

Dextran degradation and biofilm inhibition using microbial dextranases

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Abstract

Dextranase [E.C. 3.2.1.11] is a collection of enzymes that catalyze the hydrolysis of $[\alpha\text{-}1\rightarrow6]$ glycosidic bond found in dextran to produce glucose, isomaltose, and several other linear or branched oligosaccharides. The produced isomalto-oligosaccharides have a prebiotic effect by reducing the cariogenic effect of sucrose in oral cavity. Sugar cane molasses is a rich source of microbial dextranases, that are enzymes have the ability to degrade the polysaccharide dextran to low molecular weight fractions which have many therapeutic and industrial applications, Dental caries biofilm removal one of those applications. Various dextranases can be isolated from several microorganisms e.g. mold, yeasts and bacteria. These dextranases can hydrolyze dextran in an endo-wise or exo-wise fashion, to eliminate dextran synthesized by different microorganisms in mouth to prevent dental caries. Definitely, Streptococci produce an exo-polysaccharide composed of dextran, the dental plaques that is formed by *Streptococcus mutans* and *Streptococcus sobrinus* can be eliminated using dextranases enzymes that can be added to the dental products for dental caries removing.

1. Dextranases

1.1. Definition

Dextranases are enzymes that are used for dextran hydrolysis from its high molecular weight to the low molecular weight fractions [1].

Dextranase [E.C. 3.2.1.11] is a collection of enzymes that catalyze the hydrolysis of $[\alpha\text{-}1\rightarrow6]$ glycosidic bonds found in dextran to produce glucose, isomaltose, isomaltotriose, and several other linear or branched oligosaccharides. The produced isomalto-oligosaccharides have a prebiotic effect by reducing the cariogenic effect of sucrose in the oral cavity [2].

Various dextranases can be isolated from several microorganisms e.g. mold, yeasts, and bacteria. These dextranases can hydrolyze dextran in an endo-wise or exo-wise fashion, to eliminate dextran synthesized by different microorganisms in the mouth to prevent dental caries.

Streptococci produce an exo-polysaccharide composed of dextran, the dental plaques that are formed by *Streptococcus mutans* and *Streptococcus sobrinus* can be eliminated using dextranases enzymes, that can be added to the dental products for dental caries removing [3].

Dextran-degrading enzymes form a various group of different carbohydrases and transferases, that are classified into endo and exo- dextranases according to the mode of action called dextranases [4].

There are two main classes of dextranases: exo-dextranases that produce glucose or iso-maltose from the non-reducing

ends of dextran. The other is endo-dextranases, which hydrolyze the internal glycosidic bonds inside the dextran molecule, so the oligosaccharides are generated [5]

1.2. Sources

1.2.1. Microbial dextranases

Dextranase enzymes are found in numerous microbes, like molds that catalyze endo-hydrolysis of $\alpha\text{-}[1,6]\text{-D-glycoside}$ linkages at random sites in dextran to produce iso-maltose, iso-maltotriose, and a small amount of D-glucose units.

Fungi and bacteria were recognized as the main enzymatic sources able to hydrolyze dextran. Where the Japanese researchers recognized that *Penicillium lilacinum* and *Penicillium funiculosum* fungi can produce dextranase in the presence of dextran and later from the other strains from *Chaetomium gracile* and *Gibbellela funiculosum* fungi, *Penicillium luteum*, *Penicillium notatum*, and *Aspergillus carneus* mold, besides yeast like *Lipomyces starkeyi*. Also *Bacteroides oralis*, *Flavobacterium* sp., *Streptococcus sobrinus*, *Bacillus* sp., *pseudomonas* sp. *Streptococcus mutans* from bacteria [6].

Bacterial endo-dextranases are similar to fungal endo-dextranases, showing specific modes of action for each microorganism. The bacterium *Cellvibrio fulva* can hydrolyze dextran into relatively large fragments with no D-glucose units or disaccharides.

According to *Lactobacillus bifidus* can produce a mixture of oligosaccharides with no glucose units or isomaltose from unbranched dextran of *Streptomyces bovis* and branched *Leuconostoc* dextran [4].

Dextran hydrolyzing organisms have been reported that can be isolated from natural environment that is considered harsh environment, where the most important commercial source of dextranases is fungi because of its higher productivity levels of the enzyme, with enzyme stability at high temperatures [7].

1.3. Dextranases classification

According to the amino acid sequence similarity, dextranases have been classified into two families: [Glycosyl-hydrolase family GH49 and GH66], where bacterial dextranases from *Streptococcus* sp. belong to the GH66 family. The dextranases from *Arthrobacter* sp., *Penicillium* dextranases sp., and *Lipomyces starkeyi* are belong to GH49 family [7].

1.4. Dextranase applications

Dextranase is an inducible enzyme acting as an [α -1,6-glucosidic linkages] hydrolysis in dextran molecule to get smaller oligosaccharides. This process can reduce the dextran stickiness, which is suggested to solve microbe-produced dextran problems in the cane sugar industry, with the same justification, it can be used for caries prevention by inhibition of biofilm formation on teeth surface [7].

