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# LAB

- *Lactic acid bacteria (LAB)* - “milk-souring organisms.”
- Gram positive, nonsporulating, catalase negative
- Devoid of cytochromes
  - Inability to synthesize porphyrin groups, i.e. heme.
- Anaerobic but aerotolerant
- Fastidious
- Acid tolerant
- Strictly fermentative with lactic acid as the major end product during sugar fermentation

# Functions in the food chain

- Dairy starter culture
- Vegetable fermentation
- Alcoholic beverage fermentation (3/4)
- Meat fermentation
- Cereal fermentation
- Probiotics

# Lactic Acid Bacteria: Classification

- *Lactobacillus, Leuconostoc, Pediococcus, and Streptococcus*
- *Aerococcus, Alloiococcus, Carnobacterium, Dolosigranulum, Enterococcus, Globicatella, Lactobacillus, Lactococcus, Lactosphaera, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus, and Weissella.*
- The genus *Bifidobacterium*, often considered in the same context as the genuine lactic acid bacteria and sharing some of the typical features, is phylogenetically unrelated and has a unique mode of sugar fermentation.

# **Tools for LAB Classification and Identification**

- Phenotypic/biochemical characteristics
- Molecular biology approaches
  - Gene homology, 16S rDNA sequences

**Table 1** Differential Characteristics of Lactic Acid Bacteria<sup>a</sup>

Characteristic	Rods				Cocci				
	<i>Carnob.</i>	<i>Lactob.</i>	<i>Aeroc.</i>	<i>Enteroc.</i>	<i>Lactoc. Vagoc.</i>	<i>Leucon.</i>	<i>Pedioc.</i>	<i>Streptoc.</i>	<i>Tetragenoc.</i>
Tetrad formation	-	-	+	-	-	-	+	-	+
CO <sub>2</sub> from glucose <sup>b</sup>	-	±	-	-	-	+	-	-	-
Growth at 10°C	+	±	+	+	+	+	±	-	+
Growth at 45°C	-	±	-	+	-	-	±	±	-
Growth in 6.5% NaCl	ND <sup>d</sup>	±	+	+	-	±	±	-	+
Growth in 18% NaCl	-	-	-	-	-	-	-	-	+
Growth at pH 4.4	ND	±	-	+	±	±	+	-	-
Growth at pH 9.6	-	-	+	+	-	-	-	-	+
Lactic acid <sup>c</sup>	L	D,L,DL <sup>e</sup>	L	L	L	D	L, DL <sup>e</sup>	L	L

<sup>a</sup>+, positive; -, negative; ±, response varies between species; ND, not determined.

<sup>b</sup>Test for homo- or heterofermentation of glucose; negative and positive denotes homofermentative and heterofermentative, respectively.

<sup>c</sup>Configuration of lactic acid produced from glucose.

<sup>d</sup>No growth in 8% NaCl has been reported.

<sup>e</sup>Production of D-, L-, or DL-lactic acid varies between species.

# Milk

- Lactose
  - major fermentable sugar, 40–50 g/l
  - The glucose moiety of lactose is used faster than galactose moiety by lactococci
- Proteins
- Fat
- At the end of the growth phase, less than 0.5% of the lactose is used by lactococci
- The fermentation product of the lactococci is L(+)-lactic acid.

# Starter culture

- Rapid growth, dominant
  - Inhibiting spoilage and pathogenic organisms
- Manufacturing requirement

# Performance unstable

- Plasmid encoded traits
- Bacterial phage
- Others

# Plasmid

- Self-replicating
- Extrachromosomal DNA
- Present in virtually all bacterial species
- Varying in size, structure, mode of replication, ability to propagate, transferability
- Double or single stranded
- Traits to carry

# Functional Properties of Plasmids in Lactococci

- Sugar metabolism
  - Lactose; galactose
- Citrate fermentation
- Proteolytic activity
- Antagonistic substances
  - Lactococcins, diplococcin, etc.
- Phage resistance
  - R/M systems; phage absorption; abortive infection
- Exopolymer production

# Significance of proteolytic system in dairy fermentation

- Support rapid growth of starter culture
  - Dominant population, produce lactic acid
  - Inhibit pathogenic and spoilage agents
- Characteristic flavor and texture development
  - Peptides

