CYCLOLAB

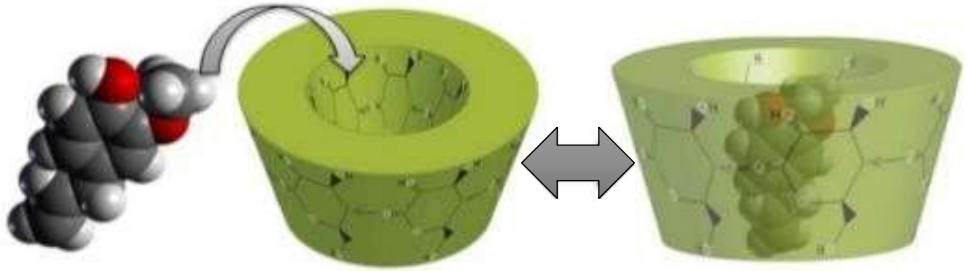
PIONEERS IN CYCLODEXTRIN-BASED INNOVATION

GETTING THE BEST OUT OF CYCLODEXTRINS

Cyclodextrins in Biotechnology

WHAT ARE CYCLODEXTRINS?

- Composed of sugars
- Cyclic molecules
- Naturally occurring compounds
- Used in food, pharmaceuticals, drug delivery, chemical industries, agriculture, etc.
- **Sub-nanometer** sized molecular containers with hydrophilic outer phase and hydrophobic interior properties
- Reversible inclusion complex formation



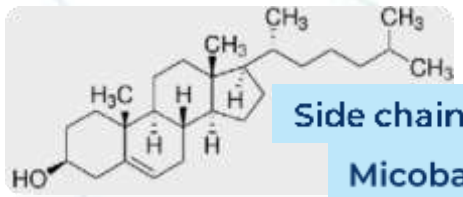
CYCLO AB

DEXOLVE™

WHY USE CYCLODEXTRINS?

- CDs enhance the solubility of complexed substrates (substitute detergents and co-solvents)
- CDs do not damage the microbial cells or the enzymes
- CDs intensify the enzymatic conversion of lipophilic substrates
- CDs improve the yield of product-inhibited fermentations
- Organic toxic compounds are tolerated by microbes in higher concentrations
- Compounds in small amounts can be isolated simply from complicated mixtures
- CD complexes can substitute mammalian serum in tissue cultures
- Unstable and/or insoluble proteins can be dissolved and stabilized in aqueous solution

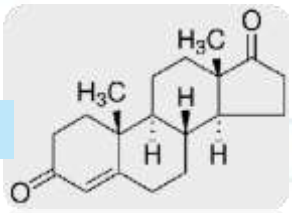
MICROBIOLOGICAL TRANSFORMATION



Cholesterol

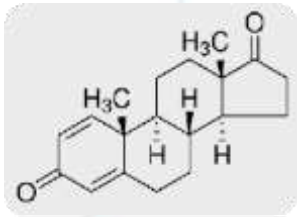
Side chains degradation

Micobacterium sp.



