

Cellulase

Introduction (source)

Cellulase refers to an entourage of enzymes produced chiefly by fungi, bacteria and protozoans that catalyze cellulolysis (i.e. the hydrolysis of cellulose).

However, there are also cellulases produced by a few other types of organisms, such as some termites and the microbial intestinal symbionts of other termites.

Several different kinds of cellulases are known, which differ structurally and mechanistically.

Cellulase



Cellobiohydrolases

whose major activity involves the cleavage of cellobiose residues consecutively from the ends of the cellulose chains

Endoglucanases

whose major activity involves the cleavage of β -glycosidic bonds in the cellulose chain

they are necessary for the efficient hydrolysis of cellulose to soluble oligosaccharides

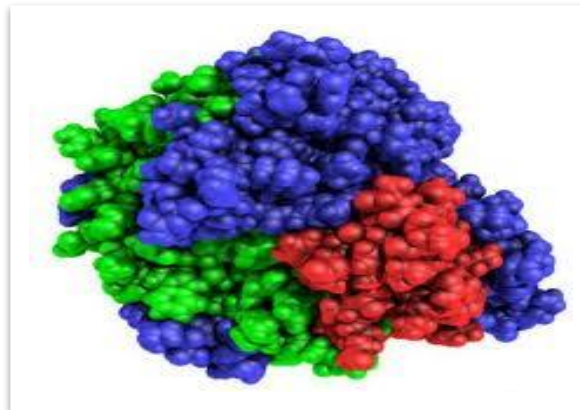
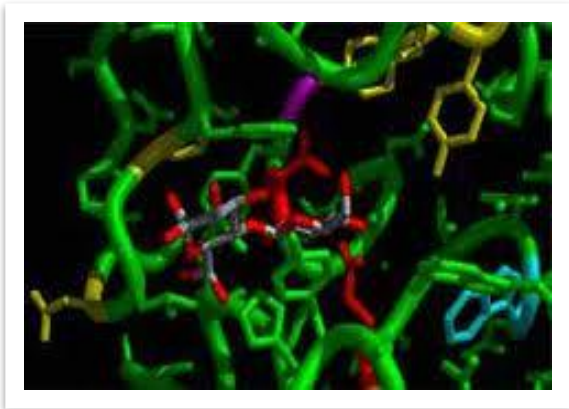
Complete vs. incomplete cellulases

- Some species of fungi and bacteria are able to exhaustively digest crystalline cellulose in pure culture are said to have complete or true cellulases.
- The majority of organisms that produce cellulases can only hydrolyze the cellulose in their diets to certain extent. they are known as incomplete cellulases.
- These cellulases unable to digest cellulose exhaustively can still generate sufficient amount of glucose for their producers. Endogenous cellulases of termites belong to this category.

Other Names

Other names for 'endoglucanases' are:

endo-1,4-beta-glucanase, carboxymethyl cellulase (CMCase), endo-1,4-beta-D-glucanase, beta-1,4-glucanase, beta-1,4-endoglucan hydrolase, and celludextrinase. The other types of cellulases are called exocellulases.



Types of reactions/ Classification

General types of cellulases based on the type of reaction catalyzed:

1. Cleaves internal bonds at Endocellulase (EC 3.2.1.4) randomly amorphous sites that create new chain ends.
2. Cellobiase (EC 3.2.1.21) or beta-glucosidase hydrolyses the exocellulase product into individual monosaccharides.
3. Cellulose phosphorylases depolymerize cellulose using phosphates instead of water.