**Cell Wall**

**Cell Membrane**

**Cytoplasm**

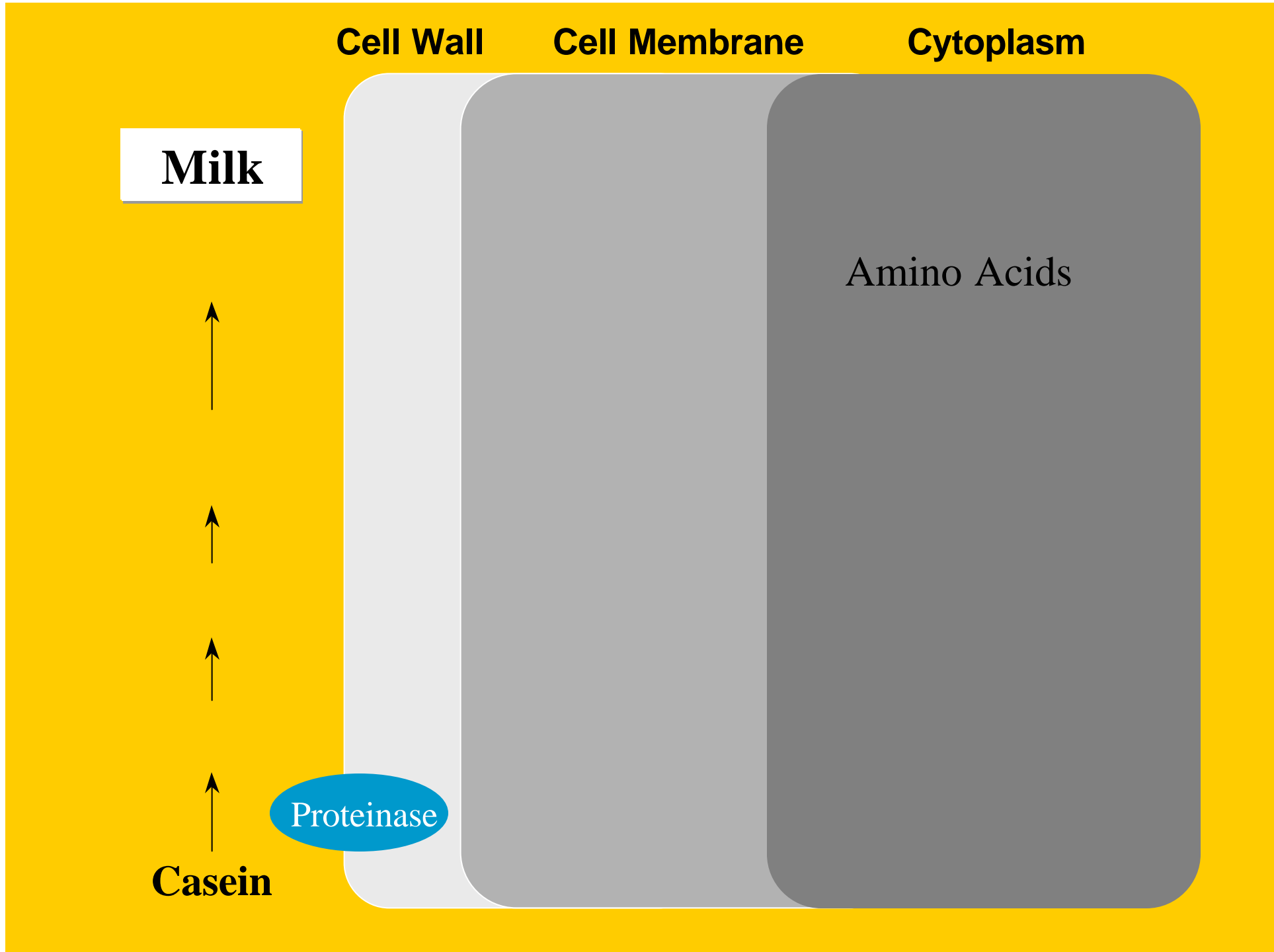
**Milk**



**Proteinase**

**Casein**

**Amino