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Cellulolytic Activities of Phytopathogenic Microorganisms

JEAN-PIERRE CHAMBOST

Laboratoire de Chimie Bactérienne, C.N.R.S., 13009 Marseille, France

Abstract

Many phytopathogenic microorganisms, causative agents for wilt or soft rot, produce cellulolytic enzymes which can be related to these symptoms. To discuss this assertion the actual mechanism of cellulose enzymatic hydrolysis should be known. Because this point is not yet clear recent models are presented and discussed.

Significant illustrations are given in which cellulase activity can be linked to the symptoms. Recent results obtained with *Erwinia chrysanthemi* could lead to a new approach of enzymatic pathogenicity.

Keywords: cellulase system, phytopathology, bacteria, fungi

Plant walls are made of intricated polymers organized in a stiff network in which hemicelluloses and pectin protect cellulose. When cells differentiate to parenchyma they surround themselves with lignin (Wardrop, 1977). This frame gives the shape to the cells and contributes to the rigidness of the plant tissue.

Wilt and soft rot symptoms result from limited hydrolysis of some wall polymers and although cellulose hydrolysis is not primarily implicated in the symptoms many phytopathogenic microorganisms produce a large amount of cellulolytic enzymes.

*Reviewed

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Table 1. Crystallinity degree of cellulose depending on the source. From Kulshreshtha and Dweltz, 1973)

	Cellulose crystallinity
Valonia	100%
Azotobacter xylinum	76%
Cotton	46%

Despite the fact that the cellulolytic system is not very efficient *in vitro* and hard to improve as it has not yet been sufficiently characterized, it is used in biochemical processes.

Some phytopathogenic microorganisms are used to analyse cellulolytic mechanism and may be one outlet for cellulase production.

To be able to relate disease symptoms to cellulolytic activity the actual mechanism of enzymatic cellulose hydrolysis should be known.

Cellulose is an homopolymer of glucose units linked together by β-1,4 linkages. In higher plants the degree of polymerisation is uniformly about 14,000 whereas in cellulose produced by the bacterium *Azotobacter xylinum* the degree of polymerization is 3,500; in commercial cellulose samples it is between 50 and 5,000.

Although the main topic of this paper is not cellulose configuration it is necessary to give some data on this matter related to the mechanism of enzymatic hydrolysis of this peculiar substrate.

In a single chain glucose molecules are associated by hydrogen bonds (Fig. 1). Moreover because of the β-linkage the hydroxyl groups of a same chain are on the same plane so that many chains can be close enough to be firmly held together by hydrogen bonds. This results in an orderly structure called 'crystalline" in a polymolecular system named fibril. However, this structure is not uniform and is deleted in some areas said to be amorphous. The crystallinity ratio in a cellulose fiber depends on the origin and the treatment to purify it (Table 1). In the fibers cellulose molecules are parallel (Chanzy and Henrissat, 1985). The structure is so tight that enzymes cannot enter into the crystal so that the crystalline structure must be broken down to complete the hydrolysis of the cellulose.

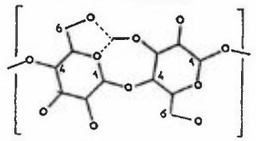

Figure 1. Hydrogen bonds in cellobiose, repetitive unit of cellulose.

1. Cellulose Enzymatic Hydrolysis: Cellulolytic Complex

Cellulose biodegradation is quite exclusively effected by microorganisms: they can be aerobes or anaerobes, bacteria, fungi and yeasts. These organisms can hydrolyse native cellulose into sugars mainly cellobiose and glucose. Several enzymes are involved in the hydrolysis but the properties of some of them are not well established so that the exact composition of cellulolytic complex is still in doubt. Two major difficulties are encountered in the analysis of this system. Firstly cellulolytic enzymes which are nearly always found in a multienzymatic complex are very hard to dissociate, making separation difficult. Moreover these enzymes are often subject to protease transformations so that it is not easy to assess if two isoenzymes with slightly different properties come from the same protein or are true different proteins. The second major difficulty is due to the absence of specific substrates making some components difficult to characterize so that they can be inferred by indirect proof only.

Three different types of enzymes are involved in cellulose hydrolysis.

The endo-β-1,4-glucan hydrolases (EC 3.2.1.4) or endoglucanases hydrolyse glucosidic linkages at random inside the cellulose chain yielding cellodextrins. These enzymes are in larger amounts than the others (Reese, 1977) and are easy to detect because they are active on a soluble chemical specific substrate, carboxymethylcellulose (CMC). So they are also referred as CMCases.

