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CANNING see **HEAT TREATMENT OF FOODS**: Thermal Processing Required for Canning; Spoilage Problems Associated with Canning.

CATERING INDUSTRY see **PROCESS HYGIENE**: Hygiene in the Catering Industry.

CELLULOMONAS

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Characteristics of the Genus

The genus *Cellulomonas* contains a heterogeneous collection of cellulose-decomposing bacteria principally isolated from soil materials. The genus *Cellulomonas* along with *Jonesia*, *Oerskovia*, and *Promicromonospora* has recently been assigned to a new family Cellulomonadaceae. Phylogenetically the family belongs to the order Actinomycetales. A combination of chemotaxonomic and morphological properties differentiate members of Cellulomonadaceae from related taxa. Despite their apparent close phenotypic relationship, members of the genus *Cellulomonas* are genotypically different though they have several common genes. Distinction

between species is based on a number of morphological and biochemical characteristics for which different schemes have been reported to identify species of *Cellulomonas*. Strains of the genus produce cellulases and hemicellulases which have several industrial applications. Intergeneric hybrids have been constructed between *Cellulomonas* and *Zymomonas* or multiple genes coding for different enzymes have been cloned in *Z. mobilis* and *Saccharomyces cerevisiae* to extend their substrate consumption efficiency and produce valuable biochemicals from inexpensive substrates.

Initially 27 putative species were collected in the genus but later on in the *Seventh Bergey's Manual of Determinative Bacteriology*, only 10 species were

recognized; the rest were found to be synonymous with these ten. The 10 species were distinguished from each other by motility, nitrate reduction, ammonia production, chromogenesis and, in the case of *C. fimi*, by the fermentation of xylose and arabinose. They were regarded as Gram-negative rods; a portion of rods is arranged at an angle to each other to give V-formations. However, only six authentic cultures were available and *C. biazotea* was regarded as the type species. Since cellulose decomposition is a feature of many other soil bacteria and fungi, therefore, this genus received only academic importance as it had no economic utility. Recently, efforts have been made to utilize cellulosic crop residues, major agro-industrial wastes and environmental pollutants, for production of value-added products. Therefore, renewed interest has developed in isolation, identification and application of *Cellulomonas* spp. in the production of glucose from such wastes. Recently, they have been isolated from rumen, activated sludge and cellulose-enriched environments.

In the eighth edition of *Bergey's Manual*, only one species was recognized. It was suggested that minor variation in chromogenesis, motility, and nitrate reductase activity, did not permit recognition of 10 species. *Cellulomonas flavigena* and not *C. biazotea* was recognized as a type species.

Later, the data from DNA-DNA homology and biochemical reaction studies recognized seven species of the genus *Cellulomonas*. Because of a substantial degree of similarity *Cellulomonas* was included in the family Corynebacteriaceae. Neighbouring groups are defined by *Arthrobacter*, *Renibacterium*, *Micrococcus*, *Stomatococcus*, *Dermatophilus*, *Brevibacterium* and *Microbacterium* and related genera. In the second volume of *Bergey's Manual of Systematic Bacteriology*, six species, namely *Cellulomonas biazotea*, *C. cellasea*, *C. fimi*, *C. flavigena*, *C. gelida* and *C. uda*, have been recognized. Distinction was based on a number of biochemical and chemotaxonomic characteristics, namely peptidoglycan type, fatty acid composition, and other conventional tests namely morphology, cultural conditions for good growth, biochemical reactions exhibited by these species and cell wall composition with respect to carbohydrates. Gram reaction in these species can some times be difficult to determine; some Gram positive strains may lose colour and may appear Gram negative.

Some authors have proposed a scheme for identification of these six species of *Cellulomonas* which can be used to tentatively separate these species. According to this scheme, *C. biazotea* can be separated because it is the only species which grows on raffinose. Among the others, *C. fimi* is lysine and ornithine positive, all other species being negative. *C.*

fimi, *C. flavigena*, and *C. uda* are *o*-nitrophenyl- β -D-galactopyranoside (ONPG) positive whereas *C. gelida* and *C. cellasea* are negative. *C. gelida* produces acids from glucose whereas *C. cellasea* does not and can be separated. Among *C. fimi*, *C. flavigena* and *C. uda*, *C. fimi* does not produce nitrate reductase. *C. flavigena* does not produce acid from lactose and can be separated from *C. uda*. According to this classification, *Cellulomonas* spp. are usually Gram-negative during the first 24 h of growth, thereafter, Gram-positive or Gram-variable staining is obtained on cells grown under optimum growth conditions. In several old cultures, Gram reaction is usually negative and cells are pleomorphic in nature and are motile.

