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DECOMPOSITION OF NATIVE CYCLODEXTRINS AND CYCLODEXTRIN DERIVATIVES BY VARIOUS *TRICHODERMA* SPECIES

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The growth and the development of thallus of 14 *Trichoderma* species have been determined by using native α-, β- and γ-cyclodextrins (CDs) and 8 different CD derivatives as a unique carbon source. The majority of species can use CD for growth, this ability was related to their intrageneric taxonomic position in order of sections Trichoderma>Longibrachiatum>Pachybasium> Saturnisporum *T. reesei* being the most and *T. virens* the least active. All CDs have been utilized but their nutritional value was significantly influenced by the chemical structure. It was found that the size of molecules had major importance than the flexibility of their rings. Among natural derivatives *Trichodermas* preferred the α- and γ-CD prior to β-CD, carboxymethylation led to reduction of the utilization of α- and γ-CDs and enhanced that of β-CD. Methylation or polymerisation of β-CD also modified decomposition rate.

Изучены рост и развитие таллома у 14 видов *Trichoderma* с использованием природных α−, β− и γ−циклодекстринов (ЦДы) и 8 разных производных ЦД в качестве единственного источника углерода. Большинство видов грибов может использовать ЦД для роста. Эта способность относится к их межродовому таксономическому положению, поскольку в порядке секций *Trichoderma>Longibrachiatum>Pachybasium> Saturnisporium*, вид *T.reesei* − самый активный, а *T.virens* − самый слабый. Все ЦДы усваивались, но их питательная ценность значительно зависит от химической структуры. Выявлено, что размеры молекул имеют более важное значение, чем пластичность их колец. Из природных производных культуры *Trichoderma* более предпочитают α− и γ−ЦД, чем β−ЦД. Карбоксиметилирование ведет к снижению утилизации α− и γ−ЦД, но повышает использование β−ЦД. Метилирование и полимеризация β−ЦД также изменяет процесс их разложения.

Ուսումնասիրվել է *Trichoderma*-ի 14 տեսակների աճը և թալոմի զարգացումը, օգտագործելով բնական α−, β− и γ−ցիկլոդեքստրինները (ՑԴներ) և 8 տարբեր ցիկլոդեքստրինների ածանցյալները որպես ածխածնի միակ աղբյուր: Տեսակների մեծամասնությունը կարող է օգտագործել ՑԴ-ն աճի համար: Այս հատկությունը վերաբերվում է նրանց միջցեղային տաքսոնոմիական դիրքին, քանի որ ըստ բաժինների *Trichoderma>Longibrachiatum>Pachybasium> Saturnisporium* հերթականության, *T.reesei* −ին ամենաակտիվ, իսկ *T.virens* ամենաթույլ տեսակն է: Բոլոր ՑԴները յուրացվել են, բայց նրանց սննդառական արժեքը բավականին կախված է քիմիական կառուցվածքից: Բացահայտվել է, որ մոլեկուլների չափերը ունեն առավել կարևոր նշանակություն, քան նրանց օղակների ճկունությունը: Բնական ածանցյալներից *Trichoderma*-ի կուլտուրաները առավել նախընտրում են α− և γ−ՑԴ, քան β− ՑԴ: Կարբօքսիմեթիլացումը տանում է α− և γ−ՑԴ -ի յուրացման նվազմանը, բայց բարձրացնում է β− ՑԴ-ի օգտագործումը: β− ՑԴ-ի մեթիլացումը և պոլիմերիզացիան նույնպես փոխում են քայքայման արագությունը:

Introduction

Trichoderma, an anamorphic Hypocreaceae (class Ascomycetes), is a cosmopolitic group of fungi, common in the environment, especially in soils. Species of this genus are important members of detritus (Papavizas, 1985), and they have been used in many human activities including commercial applications for the production of cellulolytic and hemicellulolytic enzymes and for the biological control of plant diseases (Sundheim, 1977; Naár and Kecskés, 1995, Naár and Kecskés, 1998), for the biodegradation of chlorophenols (Fava et al., 1998) and polynucleated aromatic compounds (Wang et el., 1998), and for soil bioremediation (Ye et al., 1996; Esposito and da Silva, 1998; Bardi et al., 2000). *Trichoderma* species may cause severe losses in commercially produced mushrooms (Samuels, 1996), and have been identified as causal agents of disease in immunosuppressed humans (Furukawa et al. 1998).

