# CYCLODEXTRIN NEWS

VOLUME 15. N° 11. NOVEMBER 2001 ISSN 0951-256X

## 133 ABSTRACTS IN THIS MONTH!

**Total number of publications, abstracted by CD-NEWS up to now:**

| 1985 | 1986 | 1987 | 1988 | 1989 | 1990 | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 379 | 445 | 522 | 693 | 771 | 885 | 924 | 1168 | 1033 | 1372 | 1459 | 1627 | 1464 | 1806 | 1644 | 1683 | 637 |

## BACTERIAL DEGRADATION OF CDS

Cyclodextrins are resistant to common starch hydrolyzing enzymes, to β-amylases and most of the α-type amylases as well. At the beginning of 80's among the bacterial amylases only those of *B. polymyxa* [1] and the CDase (CD hydrolyse) of *B. macerans* [2] were known to be able to degrade CDs. A *Flavobacterium* species has been selected from hundreds of starch-degrading microorganisms isolated from soil and brook water which could use CDs as sole carbon source. Its CD-degrading glucoamylase produced glucose as the final degradation product from all CD substrates [3]. Small amounts of maltose, which could be detected in the course of CD degradation, were hydrolyzed at a lower rate.

Later another *Flavobacterium* sp. was isolated from wheat bran and its CD-degrading enzyme was purified and characterized [4]. The rate limiting step of the hydrolysis was found to be the ring cleavage, the α-, β- and γCDs are quickly hydrolyzed resulting in glucose, maltose and maltotriose in long term digests.

Another gram-negative bacterium, *Klebsiella pneumoniae* is able to metabolize CDs by the concerted action of intra- and extracellular cyclodextrin glucanotransferase (CGTase), an amylomaltase-like enzyme and maltodextrin phosphorylase [5]. In the presence of glucose as acceptor the main metabolite is glucose-1-phosphate, at low glucose and maltose concentration, however, the (1-4)-α-D-glucopyranosyl-transfer reactions are catalyzed: αCD is converted to β- and γCD [6]. At low CD concentrations the cyclization and disproportionation reactions of maltooligosacharides become dominant [7].

Thus, CGTases not only transform starch into CDs, but also catalyze the transfer of CDs to appropriate acceptors and the disproportionation of linear dextrins. These three reactions are manifestations of a single catalytic activity. For instance, when αCD and maltitol as acceptor are present, αCD is transferred to maltitol and the disproportionation of functionalized dextrins carrying maltitol at the reducing end are also formed [8].

It has been shown that *Klebsiella oxytoca* can utilize starch via a novel pathway [9]. An extracellular CGTase first degrades starch into α- and βCDs which are then used as carbon and energy source. It has been proved that the CDs are transported into the cytoplasma via a specific system and that they are metabolized inside the cell [10]. After the intracellular linearization by a CDase, the maltooligosacharides formed are metabolized by the activity of maltodextrin phosphorylase and amylomaltase.

CD-degrading enzymes have been described from a series of other bacteria: *Bacillus sphäricus* [11], *B. circulans* [12], *B. coagulans* [13], *B. stearothermophilus* [14], etc. Amylases able to hydrolyze CDs were also found in *Pseudomonas spp.* [15], *B. subtilis* [16], etc.

### *Bacterial degradation in the mammalian colon*

*In vitro* studies showed that mixed cultures of colon organisms from rats and rabbits were able to degrade CDs, even the heptakis(2,6)-dimethyl βCD [17]. In a study of Antenucci and Palmer thirty *Bacteroides* strains from human colon were tested for the ability to degrade cyclodextrins *in vitro* [18]. Most (24 of 30) of the selected colon anaerobes were able to degrade α- and βCDs as evidenced by their ability to grow on CDs as the sole carbon source. More detailed investigation of CDase isolated from two selected *Bacteroides* strains showed that CDase activity was predominantly cell bound and induced by as little as 2-4-h growth on CDs. The enzymes were shown to be stable and active under pH and temperature conditions (pH 6.80; 37°C) expected in the colon environment.
The products of CD hydrolysis were distinctly different for the two *Bacteroides* strains. After 18-h incubation, HPLC analysis showed that CDase from *B. distasonis* C18-7 catalyzed the break down of αCD to glucose, maltose, and maltotriose. βCD was hydrolyzed similarly to yield a mixture of glucose, maltose, maltotriose, and maltotetraose, after 18-h incubation. In contrast, the CDase from *B. ovatus* 3524 hydrolyzed α- and βCD completely to glucose in 18 h. In this case glucose was the only degradation product during early stages of CD hydrolysis, while the CDase *from B. distasonis* C18-7 catalyzed the formation of a mixture of glucose and oligosaccharides at all stages, the relative proportions of the individual products remained unaltered. This indicates that the crude CDases from these two strains contain different enzymes.

### *Degradation by bacteria associated with plants*

Twenty-four strains in eight genera of bacteria associated with plants were examined for their ability to use the parent and methylated cyclodextrins as a sole carbon source [19]. The results of bacterial growth tests are compiled in Table 1 and 2, showing growth (+) or no growth (-).