The exo-β-1,4-glucan cellobiohydrolases (EC 3.2.1.91) or cellobiohydrolases (CBH) or exoglucanases are acting close to the non-reducing of the chain producing mainly cellobiose. Their action is recurrent so they are not active on CMC because of the substitution on the chain substrate. Related to these enzymes are glucohydrolases (EC 3.2.1.74) which split glucose from the non-reducing end. All these exo-enzymes can hydrolyse amorphous cellulose but at a slow rate.

Some β-glucosidases (EC 3.2.1.21) can hydrolyse cellobiose into glucose which is a weaker inhibitor for most glucanases compared to the disaccharide.

Each of the purified enzymes has a poorer activity on crystalline cellulose than the full system. This general observation led to consider a synergic action of the various types of the cellulolytic enzymes and successive models were proposed to relate the action of these enzymes. The mechanism of native cellulose hydrolysis is still on debate.

The first model proposed by Reese et al. (1950) (Fig. 2) was based upon the observation that there are more microorganisms able to hydrolyse amorphous

cellulose than those active on crystalline cellulose. It was concluded that the latter organisms synthesize an additional enzyme called C1 which can open the crystalline structure and was supposed to be devoid of hydrolytic activity (Reese et al., 1950).

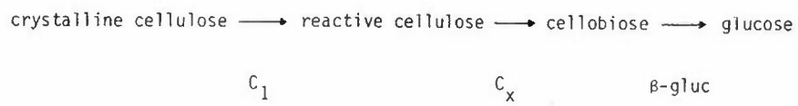

Figure 2. Cellulolytic system (from Reese et al. (1950)).

Cx was the hydrolytic factor and was easy to detect because it was active on soluble CMC where substitution prevents recurrent activity of the enzymes. It decreases sharply the viscosity of CMC solutions and does not yield mono- or di-saccharides at the beginning of the reaction. It is an endoglucanase.

The first efficient purifications of cellulase complexes raised a doubt about this model. Berghem and Petterson (1973) and Wood and McCrae (1972) simultaneously purified a protein inactive on CMC but, when added to purified endoglucanase, conferred to the mixture the ability to hydrolyse crystalline cellulose. This protein had some characteristics of the C1 factor but contrary to the model the enzyme was hydrolytic in nature. Although it was not active on CMC, excluding any endo-mechanism, this enzyme attacks cellulose by the non-reducing end removing cellobiose: it is an exo-enzyme.

Thus a new model taking into account the mode of action of both enzymes and their mutual synergism was proposed (Fig. 3). Endoglucanase acts in amorphous areas away from the non-reducing ends thereby increasing number of extremities which exoglucanase can hydrolyse.

Although this pattern is widespread it does not explain the degradation of the crystalline organization and recent results compel reconsideration of the endo-exo-mechanism as the only explanation for the ability of a couple of enzymes to hydrolyse organized cellulose.

Isolated from *Penicillium funiculosum* a glucohydrolase does not cooperate with endoglucanase from the same source although cellobiohydrolase does (Wood and McCrae, 1982). Four endoglucanases were separated from *Trichoderma Koningii* but only two of them could act in synergy with cellobiohydrolase of this fungus (Wood, 1983). To take in account this lack of cooperativeness Wood suggested the following hypothesis: since in organized cellulose, chains are firmly bound to each other by inter-chain hydrogen bonds the oxygen atoms which link two cellobiose units are alternatively

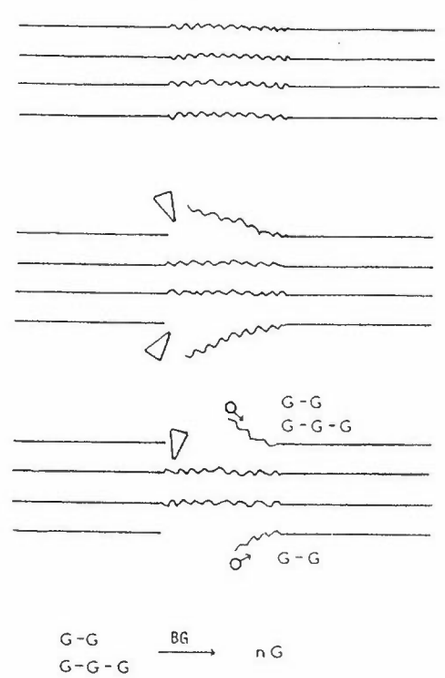

Figure 3. Endo-exo model for cellulose hydrolysis.
▷ endoglucanase
○→ exoglucanase (CBH)
BG: β-glucosidase
Acting on amorphous areas of the cellulose fibre endoglucanase creates non-reducing extremities for exoglucanase.

before and behind the crystal surface. According to the endoglucanase stereospecificity the chain end arising either fits or does not fit the exoglucanase stereospecificity (Fig. 4). This model considers the crystalline structure of cellulose but cannot explain the synergy which was observed between two cellobiohydrolases from *Trichoderma reesei* (Wood and McCrae, 1986).