Acids**



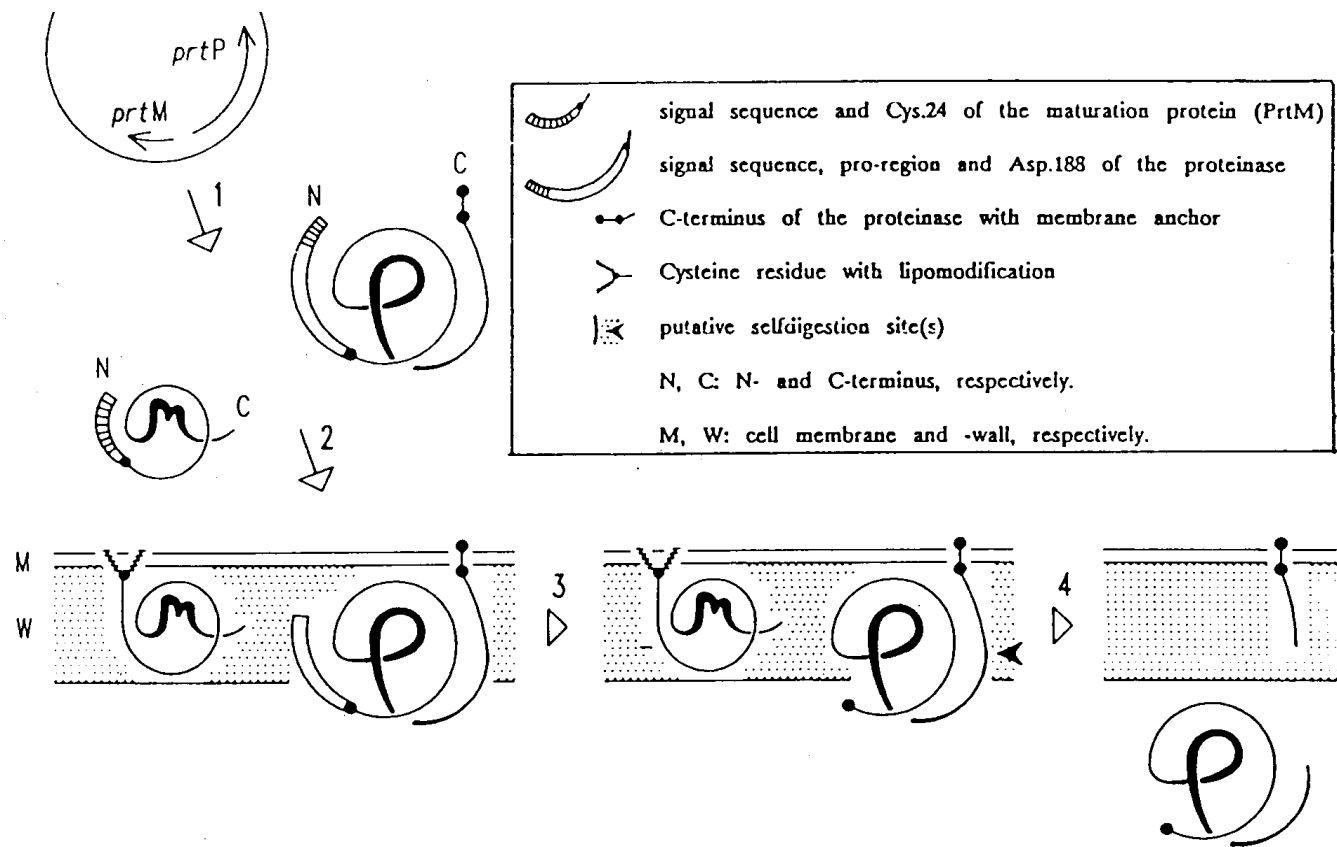
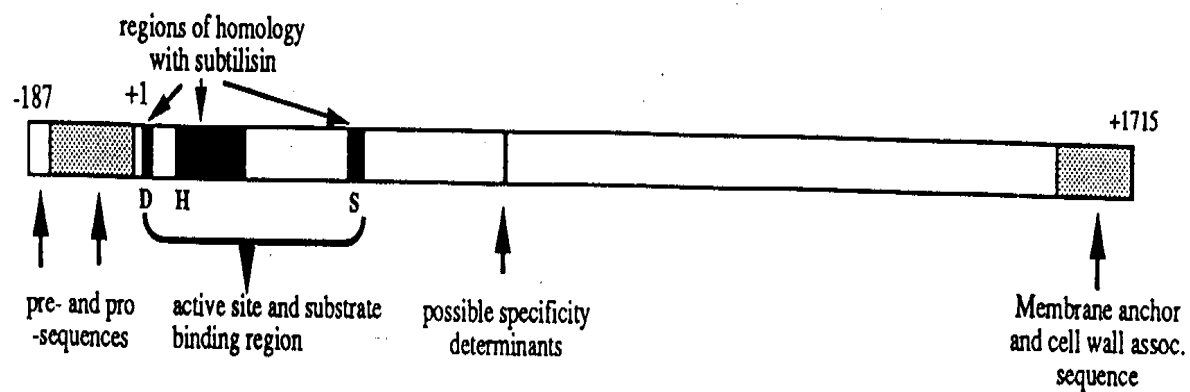
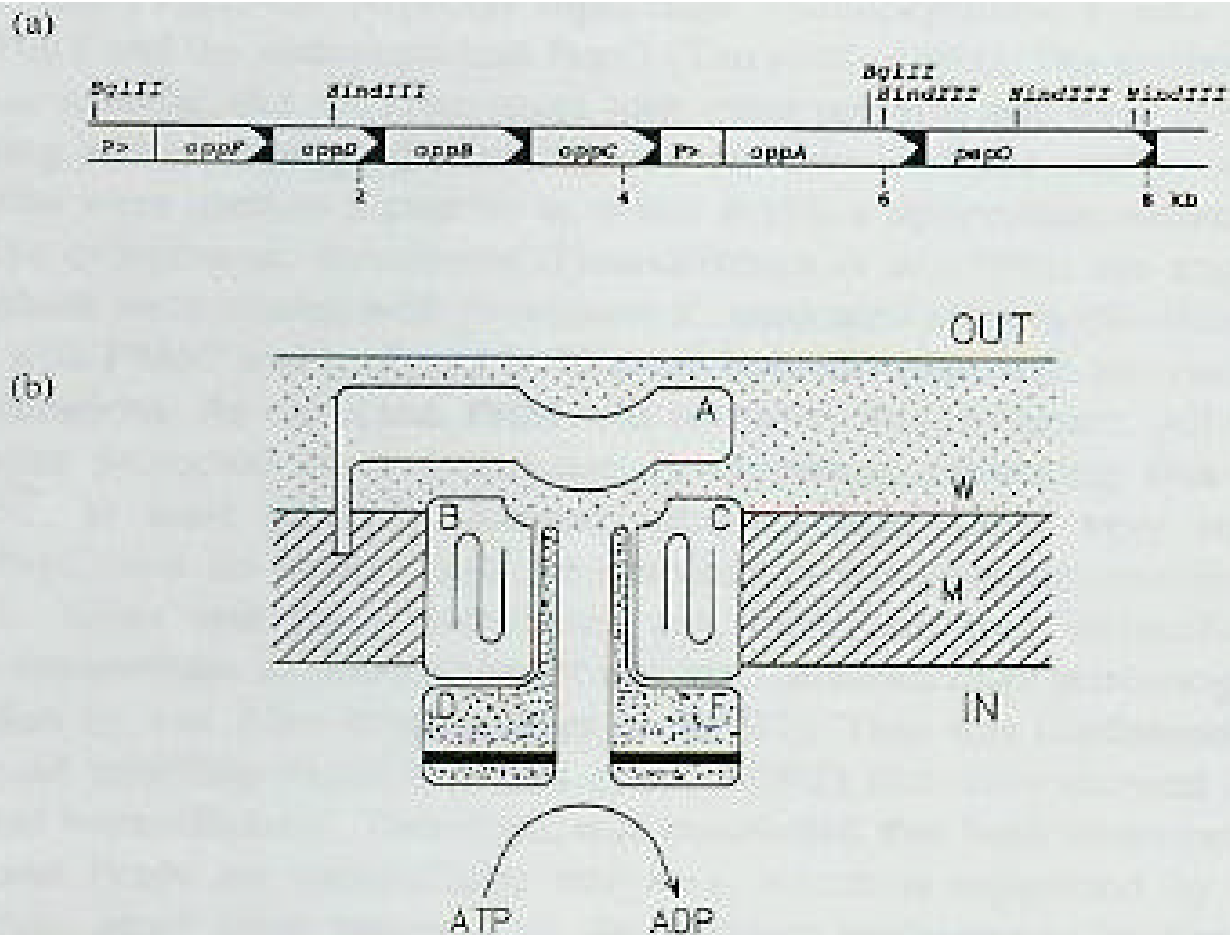


