



A review presenting production, characterization, and applications of biopolymer curdlan in food and pharmaceutical sectors

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Abstract

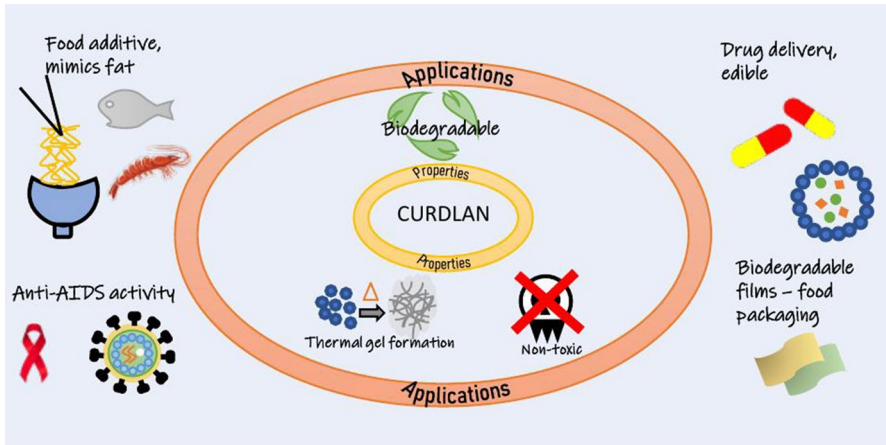
Curdlan is an exopolysaccharide, specifically a homopolysaccharide, with a high molecular weight that is made up entirely of monomeric glucose molecules connected by β -1,3-glycosidic bonds. Curdlan was first isolated in 1962 by Harada and his colleagues from *Alcaligenes faecalis* var *myxogenes* 10C3. Microbial synthesis of this curdlan is mainly associated with soil bacteria. Preliminary screening of curdlan-producing microorganisms is done on aniline blue media. The aniline blue positive microorganisms are subjected to submerged fermentation for the production of curdlan. To improve the yield of curdlan produced, various optimization techniques are employed such as Plackett–Burman, response surface methodology, and others. Curdlan can be characterized by its morphology, gel strength, its infrared, and magnetic resonances among many other characteristics. Due to its distinctive physicochemical and rheological properties, it has gained immense popularity in the food, biomedical, and pharmaceutical sectors. However, curdlan's functionality can be improved by chemically modifying curdlan to obtain grafted curdlan, hydrogels, and nanocomposites which are discussed in detail herewith. Curdlan was authorized to be used in the food industry by the United States Food and Drug Administration in 1996 and also in 1989 in Taiwan, Japan, and Korea. Over the years, many patents using curdlan have also been filed from different parts of the world. This review provides information about its structure, biosynthesis, production strategies, optimization, characterization, applications, and patents.

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Graphic abstract



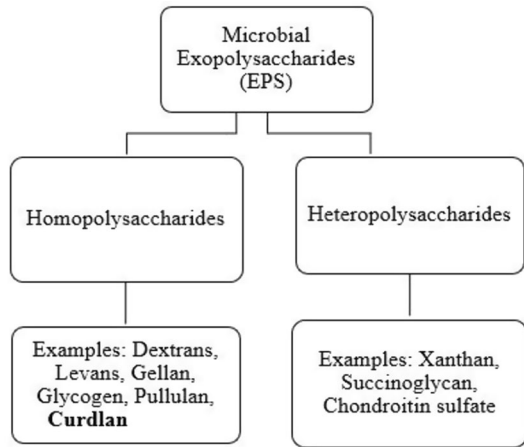
Keywords Curdlan · Exopolysaccharides · Biopolymer · Fermentation · Food applications · Pharmaceutical applications

Introduction

Microbial polysaccharides are carbohydrate polymers that have a high molecular weight. They are found in and around most microbial cells [1]. Microbial polysaccharides are divided into three categories based on their biological functions: capsular polysaccharides (O-antigen, K30), intracellular storage polysaccharides like glycogen, and extracellular polysaccharides (for instance, xanthan, levan, pullulan, etc.). Microbial exopolysaccharides (EPSs) or extracellular polysaccharides are polysaccharides formed by microorganisms and secreted outside the microbial cells [2]. The significance of microbial EPSs for the cells is mainly for the protection of the cell, the interaction between cells, and adhesion to solid surfaces [3]. Microbial EPSs are classified into two groups. They are homopolysaccharides (composed of one sort of monosaccharides) and heteropolysaccharides (composed of multiple kinds of monosaccharides) as shown in Fig. 1. [4]. One of the homopolysaccharides which have started gaining popularity in recent times is curdlan. Production of curdlan was first seen in *Alcaligenes faecalis* var. *myxogenes* 10C3 in the year 1962 by Harada and colleagues [5]. They aimed to find microorganisms that could utilize petrochemical materials, but instead they isolated this bacterium that could produce a neutral polysaccharide. This neutral polysaccharide was named ‘curdlan’ in 1966 [6]. The name curdlan comes from the word ‘curdle’ which is analogous to the property of curdlan when it is heated [7].

