CARBOHYDE

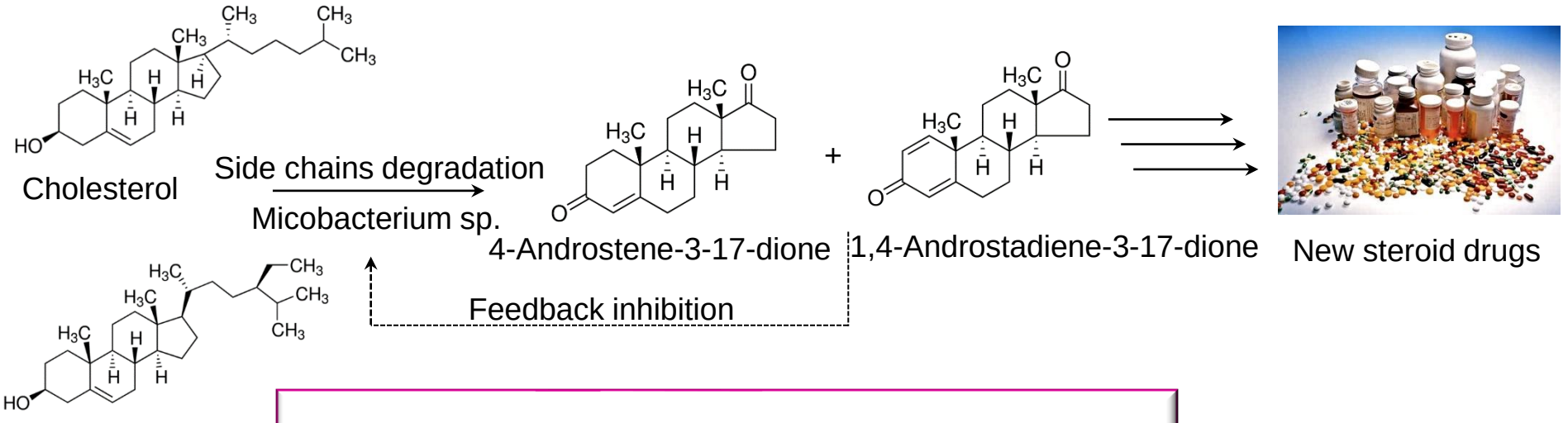
SUGAR IS LIFE

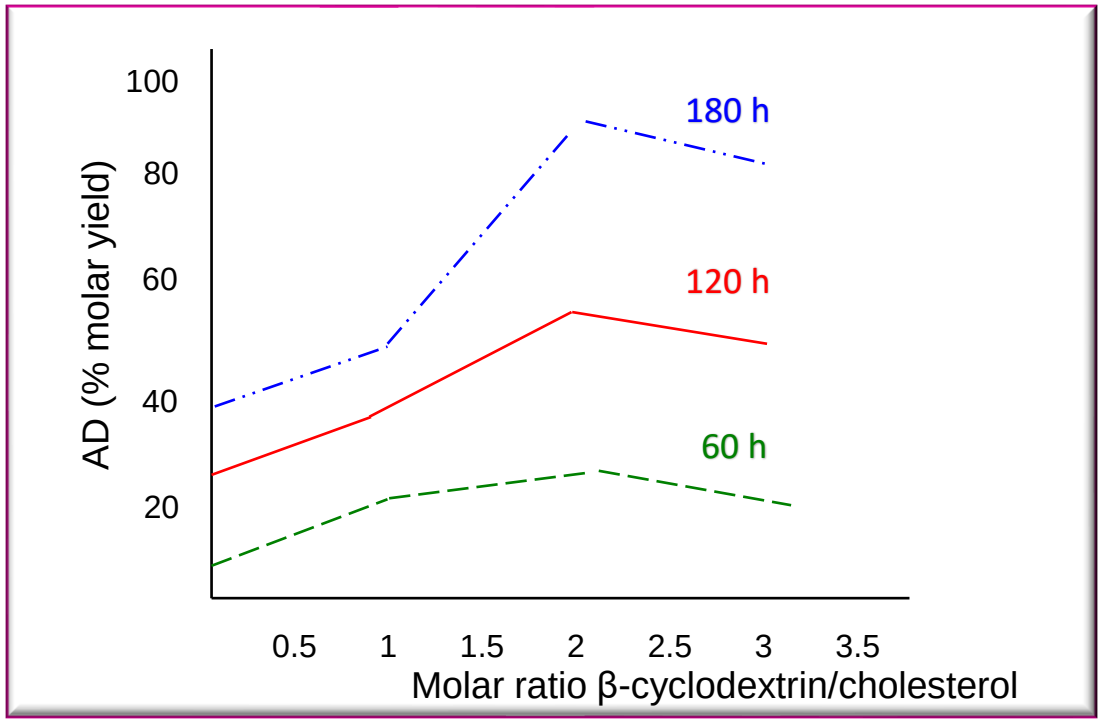
Cyclodextrins in Biotechnology

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
- CDs **increase tolerance** to organic **toxic compounds for** microbes
- **Facilitate the isolation** of compounds in small amounts 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 Substrate Conversion

