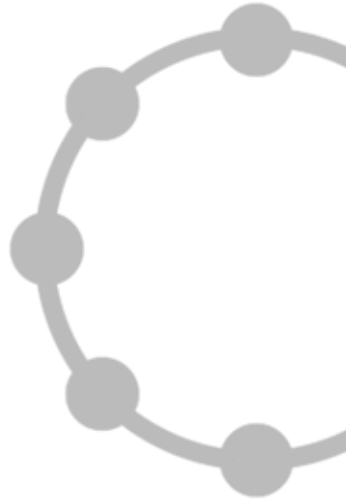




CARBOHYDE
REVOLUTION OF
CYCLODEXTRINS

Cyclodextrins in Biotechnology

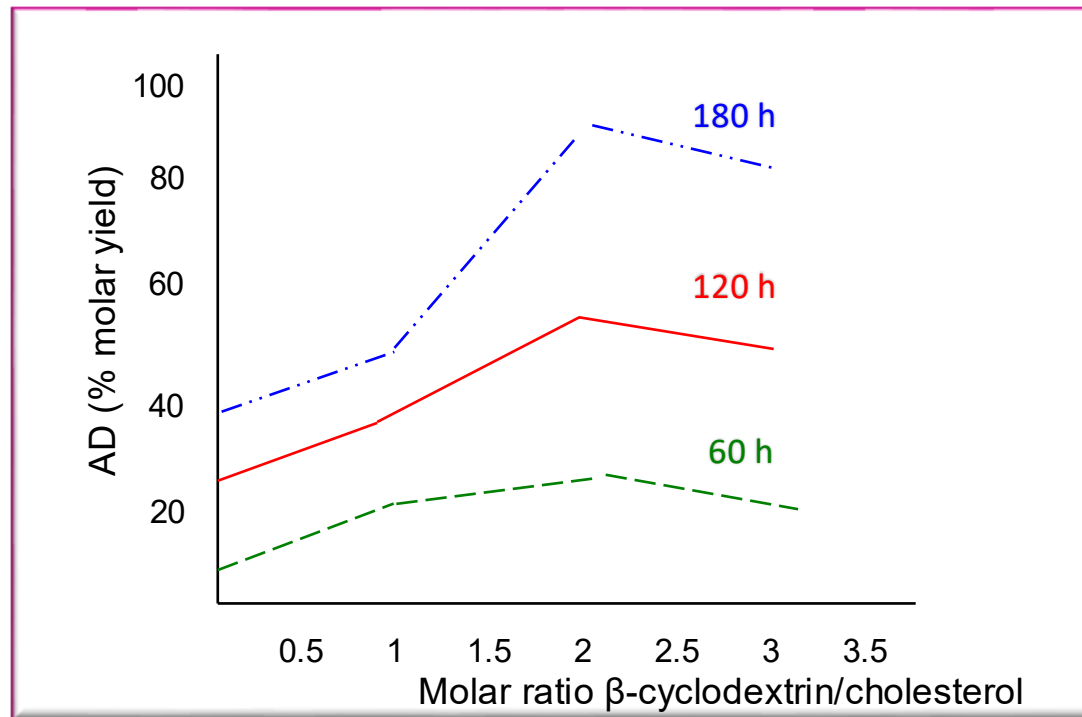
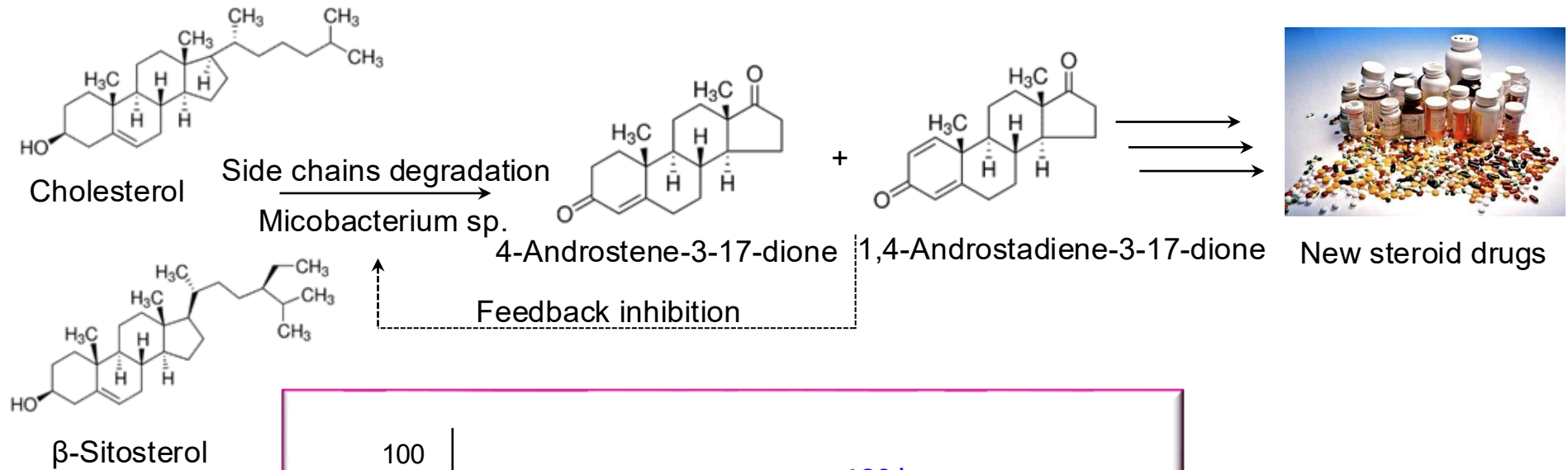