Choice of host organism

Table 1 — Major microorganisms employed in cellulase production

Major group	Microorganism		Ref					
	Genus	Species						
Fungi	<i>Aspergillus</i>	<i>A. niger</i>	40	Bacteria	<i>Acidothermus</i>	<i>A. cellulolyticus</i>	52	
		<i>A. nidulans</i>	43		<i>Bacillus</i>	<i>Bacillus sp</i>	49	
		<i>A. oryzae</i> (recombinant)	44		<i>Clostridium</i>	<i>Bacillus subtilis</i>	50	
	<i>Fusarium</i>	<i>F. solani</i>	46		<i>Pseudomonas</i>	<i>C. acetobutylicum</i>	54	
		<i>F. oxysporum</i>	47		<i>Rhodothermus</i>	<i>C. thremocellum</i>	55	
	<i>Humicola</i>	<i>H. insolens</i>	36		Actinomycetes	<i>Cellulomonas</i>	<i>P. cellulosa</i>	51
		<i>H. grisea</i>	42			<i>Streptomyces</i>	<i>R. marinus</i>	53
	<i>Melanocarpus</i>	<i>M. albomyces</i>	48			<i>Cellulomonas</i>	<i>C. fimi</i>	58
	<i>Penicillium</i>	<i>P. brasilianum</i>	38			<i>Streptomyces</i>	<i>C. bioazotea</i>	32
		<i>P. occitanis</i>	37			<i>Streptomyces</i>	<i>C. uda</i>	59
		<i>P. decumbans</i>	45			<i>Streptomyces</i>	<i>S. drozdowiczii</i>	60
	<i>Trichoderma</i>	<i>T. reesei</i>	9			<i>Thermononospora</i>	<i>S. sp</i>	61
		<i>T. longibrachiatum</i>	41			<i>Thermononospora</i>	<i>S. lividans</i>	62
		<i>T. harzianum</i>	18			<i>Thermononospora</i>	<i>T. fusca</i>	56
							<i>Thermononospora</i>	<i>T. curvata</i>

Strain engineering

- Thermostable cellulases production
- Nowadays, most of the studies about production of thermostable cellulases are focused on the utilization of cellulase-producing thermo/alkalophiles and also, on the improvement of cellulase production by optimizing its nutritional and environmental necessities or by engineering new highproducer recombinants or cellulase-producing transgenic plants, such as transgenic tobacco

Homologous overexpression in bacteria

- Some studies report the use of directed evolution techniques in combination with a rational design to overexpress cellulases in their own bacterial source. Genera such as *Bacillus* (*B. subtilis*) and *Clostridium* (*C. thermocellum*) were used as a homologous cellulases production system, their easy genetic modification and other proper features.
- However, the use of these bacteria has disadvantages such as low protein yields, high production costs or need of enriched media

Heterologous overexpression

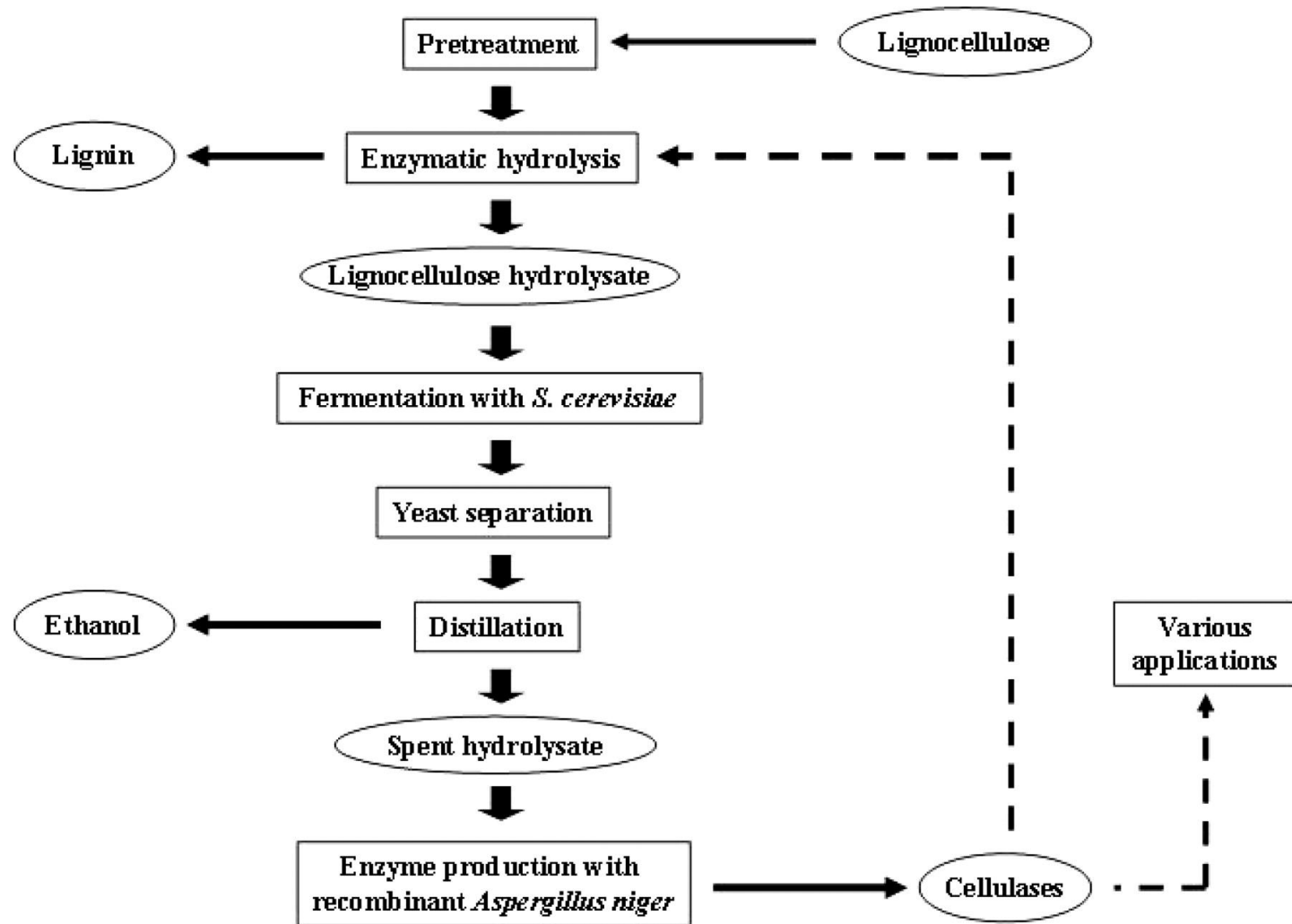
- The strategies based in heterologous expression are focused in the use of non-cellulolytic micro/organisms that have high production ratio for expressing microbial cellulases
- Bacteria such as *E. coli*, different species from the genus *Bacillus*, *Pseudomonas fluorescens*, *Ralstonia eutropha* and *Zymomonas mobilis*;
- yeasts such as *Saccharomyces cerevisiae* and *Pichia pastoris* and filamentous fungi from genera *Aspergillus* and *Trichoderma* genera
are the most used in research and industry, considered as host systems for producing recombinant enzymes. Furthermore, cell cultures of mammals, plants or insects and transgenic plants and/or animals are used for protein expression