Curdlan is an exopolysaccharide, specifically a homopolysaccharide, composed entirely of monomers of glucose connected by β -1,3 glycosidic bonds. It was produced first by *Alcaligenes faecalis* var *myxogenes* 10C3 through glucose

Fig. 1 Classification of microbial exopolysaccharides (EPSs)



fermentation. This organism was isolated from soil [8]. In comparison to other β -1,3 glucans, curdlan is unique in its structure as it is the only linear β -1,3 glucan without any branching unlike Pachyman (consists of internal 16 glycosidic linkages and four branch points) and yeast glucan (consists of β -1,3 and β -1,6 bonds) [9]. Polysaccharides possessing glucose residues are briefly tabulated in Table 1, and the chemical structure of curdlan comprising of glucose and β -1,3 glycosidic bonds is depicted in Fig. 2.

Curdlan’s molecular weight on average ranges from 5.3×10^4 to 2.0×10^6 [13]. After processing, curdlan occurs as a tasteless, colorless, odorless powder that is not soluble in water and alcohol but soluble in alkali [14]. In aqueous solutions, curdlan

Table 1 Polysaccharides with glucose residues and their structures

Polysaccharide	Location	Structure	References
Pachyman	Extracellular	β -(1,3) and β -(1,6)-linkage	[9, 10]
Glycogen	Intracellular	α -(1,4)-linkage	[11]
Cellulose	Extracellular	β -(1,4)-linkage	
Dextran	Extracellular	α -(1,6)-linkage	
Curdlan	Extracellular	β -(1,3)-linkage	
Starch-Amylose	Intracellular	α -(1,4)-linkage	[12]
Amylopectin	Intracellular	α -(1,4)-linkage	

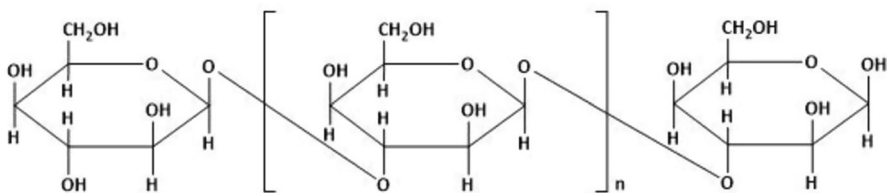


Fig. 2 Chemical structure of curdlan

forms thermo-reversible gels (low-set gels) and thermo-irreversible gels (high-set gels) [15]. When the temperature of an aqueous solution is increased to up to 55 °C and then cooled, it forms a thermo-reversible gel. However, the thermo-irreversible gel formation occurs when the solution is about 80 °C with subsequent cooling. DP or degree of polymerization of curdlan ranges anywhere between 135 and 455 [13]. Due to its distinctive physicochemical and rheological properties, curdlan has gained importance in the food and non-food industries over the past couple of years. Curdlan's biosynthesis metabolic pathway is depicted in Fig. 3.

Glucose gains entry into the cell via an active transporter, that is, either through the PEP-PTS (phosphoenolpyruvate glucose phosphotransferase) system or permease [16]. On entering the cell, the substrate gets phosphorylated into glucose-6-phosphate. Glucose-6-phosphate is converted into glucose-1-phosphate by the enzyme phosphoglucomutase followed by synthesis of UDP glucose and lipid-P-glucose from glucose-1-phosphate and catalyzed by UTP (uridine triphosphate) and UDP glucose phosphorylase. Next, the curdlan synthase enzyme