Two cellobiohydrolases — CBH I and CBH II – were purified from *T. reesei* (Henrissat et al., 1985). From electron microscopy and X-ray diffraction techniques applied to enzymatic degradation of *Valonia* cellulose, Chanzy and Henrissat observed that contrary to CBH II, CBH I binds preferentially to the crystalline surface of cellulose (Chanzy et al., 1982). So this enzyme is not a true exo-enzyme although it produces cellobiose and it is not active against CMC.

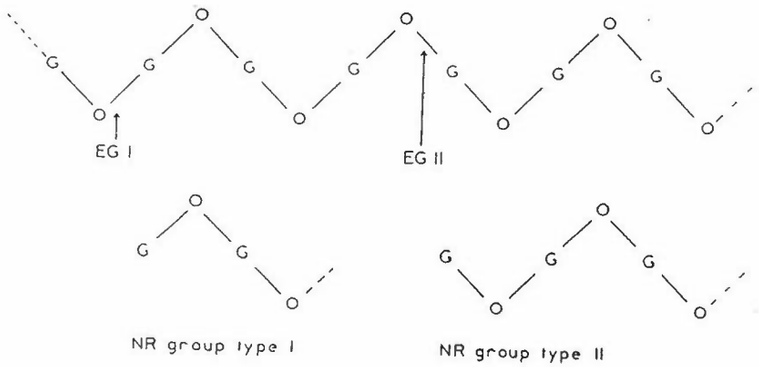

Figure 4. Stereospecificity action of endoglucanases (EG) after Wood and McCrae (1986). NR: Non-reducing group.

The authors proposed a new model (Fig. 5) in which CBH II removes cellobiose from non reducing ends. The synergy endo-exo between endoglucanases and CBH II would be effective on amorphous areas only and CBH I would be the only enzyme able to open crystalline structure.

These two last models are in agreement with the stereospecificity of some cellulases for the organized structure of cellulose. Maybe they could be combined into a new model.

2. Phytopathogenic Microorganisms Endowed with Cellulase Activity

A list of phytopathogenic microorganisms exhibiting cellulase activity cannot be exhaustive firstly because the tests used to seek cellulase activity are often restricted to CMCase. Secondly because many authors estimate that soft rot and wilt symptoms are so far related to pectin hydrolysis only that they do not care for cellulase activity (Wood, 1960). So, only significant illustrations will be given.

Phytophtora infestans has been reported to possess an incomplete cellulolytic set since it cannot hydrolyse a crystalline substrate. Bodenmann et al. (1985) partly purified one endoglucanase with low MW and two β-glucosidases poorly active on cellobiose. The properties of these enzymes were established and it was inferred that their small size could facilitate penetration and local dissolution of the host cell wall fabric.

In some more fungi cellulolytic activity has been reported: *Helmithosporum maydis, Hemiliae vastatrix* and among Fusaria *F. curcubitae* and *F. solani* whose cellulase complex were studied in some details (Wood and McCrae, 1979): three types of enzymes were isolated, cellobiohydrolase, endoglucanase and β-glucosidase. The synergy of these factors were demonstrated since the complete mixture could solubilize up to 72% of cotton against 1% only for each cellulase acting alone (Table 2).

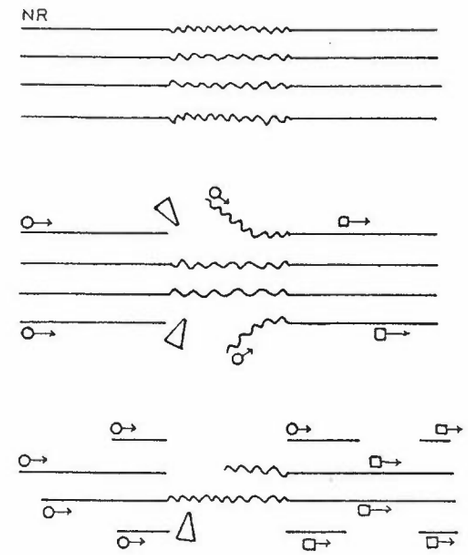

Figure 5. Model for cellulose degradation after Chanzy and Henrissat.
▷ endoglucanase
□→ CHB I
○→ CBH II
NR: Non-reducing end.
CBH II removes cellobiose from non-reducing groups either preexisting or created by endoglucanase action. CBH I binds to crystalline areas of the fibre.