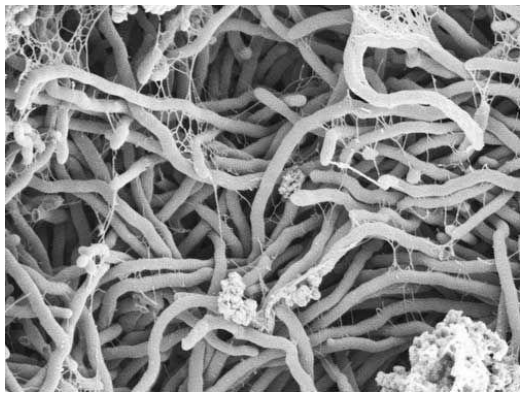




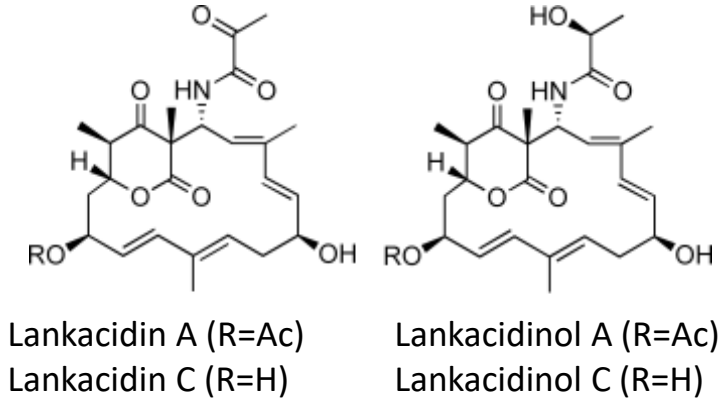
- Enhanced conversion rate
- Decreased product inhibition
- Improved product stability

Hesselink, P. G. M. et. al., (1987). In Proceedings of the 4th European Congress on Biotechnology, Vol. 2, ed. O. M. Neijssel, R. R. van der Meer & K. C. A. M. Luyben. Elsevier, Amsterdam, p. 299.

