Basic characteristics of the most important enzymes for technological applications

Oxidoreductases

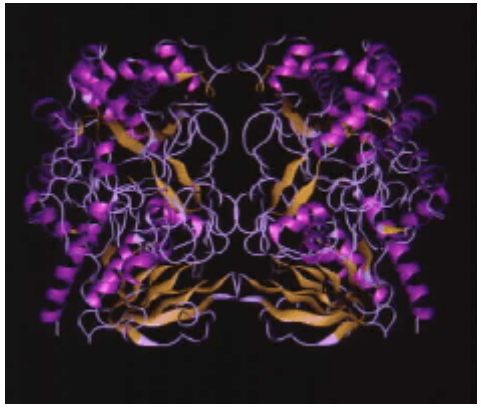
- catabolism, respiration, bioenergetics
- Mostly intracellular, bound to the structures

Glucose oxidase, peroxidase, catalase, lipoxygenase, polyphenol oxidase, lactoperoxidase, xanthin oxidase, ascorbate oxidase etc.

Glukose oxidase, β-D-glucose:O_2 1-oxidoreductase, EC 1.1.3.4 (notatin)

β-D-glucose + O_2 → δ-D-gluconolacton + H_2O_2

Present in fungi Aspergillus, Penicillium, insects etc.



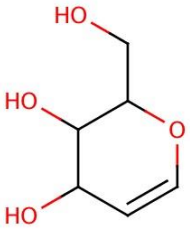
Properties: Mw 130 -175 kDa, 2 identical subunits,

Cofactor FAD (prosthetic group), Fe

Glycoprotein: 11-13 % of neutral saccharides (mannose),

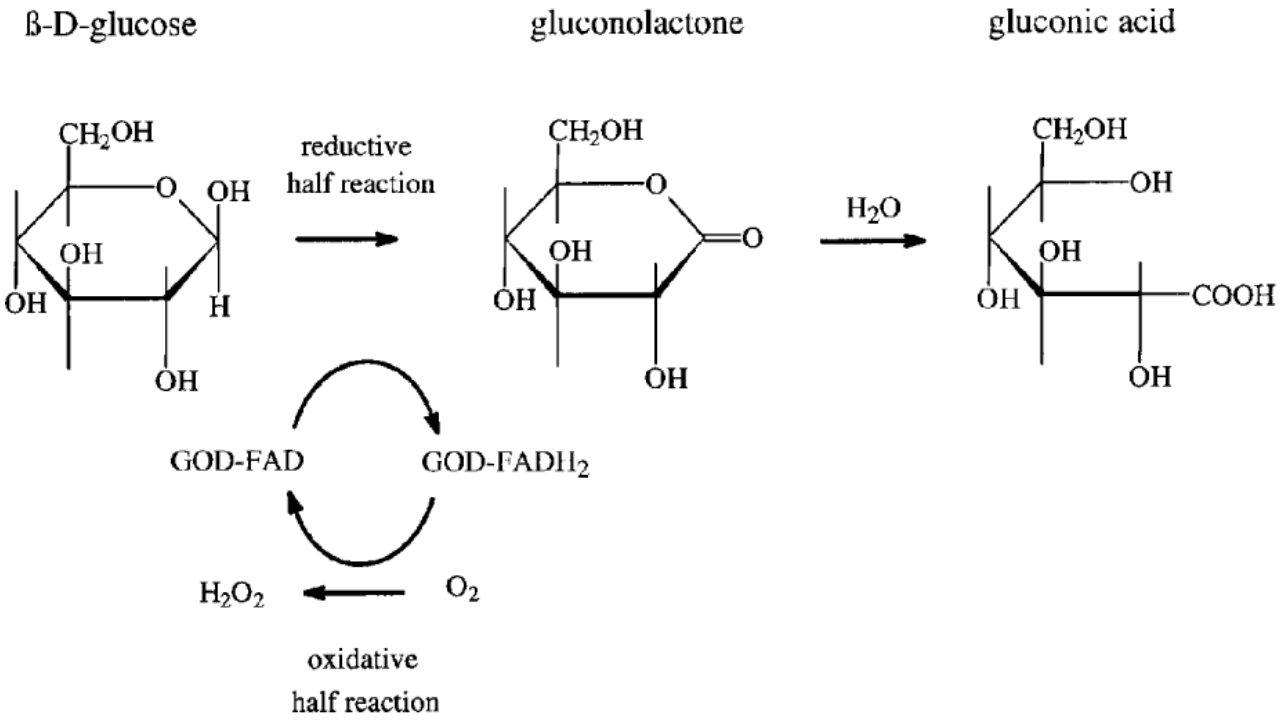
2% aminosaccharides

pH optimum: 5,5 – 5,8, pI = 4,2



Inhibitors: Ag^{2+}, Hg^{2+}, $Cu^{2+,}$ D-glucal, H_2O_2 (E-$FADH_2$ 100x higher)

Physiological function: production of H_2O_2 - antibacterial and antifungicide effect


ß-D-glucose
gluconolactone
gluconic acid
reductive
half reaction
H_2O
GOD-FAD
GOD-FADH$_2$
H_2O_2
O_2
oxidative
half reaction

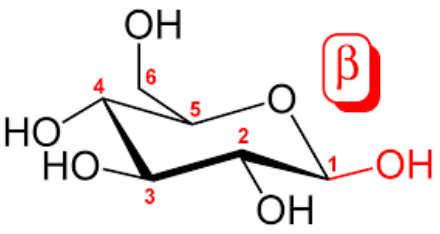
Specificity

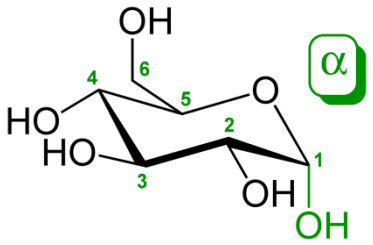
Substrate	Relative rate of oxidation (%)
β-D-glucose	100
α-D-glucose	0,64
L-glucose	0
D-mannose	1,0
D-xylose	1,0
D-galactose	0,5
maltose	0,2
melibiose	0,1
cellobiose	0,09

2-deoxy-D-glucose (20–30%), 4-O-methyl-D-glucose (15%), 6-deoxy-D-glucose (10%)

Structural characteristics of the substrate:

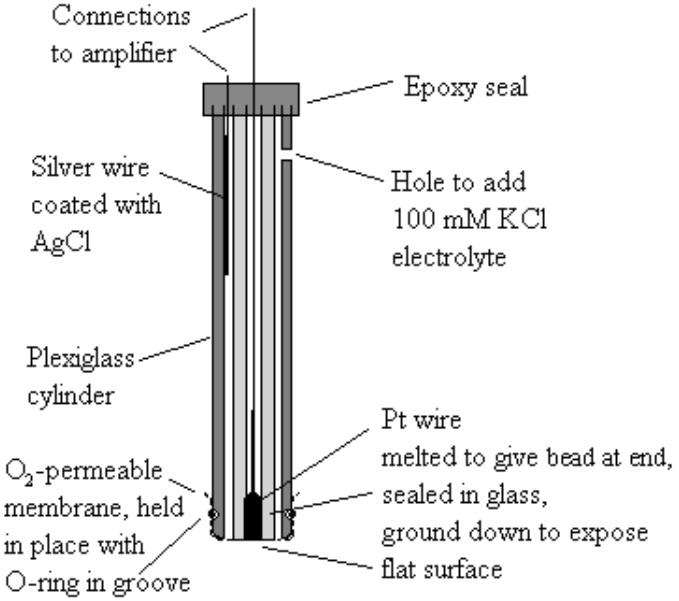
- pyranose ring in chair conformation
- equatorial –OH on C3 atom





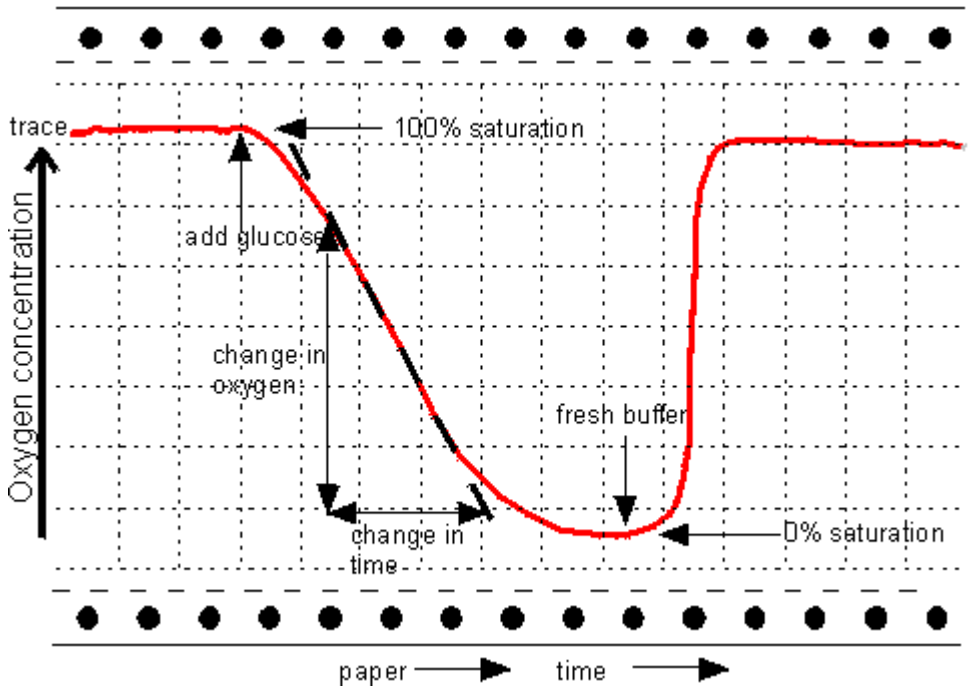
Determination of GOD activity

1. Consumption of oxygen (Clark oxygen electrode)
2. Determination of hydrogen peroxide
3. Titration of gluconic acid



Ag anode: $4Ag + 4Cl^- \rightarrow 4AgCl + 4e^-$

Pt cathode: $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$



1. Consumption of oxygen (Clark oxygen electrode)
2. Determination of hydrogen peroxide
3. Titration of gluconic acid

Classical methods: oxidation of I^-, ferrocyanide

Enzymatic – POD + chromogenic acceptor – spectormetry VIS

(o-dianisidine, 2, 2′-Azino-di-[3-ethylbenzthiazolin-sulfonate] - ABTS etc.)

Applications of glucose oxidase

- Removal of glucose (Maillard´s reactions)
- Removal of oxygen (from packed foods or beverages)
- Preparation of gluconic acid (food, concrete)
- Production of hydrogen peroxide
- Quantitative determination of glucose or other compounds which can be converted to glucose (saccharose, starch …)
- Activity determination of enzymes producing glucose (invertase, amylase etc.)

Oxidoreductases catalyzing decomposition of hydrogen peroxide

catalase peroxidase

Peroxidase:

Donor + H_2O_2 <=> oxidized donor + 2 H_2O

Catalase

$2H_2O_2 \rightarrow 2H_2O + + O_2$

Peroxidases EC 1.1.11.1 – 21 (mostly heme enzymes)

1. Animal - lactoperoxidase, myeloperoxidase – antimicrobial activity

2. Plant and microbial

Group I: intracelular (yeast cyt c POD, chloroplastic, cytosolic ascorbate POD and bacterial POD)

Group II: extracelular fungal (Fungi) LiP, MnP, LiP/MnP : ligniperdous fungi

Group III: extracelular plant (horse radish – HRP , ascorbate peroxidase - APX)

+ haloperoxidases (CPO) – formation of halogenderivatives of org. compounds - antimicrobial effect

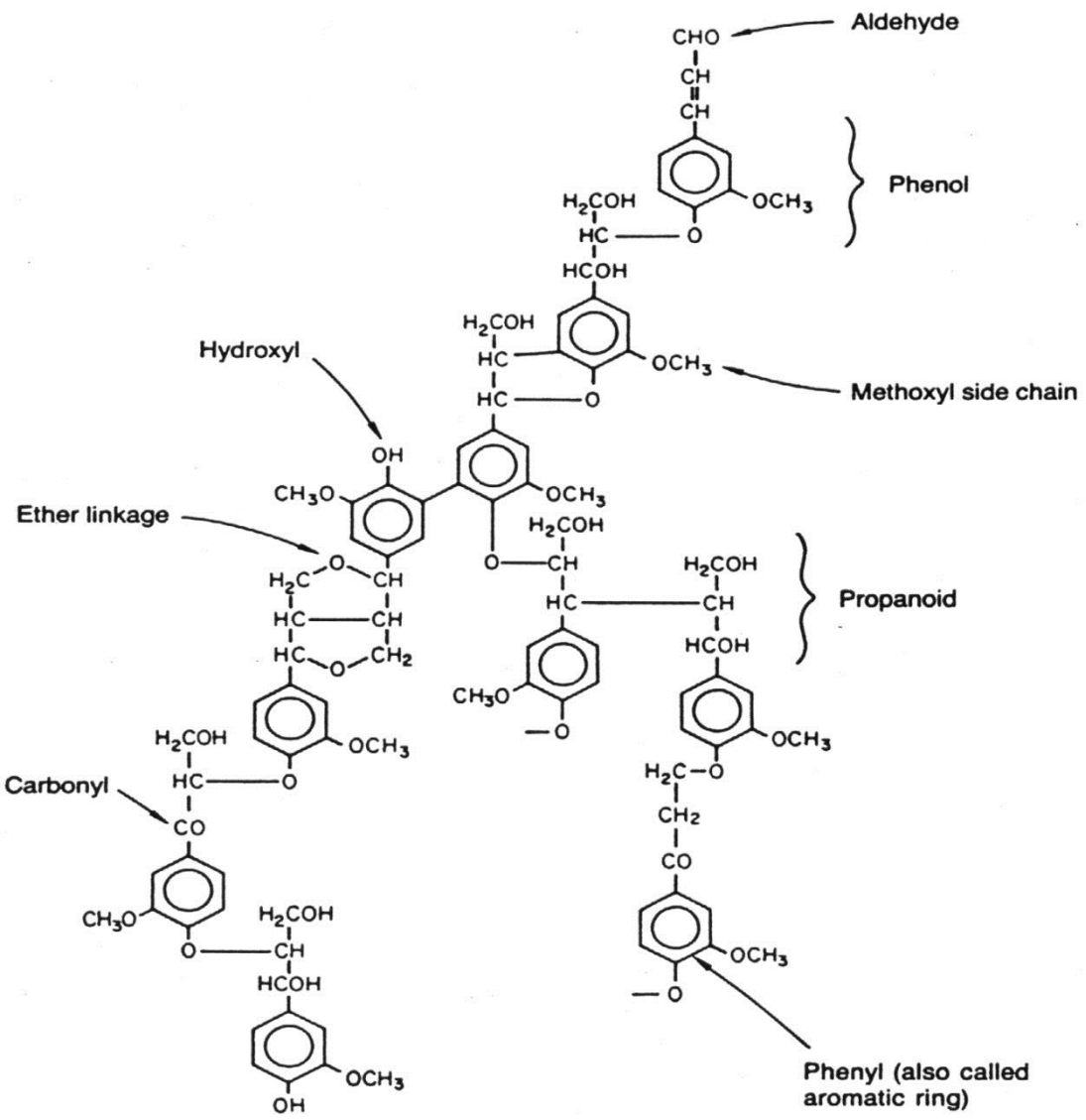
Application of peroxidases:

1. Analytics
2. label in immunochemistry
3. Biotransformations - hydroxylations
4. Biodegradation of polyphenols (dyes, PCB)
5. Biochemical changes in food raw materials

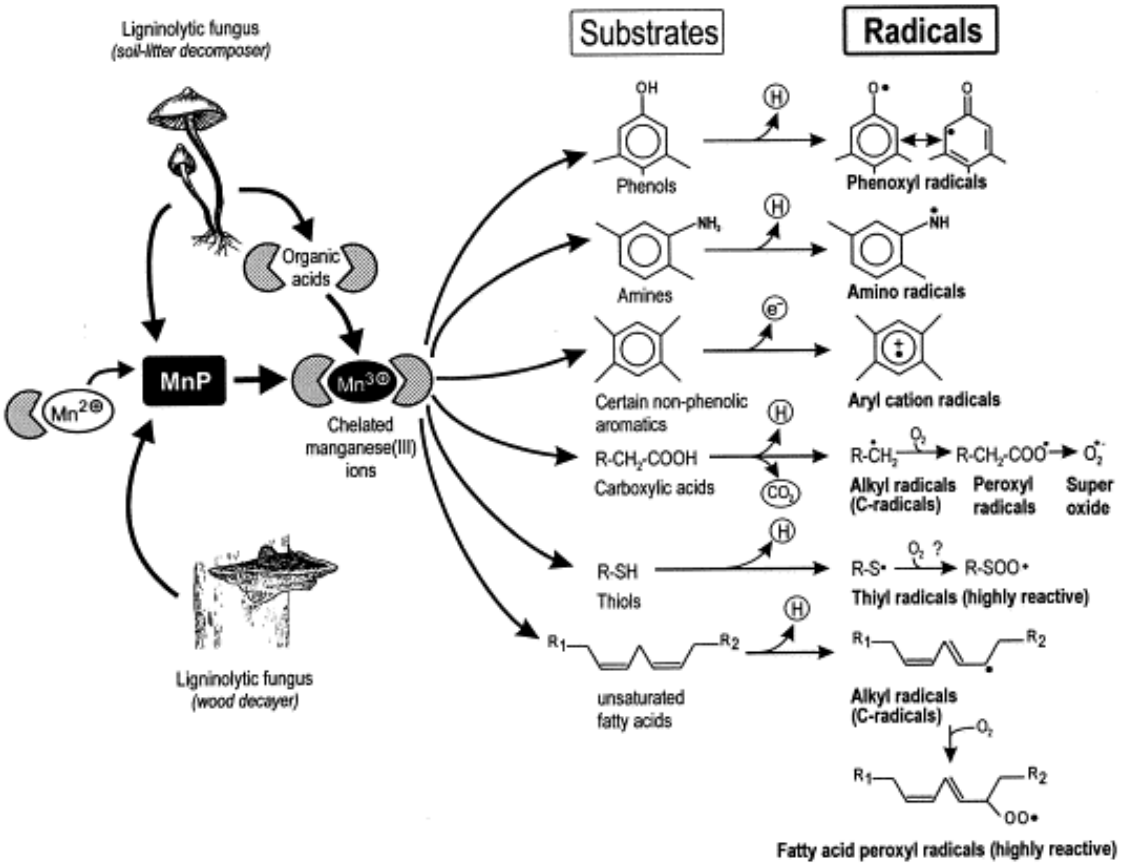
***Serpula lacrymans*-the dry rot fungus**

Gloeophyllum trabeum

Lignine structure – substrate for fungal peroxidases



Lignin and mangan peroxidases:



Low stability- heme oxidation by the reaction products - radicals
Low production – response to the low content of nutrients

Lipoxygenase, EC 1.13.11.12

Linoleate:O_2 oxidoreductase,

(Lipoxidase, caroten oxidase)

Specific for pentadiene conformation

In all eukaryots

Isoenzymes

Classified according to the site of oxidation

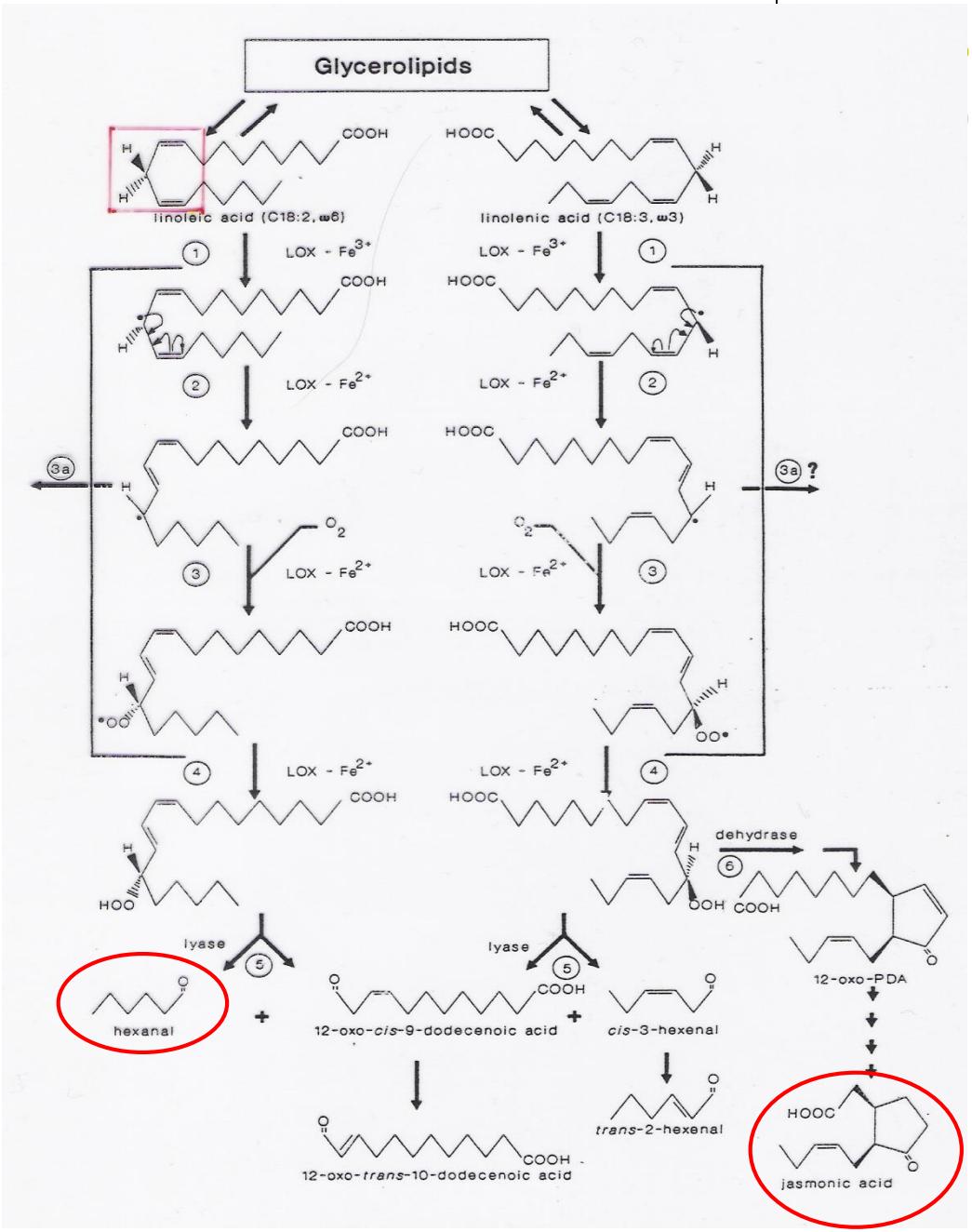
9-LOX, 13-LOX

Substrate: linoleic, linolenic, arachidonic acid

Co-oxidation reaction

Activity determination:

1. Consumption of O_2
2. UV absorbancy of dienoic structure formed (234 nm)



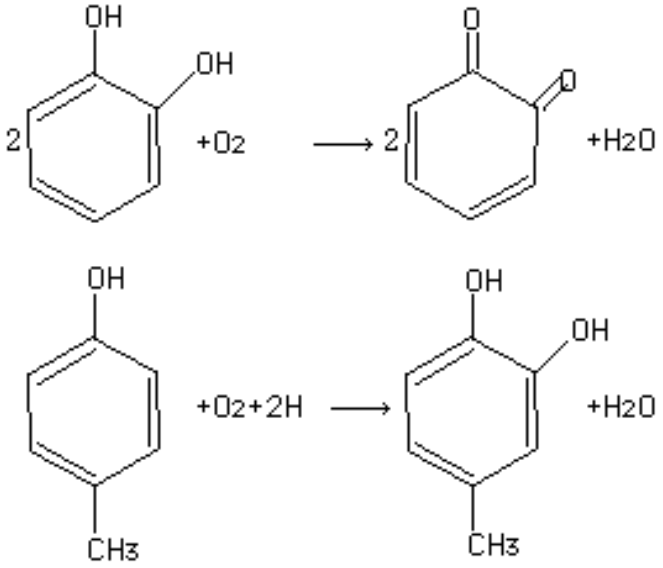
Other oxidoreductases:

Polyphenol oxidase, EC 1.10.3.1, o-diphenol: O_2 oxidoreductase

Tetramer containing 4 atoms of Cu^{2+}

Catalyzes 2 types of reactions:

1. Formation of o-quinones
2. Formation of polyphenols



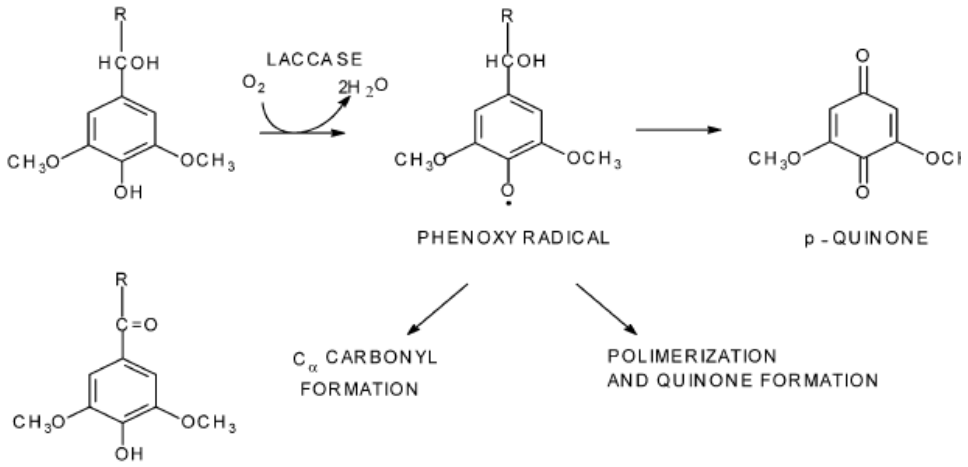
Tyrosinase, phenol oxidase EC 1.14.18.1

Catechol oxidase, cresolase …

Tyrosine + O_2 → dihydroxyphe → dopaquinone

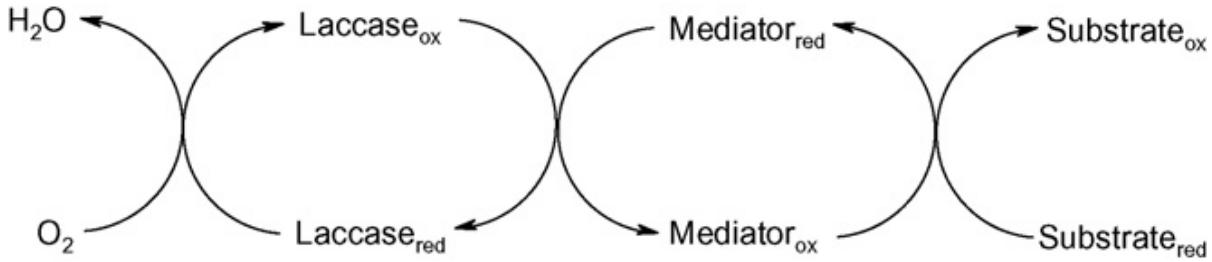
Laccase EC 1.10.3.2 – bacteria, fungi, plants