Recently, 280 physiological characters have been used in a numerical taxonomic study of 604 strains to differentiate the genera *Arthrobacter*, *Aureobacterium*, *Brevibacterium*, *Cellulomonas*, *Clavibacter*, *Corynebacterium*, *Curtobacterium*, *Microbacterium*, *Nocardia*, *Nocardioides*, *Rhodococcus*, *Terrabacter* and *Tsukamurella* by cluster analysis. A high degree of similarity between the genera *Aureobacterium*, *Cellulomonas*, *Clavibacter*, *Curtobacterium* and *Microbacterium* has been found in phylogenetic-based studies and could be confirmed phenotypically. Bacteria belonging to the genus *Corynebacterium*, including *Corynebacterium glutamicum*, and eight other species of this genus are distinctly different from *Cellulomonas* spp.

Creation of a distinctly different genus based on cellulolysis was not well accepted but further studies using modern taxonomic methods, such as molecular techniques using electrophoresis, composition with respect to peptidoglycans, fatty acid and carbohydrate profiles of cell wall, biochemical properties, DNA-DNA homology, 16S and 5S rRNA analyses, have supported the earlier conclusions on the species of *Cellulomonas*. Now the well-recognized species are *C. biazotea*, *C. cellasea*, *C. cellulans*, *C. fimi*, *C. flavigena*, *C. fermentans*, *C. gelida* and *C. uda*. They have an optimum temperature for growth of 28–33°C. They grow over a wide pH range (pH 5.5–7.8). The generation time varies between 2 and 4 h. They require nitrogen and vitamins for good growth and cellulase production. Under optimum growth conditions, cell-wall composition, peptidoglycan structure, menaquinone composition and fatty acid profiles are unique properties and can be used to separate this genus from other related genera. All species are usually nitrate reductase and catalase positive, and have yellow chromogenesis.

In the ninth edition of *Bergey's Manual of Determinative Bacteriology*, only seven species have been recognized. For their differentiation, seven tests have been proposed. The above-mentioned tests can be

easily performed in all microbiology laboratories and both can be combined for confirmatory tests.

Strains of *Cellulomonas* produce hydrolases to consume carbohydrates namely starch, xylan and cellulose. Some of these enzymes are multifunctional, possess a multidomain structure and can be induced by many substrates. They consist of a number of extracellular and intracellular enzymes produced by different species of *Cellulomonas*. Extracellular and intracellular cellulases and xylanases produced by *Cellulomonas* spp. are presented in **Table 1**. It has been suggested that cellulases of *Cellulomonas* spp. operate by a lysozyme-type reaction mechanism. An endoglucanase and an exoglucanase of *C. fimi*, like cellulases of other bacteria and fungi, consist of a catalytic and a cellulose binding domain. Each of these enzyme components can be truncated by a serine protease to separate the catalytic and cellulose-binding domains. Cellulases of *C. fimi*, *C. uda*, *C. flavigena* and *C. biazotea* have been purified to homogeneity level. The enzymes have been extensively studied for their biochemical and kinetic parameters and active site residues involved in catalysis have been identified. These active site residues can be chemically modified to suit industrial applications.

In *Cellulomonas* strain CS1-1, *C. biazotea* and their mutants, production of α -L-arabinofuranosidases (EC 3.2.1.55), β (1,4)-mananases (EC 3.2.1.78), α -D-(1,4)-galactoside galactohydrolase (EC 3.2.1.22) showing activity towards galactomannan, hemicellulase A, hemicellulase B, α - and β -glycosidases has been studied in greater detail compared with other *Cellulomonas* spp. Some species produce cellobiase activity to produce glucose from cellobiose but those which lack this enzyme, possess cellobiose phosphorylase (EC 2.4.1.20) to consume cellobiose. Cellobiose dehydrogenase (EC 1.1.99.18),

glycerol dehydrogenase (EC 1.1.1.6) and L-amino oxidase (EC 1.4.3.2) are also produced by some species of *Cellulomonas*.