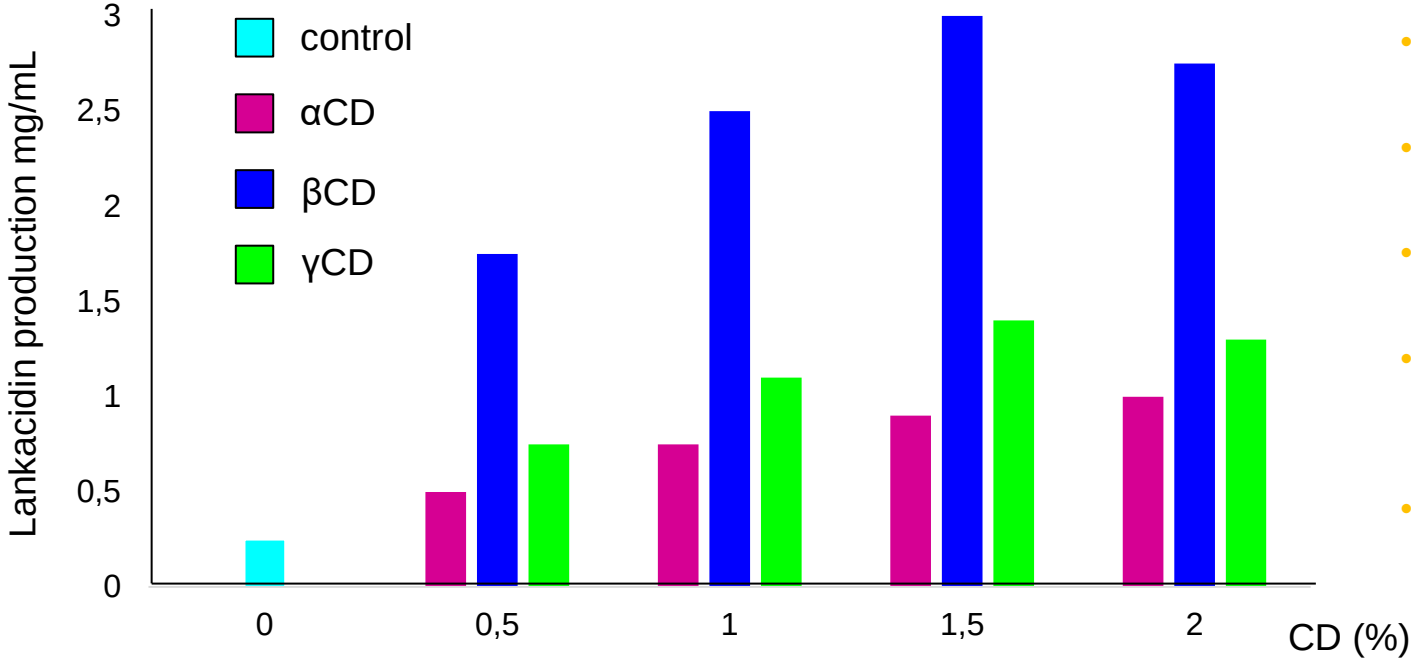
Biosynthesis by Fermentation



Streptomyces rochei volubilis



Macrolides Antitumor-Antibiotic



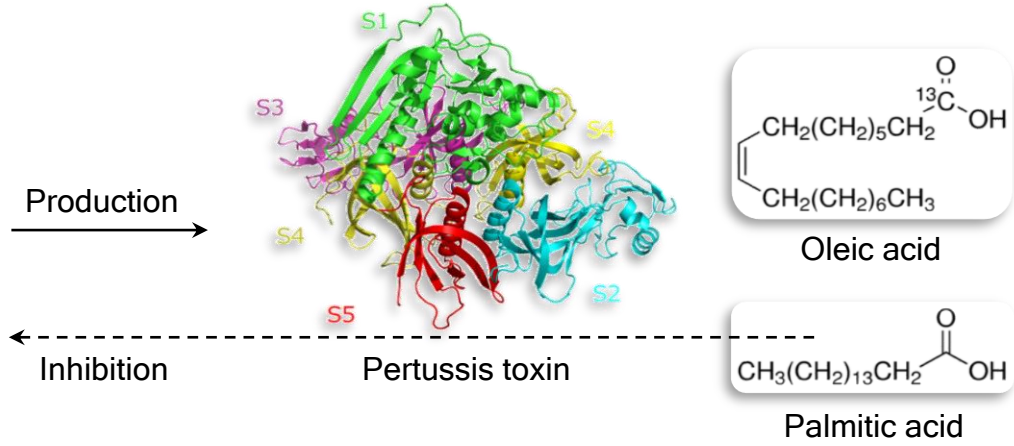
- The production of lankacidin A and C increased from 0.05 and 0.04 mM to 0.55 and 4.6 mM, respectively.
- No BCD: only 0.4 mg/ml of lankacidin C accumulated, then amount decreased slowly during fermentation.
- With BCD: lankacidin C production continued throughout fermentation and reached 3.1 mg/ml.
- BCD had no effect on microbial growth, consumption rate of carbon source and pH changes throughout fermentation. It was not consumed by the microorganism.
- The lankacidins produced existed as inclusion complexes in the culture filtrate.

Sawada, H. et.al., (1987). Appl. Microbiol. Biotechnol., 26,522.

Key-component in Pertussis Toxin production

Background

- It is commercially used in pertussis vaccine production
- Role:
  - Selective encapsulation of inhibiting by-products
  - Masking the presence of the inhibitor
  - Superior performance compared to random methyl-BCD



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

DIMEB increases pertussin toxin production 100-fold!