It is used also in dextran degradation into small-size molecules to be suitable for using as blood volume expander in hypovolemic shock. Dextranase is suggested to increase antibiotic effectiveness in case of endocarditis and for targeting therapeutic agents [7].

1.5. Dextranase activity measuring methods

Measuring the dextran-hydrolyzing enzyme activity may be difficult due to the large variability in substrates and the undefined reaction product mixture of the sugar polymers. The first method for measuring dextranase activity is based on viscous metric analysis. The second one is the nephelo metric method which detects the loss of opalescence of dextran solution, which reflects dextranase activity [4].

In the saccharogenic methods, the liberated reducing sugars in the reaction mixture are commonly assayed with the Somogyi assay using 3,5-dinitrosalicylic acid reagent, endo-dextranase activity also can be measured spectrophotometrically using chromogenic substrates. The fluorometric assay can be used in dextranase activity by combining dextran with a fluorescent dye BODIPY [4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diazas-indacene-3-propionic acid, succinimidyl ester] [4].

1.6. Factors affecting dextranase production

Carbon sources are essential nutrients for the microbial growth and accumulation of metabolite, which also influence the microbial growth and metabolism and, consequently enzyme production. Nitrogen sources are also important components for microbial cell proteins and nucleic synthesis, addition of the suitable divalent cations with suitable concentrations in culturing and fermentation can help in maintaining the active

enzyme conformation, also the temperature and pH which are two vital factors that influence the enzyme properties. The active sites in some enzymes contain amino acid groups that interact with the metallic compounds, so metals can influence the catalytic activity of enzymes by increasing or decreasing, where some metal ions cause an oxidation in the groups found in certain amino acid side chains, resulting in a strong decrease in the catalytic activity [8].

2. Biofilm

Biofilm is defined as a complex and active surface-associated community that is global in nature. The biofilm development process is considered a transformation process, where single bacterial cells form organized communities or micro-colonies by differentiation and aggregation. Bacteria, viruses, or fungi can form biofilm separately or in combined form, and the construction of a biofilm is designed according to the chemical, physical, genetic, and physiological characteristics of the present microorganisms.

Biofilms are complex, three-dimensional structures built by microbes and extracellular polymeric substances [EPS], EPS contains polysaccharides, proteins, nucleic acids, glycoproteins, and glycolipids. In some circumstances extracellular DNA.

The role of extracellular DNA is a serious component of the biofilm matrix, for providing stability of biofilm structure and saving from antimicrobial agents.

The release of eDNA is usually a result of cell lysis, the bacterial murein hydrolases or bacterial autolysin activate digestion of the cell wall, resulting in the release of DNA and other substances into the extracellular environment. Where that involved in biofilm formation by facilitating bacterial lysis, research that were done on *S. mutans* verified the part of its autolysin protein AtlA in the formation of biofilm on surfaces of polystyrene in vitro against glucans that are vital for *S. mutans* biofilm formation on dental surfaces plus polystyrene [9].

Bacterial biofilms are constructions comprising of extracellular polymeric ingredients plus single or numerous species of bacteria that form clusters that adhere to teeth surfaces, where bound water represents more than 97% of the biofilm structure. Versus to planktonic bacteria, bacteria can survive better against drastic conditions when found especially in a biofilm environment. This leads to a high level of resistance that reaches 1,000 times stronger against antibiotics than planktonic bacteria [Figure 1].

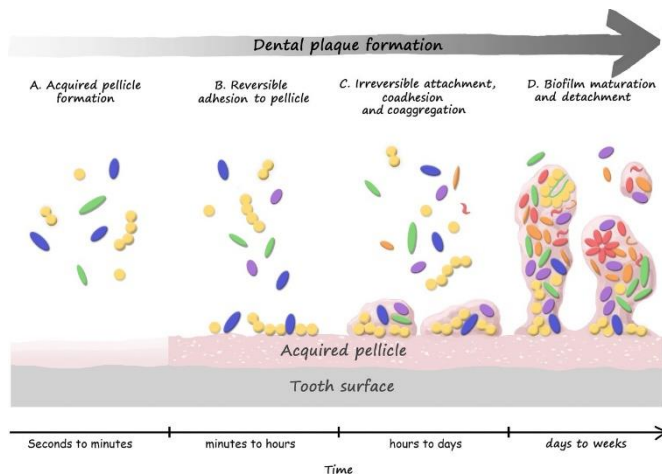


Figure 1: Illustrative representation of dental plaque development on the tooth surface. A cleaned tooth surface is very quickly covered with an acquired pellicle [A]. Commensal streptococci and other early colonizers attach reversibly to the pellicle [B]. Bacteria [mainly mutants streptococci, in the presence of sucrose and starch hydrolyses] [C]. Co-aggregation, co-adhesion, communication, and growth of attached cells, organized bacterial complexes surrounded by EPS form micro-colonies [D]^[10].

Moreover, several factors can contribute to the biofilm bacterial resistance as physical and chemical flow barriers, low sensitivity against antibiotics because of the bacterial slow growth rate when found in biofilms, in addition to non-homogeneity of structure of biofilm^[11].