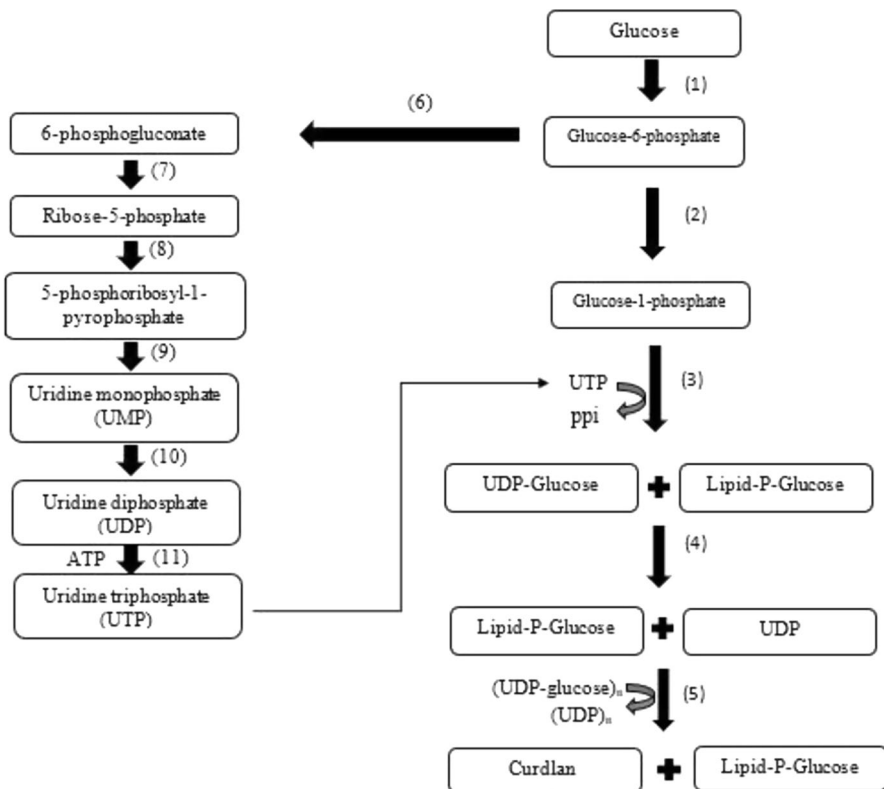


Fig. 3 Curdlan's biosynthesis metabolic pathway [4, 20]: 1: hexokinase, 2: phosphoglucomutase, 3: UDP glucose phosphorylase, 4: glycosyltransferase, 5: curdlan synthase, 6: glucose-6-phosphate dehydrogenase, 7: 6-phosphogluconate dehydrogenase, 8: ribose phosphate diphosphokinase, 9: orotate phosphoribosyl transferase and orotidine-5-phosphate decarboxylase, 10: uridylylate kinase, 11: UDP kinase

catalyzes the polymerization which will transfer one glucose molecule from UDP glucose to the nascent polymer chain, producing a UDP. UDP kinase then uses ATP either from the tricarboxylic acid (TCA) cycle or glycolysis to convert UDP into UTP [17–19]. It was found that curdlan biosynthesis requires four genes *crdA*, *crdS*, *crdC*, and *crdR*. The operon *crdASC* contains these four genes. Their roles are not known. However, it is known that the β -1,3-glucan synthase catalytic subunit is regulated by *crdS*, and *crdR* causes the expression of the operon [20].

Curdlan was approved in the food industry in 1996 by the U.S. Food and Drug Administration (FDA) [15]. Post-approval in 1996, USA launched curdlan into the market by the name Pureglucan™. It was also approved for use in Taiwan, Japan, and Korea in 1989 [21]. Major market players in producing and manufacturing curdlan are Kyowa Hakko Kirin (Japan), Shandong Zhongke Biological Technology Co. Ltd. (China), Shanghai Trustin Chemical Co. Ltd. (China), CarboMer, Inc. (USA), Sigma-Aldrich (USA), Haihang industry (China), and Fujifilm Wako Pure Chemical Corporation (Japan). A few of the patents on curdlan over the last 20 years are shown in Table 2. This table is elaborated later in the patents section following applications.

One of the key features of curdlan is that it is biodegradable as well as non-toxic to humans and the environment. Due to these characteristics, it gives rise to a multitude of applications. It is also of importance in the food and non-food industries as well, such as in the pharmaceutical and biomedical sectors. Curdlan has been utilized as a thickener and gelling in food items [13]. It has also been used as a stabilizer in food, and it can mimic fat, meat, and seafood [31]. In the pharmaceutical industry, it has been used in the encapsulation of some drugs such as theophylline, prednisolone, and indomethacin [32]. Derivatives of curdlan obtained by phosphorylation and carboxymethylation have shown great potential [21]. An example of this is curdlan sulfate which has shown anti-acquired immunodeficiency syndrome (anti-AIDS) activity [7].