Starch and cyclodextrins (CDs) can be used for the formulation of biologically active chemicals in solid state dispersons and liquid state (Schierbaum and Vorwerg, 1997). CDs are cyclic oligosaccharides commonly composed of six, seven or eight α-D-glucose units (α-, β- and γ-CD, respectively) which have an overall shape reminiscent of a truncated cone. On account of their relatively hydrophobic interiors, CDs have the ability to form inclusion complexes with a wide range of substrates in aqueous solution or heterogenic systems (Zsadon and Fenyvesi, 1981; McCray and Brusseau, 1998). This property of CDs has led to their application in areas as varied as enzyme mimics (Breslow et al., 1991; Yuan et al., 1998; Lee and Ueno, 2000), catalysis (Komiyama, 1993; Jarho, Van der Velde et al., 2000; Chou et al., 2001) and the encapsulation of drugs (Loftsson and Brewster, 1996; Thompson, 1997; Merkus et al., 1999; Loftsson and Jarvinen, 1999) and pesticides (Szejtli, 1997; Cserháti et al., 1984). Although they are frequently employed in various industrial pocesses as carriers (Duchene et al, 1999; Redenti et al., 2000) few attention has been paid to the study of their fate in biological systems (Szejtli, Gerloczy and Fonagy, 1980; Gerloczy et al., 1981; Szabo et al., 1981). It has been established that they are rapidly excreted by kidney when applied intravenously, and the rate of their intracorporal degradation is very low (Kubota et al., 2000). Bacteria associated to mammals and plants, as well as members of soil microbiota can utilize CDs (Oros et al., 1990; Pedersen et al., 1996).

The objectives of this work were the determination of the decomposition rate of native CDs and various CD derivatives by *Trichoderma* species, and the elucidation of the effect of chemical structure of CDs on the capacity of *Trichoderma* species to use them as unique carbon source.

Materials and Methods

Native α-, β- and γ-CDs (compounds I-III), their carboxymethylated derivatives (compounds IV-VI), hydroxypropylated-β-CD (VII, HP-β-CD), Dimeb (VIII = heptakis(2,6-di-O-methyl)-β-CD), Trimeb (IX = heptakis(2,3,6-tri-O-methyl)-β-CD), water-soluble β-CD polymers (X,XI) (EGP = prepared by crosslinking native β-CD molecules with ethylene glycol bis[epoxypropylether]); BGP = prepared by crosslinking native β-CD molecule with buthylene glycol bis[epoxypropylether]) were the gift of Dr.József Szejtli (CYCLOLAB Research and Development Laboratory, Budapest, Hungary). D-glucose, cellulose (Merck, Darmstadt, Germany), starch (amylum solubile), analytical grade mineral salts (Reanal, Budapest, Hungary) were used as received. The agar-agar (Oxoid No.3, Basingstoke, UK) was purified prior to use as follows: 100 g of agar-agar was suspended and steeped in 1 L of NaOH (1 N) for 18 hrs, and after washing with distilled water until neutral pH it was dried at 60-70°C.

Strains of 14 species belonging to four sections of *Trichoderma* genus: Trichoderma: *T. koningii* Oudemans [T-1], *T. aureoviride* Rifai [T-2], *T. viride* Persoon ex Gray [T-3], *T. atroviride* Karsten [T-4]; Longibrachiatum: *T. reesei* Simmons [L-5], *T. pseudokoningii* Rifai [L-6], *T. longibrachiatum* Rifai [L-7], *T. parceramosum* Bissett [L-8], *T. citrinoviride* Bissett [L-9], Pachybasium: *T. harzianum* Rifai [P-10], *T. polysporum* (Link ex Persoon) Rifai [P-11], *T. virens* (Miller, Giddens, et Foster) von Arx [P-12], *T. hamatum* (Bonorden) Bainier [P-13] and Saturnisporum: *T. saturnisporum* Hammill [S-14] were maintained on potato dextrose agar as previously described (Oros et al., 1999). Species L-5, L-8, L-9, and S-14 were taken from the collection of the Plant Protection Institute of Hungarian Academy of Sciences while species T-1, T-1, T-3, T-4, L-6, L-7, P-10, P-11, P-12, and P-13 were the gift of Dr Zoltán Naár (Esterházy Károly College, Department of Botany, Eger, Hungary). Conidia used for inoculation were produced on glucose mineral agar (GMA) amended with starch and casein (10 and 2 gL^{-1}, respectively) slains. The medium for utilization tests contained purified agar-agar, NH_4Cl, KH_2PO_4, K_2HPO_4, $MgSO_4 \times 7H_2O$ and NaCl (12, 1.0, 1.0, 0.5, 0.5 and 0.5 g per litre of tap water, respectively). Cyclodextrins or their derivatives as well as glucose were incorporated as sole carbon source into the melted medium at a concentration of 5 g per litre, prior to dispensing in 5 mm deep layer into Petri dishes (125 mm of diameter for GMA and 90 mm for the other carbon sources). The use of Petri dishes of different diamter was motivated by the different velocity of growth on glucose and CD carbon sources.