4-Androstene-3-17-dione

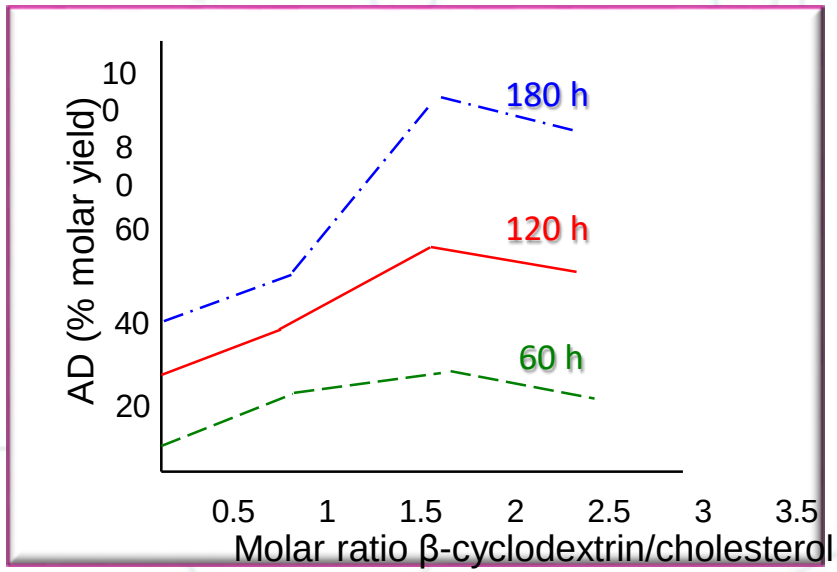
+



1,4-Androstadiene-3-17-dione

New steroid drugs

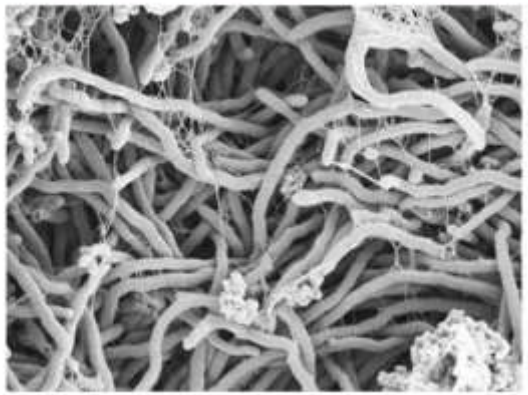
Feedback inhibition



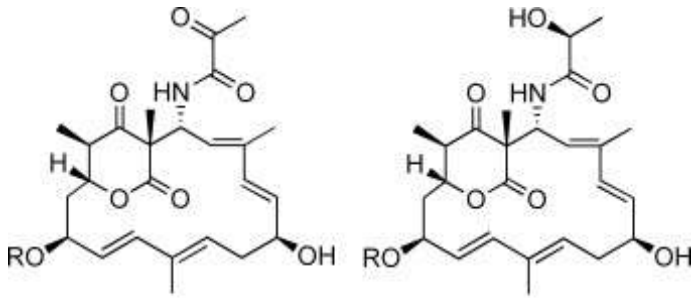
- Enhanced conversion rate
- Decreased product inhibition
- Improved product stability

Bar: Trends Biotechnol. 1989, 7, 24

BIOSYNTHESIS BY FERMENTATION



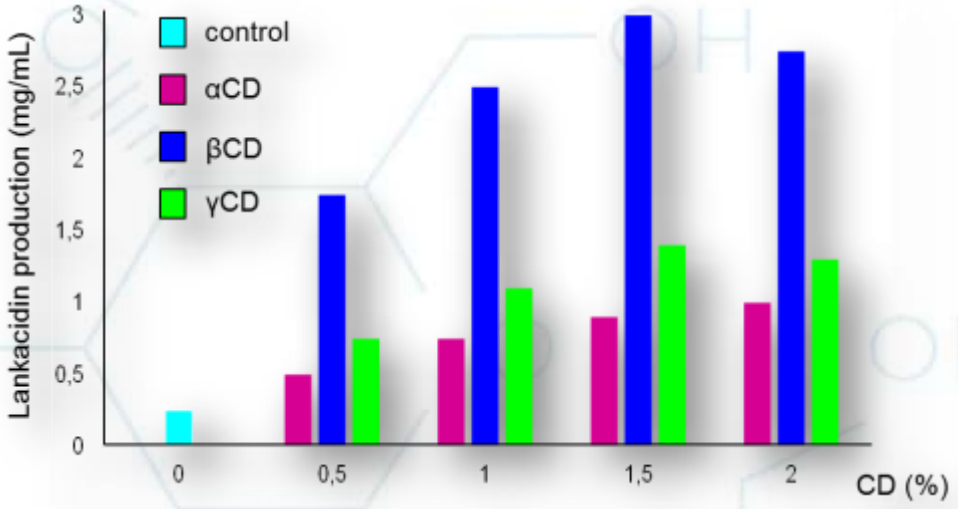
Streptomyces rochei volubilis



Lankacidin A (R=Ac)
Lankacidin C (R=H)

Lankacidinol A (R=Ac)
Lankacidinol C (R=H)

Macrolides Antitumor-Antibiotic



- Enhanced production of antibiotics
- Diminished by-products formation
- Decreased product inhibition

Sawada et al.: Appl. Microbiol. Biotechnol. 1990, 32, 556559

USE AS ABIOTIC ELICITORS

Elicitation, an Effective Strategy for the Biotechnological Production of Bioactive High-Added Value Compounds in Plant Cell Factories

Karla Ramirez-Estrada [1], Heriberto Vidal-Limon [1], Diego Hidalgo [1], Elisabeth Moyano [1], Marta Golenioswki [2], Rosa M. Cusidó [1] and Javier Palazon [1,*]