Why Use Cyclodextrins?

- 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

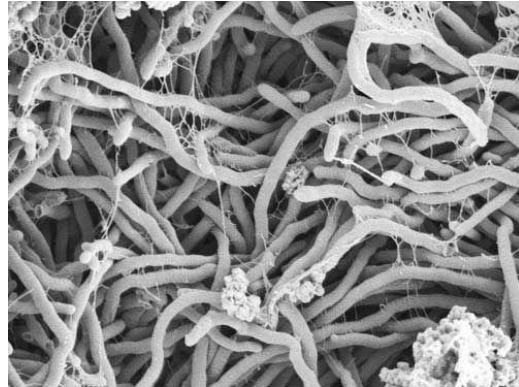


- Enhanced conversion rate
- Decreased product inhibition
- Improved product stability

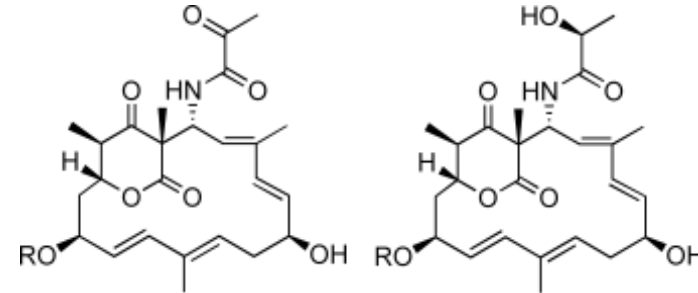
Hesselink, P. G. M. et. al., (1987). In *Proceedings of the 4th European Congress on Biotechnology*, Vol. 2, ed. O. M. Neijssel, R. R. van der Meer & K. C. A. M. Luyben. Elsevier, Amsterdam, p. 299.



