PRODUÇÃO CIENTÍFICA EM CIÊNCIAS BIOLÓGICAS

Danyelle Andrade Mota
Clécio Danilo Dias da Silva
(Organizadores)

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APRESENTAÇÃO

As Ciências Biológicas, assim como as diversas áreas da Ciência, passam por constantes transformações, as quais são determinantes para o seu avanço científico. A produção científica tem papel essencial na avaliação da ciência, pois sustenta a avaliação qualitativa e quantitativa. A avaliação da produção científica permite inferir sobre os movimentos de institucionalização e desenvolvimento da pesquisa em campos científicos, períodos e contextos específicos. Além de permitir o entendimento dos processos de produção, difusão e uso do conhecimento, também pode orientar o desenvolvimento e a adaptação de políticas científicas, tecnológicas e de inovação.

Nessa perspectiva, o e-book "Produção Científica em Ciências Biológicas", é uma obra composta de uma série de investigações e contribuições nas diversas áreas de conhecimento que interagem nas Ciências Biológicas, com uma leitura rápida, dinâmica e cheia de possibilidades de aprendizado. Assim, o e-book é para todos os profissionais pertencentes às Ciências Biológicas e suas áreas afins, especialmente, aqueles com atuação no ambiente acadêmico e/ou profissional.

Portanto, o resultado dessa experiência, que se traduz neste e-book, objetiva apresentar ao leitor a diversidade de temáticas inerentes as áreas da Saúde, Meio Ambiente, Biodiversidade, Biotecnologia e Educação, como pilares estruturantes das Ciências Biológicas. Por fim, desejamos que a obra contribua para o enriquecimento da formação universitária e da atuação profissional, com uma visão multidimensional com o enriquecimento de novas atitudes e práticas multiprofissionais nas Ciências Biológicas.

Agradecemos aos autores pelas contribuições que tornaram essa edição possível, e juntos, convidamos os leitores para desfrutarem as publicações.

Danyelle Andrade Mota
Clécio Danilo Dias da Silva

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REVIEW ON MICROBIAL LEVAN: SOURCES AND POTENCIAL USES

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ABSTRACT: Levan is an extracellular polysaccharide that consists of fructose units bound by glyosidic β-(2,6) bonds, produced by several types of microorganisms from substrates containing sucrose. Its molecular structure presents thermal stability and low viscosity, which holds interest to applications in industrial sectors. The challenge, however, is in the large-scale production, since an economic viable method has not yet been consolidated. The purpose of this work was to explore the application of microbial levan at a large scale context.

KEYWORDS: Polysaccharide, bioprocess, renewable materials

1 I LEVAN

Levan is a non-structural polysaccharide synthetized by different types of microorganisms, which include Archeas, yeast, and a wide range of bacteria, besides also some plant species. It is constituted by fructose units linked by type β-(2,6) glyosidic bonds, building compact nanospheres, which can have many potential applications. Its characteristics result in a considerably low intrinsic viscosity (ARVIDSON; RINEHART; GADALA-MARIA, 2006) and a superior structural stability when compared to homologue linear molecules (ÖNER; HERNÁNDEZ; COMBIE, 2016; SRIKANTH, 2015).

The microbial polymer is synthetized from sucrose-based substrates, usually by bacteria, such as the ones from genera *Acetobacter*, *Bacillus*, *Erwinia*, *Gluconobacter*, *Halomonas*, *Microbacterium*, *Pseudomonas*, *Streptococcus e Zymomonas* or directly from the isolated enzyme levansucrase (JAKOB et al., 2013; RUNYON et al., 2014).

In high sucrose concentration media, levan can represent up to 97% of the total biofilm exopolysaccharide (EPS) produced by *Bacillus* *subtilis* (DOGSA et al., 2013; ÖNER; HERNÁNDEZ; COMBIE, 2016). The presence of levan in these biofilms, together with other EPS, confers higher stability, as it helps to bind the microbial cells (DOGSA et al., 2013). Dogsa et al. (2013) proposed that, biologically, the conversion of sucrose to levan can make the biofilm more resistant and that the mechanism happens due to the need of *B. subtilis* to store carbon in competitive environment.

