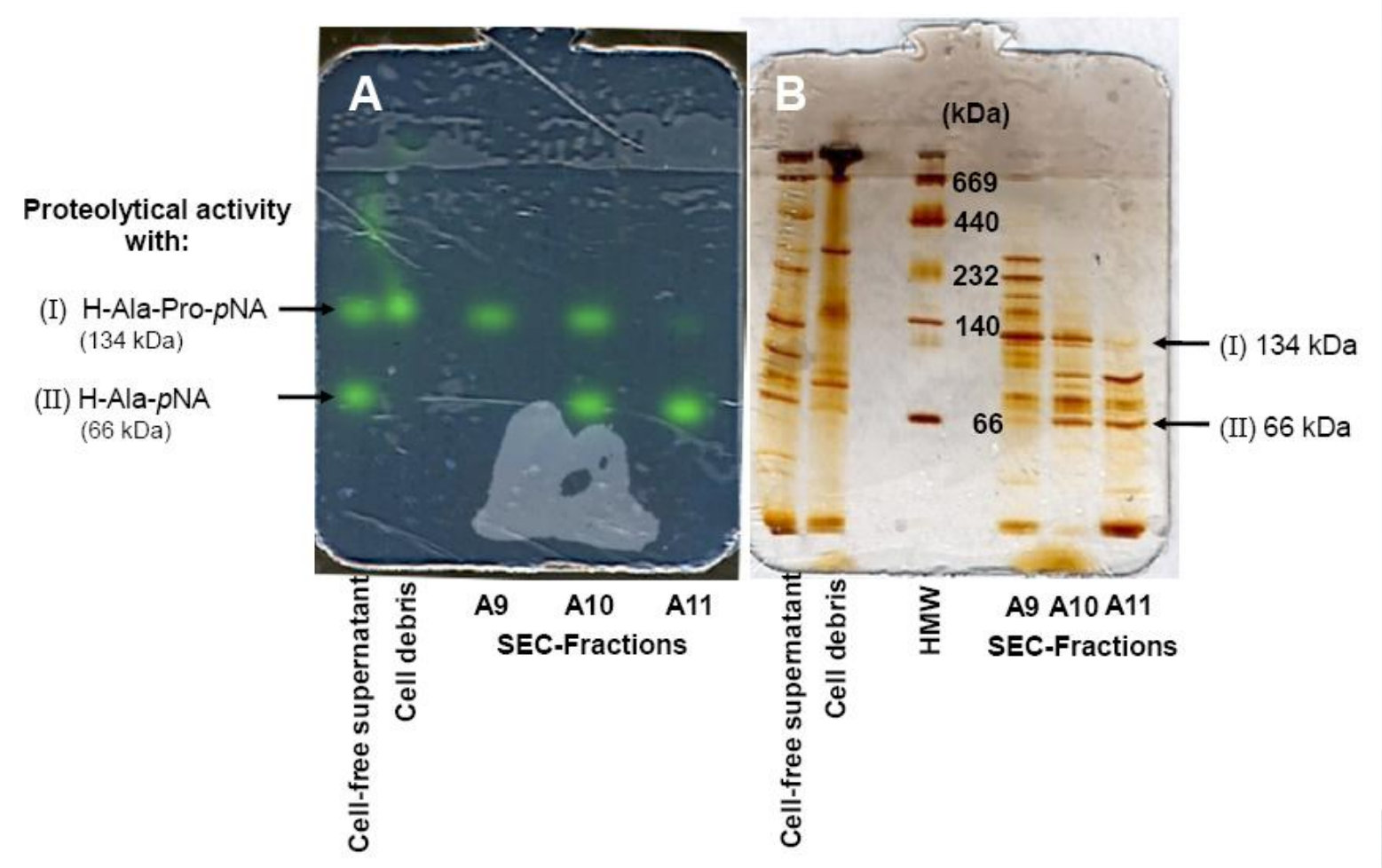
Japan: ***Calpis***

- **whole cell fermentation**

dairy products with ACE inhibitors

- **commercially available enzymes (pancreatic proteases, etc.)**
- **proteases from dairy starter cultures**

L. helveticus: proteolytic enzymes



Native-PAGE of the soluble proteolytic enzymes from *Lactobacillus helveticus*: active-staining (H-Ala-Pro-*p*NA; H-Ala-*p*NA) and silver-staining

(A: active-staining, B: silver-staining; Phast™-Gradient-Gel 8-25, GE Healthcare Bio-Science, Uppsala, Sweden).