MoA: Complexation of fatty acids (growth inhibitors) results in enhanced (100x) cell growth and toxoid production.

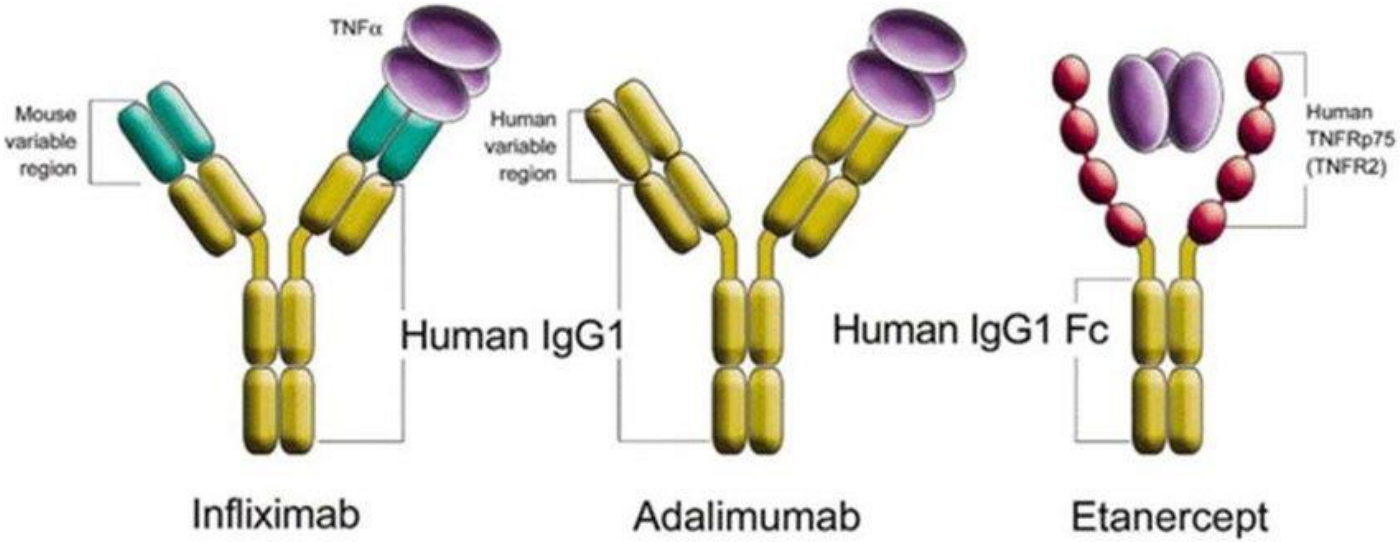
Daniela Hozbor, Maria E. Rodriguez and Osvaldo Yantorno
FEMS Immunology and Medical Microbiology 9 (1994) 117-124

Stabilizer for Monoclonal Antibodies

Open Access Article

Polysorbates versus Hydroxypropyl Beta-Cyclodextrin (HPβCD): Comparative Study on Excipient Stability and Stabilization Benefits on Monoclonal Antibodies

by Hailong Zhang [1,*], Shiqi Hong [1], Sarah Si Kai Tan [1], Tao Peng [1], Lucas Yuan Hao Goh [1], Kwan Hang Lam [1], Keat Theng Chow [1] and Rajeev Gokhale [2,*]



Physicochemical stability excipient

HPβCDs: stable under heat, autoclavation, light and oxidative stress. Chemical structure unchanged.

Polysorbates (PS): degrade under heat-stress and autoclavation severely decompose upon light irradiation and significantly hydrolyse and oxidize.