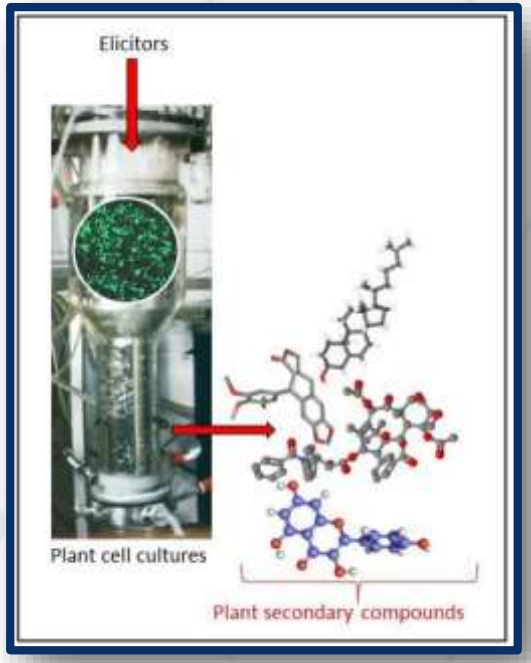
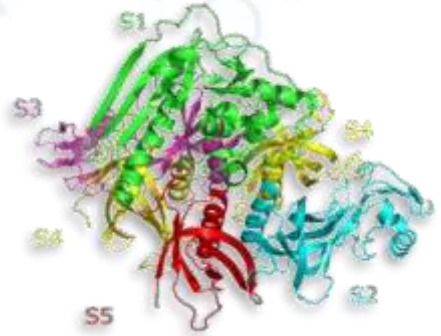


Table 2. Effect of cyclodextrins on secondary metabolite production in plant *in vitro* cultures.

Culture System	Plant Species	Secondary Metabolite (SM)	Type of SM	Reference
CS, HR	*Taxus globosa*	Taxanes	Diterpene alkaloid	[138]
	Morinda citrifolia and *Rubia tinctorum*	Anthraquinones	Phenolic compounds	[182]
	Catharanthus roseus	Vindoline, catharanthine and ajmalicine	Terpenoid indole alkaloids	[183]
	Taxus media	Taxanes	Diterpene alkaloid	[55]
	Catharanthus roseus	Ajmalicine	Monterpenoid indole alkaloid	[184]
	Vitis vinifera	Trans-resveratrol	Stilbenes	[60,185–187]
	Scutellaria lateriflora	Verbascoside, the flavones: wogonin, baicalein, scutellarein and their respective glucuronides	Phenolic compounds	[188]

CS: Cell suspensions; HR: Hairy root cultures.

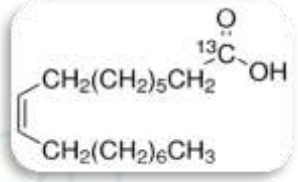
CYCLODEXTRINS AS „CATALYSTS" IN BIOTECHNOLOGY



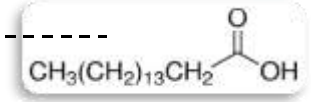
Production

Inhibition

Pertussis toxin



Oleic acid



Palmitic acid

Bordetella pertussis

Inoculum size cells in 5 µL	0	α	β	γ	DIMEB
10^3	-	-	-	-	++
10^4	-	-	-	-	+++
10^5	-	-	-	-	+++
10^6	-	++	+	+	+++
10^7	-	+++	++	++	+++
- no growth	+ < 100 colonies	++ 10^2 to 10^3 colonies	+++ full growth		

Complexation of fatty acids (growth inhibitors) results in enhanced cell growth and toxin production

DIMEB increases pertussis toxoid production 100-fold

FORMULATING VACCINES

CYCLO AB

cevac UNI L

Live freeze-dried vaccine,
Hitchner B1 strain

For the active immunization of chickens against Newcastle Disease

As an excipient , (2-hydroxypropyl)-beta-cyclodextrin is used.