Biosynthesis by Fermentation



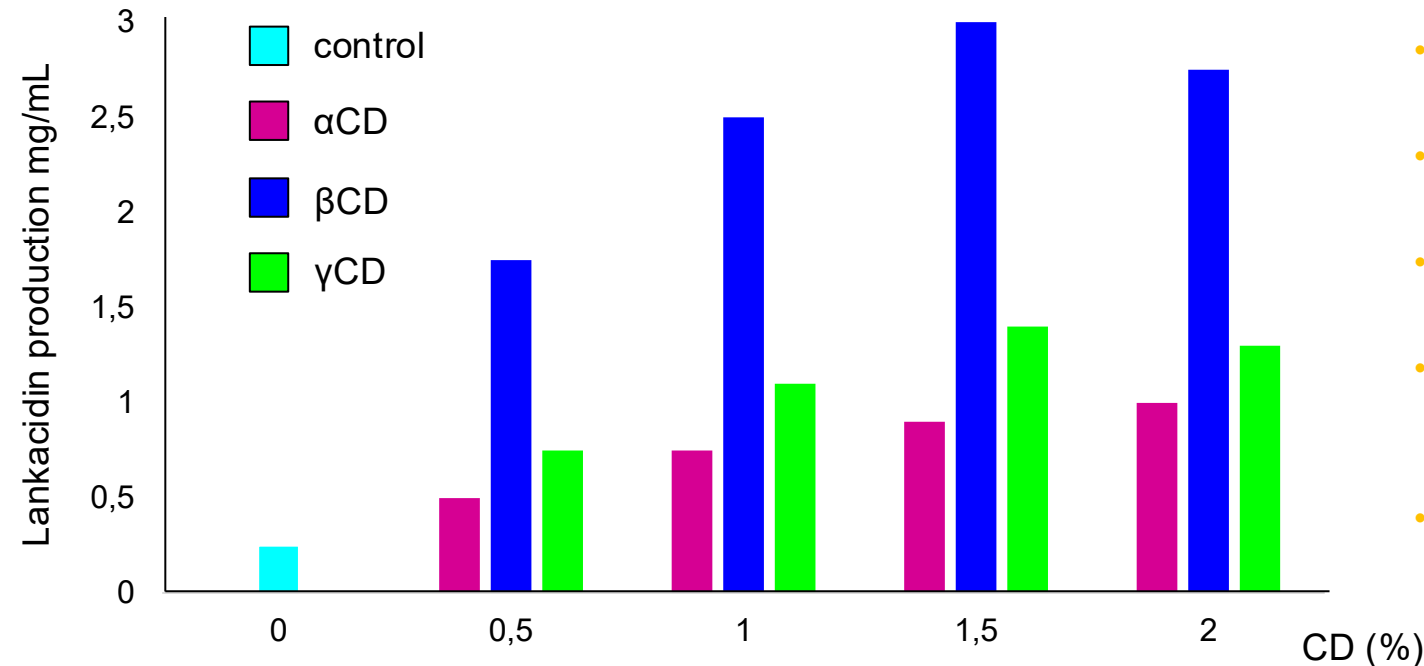
Streptomyces rochei volubilis



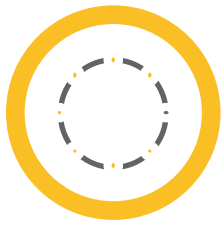
Lankacidin A (R=Ac)
Lankacidin C (R=H)

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

Macrolides Antitumor-Antibiotic



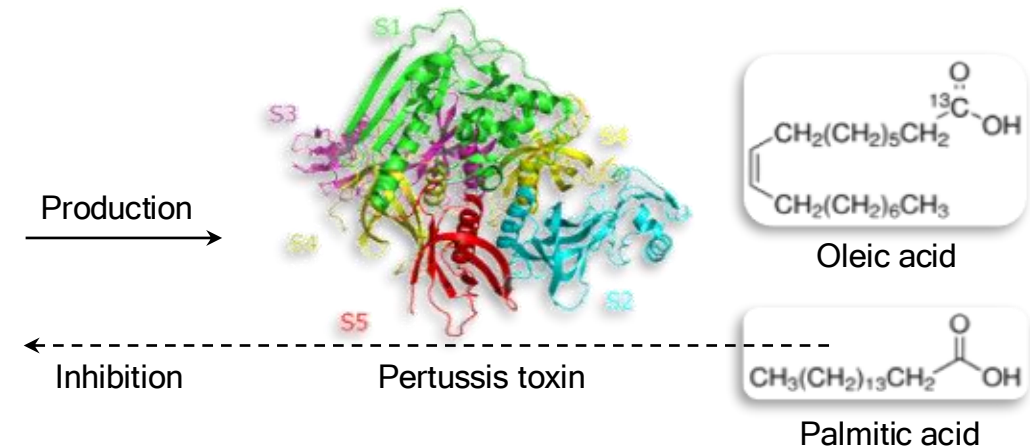
- The production of lankacidin A and C increased from 0.05 and 0.04 mM to 0.55 and 4.6 mM, respectively.
- No BCD: only 0.4 mg/ml of lankacidin C accumulated, then amount decreased slowly during fermentation.
- With BCD: lankacidin C production continued throughout fermentation and reached 3.1 mg/ml.
- 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.