Because it is an extracellular substance, it is affected by fermentation conditions like pH, oxygen concentration, bioreactor settings and composition of culture media (ÖNER; HERNÁNDEZ; COMBIE, 2016). The microbial source of the levansucrase enzyme is also an important factor that can bring different characteristics to the final product, modifying properties such as molecular weight, branching degree, diameter, intrinsic viscosity, stability and functional aspects like adhesive strength (JAKOB et al., 2013; RUNYON et al., 2014; ÖNER; HERNÁNDEZ; COMBIE, 2016).

In general terms, levansucrase originated by gram-positive bacteria, such as the one from *B. subtilis*, produce the polymer without accumulating intermediate oligofructans, unlike gram-negative species, which generate high levels of secondary products, therefore, a lower yield of levan (HOMANN et al., 2007; ADAMBERG et al., 2015; CAPUTI et al., 2013).

Fructans derived from bacteria present a higher degree of polymerization when compared to the ones originated by plants. The bacterial levan, for example, can reach higher molecular weights, even more than 500 kDa, and with a number of fructosyl units between 10^3 and 10^4, while plants produce levan with a range of 10^1 to 10^2 fructosyl units (ÖNER; HERNÁNDEZ; COMBIE, 2016).

The degree of polymerization is due to a progressive mechanism that, after enzymatic cleavage of sucrose, a new fructose unit is added to the growing chain, which is progressively elongated and that is maintained bonded to the enzyme (OZIMEK et al., 2006). Part of the released glucose is metabolized by the microorganism and used for growth, the remaining is simply accumulated in the fermentation media (ÖNER; HERNÁNDEZ; COMBIE, 2016). When the main substrate, sucrose, is extinguished, the synthetized polymer functions as a source of fructosyl units. The levansucrase enzyme breaks the glycosidic β-(2,6) bonds along the levan chain, releasing terminal fructose in a consecutive way, until the reaction stops when it meets a branch point (MÉNDEZ-LORENZO et al., 2015), represented by glycosidic β-(2,1) bonds. The branches protect the chain from hydrolysis from levansucrase itself (ÖNER; HERNÁNDEZ; COMBIE, 2016).

2 | LEVANSUCRASE

Levansucrase is an extracellular enzyme that synthetize levan from sucrose. It remains bonded to the cell surface until its release to the external environment by an enzymatic secretion route, which varies according to the host specimen. The secretion of levansucrase in gram-positive bacteria involves cleavage with precursors containing signal-peptide (VAN HIJUM, 2004; WALDHERR; MEISSNER; VOGEL, 2008).

Most of the species have a single gene responsible for the generation of this enzyme. In the *B. subtilis*, for example, the gene that codes for levansucrase is *sac*B (CRUTZ et al., 1990). In some bacteria, like in *Bacillus*, sucrose is indispensable to induct its transcription (CHOI et al., 2004; INTHANAVONG et al., 2013).

Bacteria can contain the gene for levansucrase as well as the gene that encodes for levanase, which is responsible for hydrolysis of levan molecules. Levanase production is induced by low concentration of fructose in the reactional media and it is inhibited by glucose, the primary product of sucrose transfructosylation (MENÉNDEZ et al., 2009; ÖNER; HERNÁNDEZ; COMBIE, 2016). It is known that, in the presence of fructose, levansucrase from *B. subtilis* creates a covalent fructosyl-enzyme complex, responsible for transfructosylation. This reaction occurs when the fructosyl residues is transferred to sucrose elongating the growing chan. Apart from this reaction, studies show that levansucrase itself can exhibit levanase activity as one of its intrinsic properties. It acts by utilizing levan as a substrate and, by means of an enzyme-fructosyl complex, behaves like a hydrolytic or transferase enzyme releasing fructose units (MÉNDEZ-LORENZO et al., 2015).