- Future targets for genetic manipulation and optimization will include the use of the cellulolytic system of *Clostridium thermocellum* for engineering new strains, depending of the concrete industrial application and the fully characterization of the promising thermophilic bacterium *Caldicellulosiruptor bescii*.

Table 2 — Cellulase production –Bioprocesses and organisms employed

Microorganism	Substrate	Method	Magnitude	Enzymes - Activity	Ref (s)
<i>Aspergillus niger</i> A 20	Cellulose	SmF	Shake flask	Cellobiase -27.5 U/ml	108
<i>A. niger</i> NRRL3	Wheat bran/Corn cob	SSF	Flask	Cellobiase-215 IU/g cellulose	117
<i>Bacillus pumilus</i>	CMCellulose/Glycerol	SmF	SF	CMCase-1.9 U/ml, Cellobiase - 1.2U.ml	109
<i>Bacillus sp</i> KSM N252	Carboxymethyl cellulose	SmF	Shake flask	CMCase - 0.17 U/mg protein	110
<i>B. subtilis</i>	Soybean industry residue	SSF	Cylindrical bioreactor	FPase -1.08U/mg protein FPase - 2.8 IU/gds CMCase - 9.6 IU/gds Cellobiase - 4.5 IU/gds	50 118
<i>B. subtilis</i>	Banana waste	SSF	Shake flask		
<i>Chaetomium thermophilum</i> CT2	Cellulose (sigma cell)	SmF	Shake flask	CMCase -2.7 IU/ml Cellulase -1160 ECU/ml,	111
<i>Melnocarpus albomyces</i>	Solka floc	SmF	700L fermentor	Endoglucanase -3290 ECU/ml,	48
Mixed culture: <i>T. reesei</i> , <i>A. niger</i>	Rice chaff/ Wheat bran (9:1)	SSF	Flask	FPase -5.64 IU/g	119
<i>Mucor circinelloidens</i>	Lactose	SmF	Shake flask	EGL - 0.25 U/ml FPase - 1.33 U/ml CMCase - 19.7 U/ml BGL - 0.58 U/ml	112 94
<i>Neurospora crassa</i>	Wheat straw	SmF	Shake flask		
<i>Penicillium decumbans</i>	wheatstraw/bran (8:2)	SSF SmF-Fed	SSF bioreactor	Fpase -20.4 IU/g FPase - 23 IU/ml CMCase - 21 IU/ml	120 13
<i>P. occitanis</i>	Paper pulp	batch	20L fermentor	FPase -0.55U/ml, CMCase - 21.5 U/ml, BGL - 2.31 U/ml	97
<i>P. janthinellum</i>	Sugar cane bagasee	SmF	Shake flask		
<i>Phaenerocheate chryso sporium</i>	Cellulose (Avicell)	SmF	100L fermentor	Cellulase - 29mg/g cellulose	113
<i>Rhodothermus marinus</i>	CM cellulose	SmF	150L fermentor	Endoglucanase-97.7 U/ml CMCase - 148 IU/ml Avicellase- 45 IU/ml BGL- 137 IU/ml	53 114
<i>Steptomyces sp</i> T3-1	Carboxymethyl cellulose	SmF	50L fermentor		
<i>S. drowowiczii</i>	Wheat bran	SmF	Shake flask	CMCase - 595 U/L FPase - 4.4 U/gds CBH -2.8 U/gds Endoglucanase - 987 U/gds BGL- 48.8 U/gds Cellobiase-11 mU/ml, Avicellase - 0.3 mU/ml,	60 121
<i>Thermoascus auranticus</i>	Wheat straw	SSF	Perforated Drum Bioreactor		
<i>Thermotoga maritima</i>	Xylose	SmF SmF-	Shake flask	Beta Glucosidase-30mU/ml	115
<i>Trichoderma reesei</i>	Xylose /Sorbose	Continuous	Bioreactor	FPase - 0.69 U/ml/h	100
<i>T. reesei</i>	Steam treated willow	SmF	22L fermentor Microbubble dispersion bioreactor	FPase- 108 U/g cellulose	26
<i>T. reesei</i> RUT C30	Cellulose (Avicell)	SmF	30L fermentor	FPase- 1.8U/ml	116
<i>T. reesei</i> RUT C30	Corrugated cardboard	SmF	30L fermentor	FPase- 2.27 U/ml	95
<i>T. reesei</i> ZU 02	Corn cob residue	SSF	Tray fermentor	FPase - 158 U/gDS Cellulase - 5.48 IU/ml, FPase - 0.25 U/ml	122 96
<i>T. reesei</i> ZU-02	Corn stover residue	SmF	30L fermentor	FPase - 0.88 U/ml, CMCase - 33.8 U/ml, BGL - 0.33 U/ml	97
<i>T. viridae</i>	Sugar cane bagasee	SmF	Shake flask		