Since strains of *Cellulomonas* are inhabitants of soil and decaying cellulosic substrates, they can easily be transmitted into foods. They have been found in a variety of foods, including meats, liquor of spiced olives, starchy foods and raw cow's milk. Production of toxins by *Cellulomonas* spp. is still not a well-characterized phenomenon. Therefore toxicity due to their fermentation products is not clear. Some species of *Cellulomonas* produce amylases and may contaminate starchy foods like cooked rice if stored at room temperature. They may release polysaccharides and glucose which may be used by pathogenic organisms to grow. The subsequent heat treatment, usually frying, is usually not sufficient to kill vegetative cells. Recently one species, namely, *Cellulomonas hominis* sp. nov. has been isolated from clinical specimens. Its role in pathogenicity is not well characterized. Its characterization has been based on 16S rRNA genes, phenotypic and molecular data compared with related species but its taxonomic status is to be determined by DNA-DNA hybridization studies.

Methods of Detection

Strains of *Cellulomonas* resemble many of their close relatives in the family Cellulomonaceae. Differentiation is usually done by growth on filter paper, Walseth cellulose, Sigmacell 100, or cottonwool followed by microscopic observations for mobility. The strains of *Cellulomonas* leave a yellow colony print on the surface of a cellulose medium.

Detection of *Cellulomonas* spp. in food and related specimens, as in other organisms, demands a series of tests including selective enrichment onto cellulose

Table 1 Production of cellulases and xylanases studied in different strains of the genus *Cellulomonas*

Enzyme studied	EC number	Organisms extensively studied	Comments
Cellobiohydrolase	3.2.1.91	<i>C. biazotea</i> , <i>C. fimi</i> , <i>C. flavigena</i> , <i>C. uda</i> , <i>Cellulomonas</i> CS1-1	Intra- and Extracellular production
Endo-1,4- β -D-glucanase	3.2.1.4	<i>C. biazotea</i> , <i>C. cellasea</i> , <i>C. fimi</i> , <i>C. flavigena</i> , <i>C. uda</i> , <i>Cellulomonas</i> CS1-1	Extracellular production
Filter paper activity	—	<i>C. biazotea</i> , <i>C. cellasea</i> , <i>C. fimi</i> , <i>C. flavigena</i> , <i>C. uda</i> , <i>Cellulomonas</i> CS1-1	Produced extracellularly, measured as filter paper activity (FPase)
1,4- β -Glucosidase	3.2.1.21	<i>C. biazotea</i> , <i>C. fimi</i> , <i>C. cellasea</i> , <i>C. flavigena</i> , <i>C. uda</i> , <i>Cellulomonas</i> CS1-1	Produced intracellularly
Endo-1, 4- β -xylanase	3.2.1.8	<i>C. biazotea</i> , <i>C. cellasea</i> , <i>C. flavigena</i> , <i>C. fimi</i> , <i>C. uda</i> , <i>Cellulomonas</i> CS1-1	Produced extracellularly
1,4- β -xylosidase	3.2.1.37	<i>C. biazotea</i> , <i>C. cellasea</i> , <i>C. fimi</i> , <i>C. flavigena</i> , <i>C. uda</i> , <i>Cellulomonas</i> CS1-1, <i>Cellulomonas</i> sp. NCIM	Produced intracellularly

medium, followed by plating onto cellulose agar medium made in basal salts medium (see later) containing yeast extract. Homogenates of foods are prepared in Butterfield's phosphate-buffered water at a 1:1 or 1:10 dilution or direct plate counts can be made using the selective medium. Penicillin is added to suppress fungal growth. All *Cellulomonas* spp. liberate extracellular filter paper activity, called FPase activity, which can produce a cellulose hydrolysing zone around single colonies. Some organisms also produce cell-associated cellobiohydrolase activity which can also help in clearing cellulose around single colonies. The plates are incubated at 30°C for up to 14 days for confirmation of test. In some cases, 21–30 days may be required to clear cellulose.

When a low number of strains of the genus *Cellulomonas* is expected, direct plating may not be suitable. The threshold for direct plating is a 10 cfu g⁻¹ sample. The most probable number (MNP) technique can be useful to enumerate bacteria. The MNP technique for *Cellulomonas* spp. starts with dilution of cellulose-grown cultures in triplicate. The tubes are incubated at 30°C for 72–96 h for dense growth and then plated onto cellulose agar plates for viable counts. The culture is streaked on the surface of a cellulose-agar plate to any assumptive positives. Colonies of different shape and sizes (which clear cellulose), appearing simultaneously, or those appearing at different time intervals are picked and streaked on fresh salt cellulose agar medium. Confirmation of *Cellulomonas* spp. requires completion of a number of tests (**Table 2**). Unfortunately there is no single test for unequivocal determination of *Cellulomonas* spp. They produce acetic and lactic acids from glucose under aerobic conditions (*C. biazotea*, *C. cellasea*, *C. flavigena*, *C. cellulans* and *C. gelida*) or acetic acid, lactic acid, succinic acid and ethanol under anaerobic conditions (*C. fimi*, *C. uda* and *C. fermentans*).