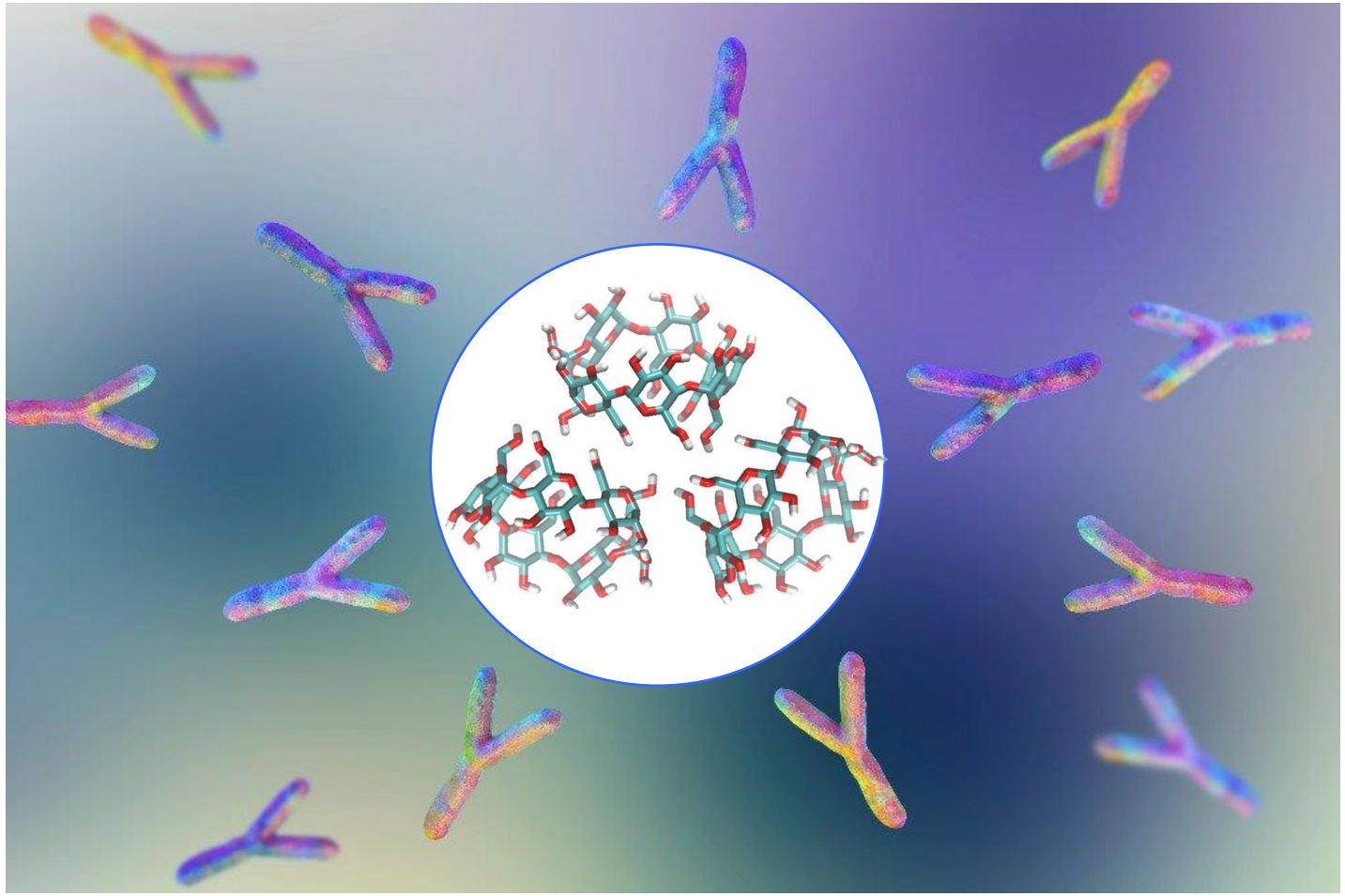
Physicochemical stability of monoclonal antibodies

HPβCD formulations: decrease in protein aggregation, superior monomer and total protein recovery compared to PS-containing formulations.

HPβCD formulations: reduce both agitation and thermal stress-induced protein aggregation and prevents subvisible particle formation compared to PS.