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



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

Inoculum size cells in 5 μ L	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!



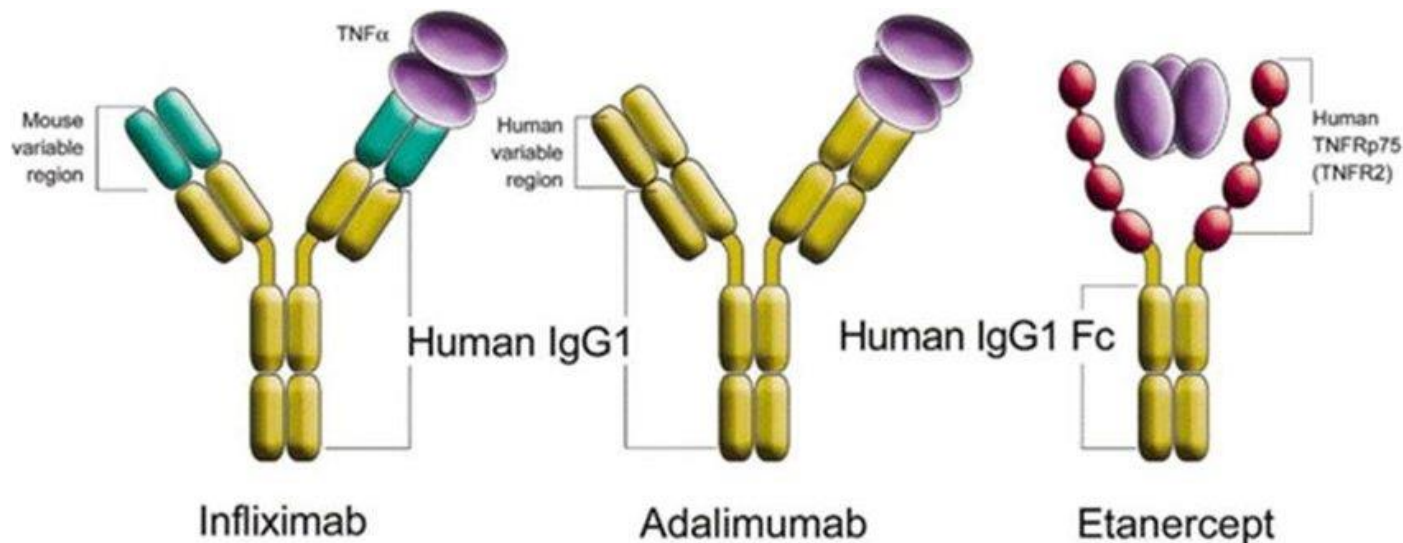
Stabilizer for Monoclonal Antibodies

Open Access

Article

Polysorbates versus Hydroxypropyl Beta-Cyclodextrin (HP β CD): 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

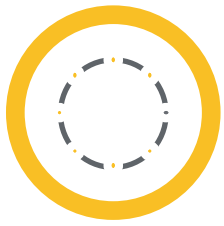
HP β CDs: stable under heat, autoclavation, light and oxidative stress. Chemical structure unchanged.

