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Research review paper

Review of Levan polysaccharide: From a century of past experiences to future prospects

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ABSTRACT

Levan is a fascinating β-(2,6)-linked fructose polymer with an unusual combination of properties characterized in this review. In nature, levan is synthesized from sucrose by a wide range of microorganisms and a few plant species. Bacterial levans often have molecular weights over 500,000 Da, are commonly branched, and form compact nanospheres offering a broad spectrum of applications. The most relevant genetic, biochemical and structural aspects of the biosynthetic enzyme levansucrase are detailed. Optimization of parameters for levan production by intact bacteria and by the isolated enzyme is surveyed. The diversity of current and potential applications of levan is illustrated by a discussion of uses ranging from personal care and aquaculture to the medical and food industries.

Contents

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1. Introduction

Levan is an unusual non-structural polysaccharide present in several microorganisms and a few plant species. Consisting almost solely of fructosyl residues linked via the β-2,6 carbons, this fructan molecule is packed into nano-sized, spherical forms, providing it with a remarkably low intrinsic viscosity (Arvidson et al., 2006) and somewhat greater stability than that of linear counterparts. Produced by either intact microorganisms in submerged cultures or by the isolated enzyme, levan is usually synthesized from sucrose, syrups or molasses. With some variability caused by production conditions, the microbial source of the levansucrase adds more differences to the molecular weight, degree of branching (Jakob et al., 2013; Runyon et al., 2014), diameter, intrinsic viscosity, stability and functionalities such as immunogenic activity and adhesive strength.

The levan story begins with natto, a traditional Japanese food that was considered to promote long life and good health. As one component of natto, levan has long attracted the interest of researchers looking for natural products with health benefits.

Lippmann is often credited with first identifying levan since he used the term "lävulan" to describe the gum recovered from molasses in the sugar beet industry (v. Lippmann, 1881). But it was the Australian bacteriologist Greig-Smith who proposed the term "levan" for the levorotation of polarized light and properties analogous to dextran in 1901 (Greig-Smith, 1901).

For more than a hundred years numerous uses have been identified for levan polysaccharide but only a few have been commercially realized simply because it has not been widely available in meaningful quantities. While levan production in the laboratory is straightforward, bottlenecks in the downstream process have hampered scale up. Technical issues associated with handling large volumes of alcohol, impracticality of dialysis on a large scale and unavailability of ultrafiltration equipment for multi-ton operations have complicated purification. Moreover, without prompt enzyme inhibition at the end of the fermentation step, depolymerization quickly decimates levan. Drying of the sticky product is another hurdle. Lyophilization and vacuum drying commonly employed in the lab (Han, 1990; Kazak Sarilmiser et al., 2015) are not readily scaled. Spray drying is widely used in industry but the adhesive strength of levan requires use of a carrier resulting in decreased product purity.

This review opens with a sketch of the functional roles of levan in nature. It then moves on to a comprehensive discussion of levan biosynthesis, detailing the most relevant genetic, biochemical and structural aspects of levansucrase enzymes. Optimization of parameters for levan production by intact bacteria and by the isolated enzyme is surveyed. The diversity of current and potential applications of levan is illustrated by consideration of uses ranging from personal care and aquaculture to the medical and food industries. The review closes with suggestions of innovative approaches to increasing productivity by levan producers and suggests heretofore untested sectors for its use.

2. Levan in nature and its function

"Fructans: beneficial for plants and humans". This title of a publication by Ritsema and Smeekens (2003) captures the spirit of the diverse levan roles in nature. This paper, along with an earlier publication that included bacteria (Vijn and Smeekens, 1999), highlight the value of the best known fructans, levan and inulin. Vereyken et al. (2003) looked at possible mechanisms for the protective effects of fructans in natural settings. They hypothesized the 5-membered ring fructans interacted with cell membranes in such a way as to stabilize them and thus seemingly protect them from damage. They found the small, flexible head groups of levan and inulin penetrated membrane surrogates unlike a 6-membered ring. It may be that this interaction provides improved membrane stability and, in turn, a higher cell survival. Researchers have identified further valuable functions described in the applications section of this review running the gamut from anti-inflammatory and anti-viral activity to films for food packaging.

Levan, often together with other exopolysaccharides, has been found to be a structural component of some biofilms in different habitats. When present in the biofilm of soil species such as *Bacillus subtilis*, levan shields the microorganisms from desiccation as the water level changes, helps glue cells in a favorable environment and protects the community from predatory organisms (Dogsa et al., 2013). Sucrose utilization by secreted levansucrase appears to be a fitness factor for the *in planta* life of several bacterial species. Levan as a biofilm component contributes to the virulence of the plant pathogens *Erwinia amylovora* and *Pseudomonas syringae* (Koczan et al., 2009; Mehmood et al., 2015). In the endophyte *Gluconacetobacter diazotrophicus*, levan in the biofilm acts as an oxygen difussional barrier helping create the microaerobic conditions required for nitrogen fixation (Hernández et al., 2000; Velázquez-Hernández et al., 2011). As a secondary but no less important role, levan in biofilms constitutes an extracellular nutrient reservoir that can be used as an energy source by bacteria under starvation conditions.

3. Biosynthesis of levan-type fructans

In nature, fructans are synthesized from sucrose by a restricted number of plant species and many microorganisms, which include *Archaea*, fungi and a wide range of bacteria. Plants synthesize fructans in the vacuole by the concerted action of various fructosyltransferases, each with its own preferential donor and acceptor substrates. Sucrose:fructan 6-fructosyltransferase (6-SFT; EC 2.4.1.10), also recognized as sucrose:sucrose 6-fructosyltransferase (6-SST) by its dual ability to use a fructan molecule or sucrose as the acceptor substrate, is responsible for the formation of β(2–6) fructosyl-fructose linkages in plant fructans. Alone, 6-SFT synthesizes linear levan chains directly from sucrose via the trisaccharide intermediate 6-kestose. Other natural fructosyl acceptors of this enzyme are 1-kestose and 6G-kestotriose (Lasseur et al., 2011; and references therein). The production of levan neoseries or mixed levan (graminan)-type fructans requires