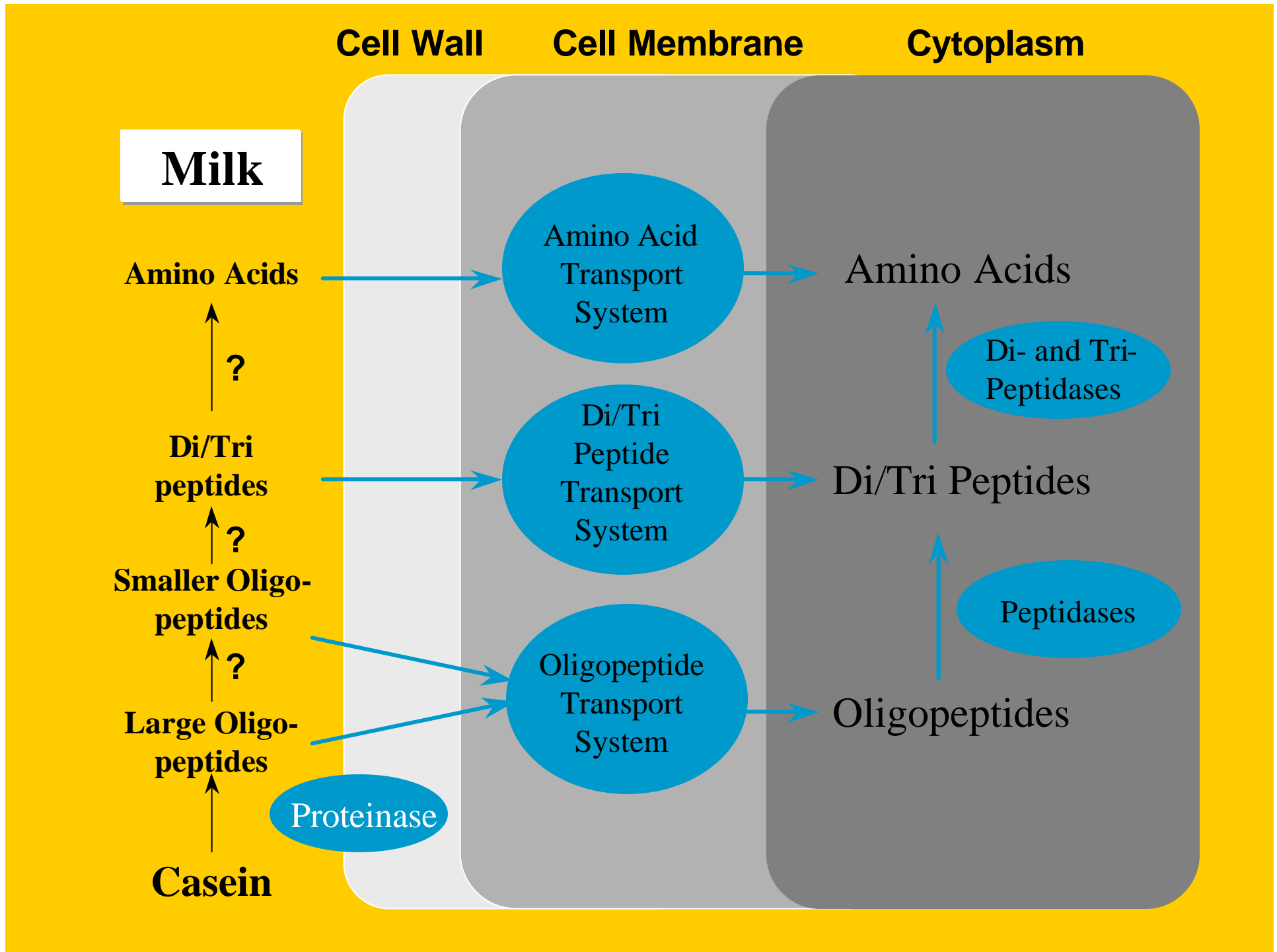
Fig. 8. The proteinase model. (1) Precursor forms of the maturation protein and the proteinase are synthesized from a proteinase plasmid carrying the *prtM* and *prtP* genes. (2) Lipomodification at position Cys.24 of pre-maturation protein and subsequent cutting by the lactococcal equivalent of *E. coli* SPase II (prolipoprotein signal peptidase) results in membrane attachment of the maturation protein. Pre-pro-proteinase is translocated across the cell membrane, cleaved by the equivalent of SPase I (signal peptidase) and remains attached to the cell membrane through the membrane anchor in the extreme C-terminus of the enzyme. (3) The maturation protein effects, in a way not yet understood, the removal of the pro-sequence by cleavage between Thr.187-Asp.188 to give a mature active proteinase (numbers refer to amino acid positions in the pre-pro enzyme). (4) Self-digestion results in the release of a C-terminally truncated proteinase (which can still be active). Incubation in  $\text{Ca}^{2+}$ -free buffer is thought to stimulate self-digestion by enhancing proteinase activity or by unmasking of self-digestion sites in the proteinase molecule.