Molecules 2022, 27(19), 6497

Monoclonal Antibody for Detection of CDs

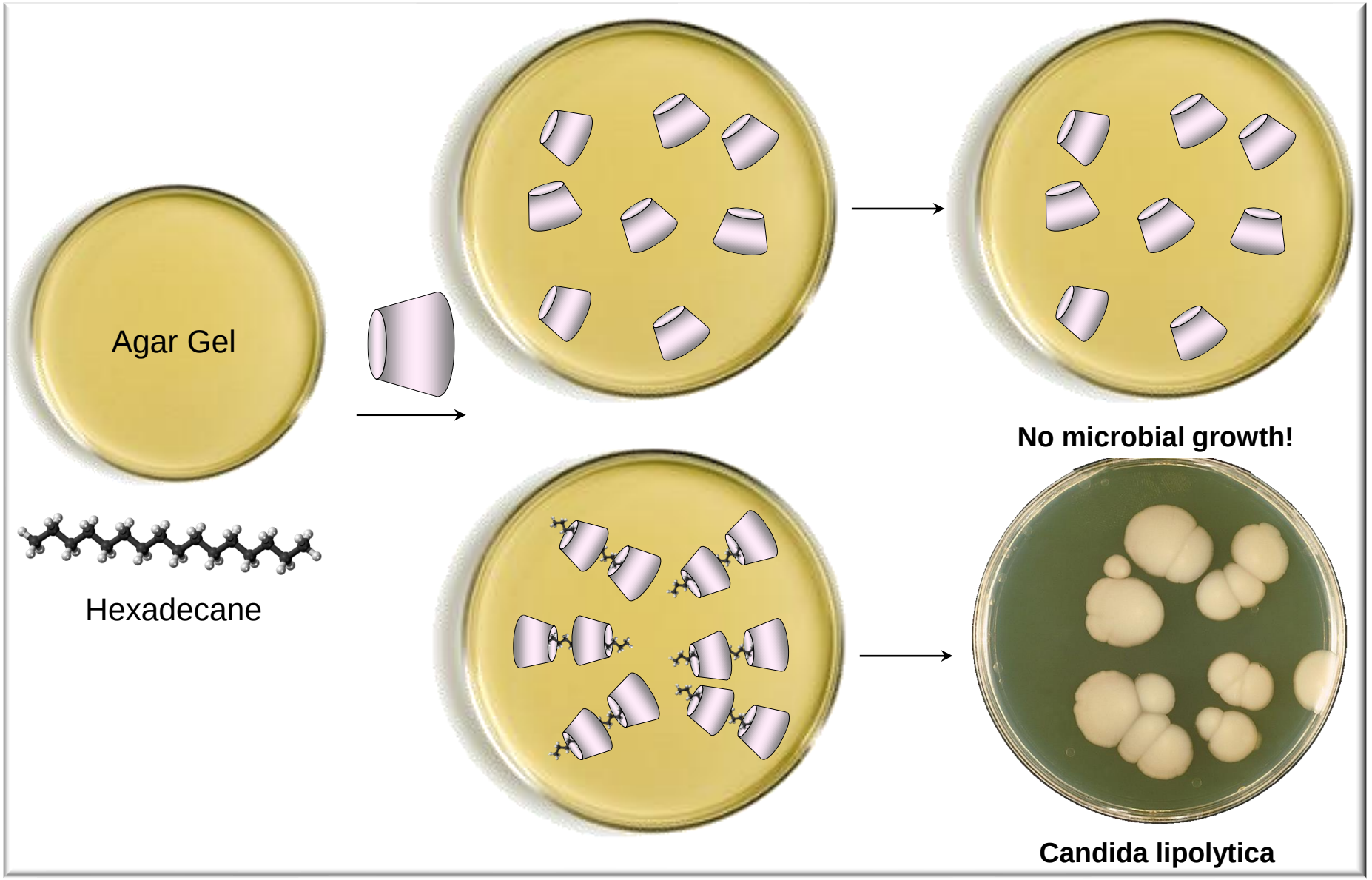


- The monoclonal antibody to BCD was generated by using a conjugate of glucosaminylmaltosyl-BCD and bovine serum albumin as an antigen.
- The monoclonal antibody was IgM/κ and reacted with β-CD with high specificity.
- The epitope recognized seemed to be located on the secondary side of the β-CD

- The immunoassay was useful to determine BCD in biological fluids such as human plasma and urine.

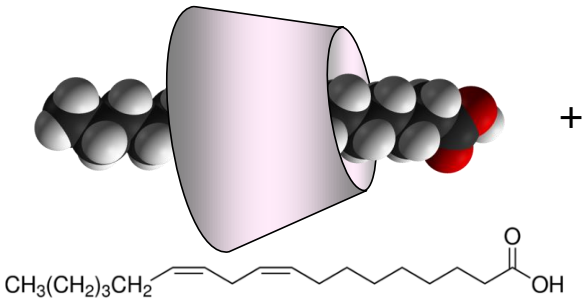
Koji Ikura et. Al., Cytotechnology 40: 23–29, 2002