Other characteristics of *Cellulomonas* spp. include reduction of nitrate to nitrite, and non-production of acetylmethylcarbinol (Voges-Proskauer negative). To assess various characteristics to confirm these species, additional tests are required.

For these tests, the strains are maintained on (preferably) Dubos salts-agar medium containing (g l⁻¹) K₂HPO₄·7H₂O 0.5, KCl 1.0, NaNO₃ 0.5, MgSO₄ 0.5, FeSO₄·7H₂O 0.1, yeast extract 2, Sigma-cell 100 10 and agar 25 g (DYEA) plates and slants. Then basal liquid medium is dispensed as 10 ml into test tubes (16 × 150 mm) and autoclaved at 15 pounds pressure for 15 min. After cooling to ambient temperature, the test carbohydrates are added aseptically to a concentration of 1% (w/v). The carbohydrates or other carbon sources are filter-sterilized with bacteriological filters (0.2 μm) if soluble or autoclaved

if not soluble. Fresh cultures of each organism are prepared in Dubos salts yeast extract-glucose medium. After overnight growth, cells are harvested aseptically by centrifugation (15 000 g, 30 min), washed twice with 0.89% NaCl solution, and resuspended in saline and their absorbance measured at 610 nm. The absorbance is then brought to McFarland tube no. 4 by dilution or by concentration. A 1 ml sample of cells is then aseptically resuspended in DYE liquid medium containing test carbohydrates at 1% final carbohydrate concentration (w/v). The cells are grown statically at 30°C. The test tubes are individually examined after each day up to 30 days of incubation. The whole experiment may be repeated three or four times for declaring negative test as compared with type cultures namely *C. biazotea* (ATCC 486, DSM 20112, NCIB 8077), *C. cellasea* (ATCC 487, DSM 20118, NCIB 8073), *C. fimi* (ATCC 484, DSM 20113, NCIB 8980), *C. flavigena* (ATCC 482, DSM 20109, NCIB 8073), *C. gelida* (ATCC 488, DSM 20111, NCIB 8076), *C. fermentans* (DSM 3133) and *C. uda* (ATCC 491, DSM 20107, NCIB 8200) available in *The Prokaryotes* (2nd edition).

Motility Motility is usually measured by stabbing the centre of a tube of semisolid growth-supporting medium and allowing the culture to grow. The cultures are incubated for 24 h at 30°C. Motile bacteria diffuse out from the stab, forming an opaque growth pattern, whereas non-motile bacteria do not diffuse out.

Hydrolysis of o-Nitrophenyl-β-D-Galactopyranoside (ONPG) Cells are streaked on the surface of DYEA plates containing ONPG in the medium and incubated at 30°C. After 3–28 days growth, the plates are spread with carbonate buffer, 200 mmol l⁻¹ (pH 8.5). The colonies are screened by measuring the diameter of the yellow halo surrounding the colonies.

Nitrate Broth A 1 ml sample of grown cultures is inoculated separately in 10 ml culture medium in test tubes and incubated at 30°C. After 24 h, nitrite production is measured by addition of α-naphthylamine and α-naphthol.

Hydrolysis of Aesculin Hydrolysis of aesculin is determined in DYE-aesculin-ferric ammonium citrate-agar medium by streaking the cells from a single colony and examining after each day of incubation. Aesculin-hydrolysing organisms produce β-glucosidase (located in the periplasmic fraction of the cell) which hydrolyses aesculin to liberate aesculin which, in turn, reacts with ferric ions to give a black precipitate. The hydrolysing efficiency can be quan-

Table 2 Biochemical and morphological tests for identification of species of the genus *Cellulomonas*