Whenever sucrose is available, the cells possess a restrict control in order to prioritize levan synthesis, instead of its degradation (VAN HIJUM, 2004; WALDHERR; MEISSNER; VOGEL, 2008). Research involving gene expression and evolutive analysis imply that synthesis and degradation of levan are, at the same time, exclusive and complementary processes, and the behavior depends on the bacterial habitat (MÉNDEZ-LORENZO et al., 2015).

3 | LEVAN APPLICATIONS

Products containing levan in their formulation are limited in the market, mainly as a consequence of the challenges encountered for production in large scale. Despite that, intrinsic properties of levan attract relevant interest of a variety of industrial sectors to the polyssacharide. In cosmetical industries, for example, wide used international tests confirm that levan meets the safety requirements to be used in cosmetics, which demonstrate that the biopolymer does not cause irritation or allergy and that it is not toxic (MONTANA POLYSACCHARIDES, 2015).

In addition to the cosmetical industry, many other applications are being explored by other market sectors, such as food and health industries. Tables 1 and 2 point some of these applications already used or in study, based on their physical, chemical and biological properties. Table 1 summarizes reports of levan's exploration in scientific articles, while Table 2 reunites some patents conceded and some solicitated for some levan applications, found in international databases. These data confirm the relevance of the polymer to diverse market areas.

Some present examples of levan producers are Natural Polymers Inc., from the United States, which utilizes *B. subtilis* as the producer microorganism, the Chinese company Real Biotech Co., which produces it from *Zymomonas mobilis,* and the Japanese company Advance Co., producing levan from *Streptococcus salivarius*. Only a few companies sell products in which levan is one of the main ingredients. An example of one that does it is the Swiss company Rahn with its levan-based products Proteolea® e o Slimexir® (ÖNER; HERNÁNDEZ; COMBIE, 2016). According to the online catalog of this company, Proteolea® slows down facial cellular aging and Slimexir® ensures reduction of fat accumulation in abdominal adipocytes, decreasing the volume of this body portion corporal (RAHN, 2018).

Industrial segment	Application	Description	Reference
Cosmetics	Moisturizing	The polymer is effective as a moisturizing lotion, it showed similar properties to hyaluronic acid.	Kim et al. 2005
	Hair resistance	Tests demonstrated a decline in lifting of hair cuticles in treatment containing levan.	Öner et al. 2016
Medicine	Healing agent	Showed that levan acts in an important step of healing burned or mechanical damaged tissue.	Sturzoiu et al. 2011
	Healing agent	Showed that levan acts in an important step of healing burned or mechanical damaged tissue.	Sturzoiu et al. 2011
	Bones regeneration	Levan methacrylate spheres combined with glass nanoparticles demonstrated considerable adhesive resistance when applied to bone renegeration.	Leite et al. 2014
	Anti-inflammatory	It has been concluded that levan can act directly in reducing stimulation of leucocyte adhesion to the cell of blood vessels.	Sedgwick et al. 1984
	Weight loss	A levan and ginseng-based diet tested in mice resulted in reduction of white adipose tissue weight, lower blood glucose in fasting, lower resistance to insulin and leptin in mice.	Oh et al. 2014
	Cholesterol reduction	Serum cholesterol was significantly decreased in animals treated with levan.	Yamamoto et al. 1999

Food	Probiotic	Levan was proven to enrich intestinal human microbiota.	Adamberg et al. 2015
	Prebiotic	Benefits to animal's intestinal microbiota were observed in comparison to prophylactic antibiotics.	Li e Kim 2013
	Shelf life	Levan was able to form a microgel with hydrocolloid properties resulting in a softer bread.	Jakob et al. 2012

Table 1. Scientific articles showing levan's potential to different application areas.

Source: Authors (2018).