To date, bacteria belonging to species of Genus *Rhizobium*, *Agrobacterium*, *Alcaligenes*, *Cellulomonas*, and *Bacillus* are known to produce curdlan [33]. The submerged mode of fermentation is the most established method for the production of curdlan using a production medium [5, 34, 35]. A problem faced during curdlan production is the high cost of the main carbon sources used for its production, i.e., glucose and sucrose. Several attempts have also been made to achieve a better yield of curdlan. Another challenge during the extraction or purification of curdlan is the recovery of curdlan with the capsular polysaccharide. However, the capsular polysaccharide, though extracellular, remains adherent to the cell wall and hence is not easily harvested [2]. Other methods of extraction such as membrane separation and solvent extraction have not been tried for the extraction of curdlan.

In this review, the production of curdlan from different microorganisms, their identification, fermentation, and extraction have been discussed. Characterization of curdlan using different techniques such as FTIR, nuclear magnetic resonance (NMR) spectroscopy, and others has also been highlighted. The main focus of this review is curdlan's wide array of applications in food and non-food industries. This review also presents details about patents on curdlan over the past few years. In this

Table 2 Patents on curdlan over the last 20 years

Year	Assignee	Title	Patent no	References
2020	Jiangnan University	Method for preparing dry rice noodles by using curdlan	CN111296740A	[22]
2020	Organo Food Tech Corp	Curdlan-containing composition and product comprising curdlan-containing composition	US10561166B2	[23]
2018	Univ Shanghai Jiaotong	Aqueous solution or hydrogel of carboxymethyl-curdlan	WO/2018/014373A1	[24]
2016	Food Industry Research and Development Institute, Industry Research and Development Institute	Substitute for fat within the meat and the forming method thereof	US9295277B2	[25]
2016	Mars Inc	Pet food	US20180343894A1	[26]
2015	University of Texas System	Methods and compositions for conformance control using temperature triggered polymer gel with magnetic nanoparticles	US20150159079A1	[27]
2007	Univ Gunma	Manufacturing method of liquid crystal gel composed of curdlan	JP2007084615	[28]
2005	Laboratoires Goemar SA	Agent for stimulating the natural defenses of plants, useful as antiviral, anti-bacterial, antifungal, and insecticide, comprises curdlan sulfate	FR2865350A1	[29]
2004	Organo Corp, Kirin Food-Tech Co, Ltd	Meat products and processed meat products coated with curdlan gel film	EPI 386547A1	[30]

context, to the best of our knowledge, this is the first review article that includes patents on curdlan.

Strategies of curdlan production

Curdlan-producing bacteria

Microbial synthesis of curdlan is mainly associated with soil bacteria [36]. Curdlan production was first observed in *Alcaligenes faecalis* var. *myxogenes* 10C3 in the year 1962 by Harada and colleagues [5]. It was named ‘curdlan’ in 1966 [6]. *A. faecalis* is a common organism in the soil. It is a gram-negative, motile bacillus.

Non-pathogenic *Agrobacterium* sp. is most widely used for curdlan production. *Agrobacterium* sp. is also a gram-negative, rod-shaped bacterium. Curdlan-producing *Agrobacterium fabrum* was isolated from the nodules of two leguminous plants, viz. groundnut and pea plant [37]. Commercial production of curdlan on a large scale is done by *Agrobacterium* sp. ATCC 31749 and *Agrobacterium* sp. ATCC 31750, a mutant of the former [20]. *Rhizobium* sp. is also known to produce curdlan [38, 39].

Another bacterium that produced curdlan was a spore-forming *Bacillus* sp. which was isolated from soil. This was the first report of *Bacillus* sp. producing curdlan [34]. *Cellulomonas flavigena* KU has been isolated from litterfall of leaves and found to produce curdlan in surplus glucose or any other carbon source [40].

Sources of curdlan-producing bacteria

Various sources have been tapped to isolate curdlan-producing microorganisms. These are shown in Table 3.

Table 3 Sources of curdlan-producing bacteria

Source	Bacteria	References
Soil	<i>Bacillus</i>	[34]
Pea plant nodules	<i>Agrobacterium fabrum</i>	[37]
Groundnut nodules	<i>Agrobacterium fabrum</i>	
Butterfly pea	<i>Rhizobium</i>	[38, 41]
Ipil-Ipil	<i>Rhizobium</i>	
Moong bean	<i>Rhizobium</i>	
Soybean	<i>Rhizobium</i>	
Cowpea	<i>Rhizobium</i>	
Black gram	<i>Rhizobium</i>	
Green gram	<i>Rhizobium</i>	
Soil	<i>Alcaligenes faecalis</i> var. <i>myxogenes</i>	[6, 42]
Soil (moist)	<i>Rhizobium radiobacter</i>	[42]

Isolation

For the isolation of bacteria from environmental sources such as soil, the spread plate method is widely used. The spread plate technique is widely used for plate counts, enrichment, selection, or screening and is used to create separate, distinct colonies. Two methods as shown in Fig. 4 are available for performing the spread plate procedure: A turntable and glass or metal rod are used in Method A, while glass beads are used in Method B. The ‘Copacabana method’ is another name for Method B [43].

