

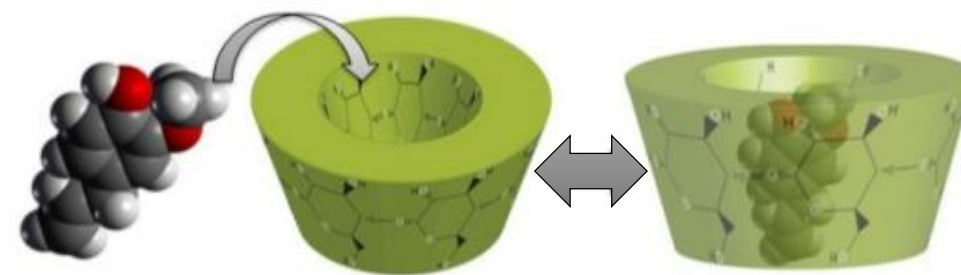
GETTING THE BEST OUT OF CYCLODEXTRINS

**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

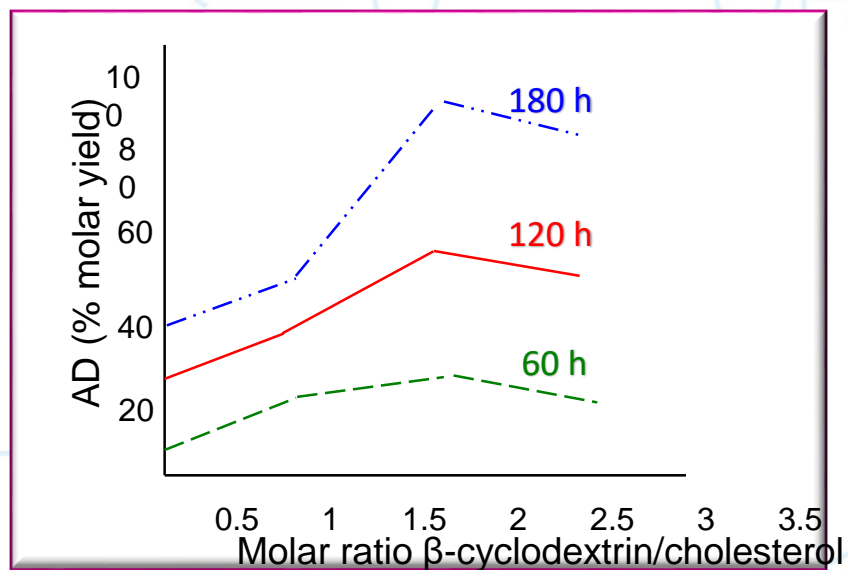
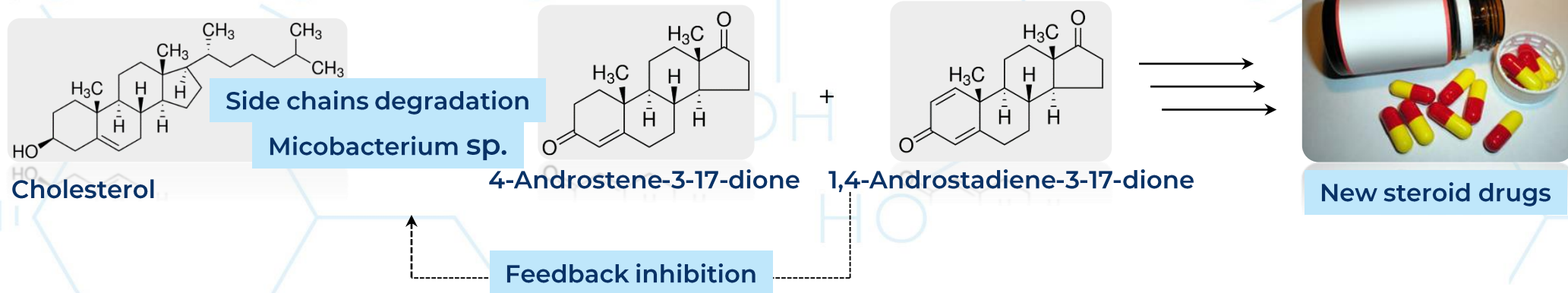


WHY USE CYCLODEXTRINS?