Segment	Application	Description	Reference
Cosmetics	Bleacher	Japanese patent conceded showing levan could be used to inhibit melanin production through significative lowering of tirosin activity.	Masayo e Takayuki 2007
Medicine	Antioxidant	Worldwide patent requested for the use of a combination of levan with ascorbic acid in order to generate a compound of high oxidative stability.	Kim et al. 2011
Food	Functional yogurt	Patent requested describing levan yogurt as a functional food.	Xiao; Feng; Lu 2014
	Fat substitute	European patent conceded to the application of levan in lactic products in substitution to the fat percentage.	Booten; de Soete; Frippiat 2002
	Healthy candies	British patent approved to produce candies from fructans, including levan with digestive benefits.	Cordero 2010
Adhesive	Binder for pelleting	Worldwide patent that utilizes levan as a binder in the pelleting process of iron ore and agglutination for cement.	Madduri et al. 2017
	Cigarette adhesive	Chinese patent of an adhesive compound of pullulan and levan as a holder to cigarette.	Lei 2016

Table 2. Patents approved and requested for levan applications in different industrial segments.

Source: Authors (2018).

4 I INDUSTRIAL PRODUCTION

Commercial production of polysaccharides is usually associated with culturing in bioreactors, in which the culture conditions can be standardized, controlled and monitored. As a result of these conditions, the synthesis of polysaccharide by microorganism sources, when compared to the one done by plants, have a lower production time and simpler extraction steps. (FREITAS; TORRES; REIS, 2017; SEVIOUR et al., 2011).

The definition of a bioprocess in industrial scale usually depends on the performance of experiments in smaller scale. The challenge of scaling-up involves mass and energy transfer, which are indispensable to maintain homogeneity of the media, specially associated to the disponibility of nutrients and dissolved oxygen used for cell growth and removal of the heat generated by microbial metabolism. The fermentation efficiency is influenced by a wide range of factors; from the right strain and components choice to possible alterations in the reactional media caused by accumulation of product (SHULER; KARGI, 2011; SEVIOUR, 2011).

Regarding levan production, according to Franken et al. (2013), the market remains unexplored due to the difficulty in developing a viable production method. Despite the known use as food additive in places like the United States, Europe and Japan, there are still limitations for industrial production and application of levan, with relies on its high cost and the lack of homogeneity in the recovering and purification steps (GUPTA et al., 2011, FREITAS; ALVES; REIS, 2014). Freitas, Alves e Reis (2011) emphasize the lack of market data referring to levan production, which points to the absence of a significative production of this exopolysaccharide in industrial and commercial scale.

The necessary downstream steps for recovery of levan are determined in accordance with the microorganism characteristics, physicochemical properties, desired purity degree and the desirable commercial application (FREITAS; TORRES; REIS, 2017; SEVIOUR et al., 2011).

Considering that levan is an extracellular polysaccharide, the first downstream step consists in biomass removal from the culture media. The standardized unit operations appropriated to cell removal are: filtration, microfiltration and centrifugation, the first two relying on particle size differences and the last one on the density difference from the mix components (ARVIDSON; RINEHART; GADALA-MARIA, 2006; DORAN, 2012). The liquid fraction containing the product of interest proceed to the following purification steps. Biomass can be recycled back to the process or directly disposed (DORAN, 2012).

After biomass separation, the polysaccharide is precipitated by a water miscible solvent in which the product is insoluble (FREITAS; ALVES; REIS, 2011). According to Tomulescu et al. (2016), levan in insoluble in organic solvents such as methanol, ketone, ethanol, n-propanol, methylketone, isopropanol, ethyl lactate and toluene. Currently, precipitation is a challenge to economic viability of the process, because the solvent:levan ratio requires excessive expenses with solvent. The most used solvent for precipitation of levan is ethanol and the necessary amount of this solvent follows a ratio of 3 or 4:1 (ethanol:levan) (ÖNER; HERNÁNDEZ; COMBIE, 2016). Therefore, the possibility of recovering ethanol from the process is being investigated and could lower down the budget of the process.