Schematic representation of the experimental approach and on-site enzyme production in a cellulose-to-ethanol process.



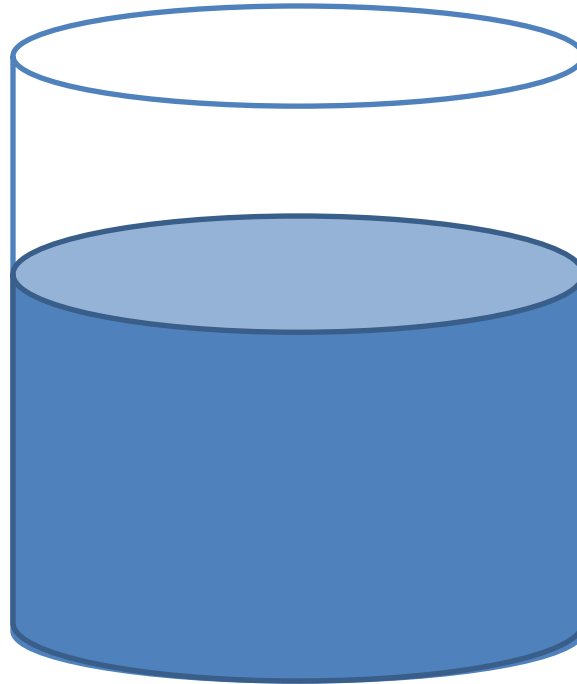
Björn Alriksson et al. Appl. Environ. Microbiol.
2009;75:2366-2374

Applied and Environmental Microbiology

Cultivation Media

Medium 1 ((without carbon source)