Substrates: 4-benzene diol , wide specificity, polyphenols, substituted polyphenols, diamines and many other but not tyrosine



Application: textile, paper, wood industry, bioremediations, wine production, canning and sugar industry

Lignine degradation by laccase:

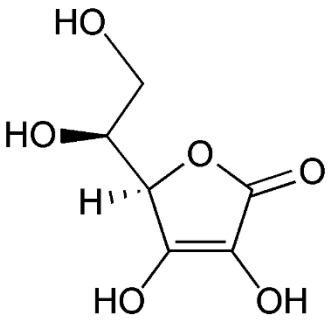


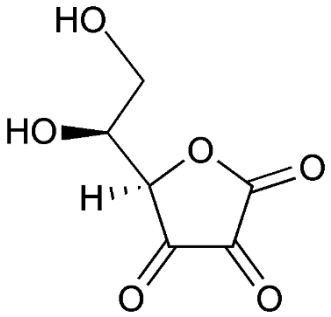
- Laccase (70 kDa) cannot penetrate into wood
- Not able to oxidize (low redox potential ~0.5 -0.8V) non-phenolic lignin units (redox potential >1.5V)
- Oxidizes only phenolic units – less than 20% in wood
- Applied together with oxidation mediator – small molecules (LMS) capable to oxidize non-phenolic components of lignin and overcome the accessibility problem

Ascorbate oxidase, EC 1.10.3.3, ascorbate:O_2 oxidoreductase

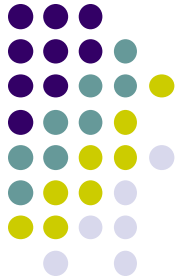
8 $Cu^{2+,}$ plants

Ascorbic acid + O_2 ----->. Dehydroascorbic acid + H_2O





Hydrolases



$$X - Y + H - OH \rightarrow H - X + Y - OH$$

Nomenclature: classification according to the type of splitted bond

In technology according to the type of the substrate:

Proteases

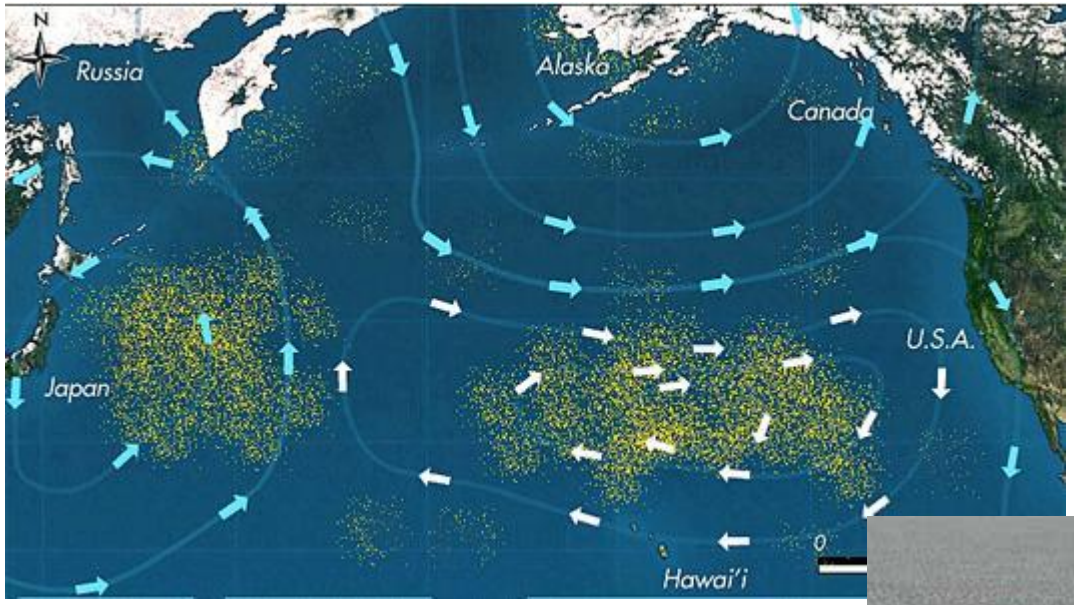
Glycosidases → Glucopolysaccharides (starch degrading enzymes)

Glycosidases → Pectic compounds, hemicelluloses, cellulose

Lipases, Phospholipases

Others - ie. esterases, aminoacylases

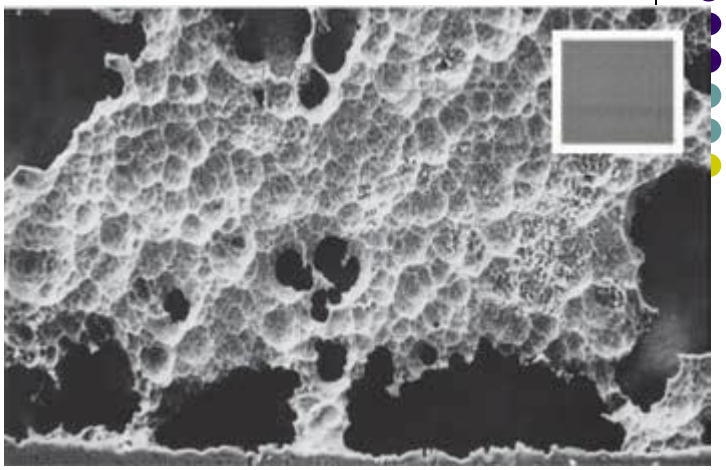
Discovery of new enzyme in 2016!!

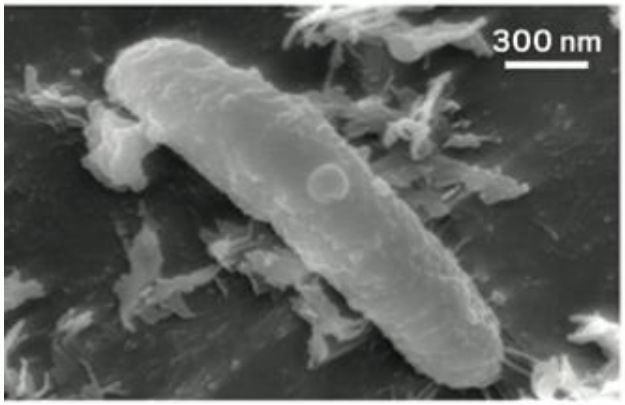


PETase EC 3.1.1.101

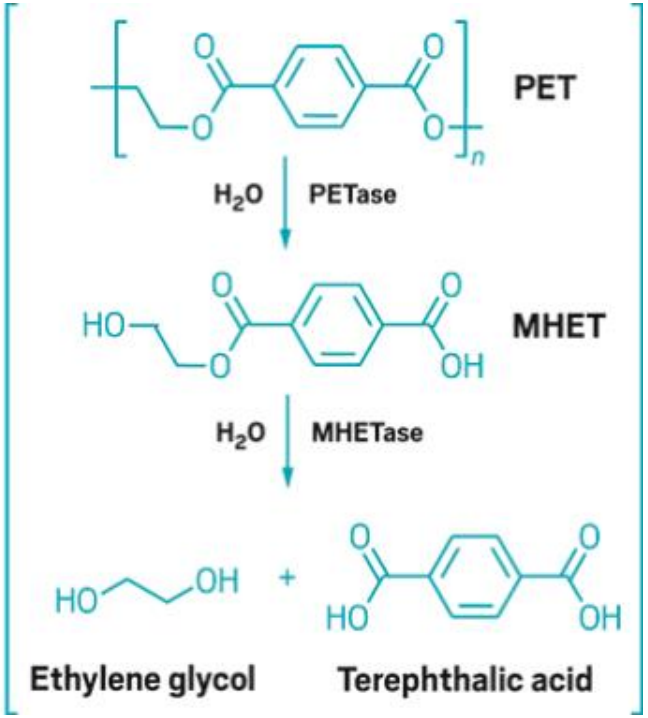
Bacteria *Ideonella sakaiensis*

Genetically engineered mutant enzyme





Ideonella sakaiensis



polyethylene furanoate - PEF

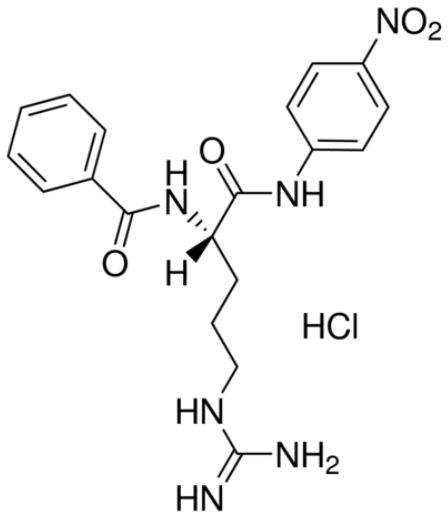
Proteases

Classified according to different aspects:

1. Origin
2. localisation in organisms, inactive forms
3. pH optimum
4. Specificity (endo, exo)
5. Mechanism of catalysis -

Activity determination:

- Natural substrates (hemoglobin, casein), UV determination
- Natural substrates with adsorbed dyes, VIS
- Synthetic substrates – BAPA, VIS
- Others – washing tests



N_α-Benzoyl-L-arginine 4-nitroanilide hydrochloride

Animal proteases

Serine - trypsin, chymotrypsin

Aspartate - pepsin, chymosin

Chymotrypsin

pH optimum: ~ 8,0

Specificity: preferentially peptide bond behind aromatic AA

Trypsin

pH optimum: ~ 8,0

Specificity: Arg, Lys

Pepsin

pH optimum 1,0 - 2,0

hydrophobic, preferably aromatic AA residues

Application: pharmaceuticals (digestives, treatment of injuries, post operational treatment etc, Wobenzym)

Chymosin (rennin), EC 3.4.23.4

Autocatalytic activation - pH 5 → chymosin

pH 2 → pseudochymosin

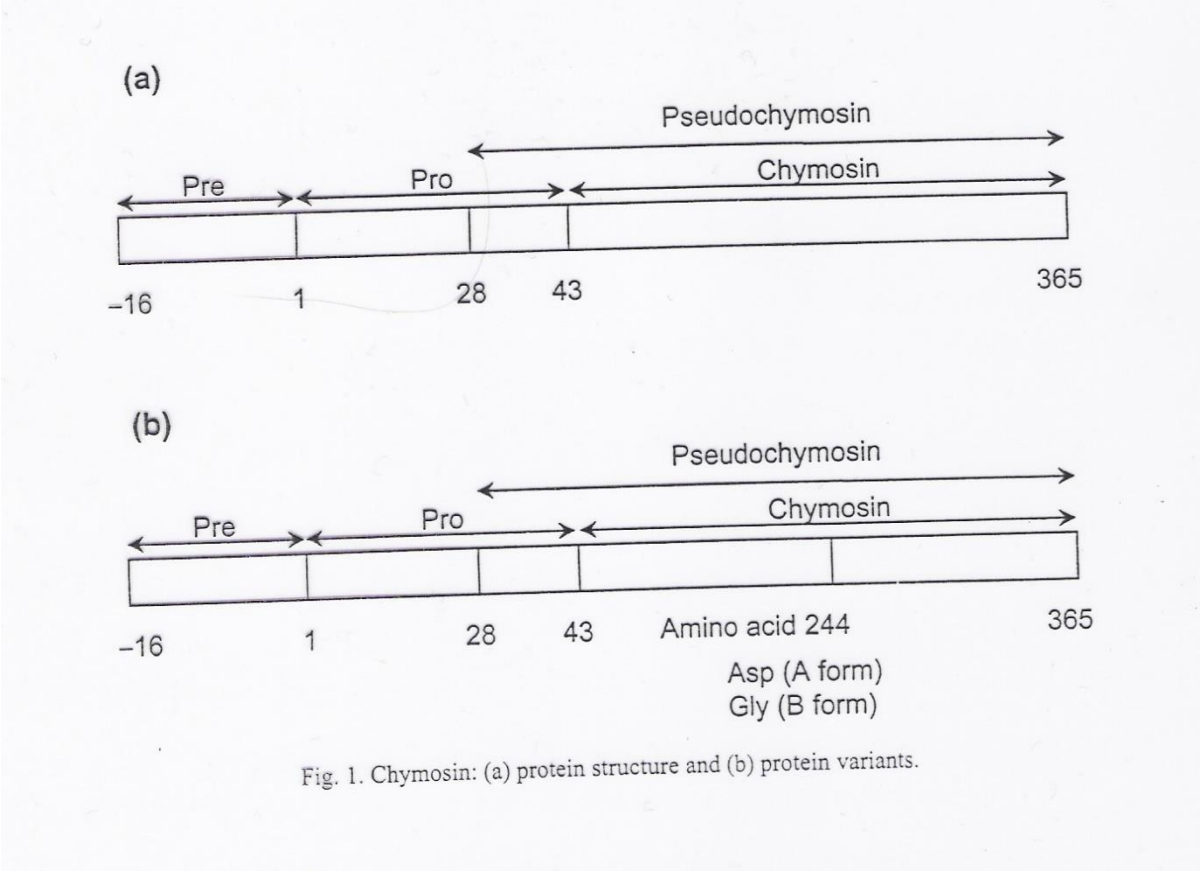


Fig. 1. Chymosin: (a) protein structure and (b) protein variants.