2.1. Biofilm inhibition enzymatic mechanism

The microbial enzymes are considered an alternative therapy for fighting cariogenic biofilm formation, by adding them to oral hygiene products, for example, mouth wash and toothpaste to support the mechanical cleaning of teeth, where enzymes can act gently and specifically on the harmful environment only. Hence they rarely affect the nature of the biological environment of the oral cavity, with the advantage of avoiding bacterial resistance to chemicals or antibiotics^[10].

Plaque deposition retardation by dextranase on teeth surface can repress the adherence of oral streptococci, that is leading to inhibition of dental caries formation.

Glucan hydrolases is a dextranase that target the biofilm components matrix by removing of the plaque by biofilm-bonding extracellular matrix degradation or by interference with sucrose-dependent bacterial adhesion that can disrupt the biofilm formation^[10].

3. Dextran

3.1. Definition

Dextran is a branched complex exo-polysaccharide molecule that is homogeneous polymer composed of D-glucose units. It has a significant number of successive α [1,6]-linkages in the main chain of the structure that reached to 50% of the whole linkages, with side-chains consist of α [1,3] and occasionally from α [1,4]- or α [1,2]- branched links. The structure of any type of dextran depends on the microbe that produce it [12].

Dextran is hydrolyzed into different small molecular weight fractions with wide industrial importance using chemical and enzymatic methods^[2].

3.2. Dextran synthesis

Naturally dextran can be produced from sucrose by dextranases enzymes that are produced by *Streptococcus*, *Lactobacillus* and *Leuconostoc* sp. or by dextrinases from malto-dextrins by certain *Gluconobacter* strains. However, in case of the dental plaque *streptococci*, the formed dextran is consists of soluble and insoluble dextran types^[12].

4. Summary

Sugar cane molasses are considered a very rich source of the beneficial microbes, that can be used in biofilm degradation of the cariogenic bacteria, where those microbes are *Bacillus*, *Pseudomonas*, *Lactobacilli* and *Streptococcus* spp. and have the ability to produce different enzymes specifically dextranase and mutanase enzymes, that included in dental caries biofilm inhibition, using the microbial enzymes in biofilm inhibition is a natural approach that save oral cavity hygiene and avoid the traditional oral chemicals side effects.

5. Future Perspective

In the future work, Bacteriophages can be mixed or combined with dextranase enzymes to increase the efficacy of bacterial killing and biofilm inhibition.

References

- Zohra RR, Aman A, Zohra RR, Ansari A, Ghani M, Qader SA. Dextranase: hyper production of dextran degrading enzyme from newly isolated strain of *Bacillus licheniformis*. *Carbohydr Polym*. 2013;92[2]:2149-53.
- Zohra RR, Aman A, Ansari A, Haider MS, Qader SAU. Purification, characterization and end product analysis of dextran degrading endodextranase from *Bacillus licheniformis* KIBGE-IB25. *International journal of biological macromolecules*. 2015;78:243-8.
- Wang D, Lu M, Wang S, Jiao Y, Li W, Zhu Q, *et al*. Purification and characterization of a novel marine *Arthrobacteroxydans* KQ11 dextranase. *Carbohydrate polymers*. 2014;106:71-6.
- Khalikova E, Susi P, Korpela T. Microbial dextran-hydrolyzing enzymes: fundamentals and applications. *Microbiology and Molecular Biology Reviews*. 2005;69[2]:306-25.
- Kim JK, Shin SY, Moon JS, Li L, Cho SK, Kim TJ, *et al*. Isolation of dextran-hydrolyzing intestinal bacteria and characterization of their dextranolytic activities. *Biopolymers*. 2015;103[6]:321-7.
- Jiménez ER. Dextranase in sugar industry: a review. *Sugar tech*. 2009;11[2]:124-34.

7. Panan Rerngsamran PT, Nilnate Assavasirijinda, Supat Chareonpornwattana,, Thaniyavarn S. Cloning, characterization, and heterologous expression of a dextranase gene from *Penicillium pinophilum* SMCU3-14 Science Asia. 2014;40:9.
8. Zhao J, Wang L, Wei X, Li K, Liu J. Food-grade expression and characterization of a dextranase from *Chaetomium gracile* suitable for sugarcane juice clarification. Chemistry & Biodiversity. 2021;18[1]:e2000797.
9. Jung C-J, Hsu R-B, Shun C-T, Hsu C-C, Chia J-S. AtlA mediates extracellular DNA release, which contributes to *Streptococcus mutans* biofilm formation in an experimental rat model of infective endocarditis. Infection and immunity. 2017;85[9]:e00252-17.
10. Pleszczyńska M, Wiater A, Bachanek T, Szczodrak J. Enzymes in therapy of biofilm-related oral diseases. Biotechnology and applied biochemistry. 2017;64[3]:337-46.
11. Welch K, Cai Y, Strømme M. A method for quantitative determination of biofilm viability. Journal of functional biomaterials. 2012;3[2]:418-31.
12. Naessens M, Cerdobbel A, Soetaert W, Vandamme EJ. *Leuconostoc* dextranase and dextran: production, properties and applications. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology. 2005;80[8]:845-60.

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