- 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

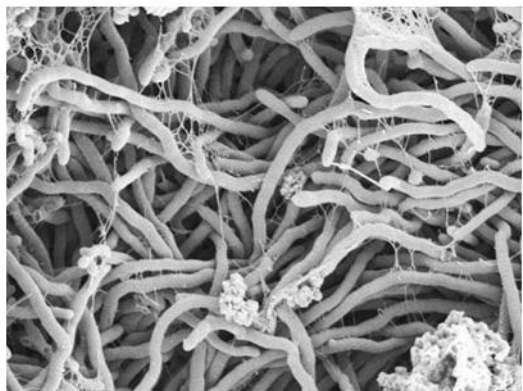


MICROBIOLOGICAL TRANSFORMATION

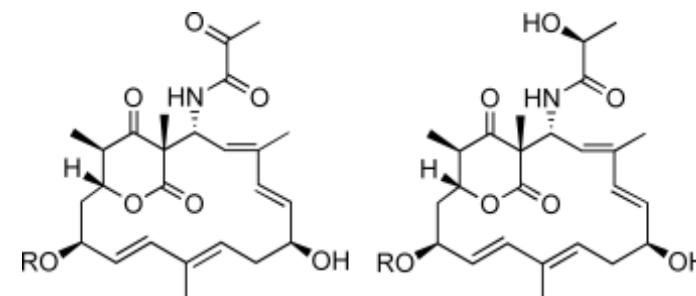


- Enhanced conversion rate
- Decreased product inhibition
- Improved product stability

BIOSYNTHESIS BY FERMENTATION



Streptomyces rochei volubilis

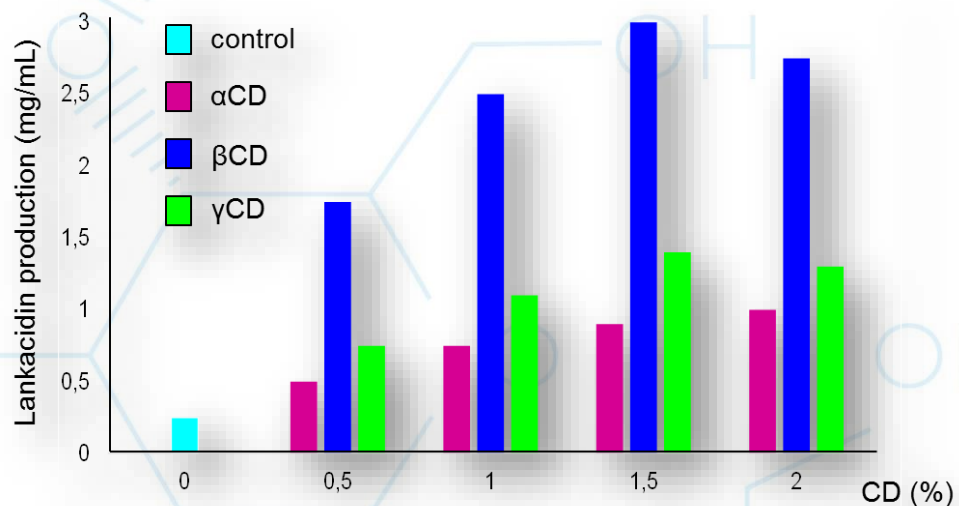


Lankacidin A (R=Ac)
Lankacidin C (R=H)

