Dextran Production

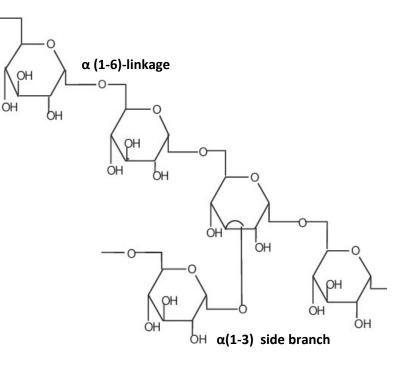
By Dr. Ekta Khare Department of Biosciences & Biotechnology, Chhatrapati Shahu Ji Maharaj University, Kanpur

History

- The name 'dextran' was first used by Scheibler in 1874 when he found that the mysterious thickening of cane and beet sugar juices was caused by a carbohydrate of empirical formula ($C_6H_{10}O_6$) having a positive optical rotation.
- Previously, in 1861, Pasteur had shown that these slimes were caused by microbial action.
- van Tieghem named the causative bacterium *Leuconostoc mesenteroides.*
- Later investigators showed that dextran can be formed by several bacterial species and that it is not a well defined substance with specific properties.

Structure of Dextran

- Dextrans are now defined as homopolysaccharides of glucose that feature a substantial number of consecutive $\alpha(1,6)$ -linkages in their major chains, usually more than 50% of the total linkages.
- These α-D-glucans also possess side-chains, stemming mainly from α(1,3)- and occasionally from α(1,4)- or α(1,2)-branched linkages.
- The exact structure of each type of dextran depends on its specific producing microbial strain and hence on the specific type of dextransucrase(s) involved.



Production

- The great majority of dextrans in nature are synthesised from sucrose by dextransucrase enzymes, secreted mainly by *Leuconostoc, Streptococcus* and *Lactobacillus* species.
- Dextrans can also be synthesised from maltodextrins by dextran dextrinase activity of certain *Gluconobacter strains*.
- Leuconostoc mesenteroides NRRL B-512F dextransucrase has received most attention and the resulting dextran is produced commercially.
- This dextransucrase is secreted in relatively large amounts into the culture supernatant with a minimum number and quantity of related contaminating enzymes (such as invertase and levansucrase) and it forms a high molecular weight, soluble dextran.

...Production

- Dextran cannot be produced by dextransucrase from glucose, mixtures of glucose and fructose or any other naturally occurring sugar; sucrose is absolutely required.
- Sucrose induces in these bacteria formation of the dextransynthesizing enzyme dextransucrase.
- Fructose, the by product of dextran synthesis, is metabolized by Leuconostoc which cannot, however, metabolize either sucrose (they have neither invertase nor sucrose phosphorylase) or dextran.
- The relatively high energy of the acetal-ketal linkage joining the glucose and fructose moieties of sucrose is utilised by the enzyme to synthesise the $\alpha(1,6)$ -linkages of the main chain.

Industrial production of Dextran from *Leuconostoc mesenteroides*

- To date, commercial dextran production is mainly accomplished by growing cultures of Leuconostoc mesenteroides. Treatment of L. mesenteroides cells with the mutagen nitrosoguanidine, resulted in the selection of a mutant (designated B-512FM) which produced 300 times more enzyme than the parent strain in media containing :
 - Sucrose
 - Organic source of nitrogen (eg. peptone)
 - Growth factors (nicotinic acid, thiamine, panthothenic acid and biotin, together with the amino acids valine and glutamic acid)
 - Trace minerals
 - Phosphate
 - Production of dextransucrase increases twofold by the addition of 0.005% calcium chloride
- The bacterium is facultatively anaerobic or microaerophilic and fermentations are not aerated.
- Fermentation conditions:
 - initial pH (typically 6.7–7.2),
 - temperature (about 25 °C),
 - initial sucrose concentration (usually 2%)
 - fermentation time (usually 24–48 h)

....Production

- Fermentation kinetics: During the first 20 h of fermentation microbial growth and production of dextran sucrase enzyme takes place. The optimal pH for enzyme production was 6.5–7.0.
 - the culture pH falls to ~5.0 because of the formation of organic acids, favourably near the optimal pH of dextransucrase. The optimal pH for enzyme activity was 5.0–5.2
 - Dextran branching appears to increase at elevated temperatures.
- Down stream processing: Dextran is harvested from the fermentation medium by alcohol precipitation and purified by further precipitation after redissolution in water.
 - Cell debris is removed by centrifugation.
- This conventional process has the disadvantage of propagating cells.
- It has long been recognised that dextran could also be produced enzymatically using cell-free culture supernatants that contain dextransucrase.
- This Conditions in the enzymic method are more constant and easier to control than in the whole-culture method; furthermore, the product is more uniform and easier to purify.
- Dextransucrase has also been immobilised and used for production.

Dextrans synthesized by *Gluconobacter* dextran dextrinase (DDase)

- Cultures of Acetobacter capsulatus NCTC 4943 (now Gluconobacter oxydans ATCC 11 894) and A. viscosus NCTC 7216 (now G. oxydans ATCC 11 895), when grown on dextrin (but not when grown on other common carbohydrates) produced abundant amounts of polysaccharide material, which had serological properties similar to dextran, produced from sucrose by L. mesenteroides.
- Gluconobacter dextran is composed of 90% $\alpha(1,6)$ -linkages and 10% $\alpha(1,4)$ -linkages.

Applications

- 1. In Medical field
- Dextran is used to decrease vascular thrombosis.
- It is occasionally used to replace lost blood in emergency situations where replacement of blood is no available.
- The iron dextran solution is applied for treatment of human anaemic deficiency.
- 2. In food Industry
- Dextran improves softness, crum texture and loaf volume in bakery products.
- Dextran is used as a stabilizer for confectionery as it prevents crystallisation, increases viscosity and maintains flavour.

Dextran prevents frozen foods from oxidation and also help to preserve texture or flavour.

3. Laboratory uses

- Fluorescently labelled dextran can be used to visualise endosomes under a fluorescent microscope as it binds to early endosomes.
- It can also be used as stabilising coating to potent nanoparticles from oxidation and improve biocompatibility.
- Commercial cross-linked dextran is known as Sephadex used for the purification and separation of proteins, nucleic acids and polysaccharides.

4. Wastewater Treatment

• Dextran offers many useful features like stable alkali and acids. It binds with metal ions in alkali pH and is biodegradable. It is used during the flocculation process in wastewater treatment.

Questions

- Write an essay on industrial production of dextran.
- Explain the fermentation conditions and kinetics of dextran production.
- Write application of dextrans.