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

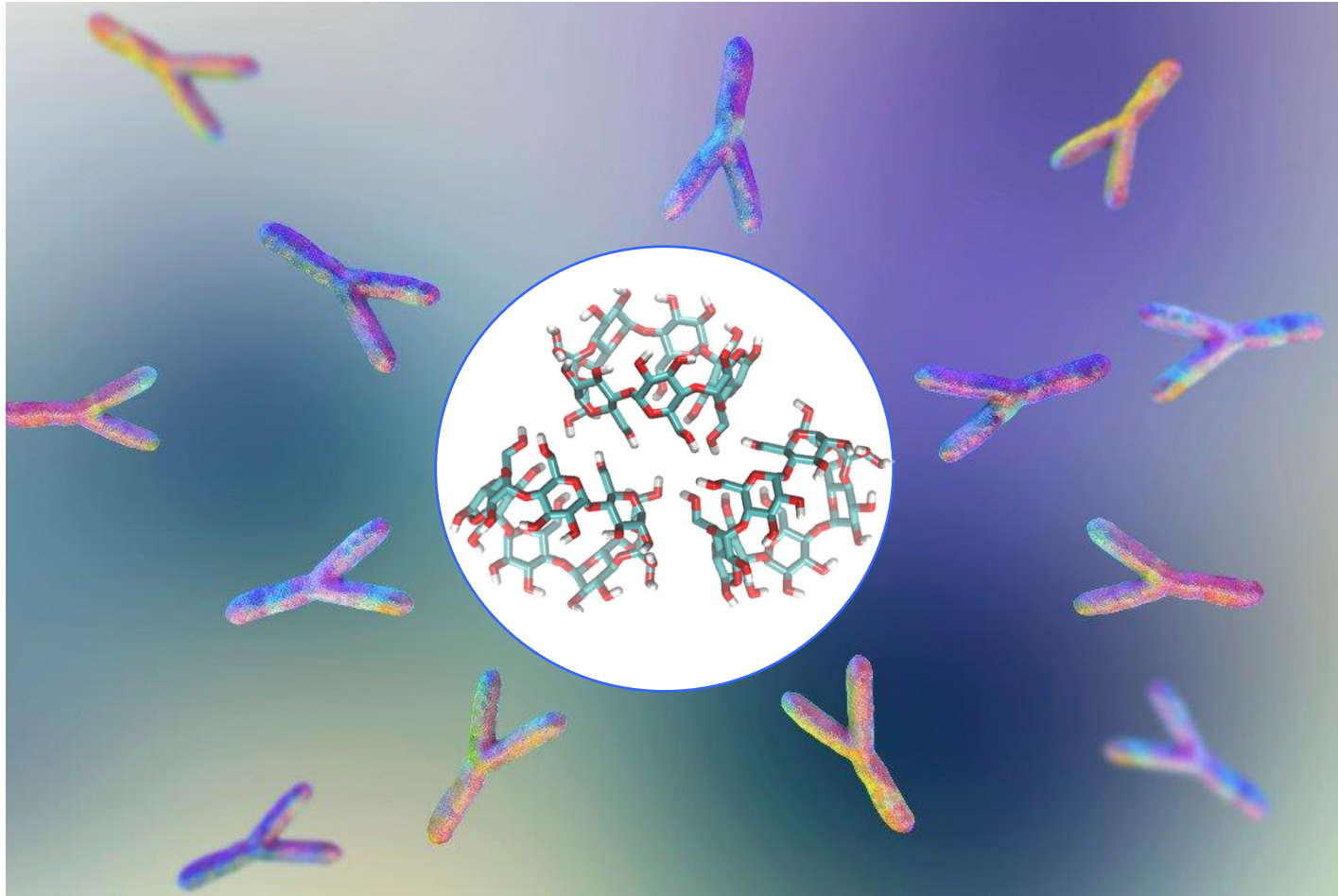
Physicochemical stability of monoclonal antibodies

