FOLIA FORESTALIA POLONICA
Seria B, zeszyt 24, 1993

COLONIZATION OF A PRETREATED *EUCALYPTUS VIMINALIS* WOOD BY *CLOSTRIDIUM THERMOCELLUM* STRAIN JG1

Julitta K. Gajewska

Warsaw Agricultural University – SGGW
Department of Agricultural Microbiology

The decay of *Eucalyptus viminalis* wood after steam – explosion pretreatment by anaerobic, thermophilic and cellulolytic *Clostridium thermocellum* bacteria during wood colonization was examined. Degradation pattern of wood vessels on basic ultramicroscopic appearances (SEM, CLSM, TEM) was discussed.

Introduction

Several bacteria can effectively hydrolyse cellulose but thermophilic bacteria appear to be most promising for single–stage biotransformation of cellulose to fuels [31, 32, 36]. The anaerobic, cellulolytic and thermophilic bacterium, has received some attention in the past to carry out a limited attack on derived forms of cellulose [18, 19]. *C. thermocellum* can convert pretreated lignocellulosic materials like aspen wood and wheat straw after pretreatment by steam explosion method (23), amorphous and crystalline cellulose [7, 8, 9, 10, 17, 19], and cellobiose [3, 30] into ethanol.

In other hand, bacteria along with fungi bring about significant losses of wood every year. Early work on bacterial decay of wood was reviewed by Holt et al. [12]. Recent studies were connected with scanning and transmission electron microscopy observations of bacterial degradation of wood [2, 20, 27]. Singh and Butcher [27] described laboratory studies using mixed cultures of pine wood degrading bacteria nad observations of decaying timbers from natural environments which have shown the degradation to be of three main types on the basis of microscopic appearances of degradation pattern. The three types were: cavitation, erosion and tunneling. Attempts to obtain degradation of lignified wood cells in the laboratory by pure cultures of bacteria have thus far not been successful.

The cellular interactions and adhesion of various *C. thermocellum* strains was investigated onto cellulose fibers and onto insoluble hemicellulose aggregates from steam – exploded wood fractions and wheat straw. As these wood fractions hot water extractions from steam – exploded birch wood were used [34]. *C. thermocellum* sporulated while attached to the fibers composed of cellulose/filter paper stripes, when pH dropped below 6,4. It was postulated that the attachment was involved in cellulose breakdown and that *C. thermocellum* bacteria gaine an advantage by remaining attached to its insoluble substrates when the environment is not suitable for rapid growth [33].

Wiegel and Dykstra [33] presented light and electron micrographs a spore – forming *C. thermocellum* cells attached to cellulose fibers. Ultrastructure of *C. thermocellum* cell surface cellulosome (a cellulose -binding, multicellulase – containing protein complex) and its interaction with cellulose were described by Bayer and Lamed [1].

Ward and Groom [29] described some drying problems with red oak *Quercus rubra* infected with anaerobic bacteria *Clostridium* sp. Bacterially infected heartwood in oak can appear sound and without dark coloration. But green lumber containing bacterial heartwood is more prone than normal, noninfected oak to develop excessive amounts of drying defects even when kiln – dried by relatively mild conventional schedules. Volume losses from kiln-drying bacterial oak are principally due to deep surface checks, honeycomb, and ring failure. Under normal conditions, commercial kiln operations will generally lose 2 to 3 precent of their oak lumber to honeycomb. Clostridial infections it was easy recognise because these bacteria can produce odors which are due to a mixture of volatile fatty acids, namely acetic, propionic, butyric, valeric and caproic [37].

Schink et al. [25] found that *Clostridium* sp. bacteria isolated from wetwood of living trees can degrade pectin in xylem tissue but not cellulose and lignin. In xylem tissue, the compound middle lamella contains relatively large amounts of pectin and is probably weakened by the enzymatic action of *Clostridium* and assotiated microorganisms found in bacterial oak. Microscopic observations of honeycomb and ring failure in the kiln-dried oak samples revealed that these ruptures commonly occured in compound middle lamellae between ray and and fiber tissue or between early wood and latewood of the previous growing season. Apparently, pectin degrading bacteria are a factor in the reduced ability of bacterial oak to withstand shrinkage stresses during kiln-draing.

In this paper were presented the results of a microscopic observations (confocal laser scanning microscope – CLSM, scanning electron microscope – SEM, and transmission electron microscope – TEM) during colonization of cellulose fibers and pretreated wood Eucalyptus viminalis by sporulated *C. thermocellum* bacteria, previously isolated from compost Dano and physiologically characterized by Gajewska [8, 9] and Gajewska and Witkowska [10].