2. Functional regions of the cell wall proteinase deduced from the nucleotide sequence of the gene. D, H, S, positions of aspartate, histidine and serine residues constituting the catalytic triad. (Based on data from [32-35,44].)



**Figure 4.3** (a) Genetic organization of the *opp* operon of *L. lactis*. The direction of transcription of the genes is indicated by the arrowheads. The size of the DNA (in kb) is shown below the bar. P>, putative promoter and its direction. (b) Tentative model of the *L. lactis* oligopeptide permease system Opp, based on the model proposed for Opp of *S. typhimurium* (Ames, 1986; Higgins *et al.*, 1990). A, B, C, D, and F are OppA, OppB, OppC, OppD and OppF, respectively. OppA is shown attached to the membrane (M) via lipid modification of its Cys23 residue. The black bars in OppD and OppF represent the conserved putative ATP binding sites. Possible energization of the uptake system through ATP hydrolysis (by either OppD or OppF, or both) is also shown. W, cell wall.



**Milk**

**Cell Wall**

**Cell Membrane**

**Cytoplasm**

**Amino Acids**



**Di/Tri peptides**



**Smaller Oligopeptides**



**Large Oligopeptides**



**Casein**

**Proteinase**

**Amino Acid Transport System**

**Di/Tri Peptide Transport System**

**Oligopeptide Transport System**

**Amino Acids**

**Di- and Tri-Peptidases**

**Di/Tri Peptides**

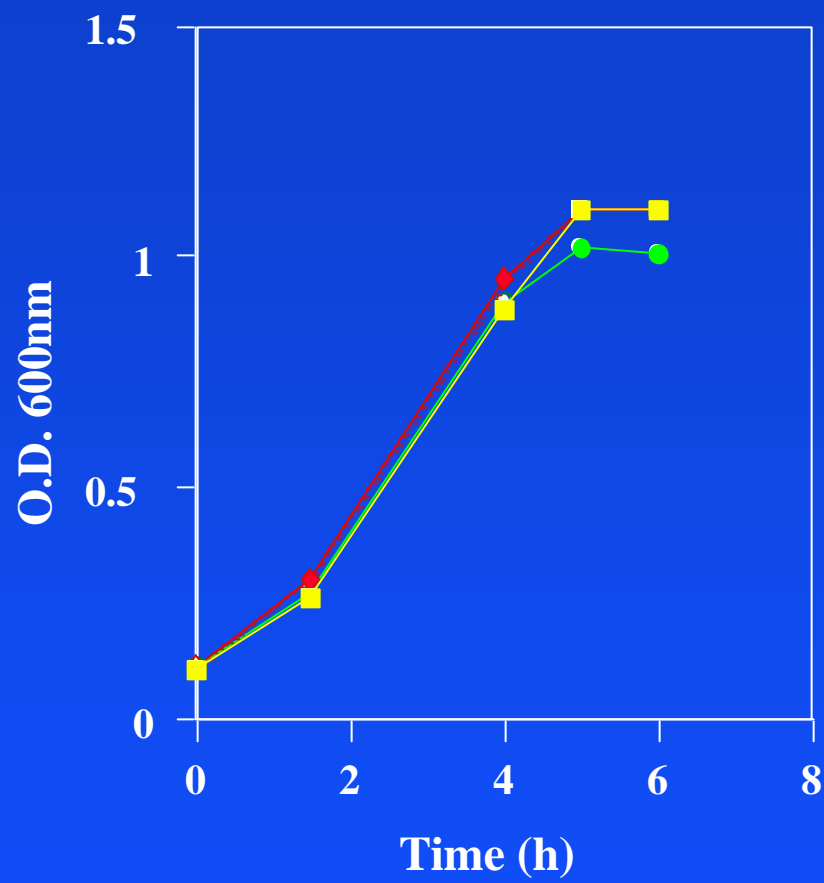
**Peptidases**

**Oligopeptides**

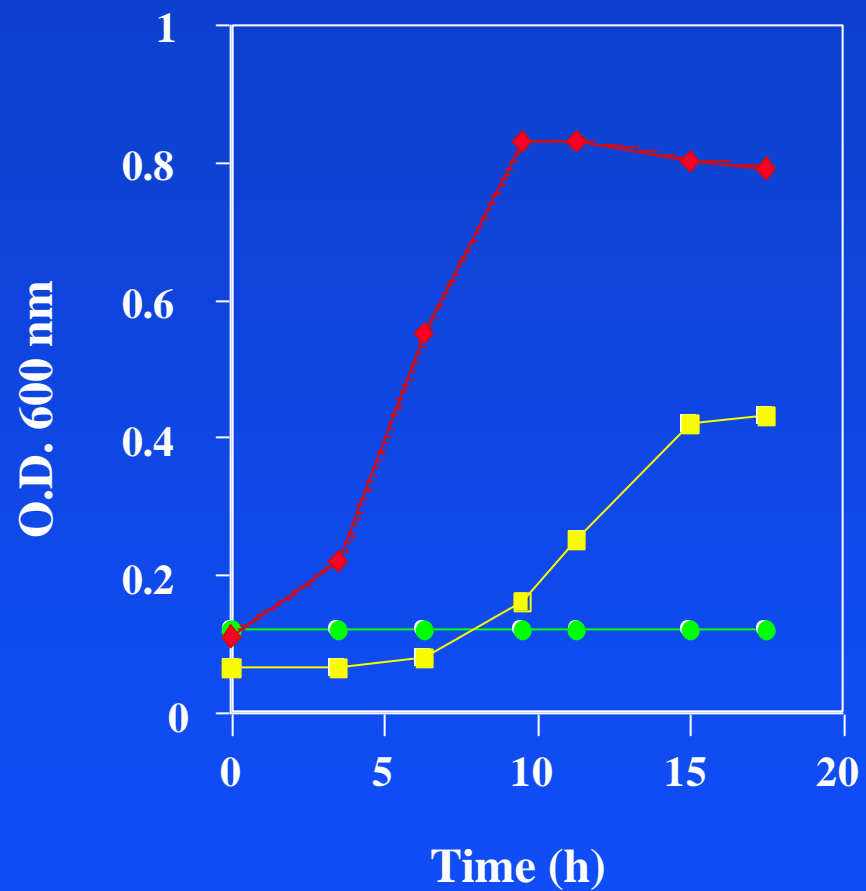
Mutant Analysis  
-identification of the pyruvate  
carboxylase pathway

# Growth in milk

- *L. lactis* C2 coagulates milk at 21°C ~48h
- KB4 slow coagulator



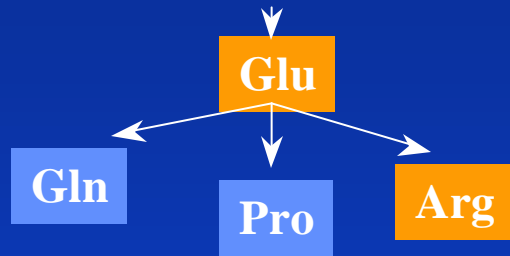
with 20 a.a



with 9 a.a

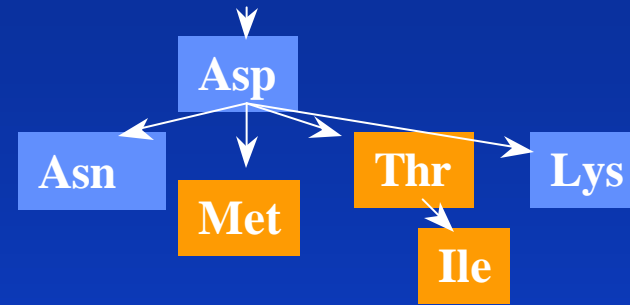
(1)

a-ketoglutarate



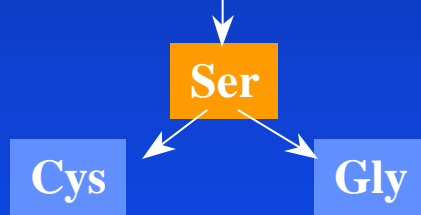
(2)

oxaloacetate



(3)