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

Macrolides Antitumor-Antibiotic

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



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

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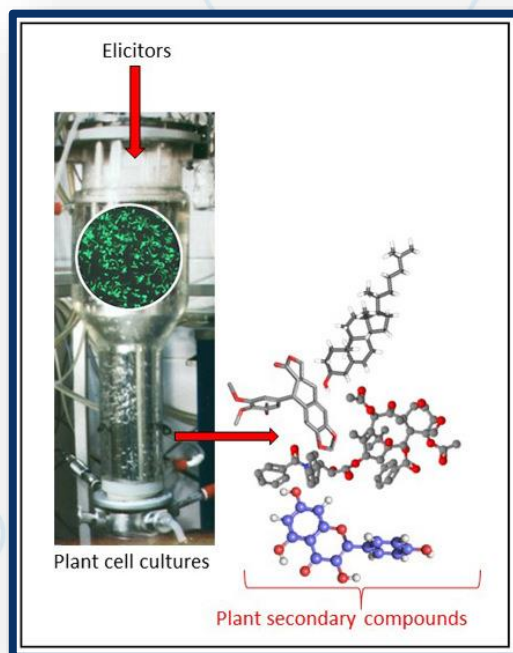
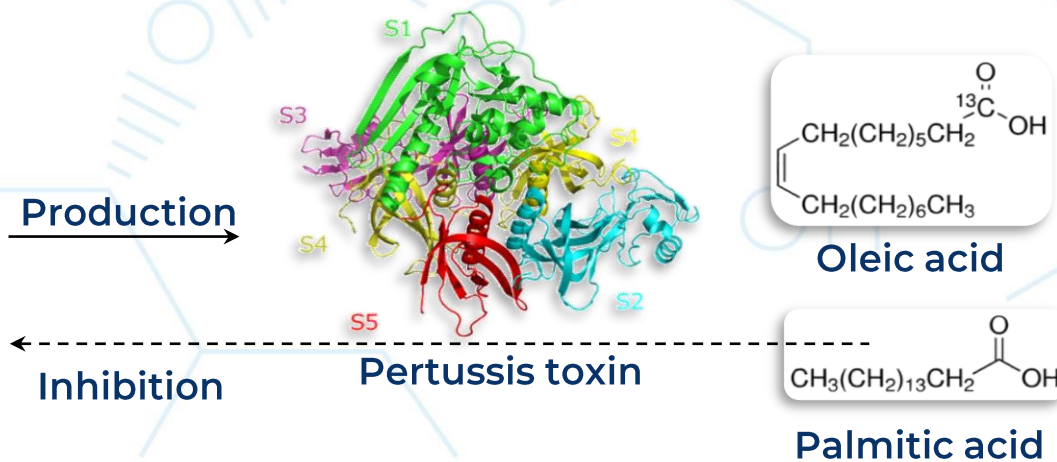


Table 2. Effect of cyclodextrins on secondary metabolite production in plant *in vitro* cultures.

Culture System	Plant Species	Secondary Metabolite (SM)	Type of SM	Reference
CS, HR	<i>Taxus globosa</i>	Taxanes	Diterpene alkaloid	[138]
	<i>Morinda citrifolia</i> and <i>Rubia tinctorum</i>	Anthraquinones	Phenolic compounds	[182]
	<i>Catharanthus roseus</i>	Vindoline, catharanthine and ajmalicine	Terpenoid indole alkaloids	[183]
	<i>Taxus media</i>	Taxanes	Diterpene alkaloid	[88]
	<i>Catharanthus roseus</i>	Ajmalicine	Monoterpenoid indole alkaloid	[184]
	<i>Vitis vinifera</i>	Trans-resveratrol	Stilbenes	[60,185-187]
	<i>Scutellaria latiflora</i>	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



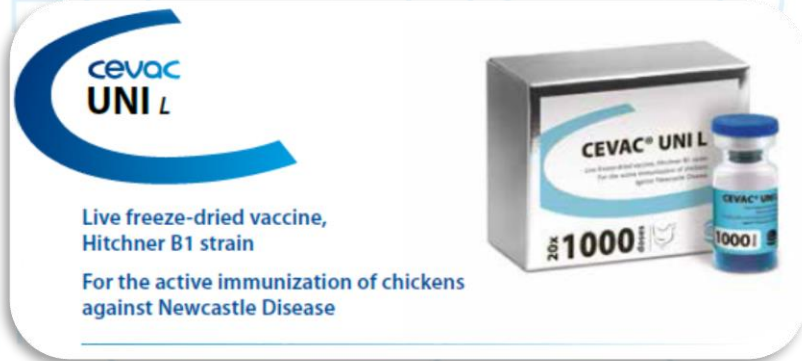
Bordetella pertussis

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



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



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.