Confirmation test	Species						
	Biazotea	cellasea	cellulans	fimi	flavigena	gelida	uda
Gram reaction	+						
Catalase	+	+	+	+	+	+	+
Motility	+	+	-	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+
Voges-Proskauer	-	-	-	-	+	+	+
Methyl red	-	-	-	-	-	-	-
ONPG	+	-	-	-	-	-	-
Glucose utilization (anaerobic)	-	-	+	+	+	-	+
Aesculin	+	+	+	-	-	+	+
Urease	+	+	+	+	+	+	+
Ammonia from peptone	-	-	+	-	+	-	+
Lactose	+	+	-	+	-	+	+
Ribose	-	-	+	-	+	-	-
Raffinose	+	-	-	-	-	-	-
Proline	-	+	+	-	-	-	-
Acid from glucose	+	+	+	+	+	+	+
Arabinose	+	+	+	+	-	+	-
Fructose	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Lysine decarboxylase	-	-	-	-	-	-	+
Arginine dihydrolase	-	-	-	-	-	-	+
Ornithine decarboxylase	-	-	-	-	+	-	+
(L)-Lactate	+	+	+	+	-	-	-
Acid from dextrin	-	-	DN	+	+	+	+

ONPG, *o*-nitrophenyl- β -*D*-galactopyranoside.
DN, not determined.

tified by measuring the zone of blackening (in mm) around single colonies. Differences in values indicate the genetic variability among species.

Hydrolysis of Carboxymethyl Cellulose (CMC) CMC is added to DYEA medium and 10 μ l of cells of equal density are poured on the surface of the plate. After growth at 30°C, CMC hydrolysis is visualized as a yellow zone when stained with Congo red followed by treatment with NaCl. Differences in the diameter of the yellow zone indicate the genetic variability among species.

Hydrolysis of Sigmacell 100 Crystalline Cellulose Hydrolysis of Sigmacell 100 is visualized by streaking the cells on the surface of a plate containing Sigmacell 100 in the top layer of DYEA and DYEA in the base layer. After incubation, the streaked area is examined every day to see the zone of Sigmacell clearance. The diameter of the zone of clearance around single colonies indicates the organisms ability to decompose cellulose. Differences in values indicate the genetic variability among species.

Acid Production from Carbohydrates A 0.1 ml sample of grown culture is inoculated into 10 ml of culture medium in a test tube containing nutrient

broth supplemented with test saccharides (glucose, D-ribose, raffinose, lactose, dextrin etc.) with an inverted Durham tube. After 24 h, phenol red is added which is red at neutral pH but changes to yellow at acidic pH (pH 6.8). The appearance of gas in the Durham tube, indicates the fermentative ability of the organism.

Utilization of L(+)-Lactate A 0.1 ml sample of grown culture is inoculated into 10 ml of culture medium in a test tube containing nutrient broth supplemented with L(+)-lactic acid. The appearance of dense growth indicates the utilization of L(+)-lactate in the fermentation medium.

Indole Production Test A 0.1 ml sample of culture is inoculated into trypticase soy broth, and incubated at 30°C. Then 10 drops of Kovac's reagent are added into dense growth and the tube is agitated gently. The appearance of a brick-red colour indicates the production of indole from tryptophan in the medium.

Utilization of Proline A 0.1 ml sample of grown culture is transferred to 10 ml of culture medium in a test tube containing nutrient broth supplemented with proline. After 24 h, proline is consumed by the organism and dense growth is visible.

Utilization of Lysine and Ornithine A 0.1 ml sample of grown culture is transferred into 10 ml of culture medium in a test tube containing nutrient broth supplemented with lysine or ornithine. After 24 h, the compounds are consumed by the organism and dense growth in each case is visible.

Applications of *Cellulomonas* spp.

Almost all species of *Cellulomonas* have been screened to produce cellulases, namely endo- β -glucanase, 1,4- β -D-glucan glucohydrolase, exocellobiohydrolase, β -1,4-D-glucosidase, endo- β -xylanases and β -xylosidase, for the utilization of agro-industrial wastes. These enzymes find application in the textile, paper and pulp industries. All cellulases and xylanases produced by *Cellulomonas* spp. have been purified to homogeneity and multiple isomers have been obtained. The cellulose-binding domain of endo- β -1,4-glucanase produced by *C. fimi* has been used to purify human interleukin-2 for clinical applications. The enzyme preparations can be used for obtaining stable botanical extracts for food/medical applications.