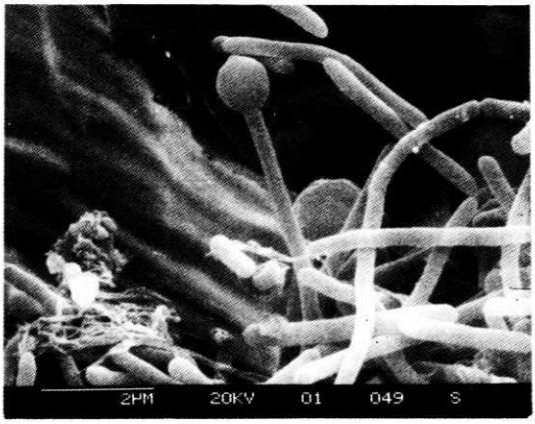

Fig. 1. Colonization of cellulose fibers of filter paper by sporulated *Clostridium thermocellum* bacteria (scanning electron microscope – SEM micrograph)

Fot. 1. Zasiedlanie włókien celulozy bibuły filtracyjnej przez przetrwalnikujące bakterie *Clostridium thermocellum* (zdjęcie ze skaningowego mikroskopu elektronowego – SEM)

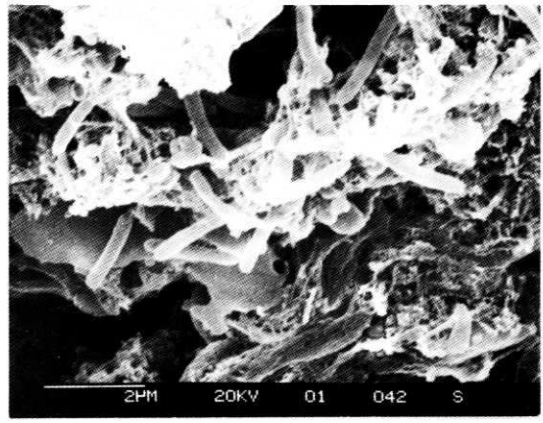

Fig. 2. Colonization of *Eucalyptus* wood after pretreatment by steamexplosion method by *Clostridium thermocellum* bacteria

Fot. 2. Zasiedlanie drewna *Eucalyptus viminalis* po wstępnej obróbce metodą eksplozyjną przez bakterie *Clostridium thermocellum* (zdjęcie ze skaningowego mikroskopu elektronowego – SEM)

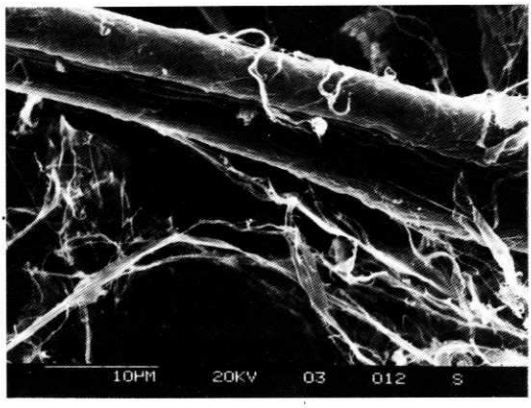

Fig. 3. Control – micrograph of cellulose fibers of filter paper from scanning electron microscope – SEM

Fot. 3 Kontrola – zdjęcie włókien celulozy bibuły filtracyjnej ze skaningowego mikroskopu elektronowego – SEM

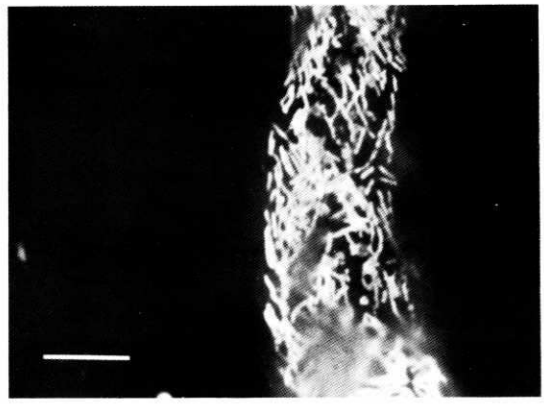

Fig. 4. Invasion (erosion) cells of *C. thermocellum* colonized inside cellulose fiber of filter paper (confocal laser scanning microscope – CLSM). Bar reprezents 10 μm