3-phosphoglucerate



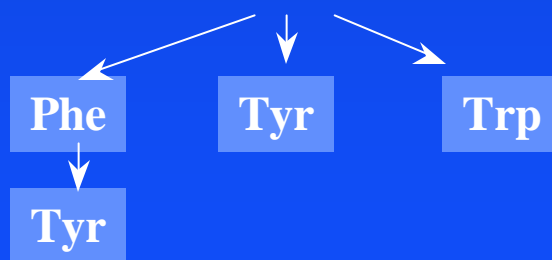
(4)

pyruvate



(5)

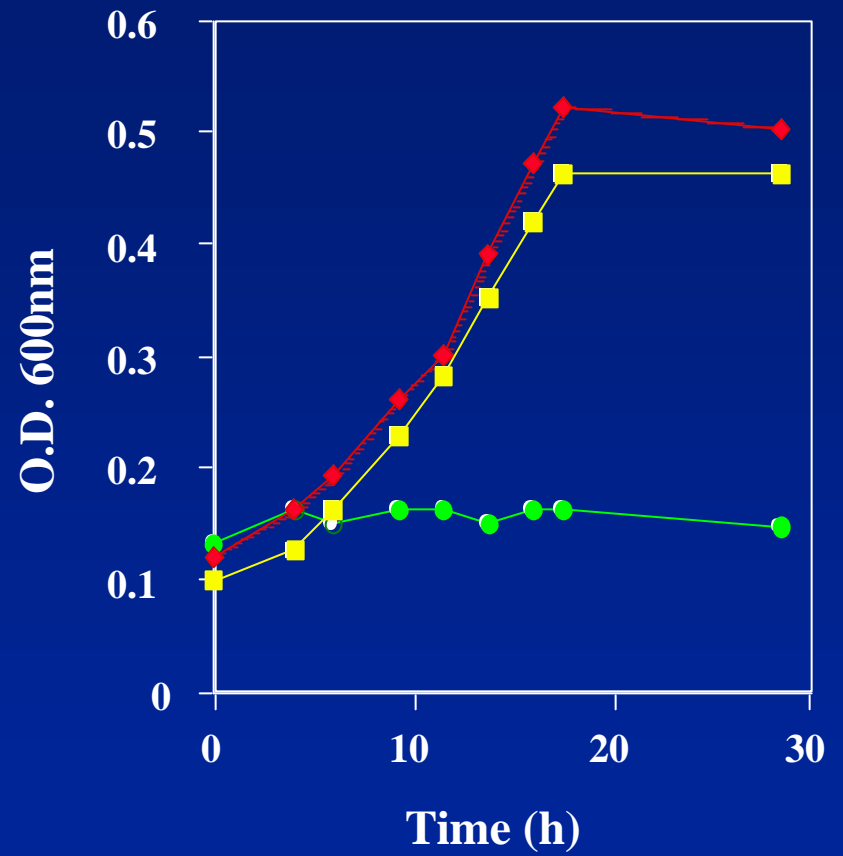
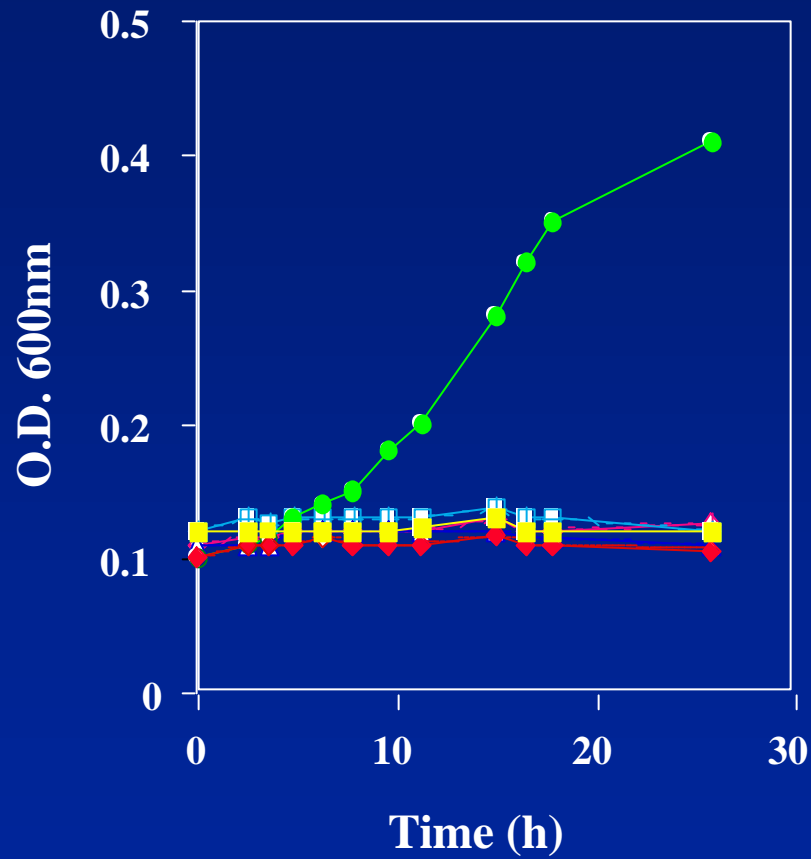
PEP + Erythrose 4-phosphate