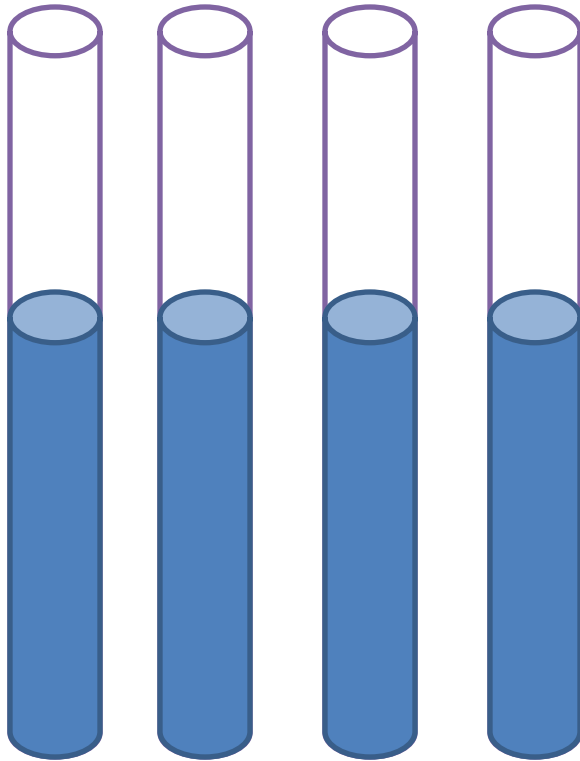
NaNO₃, 2.0, KH₂PO₄, 1.0, MgSO₄ · 7H₂O, 0.5, and (mg.L-1) FeSO₄, 10.0. The pH of the medium was adjusted to 6.5.



Medium 2

Peptone, 6.0, KH₂PO₄, 1.0, MgSO₄ · 7H₂O, 1.72, KCl, 0.5, and (mg.L-1) FeSO₄, 10.0. The pH of the medium was adjusted to 5.5.

Harvest and Separation of Enzymes

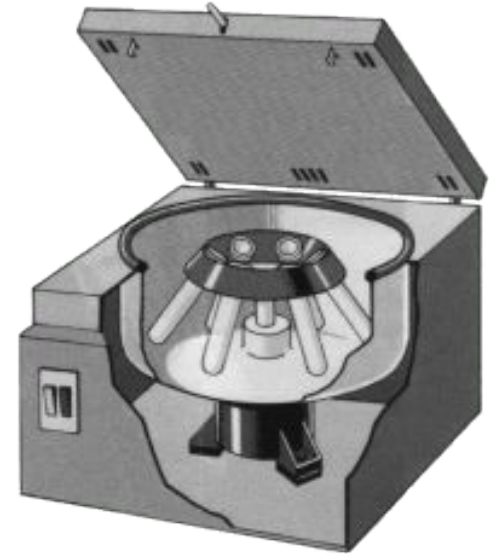


6 ml

6 ml

6 ml

6 ml



These were then centrifuged at 5000 rpm for 15 minutes and the supernatant was collected to 10 mL sterile tubes and stored at -20°C for further use in enzyme assays

Uses

Industry	Applications
Agriculture	Plant pathogen and disease control; generation of plant and fungal protoplasts; enhanced seed germination and improved root system; enhanced plant growth and flowering; improved soil quality; reduced dependence on mineral fertilizers
Bioconversion	Conversion of cellulosic materials to ethanol, other solvents, organic acids and single cell protein, and lipids; production of energy-rich animal feed; improved nutritional quality of animal feed; improved ruminant performance; improved feed digestion and absorption; preservation of high quality fodder
Detergents	Cellulase-based detergents; superior cleaning action without damaging fibers; improved color brightness and dirt removal; remove of rough protuberances in cotton fabrics; antiredeposition of ink particles
Fermentation	Improved malting and mashing; improved pressing and color extraction of grapes; improved aroma of wines; improved primary fermentation and quality of beer; improved viscosity and filterability of wort; improved must clarification in wine production; improved filtration rate and wine stability
Food	Release of the antioxidants from fruit and vegetable pomace; improvement of yields in starch and protein extraction; improved maceration, pressing, and color extraction of fruits and vegetables; clarification of fruit juices; improved texture and quality of bakery products; improved viscosity fruit purees; improved texture, flavor, aroma, and volatile properties of fruits and vegetables; controlled bitterness of citrus fruits
Pulp and Paper	Coadditive in pulp bleaching; biomechanical pulping; improved draining; enzymatic deinking; reduced energy requirement; reduced chlorine requirement; improved fiber brightness, strength properties, and pulp freeness and cleanliness; improved drainage in paper mills; production of biodegradable cardboard, paper towels, and sanitary paper
Textile	Biostoning of jeans; biopolishing of textile fibers; improved fabrics quality; improved absorbance property of fibers; softening of garments; improved stability of cellulosic fabrics; removal of excess dye from fabrics; restoration of colour brightness
Others	Improved carotenoids extraction; improved oxidation and colour stability of carotenoids; improved olive oil extraction; improved malaxation of olive paste; improved quality of olive oil; reduced risk of biomass waste; production of hybrid molecules; production of designer cellulosomes

Thank you for attention!!!