ELSEVIER

Vaccine

Volume 34, Issue 27, 8 June 2016, Pages 3191-3198

Intranasal hydroxypropyl-β-cyclodextrin-adjuvanted influenza vaccine protects against sub-heterologous virus infection

Takato Kusakabe [a, b], Koji Ozasa [a], Shingo Kobari [a], Masatoshi Momota [a, b], Natsuko Kishishita [a], Kouji Kobiyama [a, b], Etsushi Kuroda [b], Ken J. Ishii [a, b]

Suvaxyn PCV™ contains inactivated recombinant Porcine Circovirus type 1, expressing the Porcine Circovirus type 2 ORF2 protein. This vaccine is used for the active immunization of pigs over the age of 3 weeks against Porcine Circovirus type 2 (PCV2).
Sulfolipo-cyclodextrin (SLCD) is used as an adjuvant.

FORMULATING VACCINES

Cyclodextrins may increase chemical integrity of vaccine components

It has been found that cyclodextrins may protect inactivated polio virus (IPV) from D-antigen titer loss caused by the presence of thiomersal.

Studied on a limited panel of cyclodextrins, β-cyclodextrin, γ-cyclodextrin and 2-hydroxypropyl-γ-cyclodextrin provided protection of IPV from the detrimental effects of the used preservative.

PCT patent application WO2016012385 – Vaccine composition comprising IPV and cyclodextrins (Sanofi Pasteur)

FORMULATING VACCINES

Hydroxypropyl betadex is component in J&J COVID-19 vaccine

Janssen incorporated HPBCD into their monovalent Covid-19 vaccine composed of a recombinant, replication-incompetent adenovirus type 26 (Ad26) vector, constructed to encode the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spike protein.

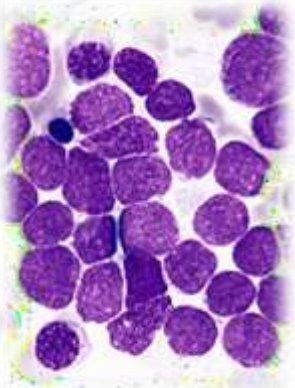
In the patent application of Janssen's HIV vaccine candidate (WO2017216288) HPBCD is appiled in 4-6% (w/w) in the product.

Possible reason: HPBCD acts as a effective protein stabilizer hindering aggregation and adsorption onto the container wall. Another possibility is cryopreservation according to a recent review article.

SERUM-FREE CULTURE MEDIA

Mycobacterium leprae

Water-soluble lipid/CD complexes



Lymphoblast cells

Szente et al.; J. Incl. Phenom. Mol. Recogn. Chem. 1993, 16, 339-354
Rajnavölgyi et al. Beilstein J. Org. Chem. 2014, 10, 3152–3160

Cultivation of non-cultivable Mycobacterium leprae

Serum substitutes for lymphoblast cells

Non-cholesterol interacting

fatty acid/CD complexes

Solubilization of lipids (fatty acids, cholesterol, phospholipids)

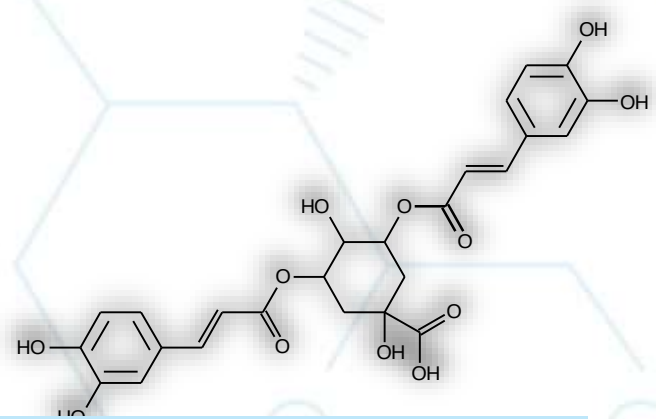
No threat of prion proteins

CYCLODEXTRIN IN ARTIFICIAL FERTILIZATION

- Improvement of the quality of semen by cholesterol supplementation with cholesterol loaded methyl BCD (cryopreservation)
- Enhancement of capacitation and fertility rate by preincubation of thawed sperms with methyl BCD

DEXOLVE™

REDUCING ENZYMATIC BROWNING OF FRUIT JUICES



chlorogenic acid
(and other polyphenols)