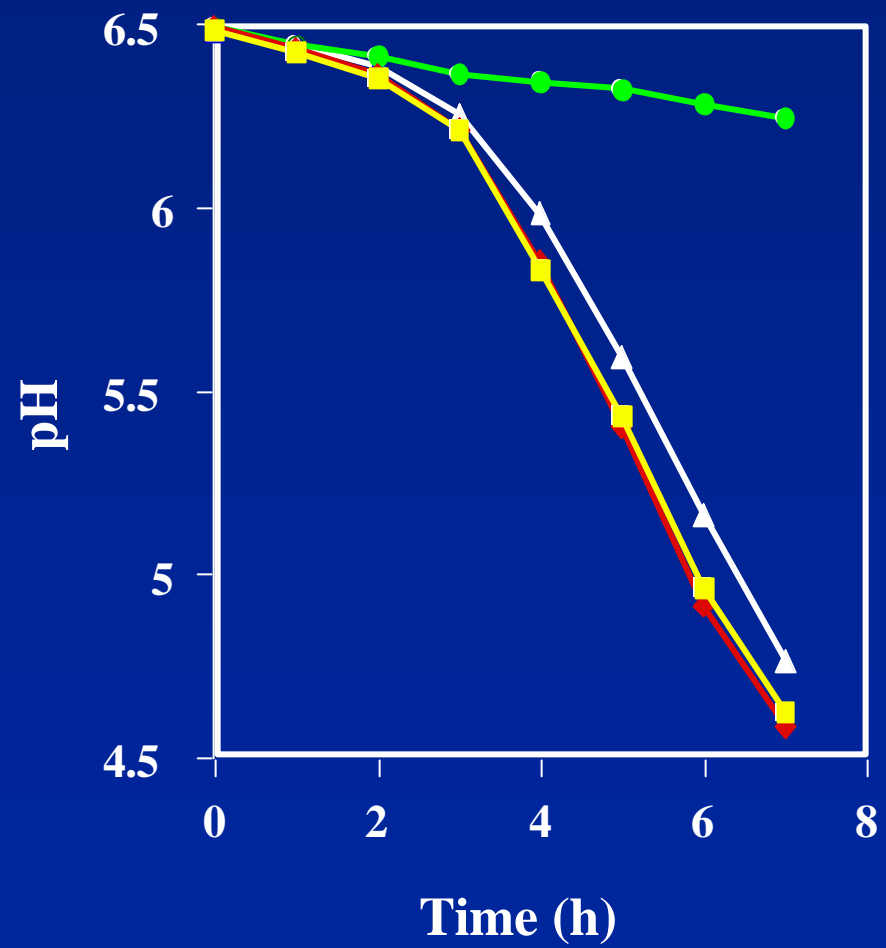


(6)

Ribose-5-P







**Table 3. Growth of *L. lactis* KB4 in a simple synthetic medium supplemented with various aspartic acid sources**

<b>Supplemented Component</b>	<b>Growth</b>
<b>Asp-Gly</b>	<b>+</b>
<b>Asp-Ser-Asp-Pro-Arg</b>	<b>+</b>
<b>Gly-Asp-Asp-Asp-Asp-Lys</b>	<b>-</b>

**Table 4. Growth of *L. lactis* KB4 in a simple synthetic medium supplemented with various precursors of aspartic acid**

<b>Supplemented Component</b>	<b>Growth</b>
<b>Oxaloacetic acid</b>	-
<b>Fumaric acid</b>	-
<b>Malic acid</b>	-
<b>Pyruvate</b>	-

**Table 5. Assay for enzymes involved in aspartic acid biosynthesis**

Strain	Enzyme	Specific Activity
<i>L. lactis</i> C2	glutamate-aspartate transaminase (GOT)	1.8 units/mg protein
	pyruvate carboxylase	54,663 DPM/mg protein
<i>L. lactis</i> KB4	glutamate-aspartate transaminase (GOT)	1.5 units/mg protein
	pyruvate carboxylase	2,959 DPM/mg protein

Pyruvate + [<sup>14</sup>C]Bicarbonate

Biotin

CoA

ATP

**Pyruvate Carboxylase**

**GOT**

[<sup>14</sup>C]Oxaloacetate

[<sup>14</sup>C]Aspartate

Glutamate

alpha-keto-  
glutaric acid

# Summary: Lactic acid bacteria

- Important starter culture
- Many traits are encoded by plasmid
- Proteolytic system important for rapid growth and fermenting products
- Maturation of PrtP requires PrtM
- Mutation analysis