Fot. 4. Inwazyjne (erozyjne) komórki bakterii *C. thermocellum* zasiedlające wewnątrz włókno celulozy bibuły filtracyjnej (konfokalny laserowy mikroskop skaningowy – CLSM). Podziałka wskazuje 10 μm

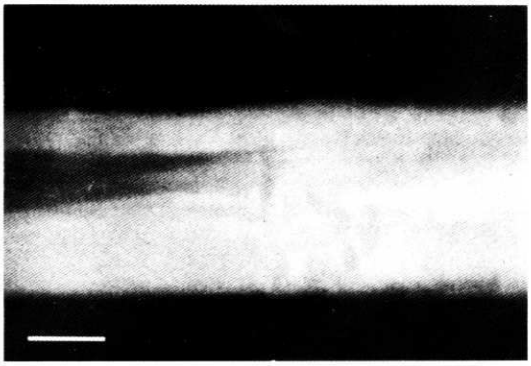

Fig. 5. Control of cellulose fiber of filter paper (confocal laser scanning microscope – CLSM). Bar represents 10 μm

Fot. 5. Kontrola – zdjęcie włókna celulozy bibuły filtracyjnej (konfokalny laserowy mikroskop elektronowy – CLSM). Podziałka wskazuje 10 μm

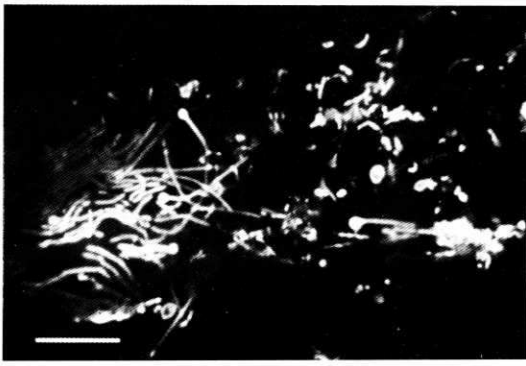

Fig. 6. Colonization of wood (*Eucalyptus viminalis*) and it's decay by erosion *Clostridum thermocellum* bacteria (confocal laser scanning microscope – CLSM). Bar represents 10 μm

Fot. 6. Zasiedlanie drewna *Eucalyptus viminalis* i jego rozkład przez erozyjne bakterie *Clostridium thermocellum* (konfokalny laserowy mikroskop skaningowy – CLSM). Podziałka wskazuje 10 μm.

Fig. 7. Division and attachment of *Clostridium thermocellum* cels to *Eucalyptus* wood after pretreatment (transmission electron microscope – TEM). Magnification : 16000 ×

Fot. 7. Podział i przyleganie komórek *Clostridium thermecellum* do drewna *Eucalyptus viminalis* po wstępnej obróbce (transmisyjny mikroskop elektronowy – TEM). Powiększenie : 16000 ×

Fig. 8. Longitudinal section throught *C. thermocellum* cells adherent to *E. viminalis* wood after pretreatment (transmission electron microscope – TEM). Magnification : 16000 ×

Fot.8. Przekrój podłużny przez komórki *C. thermocellum* przylegające do drewna *E. viminalis* po wstępnej obróbce (transmisyjny mikroskop elektronowy – TEM). Powiększenie : 16000 ×