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

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



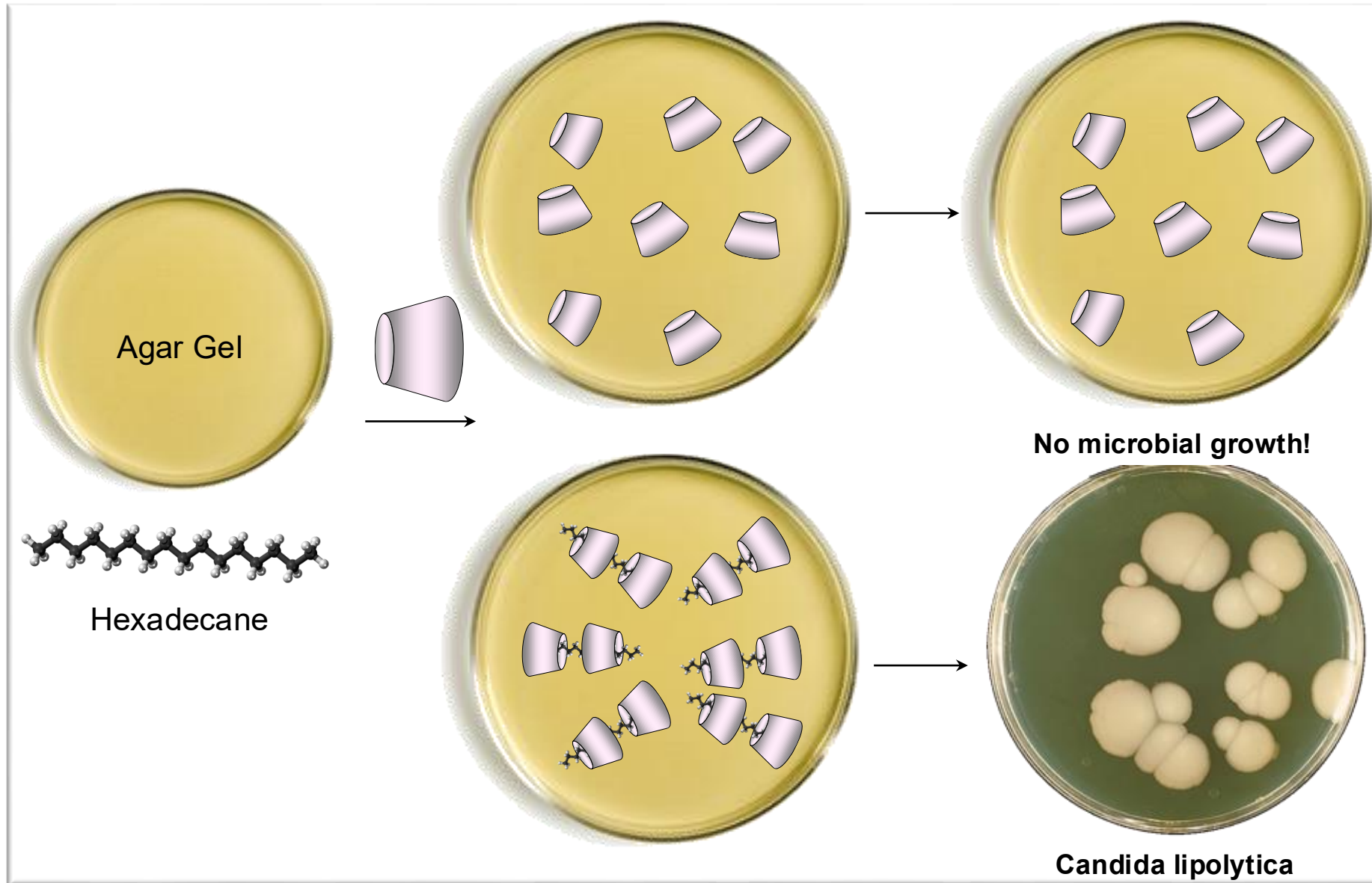
Monoclonal Antibody for Detection of CDs

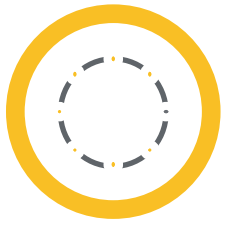


- The monoclonal antibody to BCD was generated by using a conjugate of glucosaminylmaltosyl-BCD and bovine serum albumin as an antigen.
- The monoclonal antibody was IgM/k and reacted with β -CD with high specificity.
- The epitope recognized seemed to be located on the secondary side of the β -CD
- The immunoassay was useful to determine BCD in biological fluids such as human plasma and urine.



Microbiological Cultivation





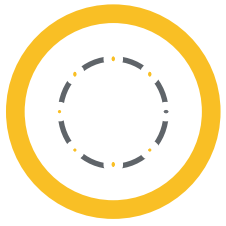
Cryoprotection

- 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.





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





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For any questions:

info@carbohyde.com