**Table 1. Growth of various plant-related gram-negative bacteria on media with CD as sole carbon source**

| No. | Species | Strain | GROWTH ON CDs | | | | |
|---|---|---|---|---|---|---|---|
| | | | αCD | βCD | γCD | DIMEB | TRIMEB |
| ***Agrobacterium*** | | | | | | | |
| 1. | **A. radiobacter** | K-84 | + | - | + | + | + |
| 2. | *A. tumefaciens* | 0 | - | - | - | - | - |
| 3. | *A. tumefaciens* | B6 | - | - | + | - | - |
| 4. | *A. tumefaciens* | C-58 | - | - | - | - | - |
| ***Bradyrhizobium*** | | | | | | | |
| 5. | *B. japonicum* | B-37 | + | + | + | + | + |
| ***Erwinia*** | | | | | | | |
| 6. | *E. atroseptica* | G-128 | - | - | - | - | - |
| 7. | *E. carotovora* | CCM1008 | + | + | + | - | - |
| 8. | *E. uredovora* | Vfr3 | + | - | - | - | - |
| ***Pseudomonas*** | | | | | | | |
| 9. | **P. lachrymans** | 31 | - | - | - | - | - |
| 10. | **P. mors-prunorm** | SR-2 | - | - | - | - | - |
| 11. | **P. phaseolicola** | 67 | - | - | - | - | - |
| ***Xanthomonas*** | | | | | | | |
| 12. | *X. alfalfae* | KX-1 | + | + | + | + | + |
| 13. | *X. campestris* | 2D 510 | + | + | + | - | - |
| 14. | *X. phaseoli var. fuscans* | ERA 4 | + | - | - | - | - |
| 15. | *X. stewartii* | B-40 | + | + | + | + | + |
| 16. | *X. vesicatoria* | 53 | + | + | + | + | + |

**Table 2 Growth of various plant-related gram-positive bacteria on media with CD as sole carbon source**

| No. | Species | Strain | GROWTH ON CDs | | | | |
|---|---|---|---|---|---|---|---|
| | | | αCD | βCD | γCD | DIMEB | TRIMEB |
| ***Bacillus*** | | | | | | | |
| 17. | *B. thüringiensis* | B-35 | + | + | - | - | - |
| ***Corynebacterium*** | | | | | | | |
| 18. | *C. betae* | CN 101B-27 | - | - | + | - | - |
| 19. | *C. fascians* | B-27 | + | + | + | + | + |
| 20. | *C. flaccumfaciens* | 8 | - | - | + | - | - |
| 21. | *C.michiganense* | 36/3 | - | - | + | - | - |
| 22. | *C. nebraskense* | CN 101 | - | - | + | - | - |
| 23. | *C. oortii* | B-11 | - | - | + | - | - |
| ***Micrococcus*** | | | | | | | |
| 24. | *M. luteus* | B-98 | + | - | - | - | - |

The data clearly show that the bacteria considerably differ in their ability to utilize CD as carbon source. *Xantomonas* spp. were the most active bacteria in degrading CDs, with *X. stewartii* and *X. vesicatoria* strains utilizing all examined CDs. Six strains were unable to grow on CD carbon source. The other strains selectively utilized one or more of the CDs.
The relative biodegradability of the various CDs differs considerably. The order of degradability of the monomers is: γCD > αCD > βCD. The methylated βCD derivatives were found to be of the lowest biodegradability.

### *Degradation in soil*

Biodegradable polymers have been produced by building in CD as a weak point accessible to enzymatic attack. For instance, a novel hydrophilic polyurethane was synthesized by chain extending of the prepolymer formed from polyethylene glycol 1000 and hexamethylene diisocyanate with βCD. Samples were buried in garden soil approximately 2-3 inches below the surface. After 10 days of soil burial the average molecular weight of the polymer was found to decrease indicating the influence of soil-borne microorganisms in initiating the degradation of the polymer [20]. The buried samples showed 52% weight loss through the dissolution of low molecular weight fragments resulted from degradation. SEM micrographs of surfaces of the buried sample show numerous holes and cracks pointing out the substantial influence of microorganisms on the sample, while the control sample is nearly homogeneous and free from cracks or pits.
The newest bioremediation technologies are based on the application of CDs to increase the bioavailability of the pollutants. The microbe population growing in the polycyclic aromatic hydrocarbons (PAH)-contaminated soil was found to be able to use βCD as sole carbon source [21], while the indigenous microflora in a polychlorinated biphenyls (PCB)-contaminated soil can utilize γCD and HPBCD as sole carbon source [22]. RAMEB is slowly mineralized in soils (8-15 % within 64 days) [23].

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## CYCLODEXTRIN RELATED SYMPOSIA

**11TH INTERNATIONAL CYCLODEXTRIN SYMPOSIUM**
**May 5th-8th 2002, Reykjavik, Iceland**

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