Polyphenol oxidase

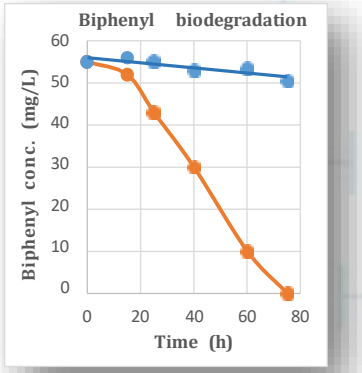
Browning

Complete inhibition of browning by soluble CDs

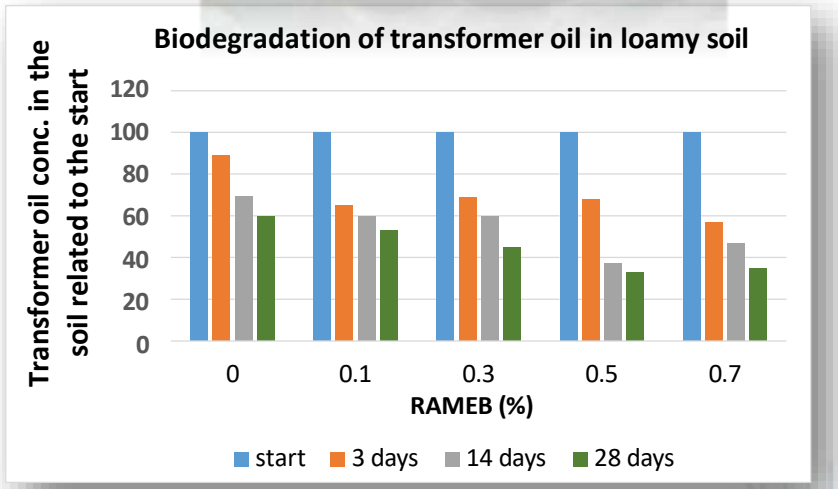
aCD > maltosyl bCD > bCD

Hicks et al.: J. Agric. Food Chem. 1996, 44, 2098-2101
Lopez-Nicholas et al.: J. Agric. Food Chem. 2007, 55, 5312-5319

CD-ASSISTED PURIFICATION OF WASTE WATER AND SOIL



- Improved solubility/ bioavailability of the pollutants
- Improved cell growth
- Protection of the biological sludge in the waste water plants
- Enhanced microbial decomposition of contaminants



Yoshii et al. Biol. J. Armenia, 2001, 18, 226-236
Fenyvesi et al. Land Contam. Reclam. 2009, 17, 585-597

WHO ARE WE AT CYCLOLAB?

The world's only all-round CYCLODEXTRIN company with experience in CD-technology since 1991

in pharmaceutical-, cosmetics-, food-, environmental- and analytical applications

Experience

Over 540 technical/scientific papers and 950 technical reports to customers

200 different cyclodextrin derivatives

130 patents/applications

40 products on the market

Drug Master Files (USA type IV) and eCTD

Over 20,000 citations to CYCLOLAB's papers

Expertise & Technology

Custom synthesis

Drug solubilization and stabilization

Further industrial applications

Cyclodextrin-related analytics

Stability testing

GMP-conform manufacturing

Feasibility studies

DEXOLVE™

CYCLOLAB SERVICE PORTFOLIO
RELATED SERVICES – R&D

Early phase drug development

Customization of CD enabled formulations

Investigation of changes in physico-chemical properties

Life cycle management

Custom cyclodextrin synthesis

Exclusive manufacture, unique synthetic routes

Self-tailored products and characteristics

In vitro bioequivalence studies

Design and performance of in vitro studies to support bioequivalence of a CD enabled formulation

Analytical services

Method development, validation; cGMP release testing of pharma grade CDs

HPLC, GC, CE, UV, MS, NMR, IR, Micro and BET content methods

Stability studies

CD-guest interaction studies

CD-based chiral separations

Assay, impurity tests

Bioanalytical investigations

IP services and consultation

30 years of experience in compilation of CD related patents (synthesis, application, etc.), patent claim analysis, consultancy in CD related projects

Over 62.000 CD related papers

DEXOLVE™

CYCLOLAB SERVICE PORTFOLIO
RELATED SERVICES – R&D

Feasibility study

Running a short feasibility study with your molecule free of charge

Proof of concept to consider CD based formulations

CycloLab Grant

CycloLab offers a unique possibility to collaborate on creating novel and interesting cyclodextrins under the terms of the CycloLab Grant

The proposal after application is thoroughly evaluated by CycloLab

If the application is approved, the cyclodextrin is provided free of charge for the beneficiary

DEXOLVE™

COMPANY CONTACTS

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DEXOLVE™