Screening and identification

Preliminary screening of curdlan-producing microorganisms is mainly done through the qualitative method on an aniline blue medium which consists of glucose, yeast extract, aniline blue dye, and agar. Isolated colonies are inoculated onto aniline blue medium and incubated for about 5 days/30°C to observe the formation of blue-colored colonies. Curdlan-producing organisms give a deep blue color owing to the β -1,3 linkages in it [44]. A quantitative method of screening is by carrying out fermentation for the production of curdlan. After screening, 16S rRNA sequencing is used for the identification of the isolates.

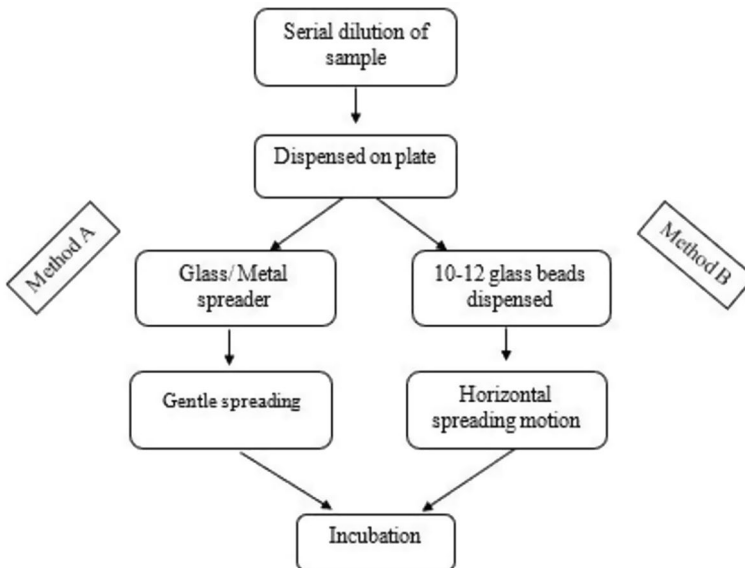


Fig. 4 Methods of a spread plate technique for isolation of bacterial strains

Fermentation

The method of fermentation most widely used for the cultivation of the above-mentioned organisms for the production of curdlan is submerged fermentation (SmF). Bacteria require high moisture content, and hence, this is the best approach. Recovery and purification of products also become easy using submerged fermentation [35]. It is clear from previous studies that two media are used, a growth media and a fermentation media [5, 34, 45, 46]. After the growth of these microorganisms in the growth media, they are transferred to the fermentation media. In this case, the fermentation media is known as the curdlan fermentation media (CFM), which is used for submerged fermentation [5]. The mode of fermentation commonly employed is shake flask fermentation. Occasionally, 5 L bioreactors have also been used [46, 47]. Curdlan is a secondary metabolite, that is, the production of curdlan starts to post the growth phase during conditions of nitrogen starvation/limitation [48].

Optimization of curdlan production

The purpose of optimization is to get maximum yield and/or productivity of curdlan by varying certain parameters such as minimal salts, carbon source, pH, nitrogen source, temperature, and hours of fermentation. To identify the significant variables affecting curdlan production, ‘one-factor-at-a-time’, Plackett–Burman design (PBD) has been used. For further optimization, the significant variables obtained were then chosen through response surface methodology (RSM) wherein central composite design (CCD) has been used to obtain a quadratic model [7]. Different strategies have been used to obtain a good yield of curdlan in the past. This is represented briefly in Table 4.

The seed culture medium is first used to culture the bacteria such that they get past the lag phase and enter the log phase of growth. Most of the studies done on curdlan production use the following common ingredients in their seed culture medium: sucrose, peptone, and yeast extract at pH 7.0 [7, 34, 45–47, 49].

Most studies prefer using sucrose as the carbon source as opposed to glucose. This is because the utilization of glucose takes place at a much higher rate as it is a monosaccharide leading to a higher demand in glucose requirement, which increases the cost as glucose is more expensive than sucrose [51].