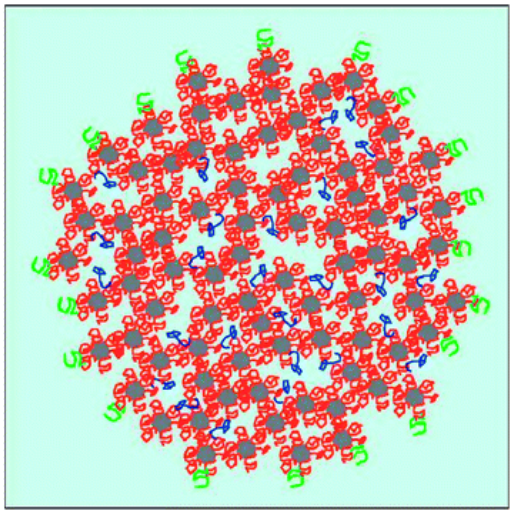
Chymosin

Aspartate protease,

pH optimum 3,5 - 6,5

Specificity: κ-casein

Casein mycelles



K-casein
β –casein
α – S1, S2

Surface of casein mycelle

-glu-…..-his-(pro-his)$_2$-leu-ser-phe-met-ala ……val

1 105 106 169

Para-κ-casein

hydrophobic

Casein macropeptide

hydrophilic

Recombinant: mRNA from calf abomasum - production MO - E.coli, B. subtilis, S.cerevisiae, K. lactis, A. niger

Plant proteases

Papain – latex from C. papaya, 5-8%, zymogen

Bromelain

Ficin - preferentially -Tyr , Phe

Actinidin

SH-proteases, wide specificity,

Cardosin (cynarase) aspartate protease

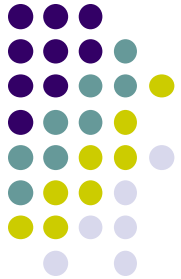
Cardoon –
***Cynara cardunculus* L**

Microbial proteases

MIKROBIÁLNÍ PROTEASY

Mikroorganismus		serinové	SH-	aspartátové	metalo -
Bacillus	alcalophilus	+			
	amyloliquefaciens	+			
	amylosaccharicus	+			
	licheniformis	+		+	
	lentus				+
	polymixa				+
	subtilis				+
	thermoproteolyticus				+
Aspergillus	flavus	+			
	melleus	+			
	oryzae	+		+	+
	niger			+	
	saitoi			+	
	sojae			+	
Endothia parasitica				+	
Mucor miehei				+	
Mucor pusillus				+	
Penicillium sp.				+	
Rhizopus delemar				+	
Clostridium sp.					+
Streptomyces griseus					+

Microbial proteases - characteristics



- Bacterial (Bacillus)

Neutral - metalloproteases, serine proteases
pH 5 – 8, low thermotolerance (advantageous for protein hydrolysates)
not inhibited by plant proteinase inhibitors (STI - antinutrational)
specificity – hydrophobic AA

Thermolysine - Zn^{2+} metalloprotease from *B.thermoproteolyticus,* stabilised by Ca^{2+} at higher temperature (1h, 80 °C, 50% of activity) – production of Aspartam

Alkaline - pH optimum ≈ 10, temp. optimum ≈ 60 ºC (suitable for biodetergents)
wide specificity (from alkaliphilic or alkalitolerant MO)

Subtilisin – alkalic serine protease from *B. amyloliquefaciens*, 27.5 kDa, type Carlsberg and BPN

cat. triade Asp32,His61,Ser221

Protein engineering: mutation of 50% of AA from 275

↑activity, – SDM Met222xSer, Ala

changing specificity by mutations in the binding site

↑thermostability – introduction of S-S bridges

↑stability in alkalic solutions

↓autoproteolytic activity

- Fungal – acidic, neutral, alkalic, metallo,

  extracellular

  wide range of pH optima 4 – 11

  wide specificity, lower thermotolerance

Aspergillus, Penicillium, Cephalosporium, Trychoderma…

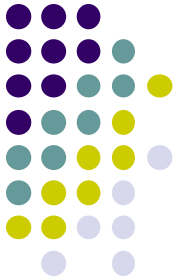
Glycosidases

Hydrolyse glycosidic bonds in homo- and heteroglycosides

Factors affecting the specificity of glycosidases

- configuration of the saccharide (D-, L-, α-, β-)
- character of the cyclic form (furanose, pyranose)
- character of aglycone, character of the atom in glycosidic bond
- size of the saccharide molecule

Determination of the glycosidase activity



- Increase of the reduction potential of the products
- Changes of the physical properties of the substrates (viscosity)
- Change of the optical rotation (polarimetry)
- Colorimetry or fluorometry using dyed polysaccharides
- Special methods (enzymatic)

Glycosidases

- Starch degrading enzymes

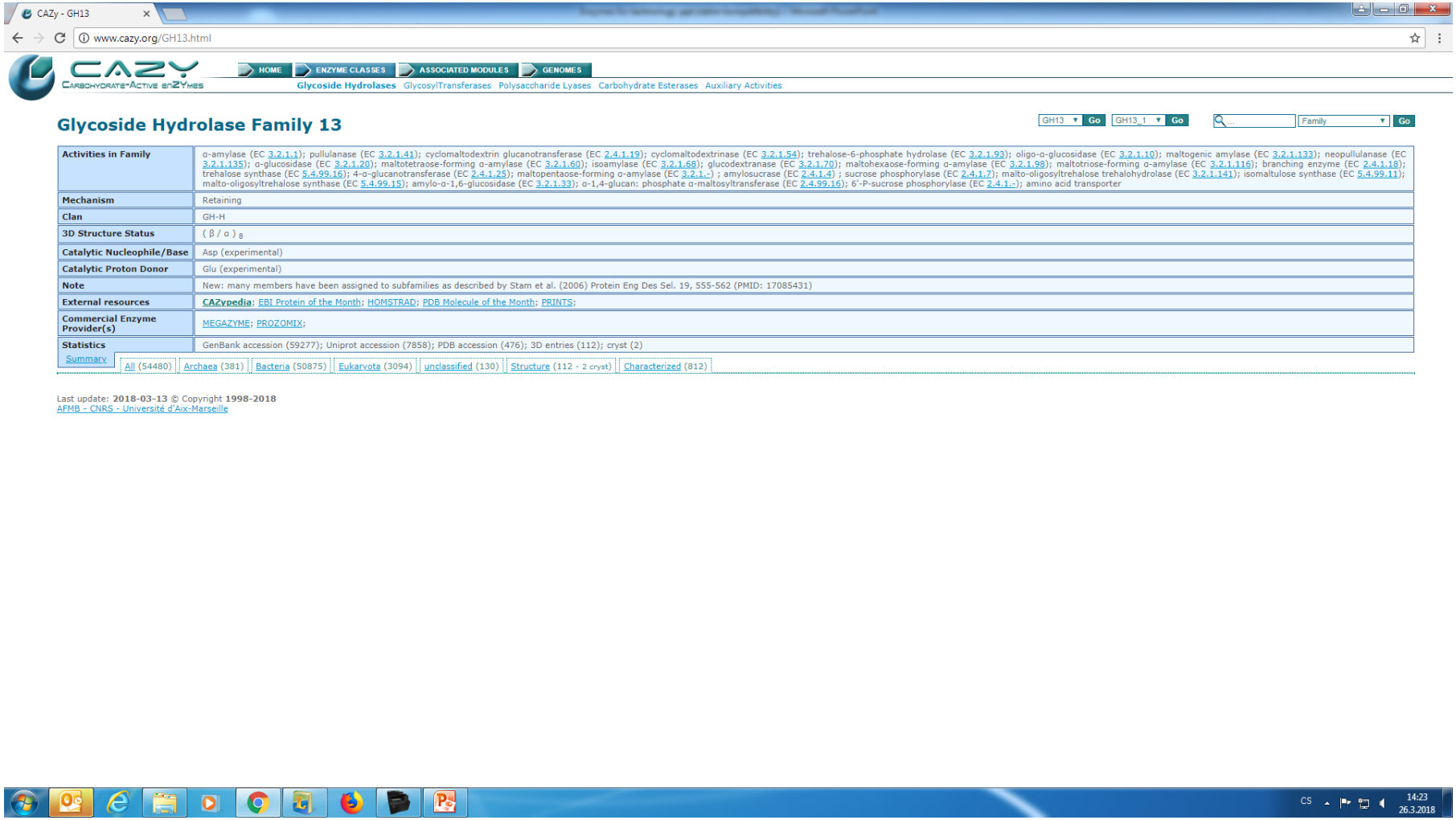
Endoamylases	α-amylase (α-1→4) GH13	Specific cleavage from non-reducing end of oligo- or polysaccharide
Exoamylases	β-amylase (α-1→4) GH14	
	Glucoamylases (α-1→4 and α-1→6)	
	α-glucosidases (α-1→4 and α-1→6)	
Debranching enzymes	pullulanases (α-1→6)	
	Isoamylases (α-1→6)	
Transferases	Cyclodextrin glycosyltransferase (α-1→4)	
	Branching enzyme (α-1→6)	

- Celulolytic enzymes
- Pectolytic enzymes
- Invertase, β-galactosidase

Glycosidases are classified according to structural similarities

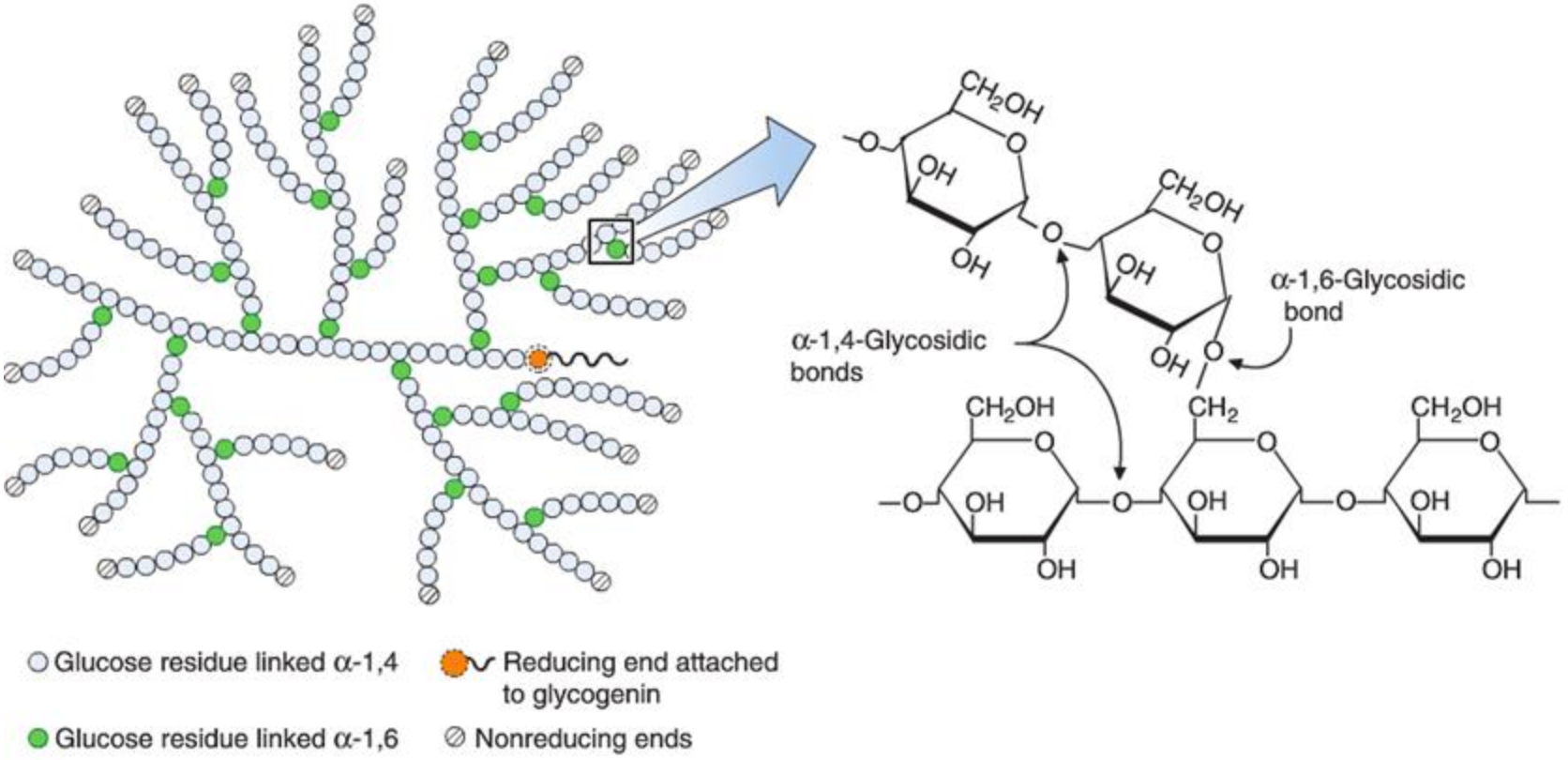
B.Henrissat: Glycosidase fymilies
Biochem Soc Trans. (1998) 26,153-6