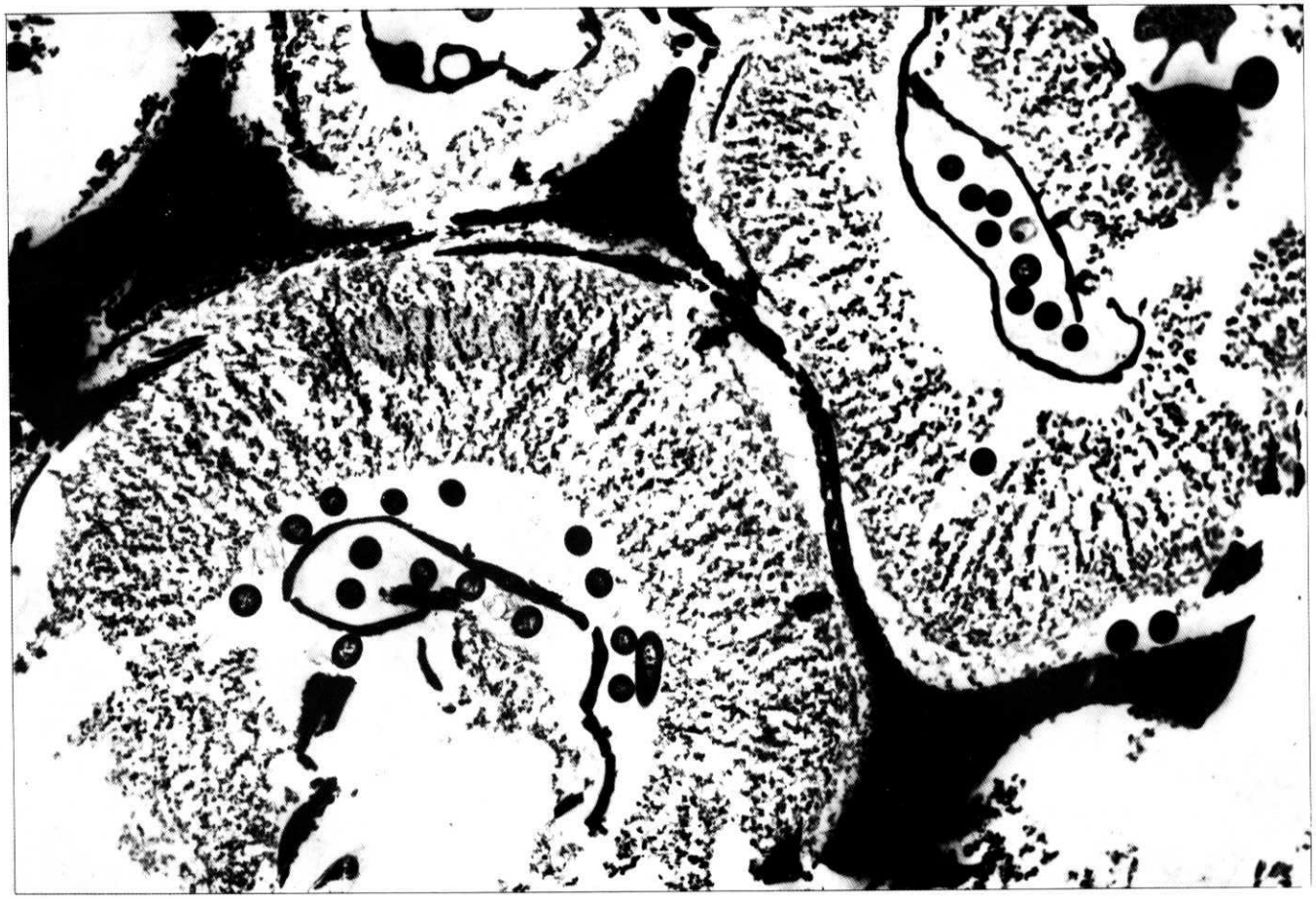

Fig. 9. Cross section of vessls of *E. viminalis* wood infected by *C. thermocellum* erosion bacteria, presented in lumen, cell wall S_1, S_2 and S_3 layers and middle lamella of wood (transmission elecuron microscope – TEM). Magnification 25 000 ×

Fot. 9. Przekrój poprzeczny przez naczynia drewna *E. viminalis* po infekcji drewna przez erozyjne bakterie *C. thermocellum*, obecnie w świetle naczyń, w warstwie S_1, S_2 i S_3 ściany komórkowej i blaszce środkowej drewna (transmisyjny mikroskop elektronowy – TEM). Powiększenie : 25 000 ×

MATERIALS AND METHODS

ORGANISM AND CULTURE CONDITIONS

Clostridium thermocellum strain JG1 was isolated previously from six months ripening compost Dano, prepared from municipal wastes in Radiowo nearly Kampinos National Park. Isolation and identification of this strain was described by Gajewska [8, 9] and Gajewska and Witkowska [10]. *C. thermocellum* JG1 strain spore-forming, thermophilic, Gram negative bacteria were isolated after heating im 100°C during 20 minutes using CM3 medium by Weimer and Zeikus [30] with own modifications, containing 0,02% sodium azide, antibiotics (penicillin, streptomycin, vancomycin and cyclohexamid), carboxymethylcellulose (CMC) (The British Drug House, Ltd.) or filter paper as carbon source. For agar plates Gas Pak anaerobic jar 150 (B.B.L. Cockeysville) was used. They were incubated four to ten days in 55°-60°C in anaerobic condition. Zones of CMC cellulolysis around colonies could be visualized by staining with Gram's iodine according with Williams [35]. For identification of *Clostridium* sp. Api 20A tests for anaerobes (Merieux, France) were used. Strain JG1 showed the highest cellulolytic activity measured by Samogy method of reducing sugars contents [24], described earlier [8].