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 applied 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.

Johnson & Johnson



Johnson & Johnson



SERUM-FREE CULTURE MEDIA



Mycobacterium leprae

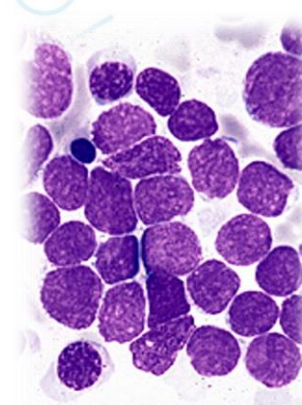
Water-soluble lipid/CD complexes

Cultivation of non-cultivable *Mycobacterium leprae*

Serum substitutes for lymphoblast cells

Non-cholesterol interacting

fatty acid/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

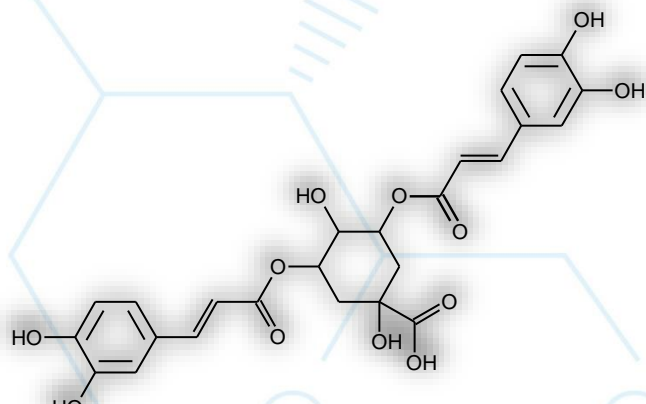
Solubilization of lipids (fatty
acids, cholesterol, phospholipids)
No threat of prion proteins

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



REDUCING ENZYMATIC BROWNING OF FRUIT JUICES



chlorogenic acid
(and other polyphenols)

Polyphenol oxidase



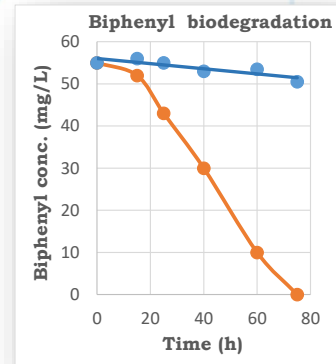
Browning

Complete inhibition of
browning by soluble CDs

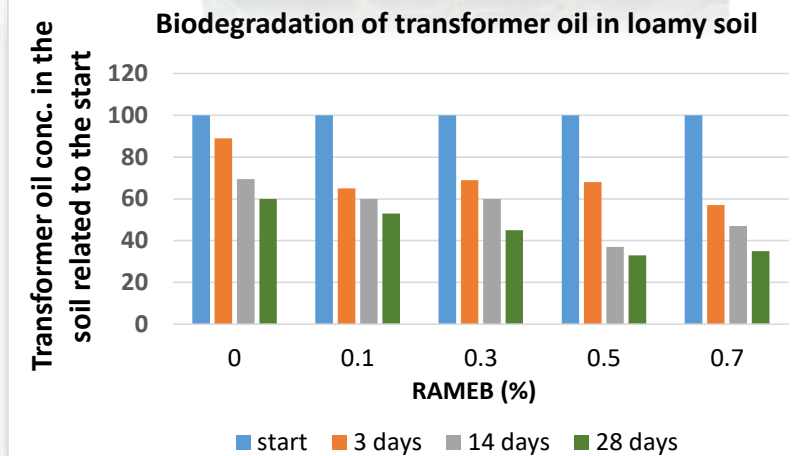
aCD > maltosyl bCD > bCD

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



WHO ARE WE AT CYCLOLAB?



The world's only all-round **CYCLODEXTRIN** company with over

40-year experience of CD-technology

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

Experience

Over 490 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



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

IP services and consultation

Custom cyclodextrin synthesis

Exclusive manufacture, unique synthetic routes

Self-tailored products and characteristics

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

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



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



CDs in BIOTECHNOLOGY

COMPANY CONTACTS

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