Microbiological Cultivation



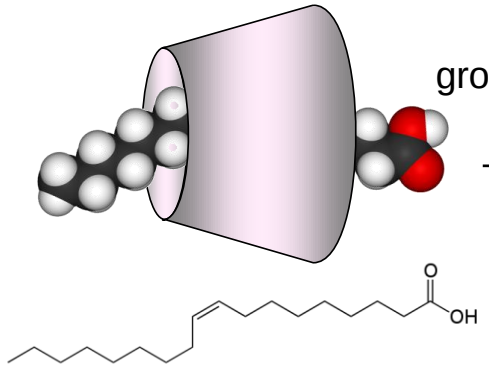
R. Bar, Appl Microbiol Biotechnol (1990) 32:470-472

Tissue Cultures



Linoleic acid/βCD complex

+



Oleic acid/βCD complex

growth-enhancing effect (10 x)

Human lymphoblast cell

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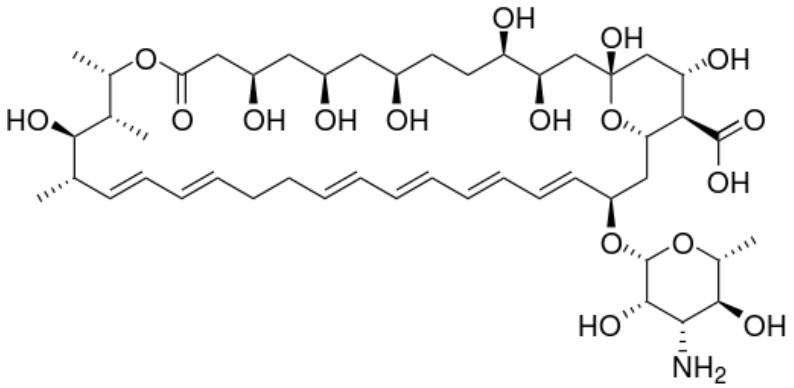
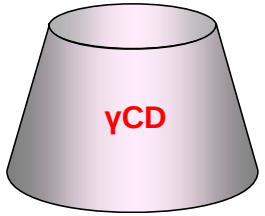
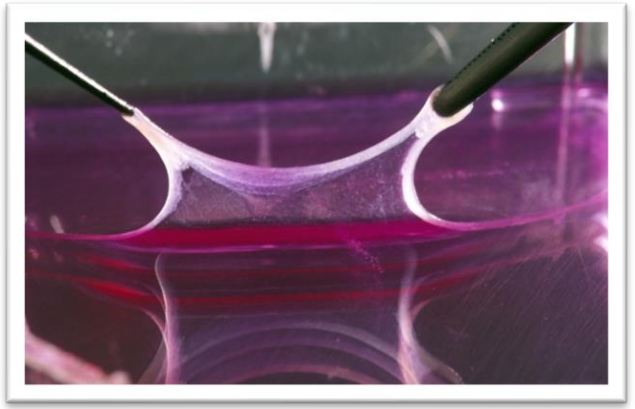


Nystatin-polyene antifungal drug
(insoluble and easely oxidized in water)

+





Mammalian tissue

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Ajinomoto Co. (1982), Jpn. Kokai, 82, 194, 787, (C.A. 98:124208)

Szejtli, J. et. Al, (1983). Hung. Teljes (Patent no.) HU 35, 172 (C.A. 103:200887)

Cryoprotection

- Sperm membrane cholesterol influences cryodamage during cryopreservation.
- Evaluation of the effect of varying cholesterol levels in on the freezeability of semen.

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

Sunita Behera et al., Vet World. 2015 Dec; 8(12): 1386–1391

Cryoprotection

J&J ad26.cov2.s: cyclodextrin as cryoprotectant

- The era of vaccines (from 2021) - changes in research focus
- EUA (FDA and EMA) in March 2021
- Janssen incorporated HPBCD into their Covid-19 vaccine
- HPBCD is used as a cryoprotectant
- MoA is unclear

CA3001050A1- Methods for Preventing Surface-Induced Degradation of Viruses Using Cyclodextrins
Braga, S.S. 2019 Biomolecules 2019 | https://doi.org/10.1016/j.cmi.2021.05.028; Braga, S.S. et al 2021 Pharmaceutics | https://doi.org/10.3390/pharmaceutics13030409