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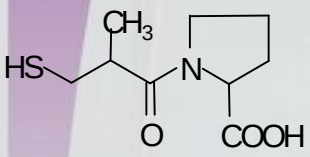
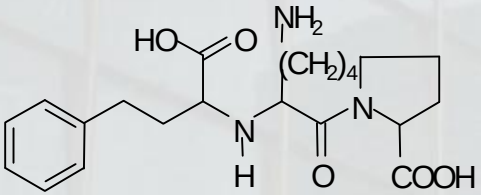
Substrate specificity

	relative activity [%]	
	whole cell	soluble enzymes
H-Ala-Pro-*p*NA	100*	100**
H-Ala-*p*NA	8.9	67
H-Pro-*p*NA	2.9	3.4
H-Val-*p*NA	0.67	3.2
	*100% = 81 nkat/mL	**100% = 60 nkat/mL

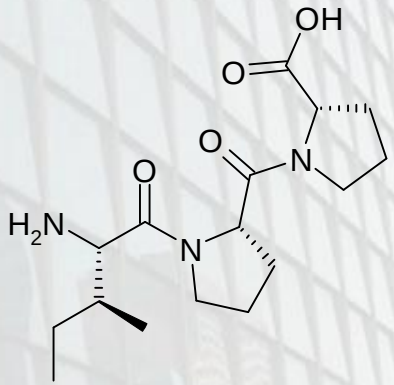


Captopril

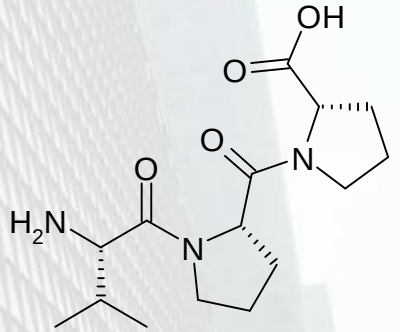


Lisinopril

Examples of drug ACE-inhibitors
[Estler and Schmidt, 2007]



Isoleucine-Proline-Proline (IPP)



Valine-Proline-Proline (VPP)

Structural formula of the bioactive, anti-hypertensive tripeptides produced in skim milk

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Sequences of whey proteins

a-Lactalbumin B (123 amino acids)

EQLTKCEVFRELKDLKGYGGVSLPEWVCTTFH
TSGYDTEAIVENNQSTDYGLFQINNKIWCKNDQ
DPHSSNICNISCDKFLNNDLTNNIMCVKKILDK
VGINYWLAHKALCSEKLDQWLCEKL

b-Lactoglobulin B (162 amino acids)

LIVTQTMKGLDIQKVAGTWYSLAMAASDISLLD
AQSAPLRVYVEELKPTPEGDLEILLQKWENGEC
AQKKIIAEKTKIPAVFKIDALNENKVLVLDTDYK
KYLLFCMENSAEPEQSLACQCLVRTPEVDDEAL
EKFDKALKALPMHIRLSFNTPQLEEQCHI

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Conclusions

- whey and its various formulations (permeates, powders, concentrates) can be regarded as versatile raw materials for further refinement by microbial cells or/and selective enzymes

- there is and probably will not be „the one big process“ but a wide varity of bright biocatalytic applications to produce new functional valuables
  - è food industry
  - è cosmetic industry
  - è pharmaceutical industry
  - è chemical industry

- entrepreneurial decisiveness is demanded from the dairy industry in order to introduce new methods and expertise and to expand their interdisciplinary activities

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