The response of fungi was characterized by measuring the linear growth of hypha (colony diameter in mm) at each day up till seven days and by evaluating the status of the thallus after 120 hrs incubation by a six fold scale (0 = no growth, 1 = tiny, non-sporulating thallus, diameter less than 10 mm, 2 = tiny thallus bearing some tufts of conidia, 10-50 mm of diameter, 3 = well developed but non-sporulating thallus, 10-50 mm of diameter, 4 = well developed sporulating thallus, diameter over 50 mm, but less intesively grew than observed on glucose, 5 = the thallus grews like on glucose containing medium). All incubations were performed at 25±2°C.

Each experiment was performed in triplicates. Two-way analysis of variation was applied for distinguishing averages of species(A)×carbon source(B) combinations. The nutritive value (bioavailability) of native CDs and CD derivatives was calculated by the spectral mapping technique (Lewi, 1976) the nutritive value of glucose being considered to be 100%. In order to compare the information content of the two methods of evaluation linear correlation was calculated between the data obtained by the methods.

Results and Discussion

Three *Trichoderma* species (*parceramosum*, *reesei* and *longibrachiatum*) produced yellow pigments on GMA (Csiktusnádi Kiss et al. 2000), and only *T. parceramosum* exhibited this property when grown on CDs. It was further established that the formation of aeral mycelium by *T. virens* was strongly depressed on media containing CDs as sole carbon source.

The average of scores characterizing the development of thallus of *Trichoderma* species in different media are compiled in Tables 1 and 2. The standard deviation of species x CD pairs was lower than 1 proving the good reproducibility of the method. The calculated F value characterizing the differences among the species x CD pairs was 347.7 indicating that the differences observed are highly significant the significance level being over 99.9%. It was not possible to reveal any connection between the intrageneric taxonomic position of *Trichoderma* species and their ability to consum CDs. The variability in activity of species depended considerably on the structure of carbon source and on the type of species as demonstrated by the relatively high coefficients of variation (C.V.(%) values).

Table 1. Development of thallus of *Trichoderma* species grown on glucose and on β-cyclodextrin derivatives as sole carbon sources. Average score values. For abbreviations see Materials and Methods

Species (A)	Carbon sources (B)					
	Glucose	β-cyclodextrin derivatives				
		Dimeb	Trimeb	HP-β-CD	EGP	BGP
T-1	3	1	1	2	1	2
T-2	4	1	1	1	1	2
T-3	5	1	1	1	1	1
T-4	5	1	1	1	1	2
L-5	3	0	1	2	1	2
L-6	5	1	1	1	1	2
L-7	4	1	1	2	1	2
L-8	5	0	1	0	0	2
L-9	3	0	1	0	0	1
P-10	4	0	1	1	1	2
P-11	3	1	1	1	1	1
P-12	5	1	1	1	1	1
P-13	3	1	1	1	1	1
S-14	3	0	1	1	1	1
C.V.(%)	23	77	0	57	42	63

C.V.(%) =variability of utilization

Table 2. Development of thallus of *Trichoderma* species grown on native and carboxymethylated α-, β- and γ-cyclodextrins as sole carbon sources. Average score values.