Extraction and purification of curdlan

The most frequently used methods for biopolymer recovery are adsorption, chromatography, membrane separation, precipitation, and solvent extraction [52]. Recovery of the biopolymer is followed by purification. Purification methods include electrodialysis, reverse osmosis, distillation, ozone purification, and purification using supercritical fluids [53, 54]. Precipitation is widely used to extract curdlan from the fermentation media. This method improves the recovery in terms of accessibility and filterability. The usual steps involved in extraction by

Table 4 Overview of fermentation process conditions and yield

Bacterial strain	Carbon source	Process conditions	Yield (g/L)	References
<i>Bacillus</i> sp.	Sucrose	180 rpm, 20 h, 30 °C, pH 7.0	3	[34]
	Glucose	180 rpm, 96 h, 30 °C, pH 7.5 Inoculum size: 10%	7.13	
<i>Agrobacterium</i> sp. ATCC 31,749	Sucrose	200 rpm, 168 h, 30 °C	40.2	[45]
<i>Agrobacterium</i> sp. ATCC 31,750	Sucrose	120 h	60	[47]
	Sugarcane molasses	Inoculum size: 5%	42	
<i>Agrobacterium</i> sp. ATCC 31,749	Glucose	200 rpm, 120 h, 30 °C	5.73	[50]
<i>Agrobacterium</i> sp. ATCC 31,749	Corn distillers solubles	Inoculum size: 5%	7.72	
	Glucose syrup from Cassava, Glucose syrup from maize, High maltose syrup from maize	120 rpm, 15 d, 30 °C	85% yield with maize maltose	[49]
<i>Agrobacterium</i> sp. IFO 13,140	Sucrose	250 rpm, 5 d, 30 °C	50% yield with maize glucose	[7]
<i>Alcaligenes faecalis</i>	Sucrose	Inoculum size: 10%	5.02	
	Orange peel hydrolysate	200 rpm, 120 h, 32 °C for shake flask 600 rpm, 32 °C, 5 L bioreactor Inoculum size: 8%	9.04 23.24	[46]

the precipitation method are as follows: centrifugation, alkali solubilization, and precipitation by acid addition. Fermentation media is subjected to centrifugation at 8000–10,000 rpm, and the supernatant is discarded. This is followed by solubilizing the sediment (which consists of curdlan and cells) by the addition of NaOH and centrifugation. And finally, HCl or acetic acid is added to the supernatant leading to precipitation of curdlan, which is then washed with water and dried or lyophilized [31, 34, 37, 49]. The precipitation method is a modification of the method employed in industries for the purification of curdlan. In industries, the curdlan that is produced is purified by solubilizing it in a solution of a strong alkali followed by spray-drying it; then it is neutralized by washing with water [15].

Since curdlan is extensively used in food, care must be taken that it is purified enough to be used as an additive. Once the curdlan is obtained in the supernatant, before precipitation, it was dialyzed using water for 24 h at 4 °C under stirring conditions, then the curdlan was precipitated. The precipitants were then washed multiple times with acetone and deionized water. The purified curdlan was then loaded on high-performance liquid chromatography (HPLC) and compared with standard curdlan [46]. In contrast to this, the precipitated curdlan was harvested and then given a water wash to remove any salts that might have been present. The precipitate was then suspended in the water again and loaded onto HPLC which was equipped with a gel permeation column [39]. A basic framework of the downstream processing of curdlan is portrayed in Fig. 5.

Characterization of curdlan

Characterization is the study of certain properties of the obtained product to understand its properties. In the case of curdlan, these factors include dry cell mass, concentration, average molecular weight, thermal gel formation, monosaccharide composition, morphology, water holding capacity, oil holding capacity, and infrared spectrum. These are shown briefly in Table 5.

Applications

Microbial exopolysaccharides are gaining immense importance due to their applications in various sectors due to their biodegradability, cost-effectiveness, and abundance [36, 58]. Curdlan is one such exopolysaccharide and biopolymer which has applications in fields such as the food industry, biomedical industry, and pharmaceutical industry. Curdlan is tasteless, colorless, and odorless, and in aqueous solutions, two kinds of gels are formed, viz. thermo-reversible (low-set) gel and thermo-irreversible (high-set) gel [15]. When the temperature of an aqueous solution is increased to up to 55 °C and then cooled, it forms a thermo-reversible gel, whereas the thermo-irreversible gel formation occurs when the solution is approximately at 80 °C with subsequent cooling [13].