Ultrafiltration or dialysis are needed for purification of levan. Ultrafiltration allows the separation on nanometric components and, because it is very selective for particle size, it results in a high purity degree for the final product. Contaminants can also be removed by dialysis, in which particles are separated by a diffusion membrane in concentration gradients (TERMIZI, 2006). Although these two methods are presented as efficient for levan purification, when it comes to industrial scale, the challenge relies on the viability of big size equipment's for the processes (ÖNER; HERNÁNDEZ; COMBIE, 2016; SHIH et al., 2005, 2011)6.

After purification step, levan can go through a drying step. The choice of the drying method requires evaluation of costs and parameters of the process. As showed in Table 3, despite the advantage of utilizing low temperatures, since the process is based on sublimation of the sample in temperatures below 0 and with low pressure, avoiding thermal degradation of the polysaccharide, the energetic demand and the lack of homogeneity in high volumes make the drying process inviable to be applied to normal industrial volumes (PHARMACEUTICAL TECHNOLOGY EUROPE, 2010). Other methods can be studied in order to turn the levan drying step possible, as shown in Table 3.

	Principle	Advantages	Disadvantages
Lyophilization	Freezing and sublimation (from gas to solid) of the product in pressure and temperature lower than the triple point (AGRAWAL et al., 2016).	Minimal degradation of the product (AGRAWAL et al., 2016).	High energetic waste, lack of homogeneity in large volumes (PHARMACEUTICAL TECHNOLOGY EUROPE, 2010).
Spray Drying	Transformation of fluid into powder, by means of atomization by a hot gas flow (SOSNIK, SEREMETA, 2015).	Quick method and good control of the particles size (SOSNIK, SEREMETA, 2015).	High energetic waste, high maintenance cost and need for a carrier compound (EHOW UK, 2017; ÖNER; HERNÁNDEZ; COMBIE, 2016)
Oven	Removal of water by heating (CECCHI, 2003).	Relatively quick method (ANIL, KIM, VENKANTESAN, 2017).	Decomposition of the polysaccharide structure and hardening of its surface (ANIL, KIM, VENKANTESAN, 2017).

Table 3. Characteristics of some drying methods for polysaccharides.

Source: Authors (2018).

5 I ADHESIVES

5.1 Synthetical adhesives

The adhesive and sealants market represented, worldwide, a total of 49,50 billion dollars in 2016 (MARKETS AND MARKETS, 2017). The estimated worldwide consumption of adhesives is close to 14 million tons per year (CERESANA, 2017). These materials can be applied into many types of industry, as example of paper and pulp, building, wood, transportation, health and cosmetical, consumer goods and others (MARKETS AND MARKETS, 2017). According to market analysts, it is a growing sector, with expectations to have a Compound Annual Growth Rate (CAGR) close to 4-5% in 2016-2022 period and, according to Grace Matthews director in an interview to Coatings World magazine, the share of adhesives reaches up to 90%, while only 10% represents sealants, which differ from adhesives for being applied to simply seal one surface, while adhesives glue two surfaces

(COATINGS WORLD, 2016; TECHNAVIO, 2016; MARKETS AND MARKETS, 2017).

The employed technology for adhesive synthesis can vary between synthesis with water, with solvent, hot-melted or with reactive substances (MARKETS AND MARKETS, 2017). The water-based one is starting to gain space and replacing the solvent-based technologies, a tendency observed because of the concern of using more eco-friendly raw material and because they emit none or lesser amounts of VOCs (Volatile Organic Compounds) (TECHNAVIO, 2016; HENKEL). Moreover, most of the commercialized adhesive derive from non-renewable raw material, like polyvinyl acetate, which dominates de market, opoxy, polyurethane, formol formaldehyde and urea formaldehyde, all coming from petrochemical sources (PATEL; MATHIAS; MICHAUD, 2013). In this context, the demand for bioadhesives is emerging since products that don't damage the environment are gaining space and people are becoming aware about the adverse effects of some synthetic materials (NASDAQ, 2018; TRANSPARENCY MARKET SHARE, 2018).