MEDIA AND ANAEROBIC CULTURE METHODS

Cells were routinally grown during four to ten days on CM3 medium [30] and CM4 medium by Herrero et al. [11]. Solid media supplemented with 1.8% agar Difco. Cell growth was initiated with inoculum size that was always above 5 – 10%. As a carbon and energy source to these basal media stripes of filter paper or samples of wood (100 mg per 10 ml of medium) were added. After the autoclaving, the cultivation was carried out in a test tube 16 mm × 180 mm with neoprene stoppers. All cultures were grown anaerobically at 55°C in N_2 oxygen free atmosphaere. Microcrystalline cellulose (Avicel) was used only in solid media. Petri dishes were incubated in Gas Pak 150 jar (BBL). Culture purity was assays by microscopic examination of cellulose agar plates for non cellulolytic colonies. In second cases, 1 ml of culture of *C.thermocellum* was serially diluted with same liquid medium and 0.1 ml was spreaded on agar plates with microcrystalline cellulose. Petri dishes were incubated anaerobically in Gas Pak 150 during ten days. Colonies were picked to fresh CM3 medium [30] or CM4 medium [11] with filter paper and after incubation in anaerobic condition they were stored at 4°C. For microscopic observations cells with cellulose or wood were harvested by centrifugation at 1700 × g (average) for five min. The pellets were suspended in 0.1% phosphate buffer, pH 7.2 and centrifuged as before. This washing procedure for cultures and controls were repeated three times. Suspensions of washed cells were used for fixations and microscopic observations.

CELLULOSE AND WOOD MATERIALS

- filter paper Whatman No 1 (stripes weight – 100 mg) in each tube with 10 ml of liquid medium,
- microcrystalline cellulose (Avicel) – 1% in solid medium,
- steam exploded Eucalyptus viminalis wood (water washed fraction of wood- 1% in liquid medium, 210°C per 50 sec., 1% SO_2, v/v) prepared by L. Ramos, Forest Products Biotechnology, UBC, Vancouver, Canada; approx. moisture content: 72%, approx. cellulose content: 58% (personal information).

Methods for transmission electron microscope (TEM) observations. These methods were described by Shelton et al. [26] with own modifications. For thin section electron micrographs, cells were fixed overnight (4°C) in 2% glutaraldehyde and 0.1 M phosphate buffer (pH 7.2). Cells were centrifuged and washed in 0.1 M phosphate buffer (pH 7.2) twice. Cells were then embedded in 1.8% agar Difco and postifixed for two hours at room temperature in 1% osmium tetraoxide in phosphate buffer 0.1 M (pH 7.2). Samples were dehydrated through an ethanol series followed by propylene oxide, then were embedded in Epon-Araldite, and Epon-Araldite+DMP 30 epoxy resin. Thin sections were poststained with 0.5% uranyl acetate and Reynolds lead citrate. Whole cell electromicrographs were obtained by floating Parlodian coated copper grids (300 mesh) on a drop bacterial suspension for 1 min. Grids were blotted and then were negatively stained form 12 min. with 2% uranyl acetate. Electron micrographs were taken on Zeiss EM 10 A transmission electron microscope.

METHODS FOR SCANNING ELECTRON MICROSCOPE (SEM) OBSERVATIONS

The same samples of *C.thermocellum* culture (strain JG1), used in TEM methods, after dehydratation in 100% ethanol were critical point dried in Critical Point Dryer CPD 020 Balzers Union. After gold coating, cultures and controls (filter paper and wood) were observed in Cambridge 250 scanning electron microscope, according with Sleat et al. [28].

METHODS FOR CONFOCAL LASER SCANNING MICROSCOPE (CLSM) OBSERVATIONS

It was used a method by Strugger, with own modification [10]. Cultures in CM4 medium and controls were contrifugated and pellets were washed two times in phosphate buffer, pH 6.0. Next, 100% absolute ethanol was added for 30 min at room temperature. After centrifugation pellets were washed twice with phospate buffer, pH 6.0, and 0.1%. Solution of acridine orange was added for 15 min. Samples were washed three times with phosphate buffer, pH 6.0. One drop of formaldehyde 37% solution was added to 1 ml of suspenison and stored 24 hours at 4°C. Samples were centrifuged and were washed in phosphate buffer, pH 6.0. One drop of suspension was added on slide surface to one drop of glycerol and over slip was pressed firmly onto slide surface.