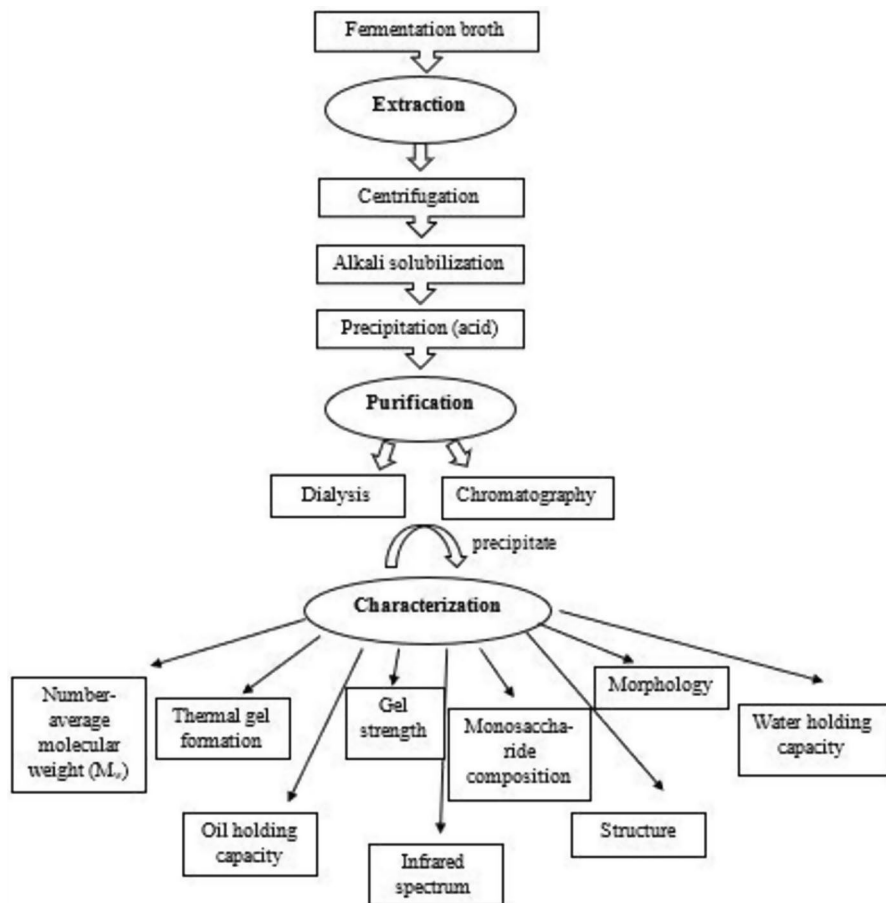


Fig. 5 Downstream processing of curdlan

Food industry

In 1996, the U.S. Food and Drug Administration (FDA) approved curdlan for use in the food industry [15]. Post-approval in 1996, USA launched curdlan into the market by the name Pureglucan™. It was also approved in Japan, Taiwan, and Korea in 1989 [21]. Curdlan can be incorporated into food by two methods. One is the direct addition of curdlan to already existing food in very low quantities (<1%), and the other is to make new types of food products where it is used in much higher quantities [13]. High-set gels of curdlan are widely used as compared to the low-set gels. Due to curdlan's impeccable rheological properties, it has been rendered as a gelling and thickener in the food industry [13]. It has also been used as a stabilizer in food, and it can mimic fat (when hydrated and heated, mimics mouth feels of fatty products), meat, and seafood too [31]. Its use is being evaluated in fat-replacement studies for frankfurter-type cooked sausage, meat patties, and other processed meat

Table 5 Properties, methods, and findings of characterization

Property	Method	Findings	References
Number-average molecular weight (M_w)	Gel permeation chromatography, high-performance liquid chromatography	1.6×10^5 — 6.6×10^5 Da	[7, 39, 55]
Thermal gel formation	Lyophilized powder dispersed in water, stirred in magnetic stirrer to get a uniform suspension, and heated at certain temperatures to form a gel	Low-set gel: 65 °C/1 h High-set gel: 95–100 °C/10 min to 1 h	[15, 42]
Gel strength	Texture analyzer used	77 ± 2 to $83 \pm 1 \times 10^3$ N 589 g/cm^2	[15, 42]
Monosaccharide composition	Hydrolysis is done using trifluoroacetic acid (TFA). Hydrolysate subjected to TLC. Separation using acetonitrile/water. Plates are sprayed with sulfuric acid–methanol and heated. Brown-black spots are seen which were compared with glucose standard Acid hydrolysis using 3 N HCl followed by 3,5-Dinitrosalicylic acid (DNSA) method and HPLC	Curdlan is made exclusively of only glucose units as the hydrolysate gave only one spot which corresponded to the glucose standard A peak at 6.02 min—corresponds to glucose	[55] [34]
Morphology	Scanning electron microscope (SEM) at a voltage of 15 kV	Commercial curdlan: Granules are collapsed or invaginated, measuring 10–100 μm in diameter Curdlan (lyophilized) produced by <i>Agrobacterium</i> IFO 13,140: flaked with irregularities	[15] [15, 56]
Water holding capacity (WHC)	Diluted in distilled water, homogenized and centrifuged. WHC is defined as a gram of water absorbed per gram of curdlan	Commercial curdlan: $4.6 \pm 0.4 \text{ g/g}$ Commercial pre-gelled: $2.20 \pm 0.08 \text{ g/g}$ Microbial (pre-gelled): $3.6 \pm 0.3 \text{ g/g}$	[15, 56]
Oil holding capacity (OHC)	OHC is a gram of oil absorbed per gram of curdlan	Commercial curdlan: $0.62 \pm 0.08 \text{ g/g}$ Commercial pre-gelled: $4.4 \pm 0.2 \text{ g/g}$ Microbial (pre-gelled): $8.7 \pm 0.1 \text{ g/g}$	[15, 56]