Table 2. Cotton solubilization efficiency of *F. solani* enzymes. (from Wood et al., 1983).

	Cotton solubilization %
CBH	1
Endoglucanase	1
β-glucosidase	0
Endo + CBH	53
Endo + β-glucosidase	6
Endo + CBH + β-glucosidase	72
Unfractionated enzymes	71

Recently, Wood and McCrae (1986) were able to purify to the homogeneity a set of glucanases from *Penicillium pinophilium* with properties fitting their synergistic model (Wood, 1983).

For a long time phytopathogenic bacteria were considered as non cellulolytic (Burkholder, 1948). Then it has been shown that supernatant of the medium of one strain of *Erwinia carotovora* could slowly hydrolyse CMC (Ammann, 1952). The same observations were made with *Xanthomonas* sp. (Goto and Okabe, 1958) and *Pseudomonas solanacearum* although this strain cannot use cellobiose or CMC or cellulose as a carbon source (Kelman and Cowling, 1965). However in plants infected by this strain the cellulose DP decreases. It was proposed by Kelman and Cowling (1965) that cellulase facilitate penetration of the bacterium into host tissue.

Few *Pseudomonas* are producing cellulase. *P. syringae* and *P. caryophylli* should be mentioned (Gross and Cody, 1985).

Garibaldi and Bateman (1970) reported an endoglucanase produced by *Erwinia chrysanthemi*.

To analyse cellulolytic systems in bacteria we took advantage of some genetic techniques which can be used with *Erwinia chrysanthemi* (Boyer et al., 1984). Thus we were able to isolate two different endoglucanase genes belonging to two operons (Barras et al., 1984; Boyer et al., 1986). The product of these genes has been characterized (Table 3). In the strain under study the product of one gene only was in sufficient amount to be purified. The production of the endoglucanase is constitutive and submitted to catabolite repression. The same genes were found in two other strains although their expression was very low. Genes encoding other co-operative cellulase activities could be present in *Erwinia* chromosome but their expression is too low to detect any activity.

The role of cellulase in diseases is not clear cut yet. The direct observation of the degradation of the intricated polymers in infected plants could be worthwhile. The details of the relations between plants and the causal agent are not known enough. For instance the host could release a signal making the pathogen expressing a complementary part of the cellulolytic system. As reported earlier, even if cellulase is not a compulsory agent in wilt and soft rot symptoms it could intervene in some cases in extending maceration of host tissue late in infection.

The simultaneous action of endopolygalacturonase and cellulase on washed carrot is more efficient than polygalacturonase alone (Sreenath et al., 1984). Pleiotropic mutants of *Erwinia* simultaneously impaired in secretion of both

Table 3. Comparison between two *Erwinia chrysanthemi* endoglucanases.

	Cel Z	Cel Y
Adsorption on DEAE-trisacryl at pH 8	+	−
Activity on CMC	+	+
Activity on umbelliferyl cellobiosides	+	−
Activity optimum pH	6.5 to 7.5	5 to 5.5
Molecular Weight	45,000	35,000
Immuno-precipitation by antibodies raised against supernatant culture	+	+
Immuno-precipitation by antibodies raised against purified CelZ protein	+	−

pectinase and cellulase were obtained (Andro et al., 1984). These observations could mean that both activities are related to pathogenicity.

It should be mentioned that fungi differ markedly from bacteria in their penetration mode. Often fungi penetrate through the intact surface of the plant by use of ezymatic or physical processes. It has been recently reported that cellulase is associated with ungerminated spores of *Botrytis cinerea* suggesting a role in penetration (Verhoeff et al., 1983). On the contrary bacteria penetrate plants only through a wounded tissue. Unlike bacteria phytopathogenic fungi seem to possess a complete and efficient cellulase system which may be essential for the process of infection.

On the other hand bacteria cellulase seems to be more involved with wilt than with soft rot.

The observation of pathogenic behaviour of very specific mutants obtained through molecular biology methods could lead to a new approach of enzymatic pathogenicity.

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