RESULTS AND DISCUSSION

Scanning electron microscope [SEM] observations has shown an attachment of cells of pure culture anaerobic thermophilic cellulolytic *C.thermocellum* JG1 strain to cellulose fibers (Fig. 1) and *E. viminalis* wood after pretreatment (Fig. 2), when cells grown onto CM4 medium during four days in 55°C. The control of cellulose fiber presents Fig. 3. Electron micrographs presented colonization process of vegetative cells and terminally sporeforming cells. Spores were formed typically for genus *Clostridium* (*plectridium* type). During colonization of wood *E.viminalis*, detachment of cells and their division on substrate was also obserwed. It was noted that spores of *C.thermocellum* bacteria can germinate within sporangium (not shown data). This type of germination significally differs from *Bacillus* germination. Spores of *Bacillus* sp. released from the mother cells prior to germination [6, 21, 22].

In the present work was provided the additional evidence for the colonization of cellulose fibers and wood, not only on the surface but inside, too. Laser technic in confocal laser scanning microscope (CLSM) was a key in those examinations. Laser micrographs Fig. 4 revealed the process of attack and penetration those anaerobic cells inside fiber of filter paper in comparison with control fiber (Fig. 5) and full decomposition of pretreated wood of *E. viminalis* (Fig. 6). Inside both substrates, sporulation of cells was very intensive. Cells inside substrates, after staining with acridine orange were good seeing in light orange colour and out-of-focus regions of the sample appear black. In addition, the destruction of substrates was observed, as result of cellulolytic activity by these type of invasion bacteria.

During transmission electron microscope (TEM) examinations of samples from transverse section of *C. thermocellum* cells growing in CM4 medium with steam exploded *E. viminalis* wood during 4 days in it was possible to note attachment of cells to substrate, and detachment, too (Fig. 7). On Fig. 8 longitudial section along bacterial cells and wood microfibrilles revealed presence of erosion bacteria in corridors. The position of bacteria is mainly in pararelly site to the "wall" of corridors, however a single bacteria have got a different directions. Zones arround the cells without electron density indicate a cellulolytic activity of cells inside wood. On basis electron micrograph from Fig. 8. We can observed division of cells inside wood. After analyses of electron micrograph from Fig. 9 it was possible to suppose, that these bacteria could attack the cell wall of wood vessels from lumen outward. During the attack long channels or throughs slighty langer than bacterial diameter were produced. *C. thermocellum* cells penetrated from the lumen, progressing outward, e.g. towards the middle lamella, through the S_3, S_2 to S_1 layer. Additionally, decomposition of the middle lamella was observed. The cellulosomes, with clear zones arroud them, nearly these erosion bacteria inside wood, were visible on the same photos, but with bigger magnitude [10]. Recently appeared a few publications about importent role of cellulosomes in cellulose decay [1, 4, 5, 10, 13, 15, 16].

It has be difficult to shown bacterial decay of wood by pure culture of anaerobic strains. These results indicate the important role of anaerobic cellulolytic *Clostridium thermocellum* bacteria which can colonize and decompose wood very quickly in 55°C in pure culture. Possibility of sporulation of *C. thermocellum* by erosed cells have negative influence in stroing process of wood in anaerobic conditions, because thermoresistant spores can survave longer time inside wood and can be a cause of next bacterial infections. Complete sporulation while attached to cellulose fibers should be an importent factor for their successful survival. In other hand, a cellulolytic activity and wood decay has very important role in an agricultural environments, e.g. composting and organic matter decomposition.