5.2 Bioadhesives

Biopolymers can have properties useful for the developing of bioadhesives, because some have a high adhesion strength. Also, these materials meet the market tendencies, in a way that they not only reduce the use of VOCs and toxic compounds and of petrochemical sources, but also that their products cause less environmental impact (SMITH; CALLOW, 2006). For these reasons, a growth in the use of these substances is expected in the bioadhesives market, with a Compound Annual Growth Rate (CAGR) close to 13%, considering the period of 2017-2022. A value close to 3 billion American dollars is expected to represent this segment in 2022 (RESEARCH AND MARKETS, 2017).

The most expressive markets for bioadhesives are paper and packaging, health, construction and personal care industries, highlighting the health sector, which has invested substantially in research and development for the fabrication of these products (GRAND VIEW RESEARCH, 2016).

Nowadays, bioadhesives from plants and animals are utilized, with plants being the most common ones. The main raw material derived from plants are starch, soy and corn, while the main ones derived from animals are bones, skin, hides, leather and other parts rich in collagen (GRAND VIEW RESEARCH, 2018). As an example of vegetable material, starch bioadhesive can be cited, which is a biodegradable, atoxic and of easy access material. Starch is capable of suffering alterations in its structure, such as gelatinization and crosslinking, giving it competitive adhesive properties when compared to synthetical adhesives, like the urea formaldehyde ones, largely utilized in wood products (ZHAO et al., 2018). Another example is the chitosan bioadhesive, material that comes from the exoskeleton of Crustacea. Its adhesive property has been proved, as well as antibacterial and hemostatic properties, being interesting for applications in wounds and burns, replacing the ones currently used for the same purpose, like cyanoacrilates, that present certain toxicity (HAAG, 2006; LU et al., 2018).

Adhesives are used in medical applications as implants, tissue glue, healing agents of wounds and in the fabrication of materials and equipments (GRAND VIEW RESEARCH, 2017). Through this branch, various requirements must be attended. Biocompatibility, non-toxicity, adhesion to organic and inorganic structures, compatibility with sterilization methods, non-inflammable and antimicrobial characteristics are essential for the success of the products (JEREMY COOLER, 2011).

Usually, conventional adhesives are not efficient to medical applications, especially the ones destined for internal use. This is due to the liberation of toxic products that compose these adhesives (BLOOD WEEKLY, 2013) and to biological incompatibility. Bioadhesives represent an alternative to overcome these types of problems, therefore, their investment among the health industry grows substantially, aiming their application in cirurgical instruments, curatives and equipments that may be in contact with the skin. The replacement of sutures for bioadhesives represents a major potential application, because it can lower the chance of infection and contamination, it is easier to handle when comparing to the synthetical threads and there is no need to remove the bioadhesive after closing the woung, as the material is biodegradable (HAAG, 2006). Additionally, some biopolymers, as is the case with levan, also present healing, anti-inflammatory and antibacterial properties (SEDGWICK et al., 1984; STURZOIU et al., 2011; GOMES et al., 2018; GRAND VIEW RESEARCH, 2018; XU et al., 2018).

When it comes to replacement of sutures, sulfated levan showed itself to be capable of elevate the resistance of chitosan and alginate films, besides elevating their adhesion strength (STURZOIU et al., 2011). *In vitro* studies of the proliferation of muscle cells adhered to the chitosan-alginate-levan film showed that the cells keep multiplying while bound to the film, proving its non-toxicity and good adhesion. This application shows one potential for utilization of biopolymers, such as levan, while also point a solution against the limitation of the resources currently applied in the medical environment (GOMES et al., 2018).

6 I CONCLUSION AND UPCOMING PERSPECTIVES

Levan presents promising biochemical characteristics for application in a wide range of industrial sectors. However, only a few researches and applications present this substance as a main component of their formula or study object. Despite that some scientific workpapers point to levan's anti-inflammatory and antimicrobial properties, these properties still need to be proved through clinical tests before the wide exploration of the biopolymer in medical applications. A challenge is also the dissemination of a process capable of overcoming the difficulties currently encountered in the large-scale production of levan.

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