Food Applications

Cellulases and xylanases produced by *Cellulomonas* spp. have not been used commercially but, like fungal enzymes, they can be applied in baked foods, fruit processing, preparation of dehydrated vegetables and food products, preparation of essential oils and flavours for the food industry, and starch processing. Concentrated enzyme preparations have been used in making jams, baby food, foods for invalids and fruit juices. Hemicellulase-rich cellulases are implicated in degumming of coffee extracts and making instant coffee. Endoglucanase has been used to remove turbidity of glucan present in wines and beers. In a well-balanced enzyme preparation, cellulosic materials are hydrolysed to monomeric sugars which may be used for production of food-grade products namely, lactic acid, acetic acid and xylitol.

Cellulomonas fimi, *C. biazotea*, *C. flavigena* and *C. uda* have been used alone or in combination with yeasts or bacteria for the production of single cell protein, to enhance the digestibility and nutritive values of poultry and animal feeds. In South Korea, farmers add inoculum of *Cellulomonas* spp. to rice straw or wheat straw to improve taste, digestibility and protein content. The organisms have been found to have a balanced level of all amino acids. This has been observed in other species of *Cellulomonas* and with *C. biazotea* grown alone or with *Saccharomyces carlsbergensis* in combined saccharification and fer-

mentation studies (Table 3). These organisms may serve as a good source of amino acids in amino acid-deficient poultry/animal diets. Cellulaseless enzyme preparations rich in xylanases and glycosidases are useful in the reduction of chlorine and chlorine dioxide consumption in the paper and pulp industry to abate environmental pollution due to tetrachloro compounds of lignin precursors present in waste waters. Endoglucanases are used in oil drilling where guar gum or CMC is used for improved oil recovery. They are also used for plant cell wall destruction to produce plant protoplasts for genetic manipulation for use in the production of transgenic cereal crops with improved grain productivity and protein content.

Cellulomonas spp. have been considered as potential candidates for waste disposal, composting of grasses, pith, dried palm oil effluents and shredded newspapers. Mixed cultures of *Cellulomonas* spp., *Desulfovibrio vulgaris* and *Methanosarcina barkerii* were found to be highly efficient at producing methane from cellulose and xylan. A mixed culture of *Cellulomonas* spp. and *Rhodospseudomonas capsulata* has been used to produce hydrogen as a source of energy. Similarly, a mixed culture of *Cellulomonas* mutant and *Azospirillum brasilense* has been used to enhance the nitrogen-fixing ability of the latter.

The cellulosic hydrolysates have been used to produce ethanol using *Saccharomyces cerevisiae*, a thermotolerant yeast strain of *Kluyveromyces marxianus* or *Zymomonas mobilis*. Strains of *Cellulomonas* possess multiple genes for the production of cellulases and xylanases. These genes have been

Table 3 Amino acid analyses of *Cellulomonas biazotea* (C), *Saccharomyces carlsbergensis* (Y) alone and in combination (C + Y)

Amino acid (g per 100 g dry cells)	C	Y	C + Y
Aspartic acid	2.92	2.59	2.87
Threonine	1.20	1.30	1.25
Glutamate	2.69	2.29	2.45
Serine	1.80	1.20	1.60
Proline	1.85	1.80	1.60
Glutamine	0.99	0.77	0.89
Alanine	2.05	1.97	0.50
Tyrosine	0.39	0.80	0.50
Valine	1.68	1.13	1.28
Methionine	0.71	0.20	0.48
Isoleucine	0.85	1.05	0.88
Leucine	2.03	1.80	1.90
Threonine	1.45	1.20	1.30
Phenylalanine	1.00	0.81	0.76
Lysine	0.99	1.61	1.45
Histidine	0.34	1.30	0.81
Arginine	0.92	0.66	0.73
Cysteine/cystine and others	3.14	2.52	3.26

cloned in *Escherichia coli*, *S. cerevisiae* or *Zymomonas mobilis* for the purification of the individual components so as to understand the structure–function–relationship of cellulases, to hyperproduce one enzyme component in greater quantity, and to enhance the substrate-consumption efficiency of non-cellulolytic organisms or to produce ethanol from cellulosic wastes. The rDNA methodology also directly aids in the development of more active cellulases. The application of cellulases through improvement and/or hyperproduction of specific components can permit them to be mixed optimally for routine and new industrial applications.

See also: **Single Cell Protein:** Yeasts and Bacteria.

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CEREALS see **SPOILAGE OF PLANT PRODUCTS:** Cereals and Cereal Flours.

CENTRIFUGATION see **PHYSICAL REMOVAL OF MICROFLORAS:** Centrifugation.