Received in April 1993

REFERENCES

1. Bayer E. A., Lamed R.: Ultrastructure of the cell surface cellulosome of *Clostridium thermocellum* and its interaction with cellulose. J. Bacteriol., 1986, 167 (3): 828-836.
2. Blanchette R. A., Nilsson T., Daniel G. F.: Biological degradation of wood. In: Advances in Chemistry, Series No 225, Archaelogical Wood: Properties, Chemistry and Preservation (Eds. R. S. Rowell and R. J. Barbour). Americ. Chemical Society, Washington, D. C., 1990.
3. Brenner D. and Johnson B. F.: Relationship between substrate concentration and fermentation product rations in *Clostridium thermocellum* cultures. Appl. Environ. Microbiol., 1984, 47(5):1126-1129.
4. Cavedon K., Leschine S. B., Canale-Parola E.: Cellulase system of a free-living, mesophilic *Clostridium* (strain C7). J. Bacteriol., 1990, 172 (8): 4222-4230.
5. Cavedon K., Leschine S. B., Canale-Parola E.: Characterization of the extracellular cellulase from a mesophilic *Clostridium* (strain C7). J. Bacteriol., 1990, 172 (8):4231-4237.
6. Chapman G. B., Zworykin K. A.: Study of germinating *Bacillus cereus* spores employing television microscopy of living cells and electron microscopy of ultraphyn sections. J. Bacteriol., 1957, 74:126-132.
7. Freier D., Mothershed C. P., Wiegel J.: Characterization of *Clostridium thermocellum* JW 20. Appl. Environ. Microbiol., 1988, 54 (1): 204-211.
8. Gajewska J. K.: Cellulolytic and physiological properties of *Clostridium thermocellum* isolated from Dano compost. Materials of XXI Symposium of Polish Microbiological Society, September, 1987: p. 418, Olsztyn.
9. Gajewska J. K.: Decomposition of biopolimers: cellulose, hemicellulose (xylan) and lignocellulosic substrates by anaerobic thermophilic *Clostridium thermocellum* strain JG1. Post doct. thesis, 1993, SGGW, Warsaw, in press.
10. Gajewska J. K. and Witkowska E.: Fermentation of cellulose by *Clostridium thermocellum*. Second Int. Symp. on "Overproduction of microbial products". Abstract Book, June, 1988, Česke Budejovice.
11. Herrero A. A., Gomez R. F., Roberts M. F.: Development of ethanol tolerance in *Clostridium thermocellum*. Effect of growth temperature. Bioch. Biophys. Acta, 1982, 693: 195-204.
12. Holt D. M. and Furtado S. E. J.: Bacterial breakdown of wood in aquatic habitats. Record of the Annual Convention of the British Wood Preserving Association. 1979: 13-24.

13. Kobayashi M. P., Romaniec M. P. M., Fauth U., Demain A. L.: Subcellulosome preparation with high cellulase activity from Clostridium thermocellum. Appl. Environ. Microbiol. 1990, 56 (10): 3040 - 3046.
14. Lamed R., Naimark J., Morgenstern E., Bayer E. D.: Specialized cell surface structures in cellulolytic bacteria. J. Bacteriol., 1987, 169 (8): 3792 - 3800.
15. Lamed R., Setter E., Bayer E.: Characterization of a a cellulase – containing complex in *Clostridium thermocellum*. J. Bacteriol., 1983, 156: 828 - 836.
16. Morag E., Bayer A. E., Lamed R.: Relationship of cellulosomal and nocellulosomal xylanases of *Clostridium thermocellum* to cellulose – degrading enzymes. J. Bacteriol., 1990, 172 (10): 6098 - 6105.
17. Mori Y.: Characterization of symbiotic coculture of *Clostridium thermohydrosulphuricum* YM 3 and *Clostridium thermocellum* YM4. Appl. Environ. Microbiol., 1990, 56 (1): 37 - 42.
18. Ng T.K. and Zeikus J.G.: Comparison of extracellular cellulase activities of *Clostridium thermocellum* LQRJ and Trichoderma reesei QM9414. Appl. Environ. Microbiol., 1981, 42: 231 - 240.
19. Ng T.K., Weimer P.J., Zeikus J. G.: Cellulolytic and physiological properties of *Clostridium thermocellum*. Arch. Mikrobiol., 1977, 114: 1 - 7.
20. Nilsson T. and Daniel G. F.: Bacterial attack of wood cell walls. In Biodeterioration 7: Proc. of the Int. Biodeterioration Symp. (7 th). Eds. Houghton D. R., Smith R. N., Eggins H. O. W., 1987, Cambridge, England.
21 Robinov C. F.: Morphology of bacterial spores, their development and germination. In Gunsalus I. C., Stanier R.Y. (eds.). The bacteria, 1960, 1: 207 - 248. Acad. Press Inc., New York.
22. Rousseau M., Flechon J., Hermier J.: Étude au microscope électronique de la germination de la spore chez Bacillus subtilis. Ann. Inst. Pasteur, 1966, 111: 149 - 160.
23. Saddler J. N. and Chan M. K. H.: Conversion of pretreated lignocellulosic substrates to ethanol by *Clostridium thermocellum* in mono and co-culture with *Clostridium thermosaccharolyticum* and *Clostridium thermohydrosulphuricum*. Can. J. Microbiol., 1984, 30 (2): 212 - 220.
24. Samogy M.: Notes on sugar determinations. J. Biol. Chem., 1952, 195: 19 - 23.
25. Schink B. J. C., Ward J. C., Zeikus J. G.: Microbiology of wetwood: importance of pectin degradation and *Clostridium* species in living trees. Appl. Environ. Microbiol., 1981, 45 (3): 526 - 532.
26. Shelton D. R., Tiedie J. M.: Isolation and characterization of an anaerobic consortium that mineralizes 3-chlorobenzoic acid. Appl. Environ. Microbiol., 1984, 48 (4): 840 - 848.
27. Singh A. P. and Butcher J. A.: Bacterial degradation of wood cell wall: a rewiew of degradation patterns. Doc. No: IRG (WP) 1460.
28. Sleat R., Mah R.A., Robinson R.: Isolation and characterization of an anaerobic cellulolytic bacterium, *Clostridium cellulovorans* sp. nov. Appl. Environ. Microbiol., 1984, 48: 88 - 93.
29. Ward J. C. and Groom D. A.: Bacterial oak: drying problem. Forest Products. J. 1983, 33 (10): 57 - 65.
30. Weimer P. J. and Zeikus J. G.: Fermentation of cellulose and cellobiose by *Clostridium thermocellum* in the absence and presence of *Methanobacterium thermoauthotrophicum*. Appl. Environ. Microbiol., 1977, 33(2): 289 - 297.
31. Wiegel J.: Formation of ethanol by bacteria – a pledge for the use of extreme thermophile anaerobic in bacteria in industrial ethanol fermentation processes. Experientia, 1980, 36: 1434 - 1442.
32. Wiegel J.: Ethanol from cellulose. Experientia, 1982, 38: 151 - 155.
33. Wiegel J. and Dykstra M.: *Clostridium thermocellum*: adhesion and sporulation while adhered to cellulose and hemicellulose. Appl. Microbiol. Biotechnol. 1984, 20: 59 - 65.
34. Wiegel J. and Plus J.: Production of ethanol from hemicelluloses and hardwoods and annual plants. In: Strub A., Chartier P., Schleser G. (eds.). Energy from biomass, 2nd E. C. Conference. Appl. Sci. Publishers, London, New York, 1990, 863 - 867.