Species (A)	Carbon sources (B)					
	Native			Carboxymethylated		
	α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD
T-1	2	0	2	1	1	1
T-2	2	1	2	1	2	1
T-3	2	0	2	1	2	1
T-4	2	2	2	1	2	1
L-5	2	2	3	1	2	1
L-6	2	2	3	1	2	1
L-7	1	1	1	1	1	1
L-8	1	2	3	1	1	1
L-9	2	2	2	0	1	1
P-10	1	1	3	1	1	1
P-11	1	2	1	1	1	1
P-12	1	1	1	1	1	1
P-13	0	2	1	1	1	1
S-14	1	1	1	1	2	1
C.V.(%)	45	55	43	29	36	0

C.V.(%) =variability of utilization

The dependence of the colony diameter of *Trichoderma viride* on the incubation time and the character of carbon source is shown in Fig.1. It can be established that the decomposition of β-CD starts after a considerable lag period similarly to other natural carbohydrate polymers. Carboxymethylation markedly modified the rate of colony growth the highest effect was observed in the case of β-CD. These effect are less marked for native α-

and γ-CDs. This discrepancy can be tentatively explained by the fact that in the β-CD molecule a complete secondary belt of hydrogen bonds is formed between the C-2 hydroxyl group of one glycopyranose unit and the C-3 hydroxyl group of the neighbouring glucopyranose unit, so that β-CD is a rather rigid structure making the molecule less liably to enzymatic attack.