Carbohydrate-Active enZYmes Database
http://www.cazy.org

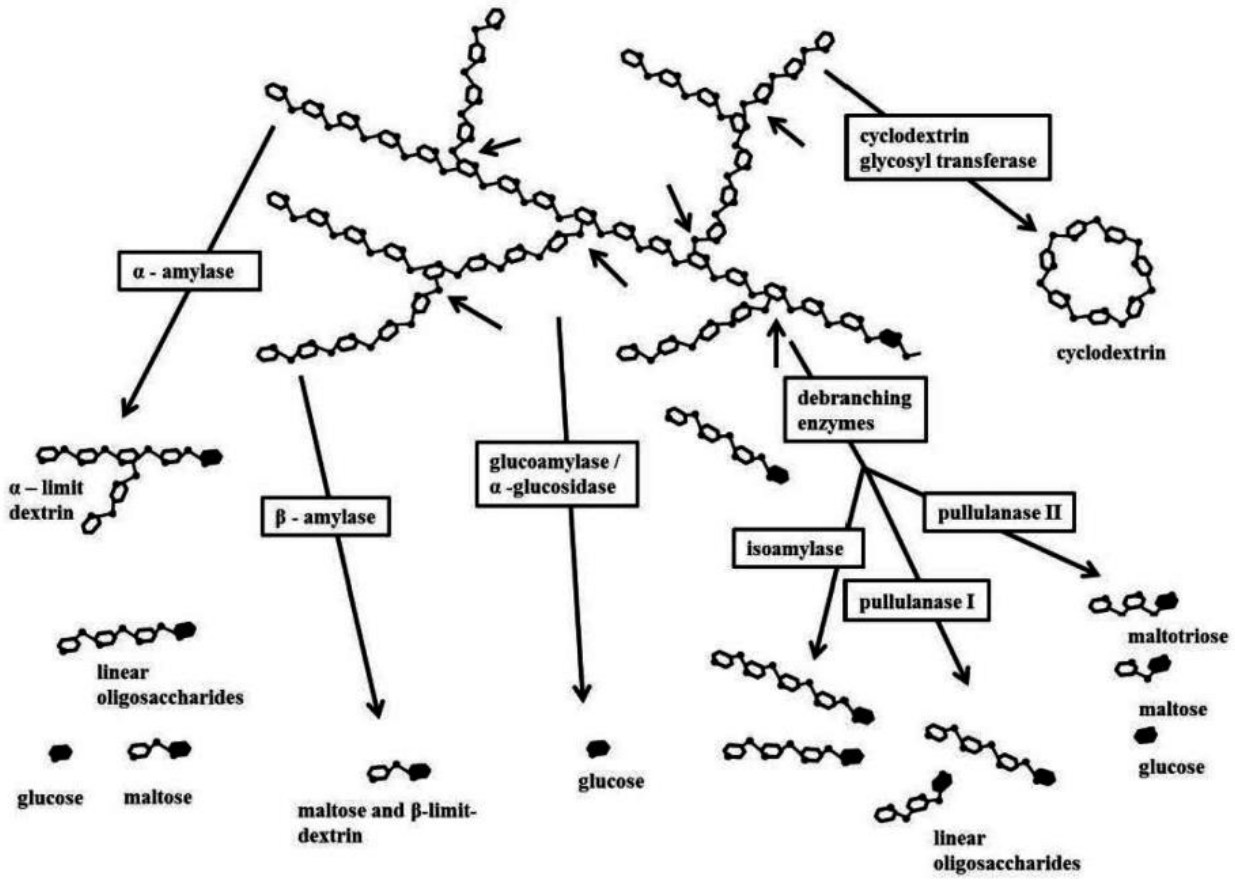


Strach degrading enzymes

Amylose – linear
Amylopectin branched



Starch degrading enzymes



Alpha- and beta – limit dextrins

Sources and properties of amylases

Plant α-amylase	**Plant β-amylase**	**Bacterial α-amylase**	**Fungal α-amylase**	**Gluco-amylase**	**Mammalian α-amylase**
Barley and malted barley	Barley and malted barley	*Bacillus amyloliquefaciens*	*Aspergillus oryzae*	*Aspergillus niger*	Saliva
Wheat and malted wheat	Soybean sweet potato	*Bacillus subtilis*	*Aspergillus candidus*	*Rhizopus delemar*	Pancreas
Malted sorghum	Wheat	*Bacillus licheniformis*		*Rhizopus niveus*	
		Pseudomonas stutzeri (G4 amylase)			

Bacterial - thermostable (110 °C), pH - neutral, $Ca^{2+,}$ stabilised by substrate

Fungal - lower temperature stability, specificity of β-amylase

Debranching enzymes

R-enzymes (plant, animal)

Pullulanases (microbial)

Type I specific for

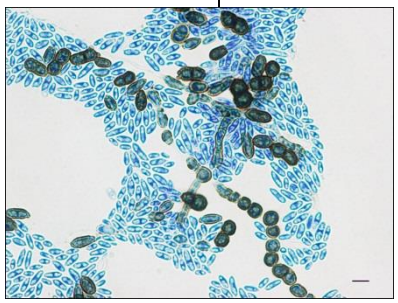
α-1,6 glycosidic bonds

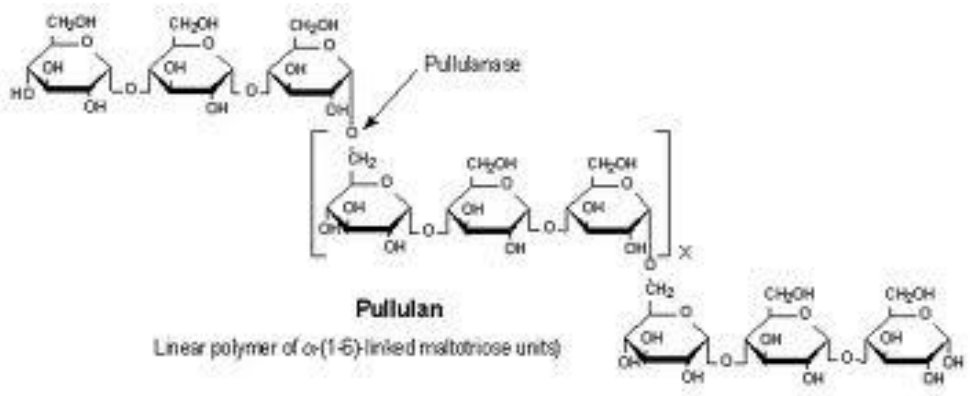
Type II specific for α-1,6 and α-1,4

glycosidic bonds

Aureobasidium pullulans

Yeast like fungus





Transferase activity ….

1. Glycosidases in general
2. Amylomaltase (EC 2.4.1.25, forming α-1,4 bonds)
3. Branching enzyme (EC 2.4.1.18, forming α-1,6 bonds
4. Cyclodextrin glycosyltransferase, (EC 2.4.1.19) *B.macerans*

Cyclodextrins:

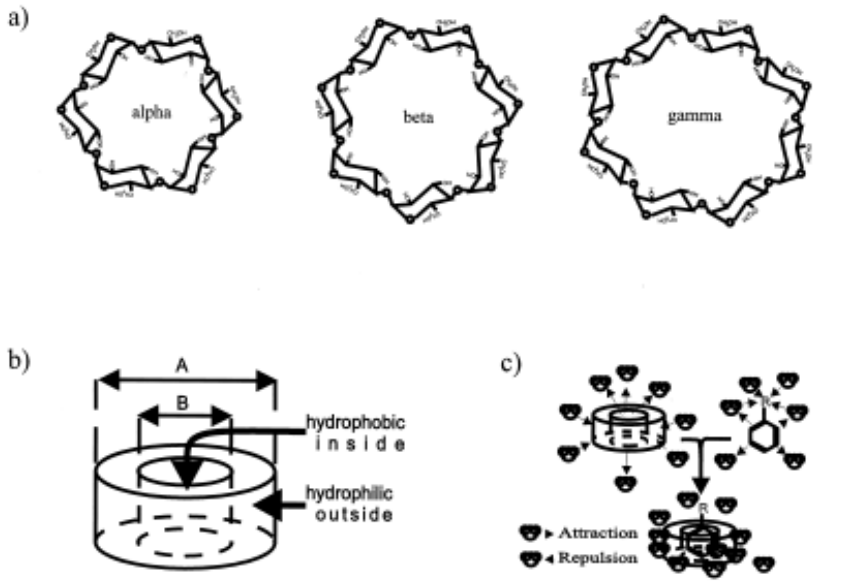


Fig. 1. Structure and properties of cyclodextrins. (a) α-, β-, and γ-cyclodextrins; (b) three-dimensional form and properties of cyclodextrins (for sizes of A and B, see Table I); (c) formation of inclusion complex of a cyclodextrin with a hydrophobic molecule. (Reproduced from Pennings [139].)

Effect of cyclodextrins on the incorporated molecules:

Stabilisation

Lower volatility

Change of chemical reactivity

Increased solubility

Change of sensoric properties

Application:

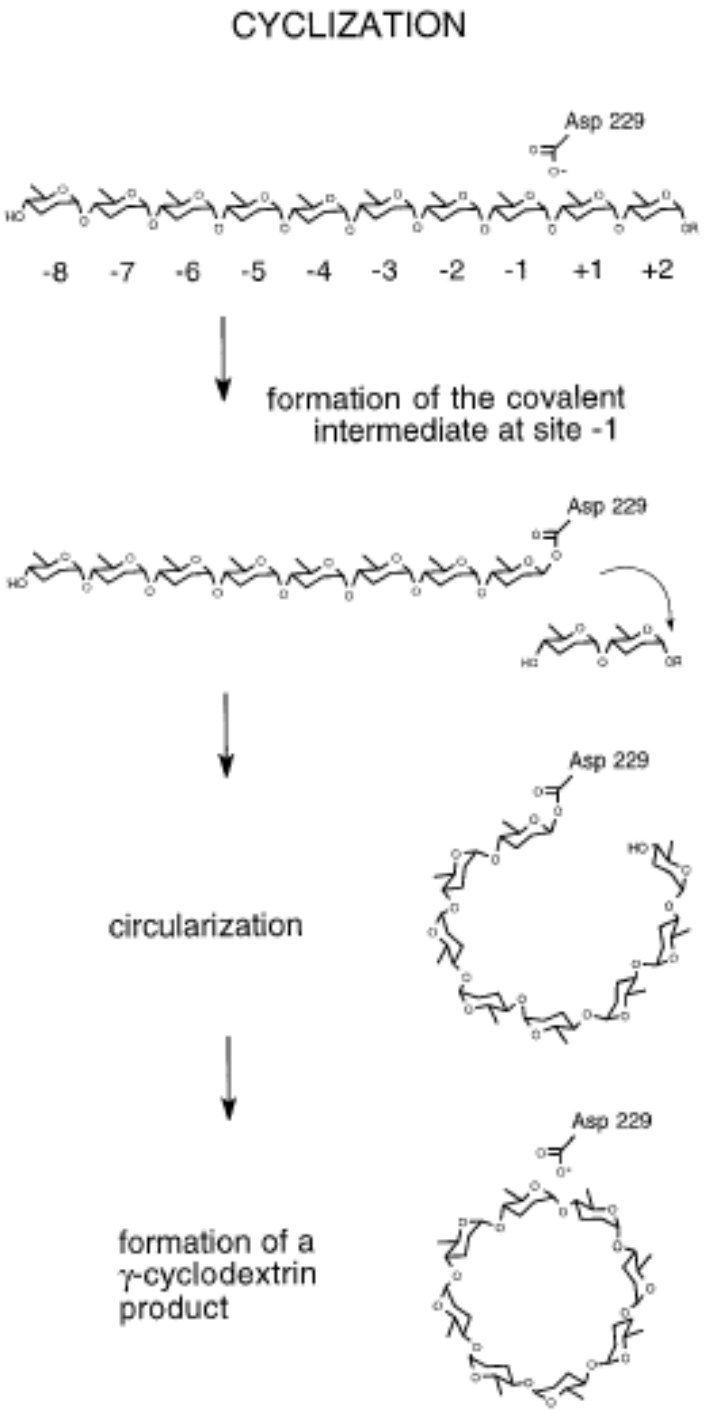
Analytical chemistry

Production of pharmaceuticals (prolonged effect)

Food industry (additives, aromas etc..

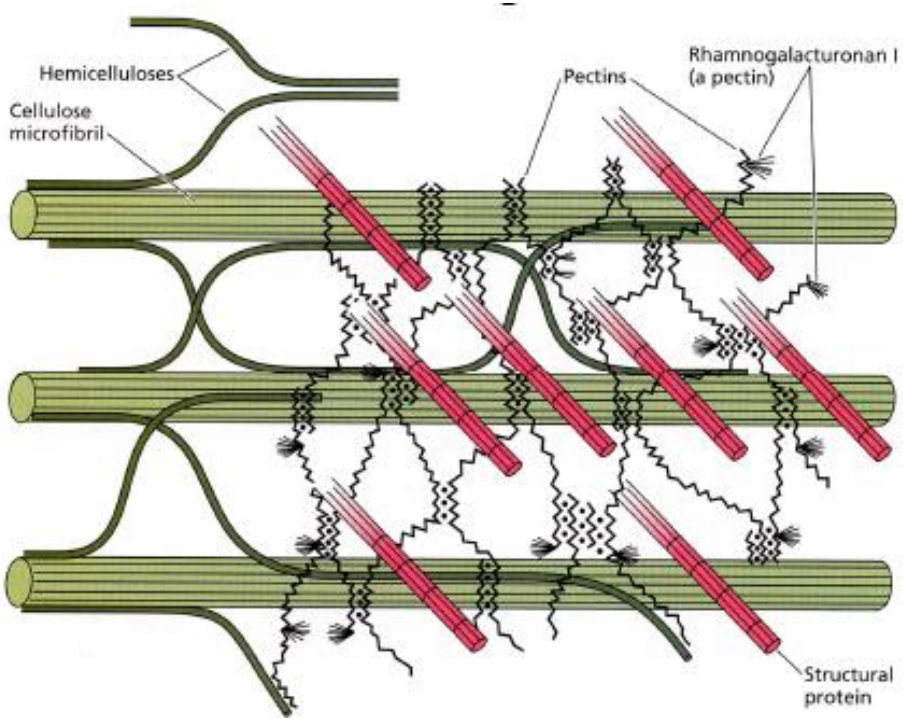
Cosmetics

Reaction catalyzed by CGTase



Enzymes degrading polysaccharides of the plant cell walls

Components of the plant cell walls:

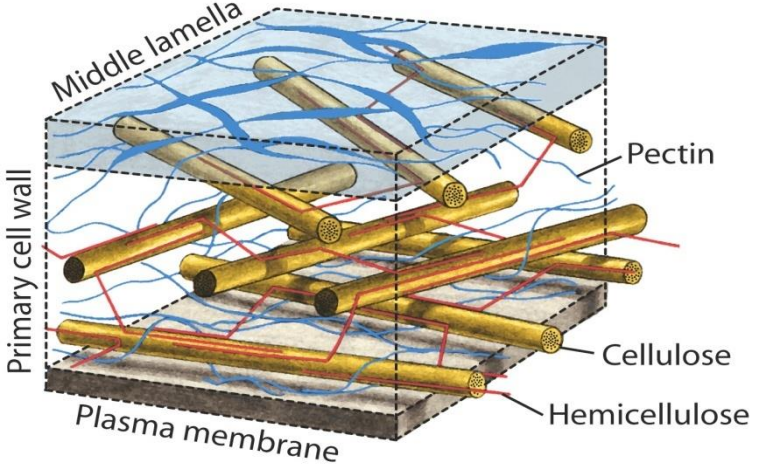


Cellulose

Hemicelluloses

Pectin

Structural protein - extensin



Pectic compounds – structure:

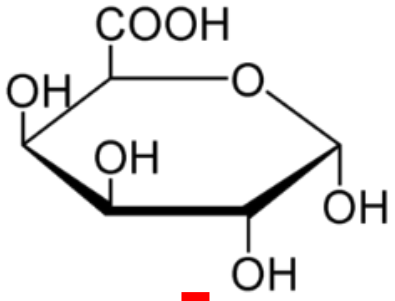
1. Homopolygalacturonan
2. Rhamnogalacturonan I
3. Rhamnogalacturonan II

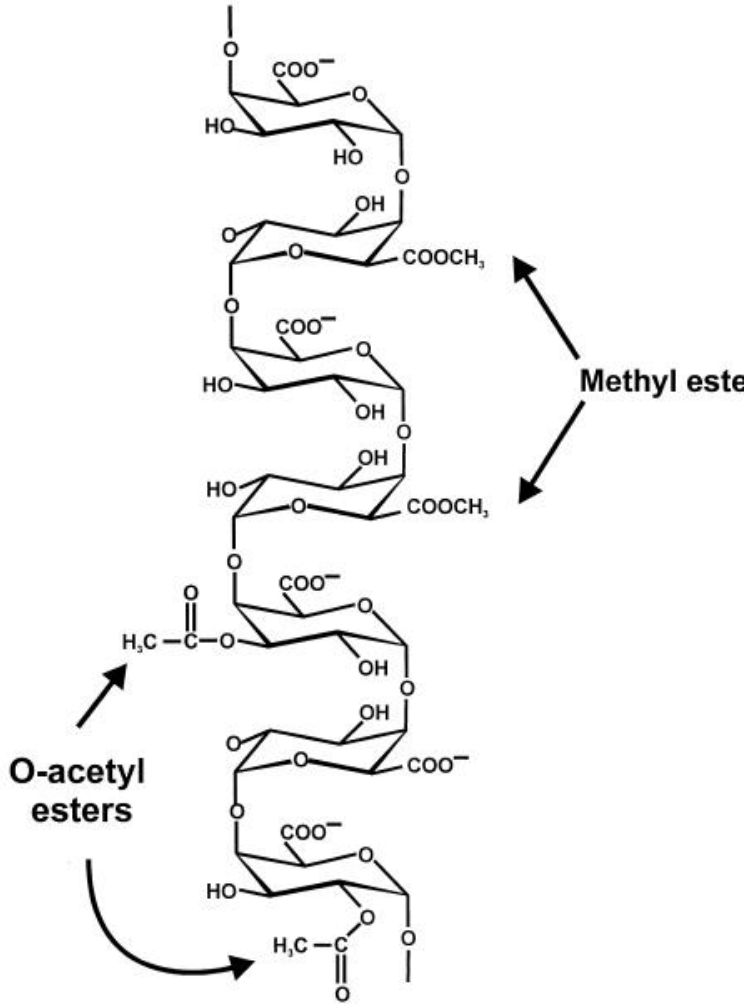
Homogalacturonan (HG) - linear galacturonic acids,

α-1,4- bonds

~ 200 monosacch.units

Partially esterified

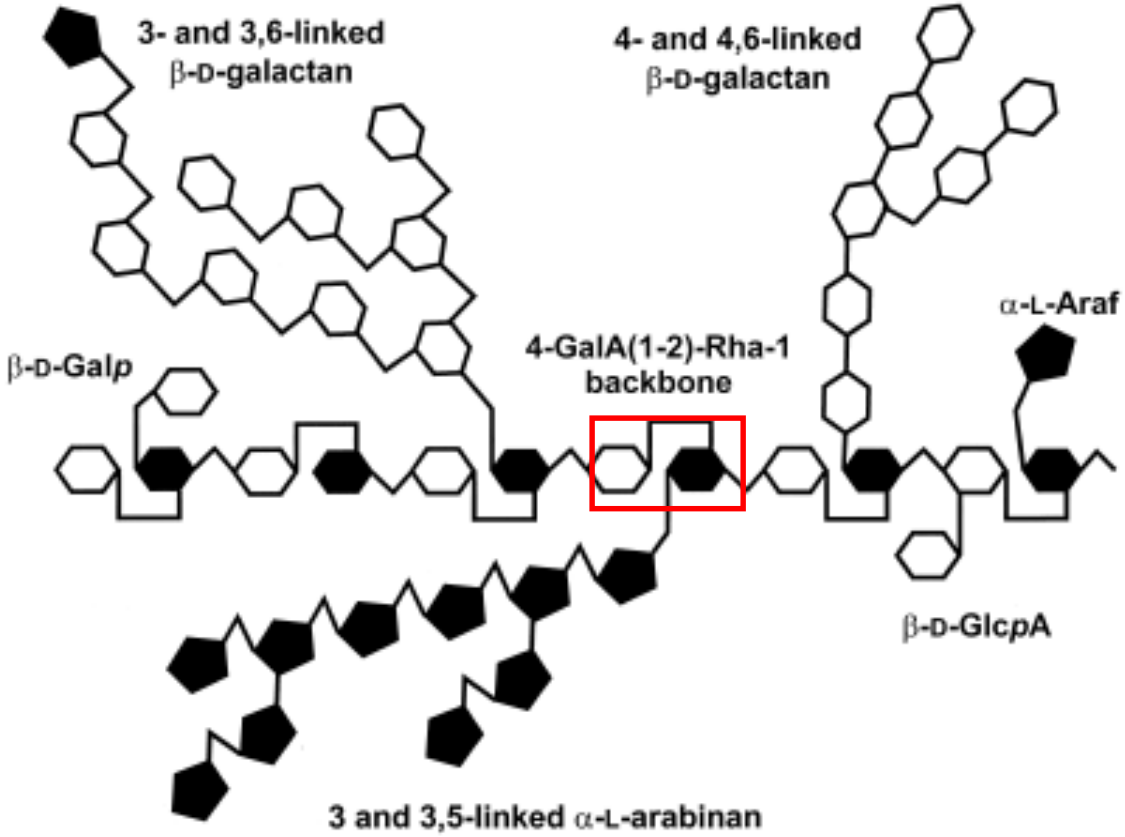




Rhamnogalacturonan I - branched,

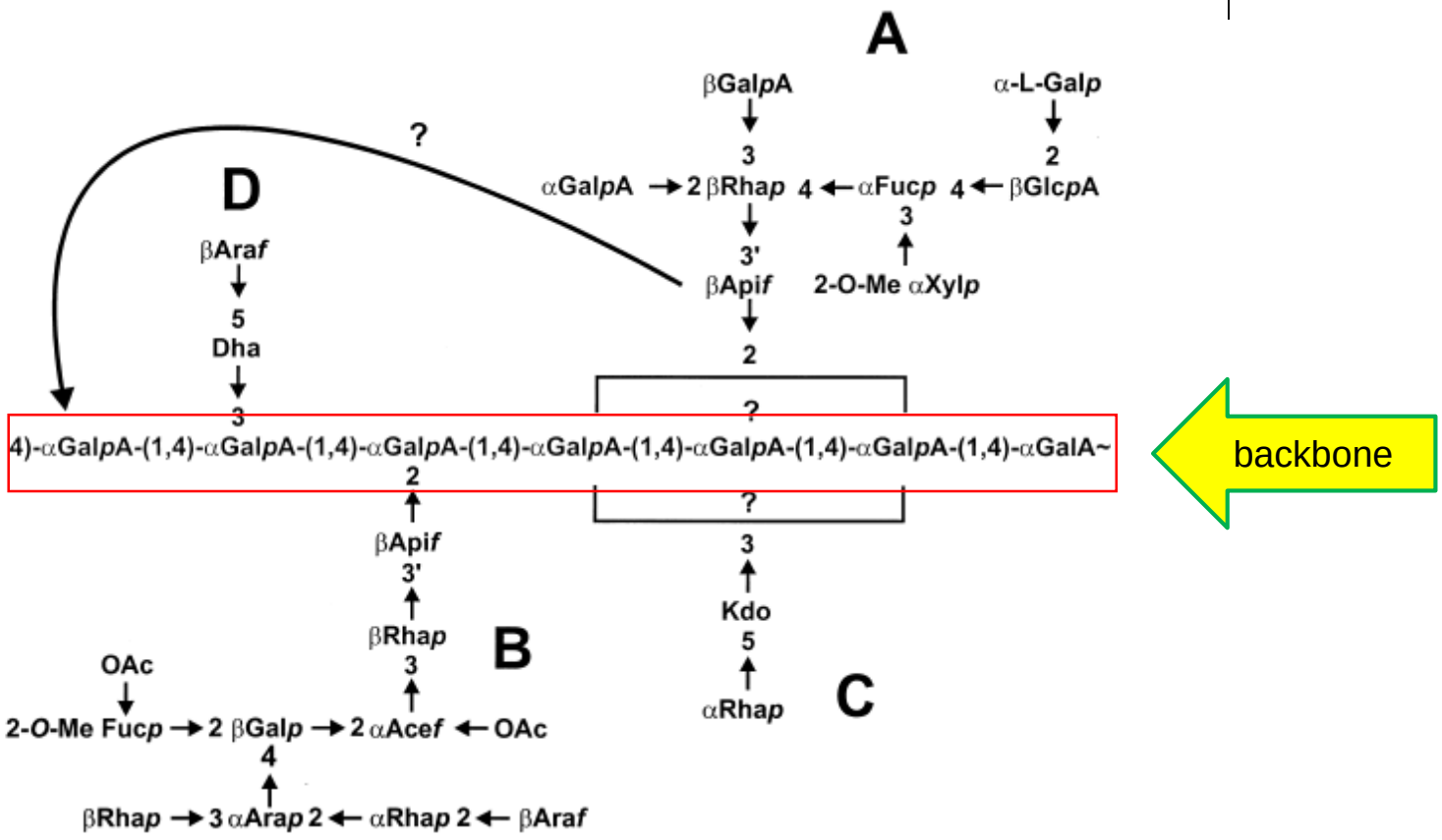
backbone [→4)-α -D-Gal*p*A-(1→2)- α -L-Rha*p*-(1→]

Branching on C4 Rha



Rhamnogalacturonan II, branched

Backbone: polygalA, 4 types of side chains



Kdo = 3-deoxy-D-*manno*-octulosonic acid

Pectine structure

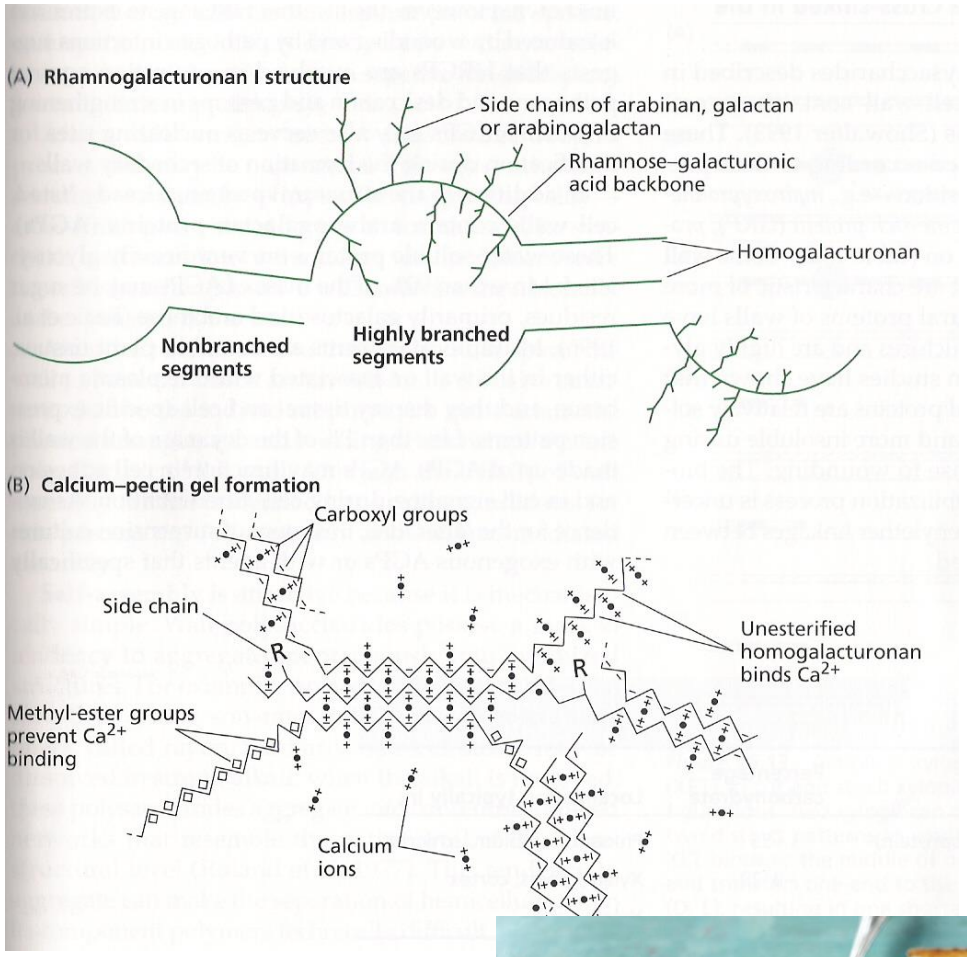
Mh:

Apples, lemon: 200 - 300 kDa

Pairs, plums: 25 -35 kDa

Oranges: 40 - 50 kDa

Sugar beet: 40 - 50 kDa



Pectic compounds – classification (in food industry)

1. Protopectin – insoluble in water, present in the intact tissue (non-ripened fruit), limit hydrolysis leads to pectin and pectic acid
2. Pectinic acid – polygalcturonan with the degree of esterification less than 75%
3. Pectin – polymethylgalacturonan, at least 75% of carboxylic groups are esterified
4. Pectic acid – soluble homopolygalacturonan, practically non esterified

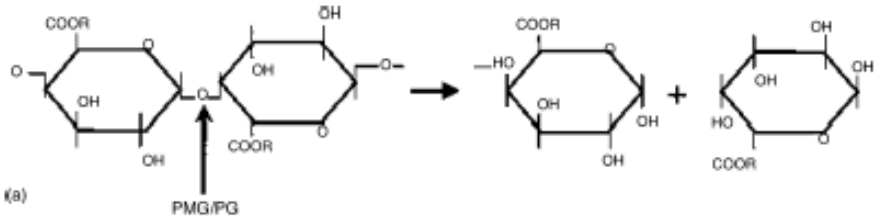
Fruit ripening

Immature (firm texture)

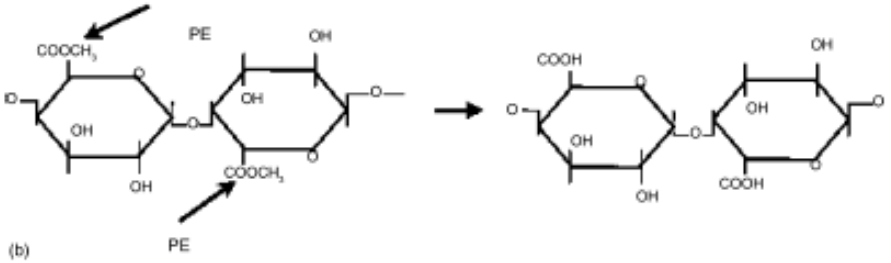
Mature (mashy)

Reactions catalyzed by pectolytic enzymes

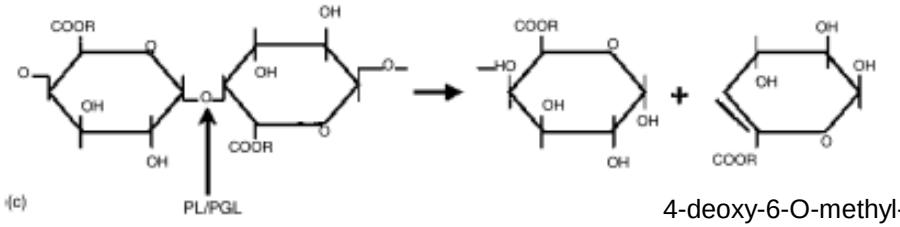
Polygalacturonases – depolymerizing e.



Pectin esterases

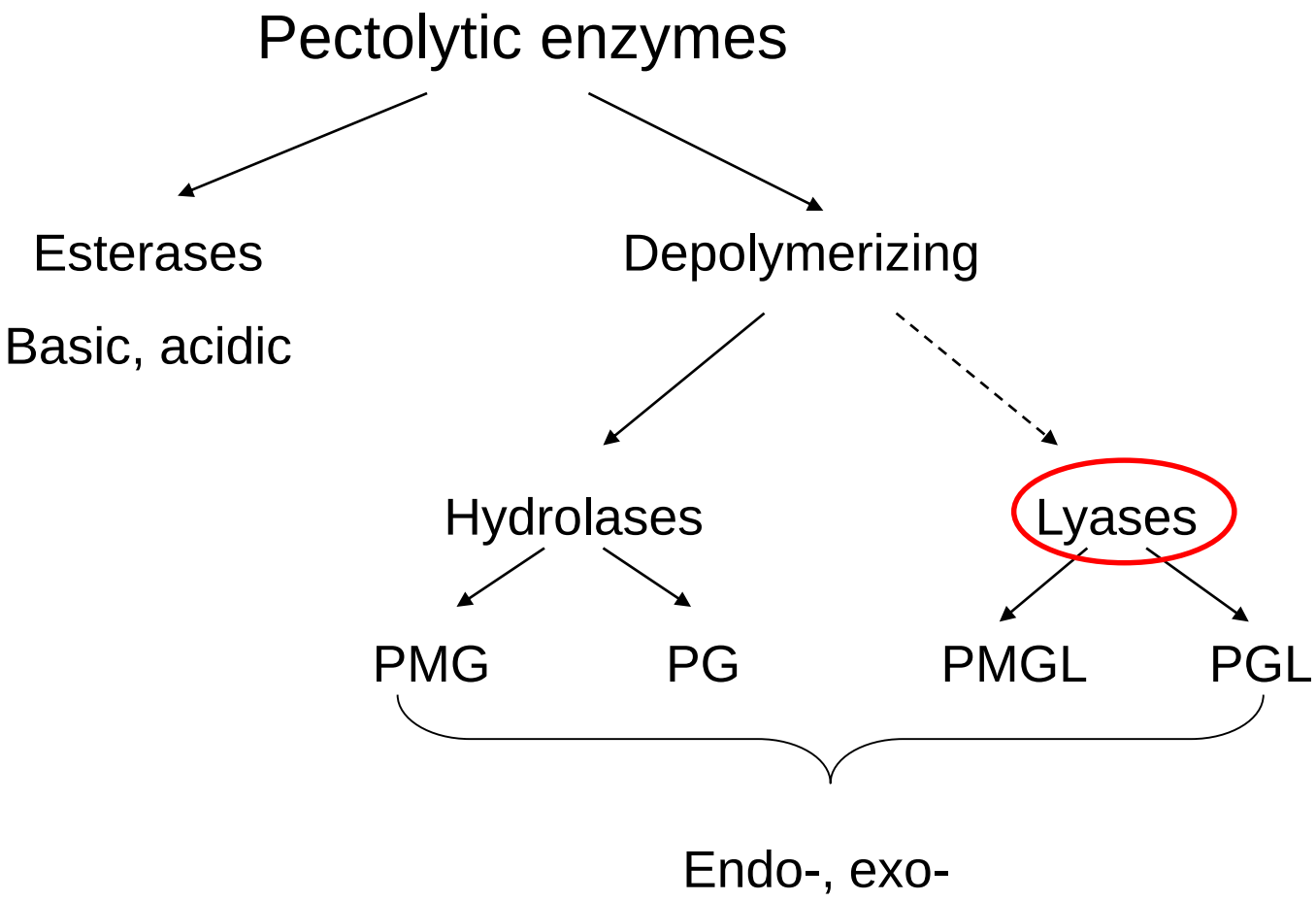


Pectin lyases - depolymerizing



4-deoxy-6-O-methyl-alpha-D-galact-4-enuronosyl

Degradation of pectic compounds is functional in physiological processes – most important is fruit ripening



favoured substrates: PMG- Polymethylgalacturonan, PG -polygalacturonan –

Depolymerazing enzymes

1. Polygalacturonases (according to preferred substrate – pectin resp. pectic acid)

pH-optimum 4 -5

Endopolygalacturonases	x	exopolygalacturonases
Decrease of viscosity		digalacturonic acid (bacterial)
higher oligogalacturonides		galacturonic acid (fungal)
		able to cleave digalacturonans

2. Lyases

pH optimum 8 - 9

fungal, bacterial, plant

Activity determination: ΔA_{235}

Substrate – highly esterified pectin

Pectin esterases

Specificity: D-methylgalacturonans, free carboxyl in the vicinity

Basic (pH optimum 7 - 8)

- plant
- microbial
- fungal

deesterification in blocs

Inhibited by reaction product (pectic acid)

X

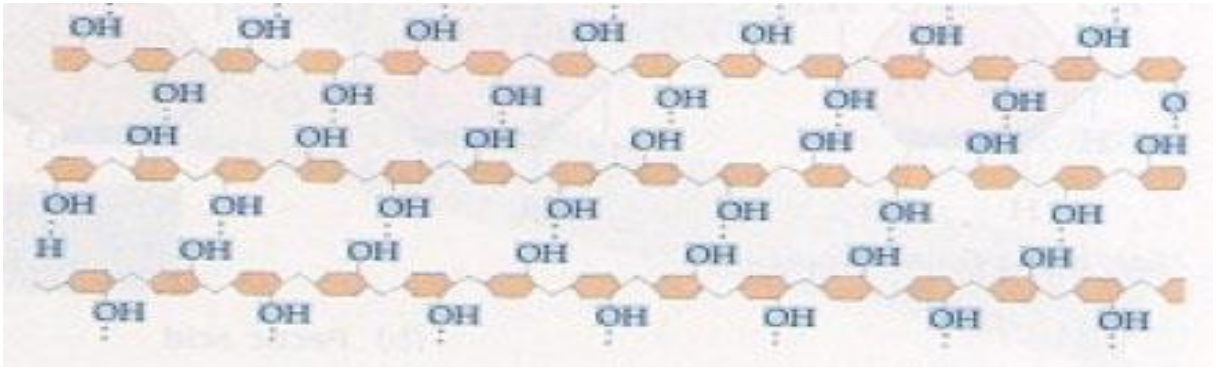
Acidic (pH optimum 4 - 6)

- fungal

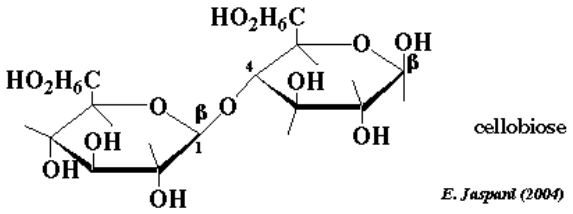
deesterification proseeds statistically

Esterases don´t decrease the viscosity of pectins in solution, on the contrary increase of viscosity in the presence of Ca^{2+} occurs

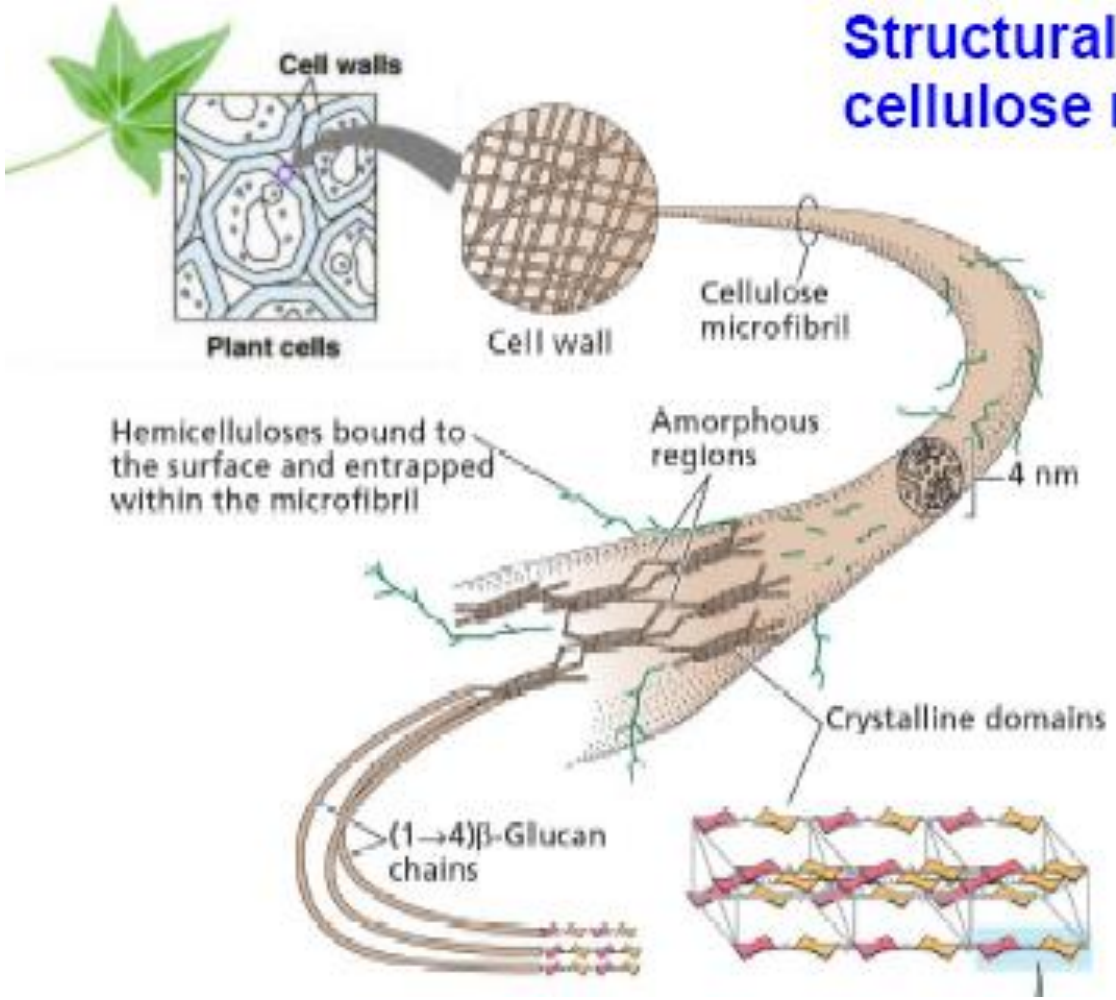
Cellulose structure



Cellobiose – structural unit

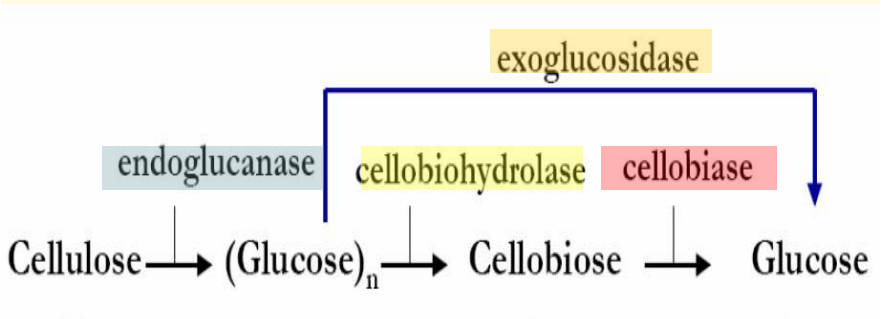


Structural model of a cellulose microfibril



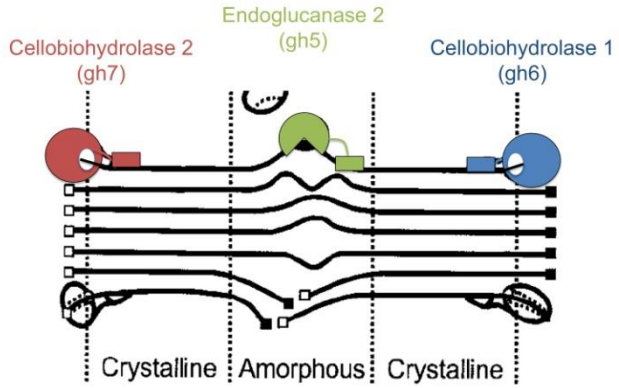
Celulolytic enzymes

multicomponent enzyme system:



glycoprotein

2 domains: catalytic + binding



Current model for enzymatic degradation of cellulose

Main producer : Trichoderma reesei

Problem: glucose and cellobiose are inhibitors of cellulase system

Hemicelluloses

(soluble in diluted alkali)

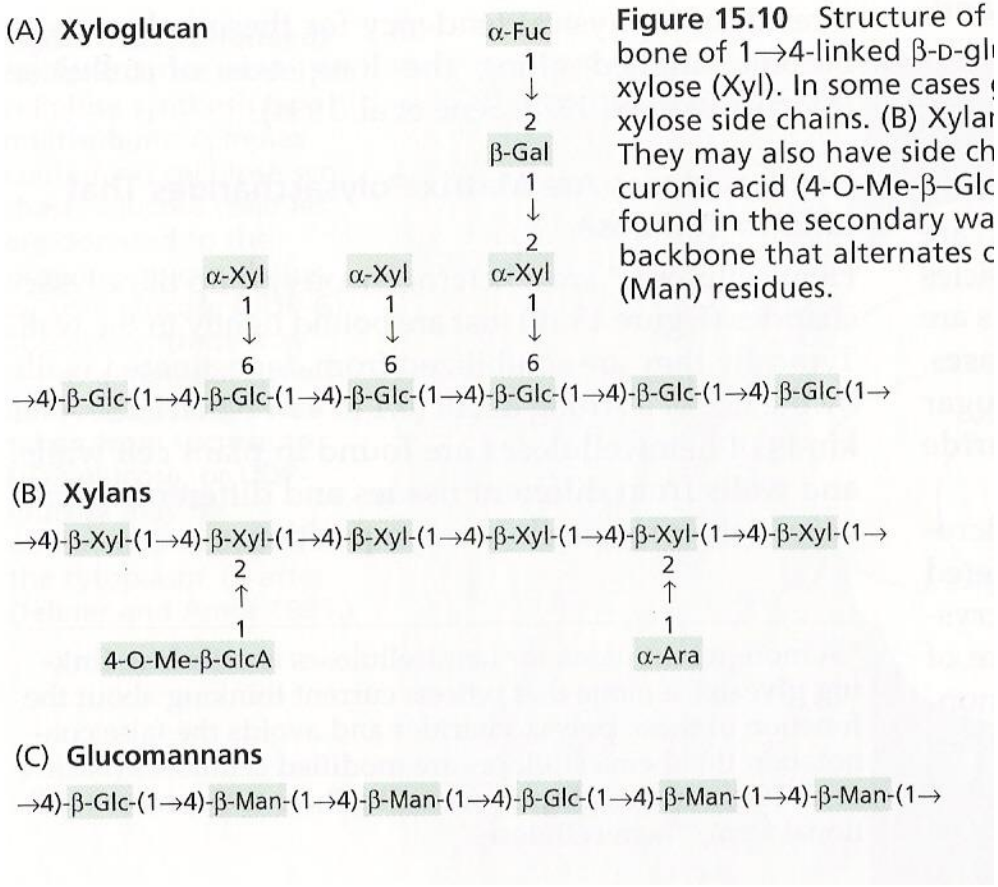
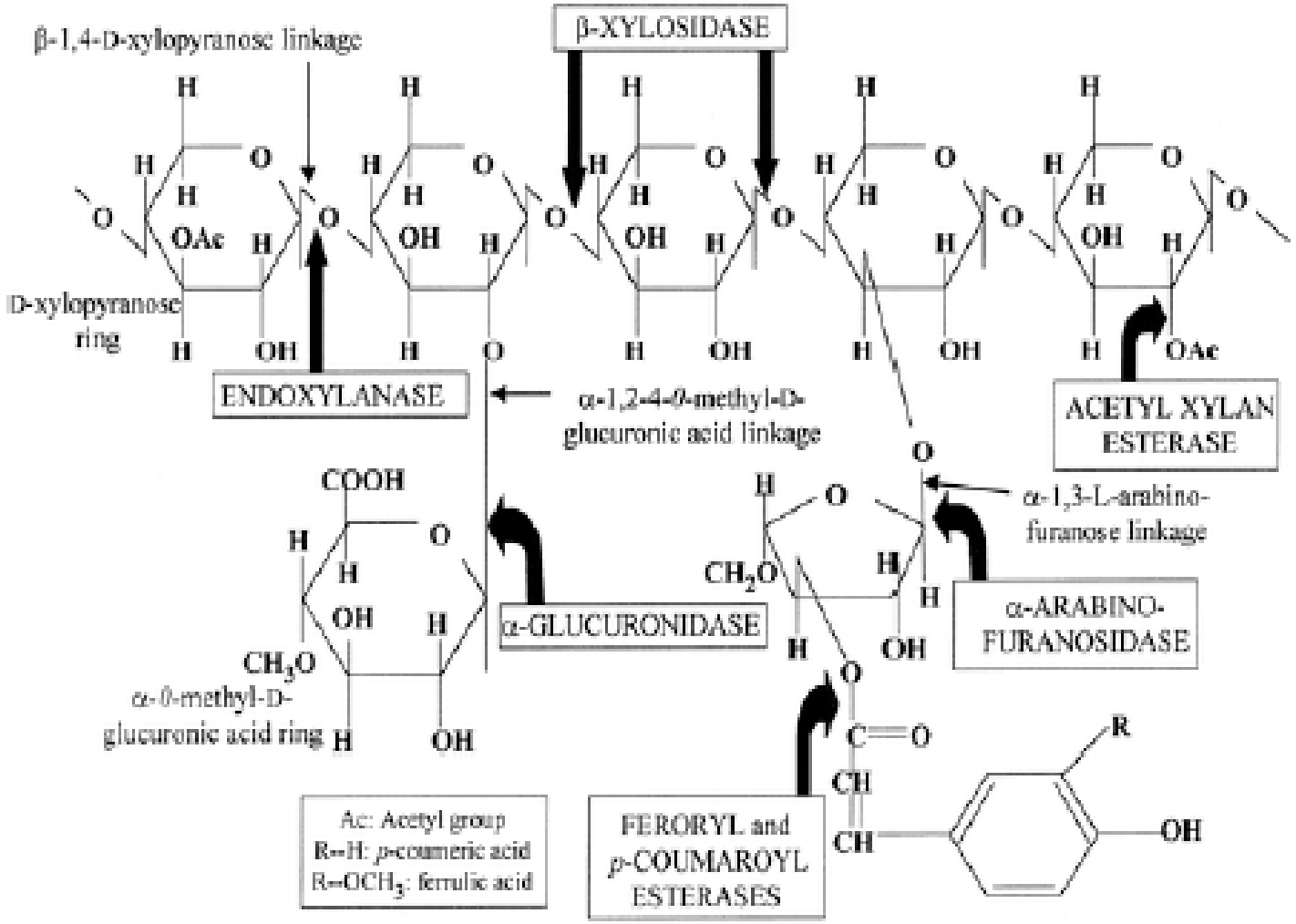


Figure 15.10 Structure of
bone of 1→4-linked β-D-gl
xylose (Xyl). In some cases
xylose side chains. (B) Xyla
They may also have side ch
curonic acid (4-O-Me-β–Glc
found in the secondary wa
backbone that alternates c
(Man) residues.

Hemicelullases



β-galactosidase - (EC 3.2.1.23)

Lactose ⟶ D-galactose + D-glucose

Sources: bacteria, fungi, yeasts

Fungal: - acid pH 2,4 - 5,4, 50°C

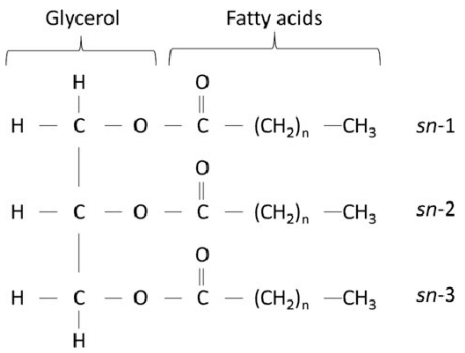
Psychrophilic microorganisms

Degradation of lactose in milk products

Transglycosylation reaction - biotransformations

Lipases

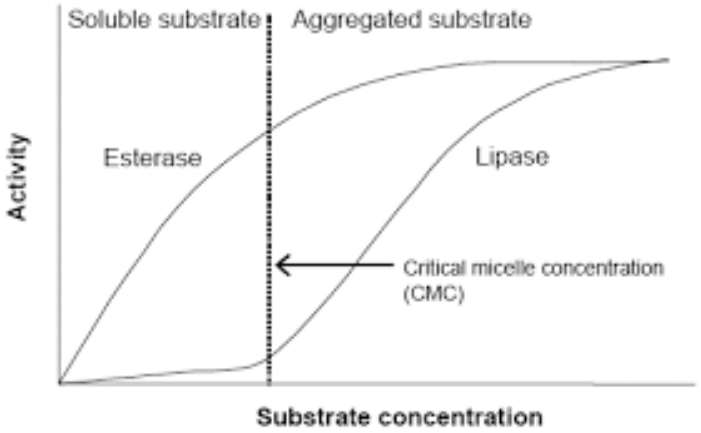
TAG → DAG (1,2 or 1,3) → MAG → FA + glycerol



Animal, plant, microbial

Active on the oil-water interface (CMC – critical micelle concentration)

mechanism - catalytic triad similar to the serin proteases



Highly stereospecific (biotransformations)

Very low specificity to FA and position of ester bond in triacyl glycerol

Some bacterial lipases specific for position 2

Reactions catalysed by lipases

Hydrolysis :

Ester synthesis :

Acidolysis :

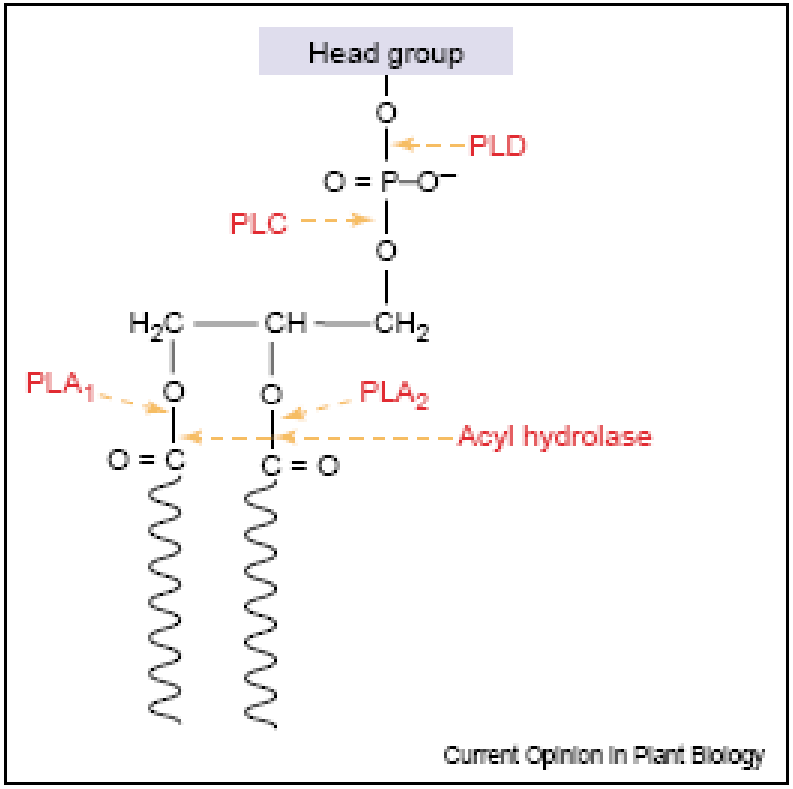
Interesterification :

Alcoholysis :

Aminolysis :

Phospholipases

Animal, plant, microbial



Reaction products:

PLD: PA + polar head

PI-PLC: DAG + IP_3

PC-PLC: DAG + P-Cho

PLA: FA + lysoPL

PA – low solubility, oil spoilage

lysoPL - surfactants

Transphosphatidylation reaction of PLD