35. Williams A. G.: Staining reactions for the detection of hemicellulose degrading bacteria. FEMS Microb. Lett., 1983, 20: 253-258.
36. Yu E.K.C., Chan M.K.H., Saddler J.N.: Butanediol production from lignocellulosic substrates by *Klebsiella pneumoniae* grown in sequential co-culture with *Clostridium thermocellum*. Appl. Microbiol. Biotechnol. 1985, 22: 399-404.
37. Zinkel D.F., Ward J.C., Kukachka B. F.: Odor problems from some plywoods. Forest Prod. J., 1969, 19 (12):60.

Authors address:
Dr Julitta K. Gajewska
Katedra Mikrobiologii Rolniczej SGGW
ul. Rakowiecka 26/30, 02-528 Warszawa

ZASIEDLANIE DREWNA *EUCALYPTUS VIMINALIS* PO WSTĘPNEJ OBRÓBCE PRZEZ *CLOSTRIDIUM THERMOCELLUM* (SZCZEP. JG1)

Streszczenie

W pracy wykazano, że bakterie *C. thermocellum* w czystej kulturze, izolowane z kompostu Dano (szczep JG1) powodują degradację drewna eukaliptusa (*Eucalyptus viminalis*), po wstępnej obróbce metodą eksplozyjną w krótkim czasie (4 dni). Na powierzchni oraz wewnątrz ligninocelulozowych substratów obserwowano przetrwalnikowanie i kiełkowanie przetrwalników oraz podziały komórek bakterii. Na podstawie obserwacji w skaningowym mikroskopie elektronowym – SEM, konfokalnym laserowym mikroskopie skaningowym – CLSM i transmisyjnym mikroskopie elektronowym – TEM, można było wyróżnić kilka etapów podczas procesu zasiedlania drewna przez te bakterie erozyjne: 1) przyczepienie się do substratu, 2) rozkład ligninocelulozowych substratów, 3) penetracja do zewnątrz włókien celulozy oraz naczyń drewna, 4) odczepienie się od substratu. Również przyjęto próby określenia mechanizmu rozkładu naczyń drewna na podstawie badań ultramikroskopowych.

Acknowledgement

The author, respectively, Dr. and Mrs. A. S. Dekaban Foundation, Dr. V. Runeckles, Charmain Dekaban Advisory Committe, and Dr. S. Berch, Dr. A. Amarasighe, Dr. L. Ramos, Mr. M. Weiss, Ms. S. Wielesko from University of British Columbia, Vancouver, BC, Canada, is gratefully acknowledged.