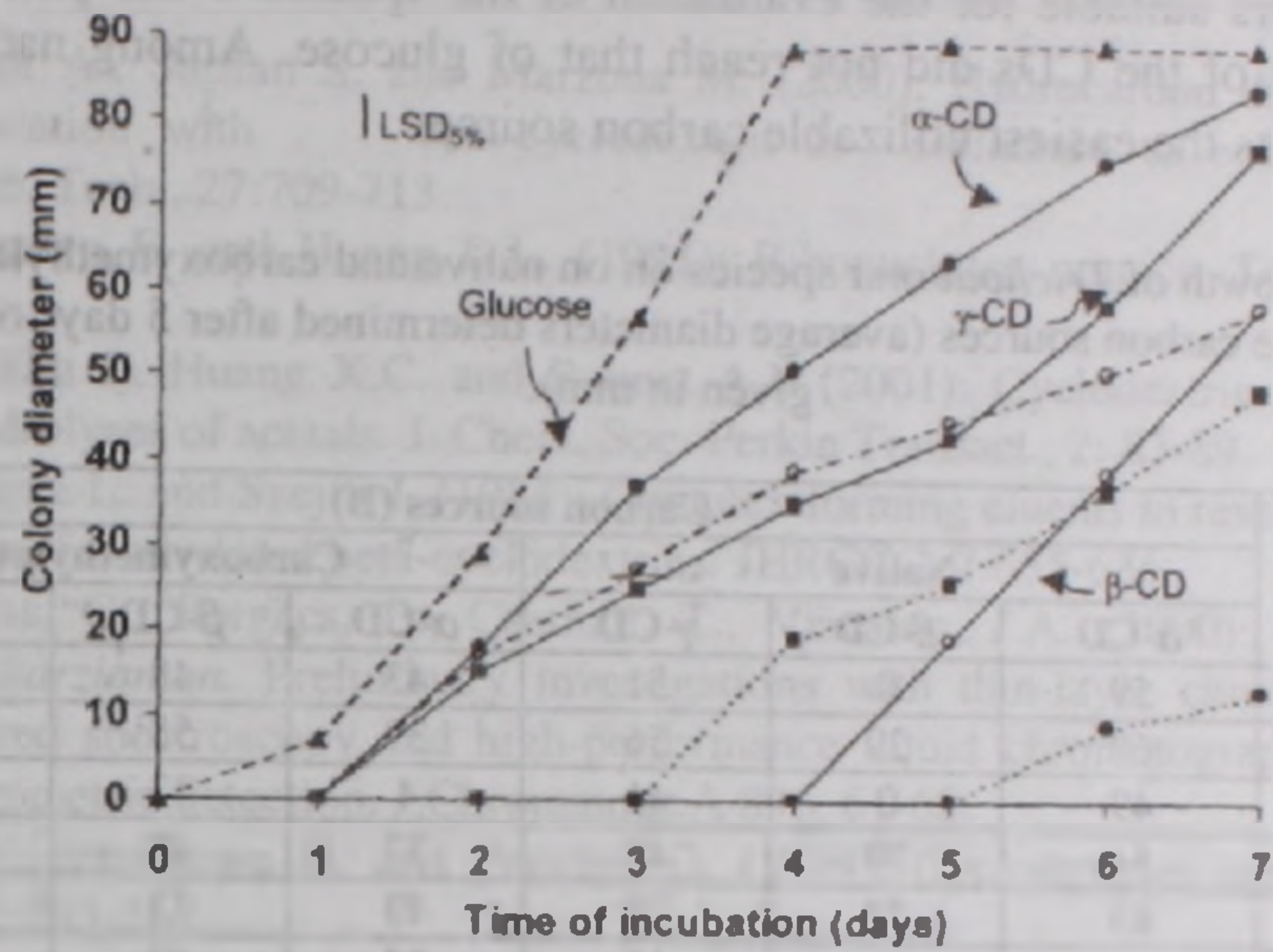

Figure 1. Dependence of the colony diameter *Trichoderma viride* on the incubation time and character of carbon source. Natural α-, β- and γ-cyclodextrins and their carboxymethylated derivatives are depicted by solid and dotted lines, respectively.

Table 3. Linear growth of *Trichoderma* species on glucose and on β-cyclodextrin derivatives as sole carbon sources (average diameters determined after 5 days of incubation are given in mm). For abbreviations see Materials and Methods.

	Carbon sources (B)					
		β-cyclodextrin derivatives				
Species (A)	**Glucose**	**Dimeb**	**Trimeb**	**HP-β-CD**	**EGP**	**BGP**
T-1	59	27	29	38	55	64
T-2	99	25	30	39	43	76
T-3	89	37	13	47	33	4
T-4	73	0	22	40	45	49
L-5	99	0	33	57	55	69
L-6	116	26	37	39	45	74
L-7	114	33	49	51	47	69
L-8	97	0	6	0	13	2
L-9	121	0	10	0	39	29
P-10	103	37	39	37	43	77
P-11	35	24	38	31	37	43
P-12	89	3	25	39	7	60
P-13	73	0	24	49	49	56
S-14	27	0	25	27	37	34
C.V.(%)	23	104	44	48	36	50
PBA (%)	100	16	25	27	22	40

C.V.(%) = variability of utilization; PBA (%) = potencial of bioavilability (related to the utilization of glucose, calculated by the spectral mapping technique).

The average colony diameters of *Trichoderma* species determined after five days of incubation in different media are compiled in Tables 3 and 4. The standard deviation of species x CD pairs was 5.1. higher than in the case of the average score values. The calculated F value characterizing the differences among the species x CD pairs was high also in this case (F_{AxB} = 160.6, significance level over 99.9%) indicating that the measurement of the colony diameters suitable for the evaluation of the species x CD pair intercations too. The nutritive value of the CDs did not reach that of glucose. Among native CDs and CD derivatives γ-CD was the easiest utilizable carbon source.

Table 4. Linear growth of *Trichoderma* species on on native and carboxymethylated α-, β- and γ-cyclodextrins as sole carbon sources (average diameters determined after 5 days of incuba- tion are given in mm).

Species (A)	Carbon sources (B)					
	Native			Carboxymethylated		
	α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD
T-1	59	0	51	43	48	19
T-2	69	29	56	39	58	18
T-3	49	0	41	4	43	21
T-4	54	39	49	37	49	9
L-5	63	22	75	49	73	37
L-6	49	0	47	33	49	45
L-7	73	49	46	47	57	8
L-8	64	0	59	20	55	43
L-9	46	45	19	3	59	4
P-10	73	49	63	56	59	49
P-11	39	56	37	14	42	14
P-12	66	50	35	20	44	14
P-13	59	45	39	33	39	30
S-14	18	72	29	57	18	64
C.V.(%)	45	55	43	29	36	0
PBA (%)	36	35	49	24	36	25

C.V.(%) = variability of utilization; PBA (%) = potencial of bioavilability (related to the utilization of glucose, calculated by the spectral mapping technique).

Significant linear relationship was found between the average score values and the colony diameters determined after 5 days of incubation the coefficient of correlation being 0.7455 indicating a significance level higher than 99%. This finding indicates that the information content of the two methods of evaluation are similar. However, the ratio of variance explained is fairly low (55.58 %) suggesting that the inclusion of both sets of data in future quantitative structure-decomposition rate studies is justified.

Conclusions

It has been established that *Trichoderma* species are potent deteriorators of native CDs and their derivatives and they may promote the elimination of this class of compounds from any organic and inorganic matrices. As the decomposition rate considerably depends on both the type of *Trichoderma* species and the chemical structure of CDs an adequate selection of species for the decomposition of a given CD is necessary.

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