Table 5 (continued)

Property	Method	Findings	References
Infrared spectrum	Fourier transform infrared spectrophotometer (FT-IR)	The absorption band at 890, 1080, and 1160 cm^{-1} indicates the presence of β -(1,3)-glucan linkages. Bands between 990 and 1200 cm^{-1} are indicative of COC and CC stretching. Bands between 1200 and 1440 cm^{-1} attributed to CH and OH bending. A band at 1457 cm^{-1} corresponds to CH_2 in-plane bending	[7, 15, 31, 37, 42]
Structure	H NMR and C NMR spectroscopy	H NMR: 7 protons ~ 3.0 and 4.0 ppm C NMR: 6 resonances ~ 103.8, 74.2, 87.32, 68.88, 77.2, 61.25 ppm (C-1, C-2, C-3, C-4, C-5, C-6) represent the β -(1,3)-D-glucan backbone	[6, 7, 38, 41, 55, 57]

products. It was discovered that replacing fat, which is currently at 20% in full-fat meat patty formulations, with 0.25 percent curdlan, and around 5% water in lean meat is adequate [59]. Since curdlan improves the textural quality, thermal stability, and water-retention capacity of food, it has been used in foodstuffs such as noodles, frozen foods, sauces, processed/packaged food [7]. In Japan, curdlan is widely employed in producing noodles. Curdlan's thixotropic properties are useful in sauces and gravies. The recommended amount for usage, in this case, is 0.3–0.5% [59].

Curdlan can also be used in food in its gel form as the gels are tolerant to freeze-thawing, have no color, no taste, and no odor. So, it can be used to impart any color or taste of choice. This can be applied in the production of vegan meat wherein curdlan can imitate meat, poultry products, and seafood. In the case of meat and seafood, gels mimic the texture and their structure, e.g., in vegan sausages and ham, crabmeat, and prawns [13]. Other vegetarian foodstuffs such as tofu can be made using curdlan which will not only mimic tofu but also make it freeze–thaw stable which is currently a problem in tofu food items. At present, curdlan comprising tofu noodles are in use in Japan and Korea [59]. Curdlan gels can also be utilized to make flavored jelly-like food products and confectionaries which can be served either hot or cold. In the case of food packaging, the most commonly used material is plastic which has advantages such as low cost, great mechanical qualities. But, the main disadvantage of using plastic is the environmental hazard that it poses due to its low biodegradability. Xanthan gum, due to its pseudoplastic behavior, has gained attention for food packaging [58]. In a recent study, edible biodegradable films were made from gums of xanthan and curdlan for food packaging [60].

The grafting of phenolic compounds onto polymers enhances their biological activities as compared to free phenolic compounds. Ferulic acid has been grafted onto curdlan successfully. These ferulic acid-grafted curdlan conjugates possess more radical scavenging ability along with antioxidant property [61]. These conjugates could be studied and developed as antioxidant agents in the food industry. The ferulic acid-grafted curdlan conjugate was used to stabilize the oil/water emulsion of beta-carotene which, in turn, led to higher bioaccessibility of the beta carotene. Hence, this conjugate can be developed to be used as a stabilizer in food emulsion delivery systems [62].

Nutrition studies on curdlan have shown that it does not have a caloric value. Therefore, it can be utilized in low-calorie food which will help calorie-conscious and diabetic people [6]. As mentioned earlier, it also helps in reducing the fat content of certain food products. Considering these health benefits along with the varied applications in the food industry, curdlan can help in creative and versatile food systems.

Biomedical and Pharmaceutical industry

β -glucans have always shown a positive effect on the human immune system being antitumoral and antimicrobial [36]. Curdlan, too, has shown several medicinal and pharmaceutical applications. Curdlan triggers some specific antimicrobial responses like phagocytosis and the formation of ROS (reactive oxygen species). It also stimulates downstream signaling in cells like neutrophils, dendrites, and

macrophages which, in turn, activate NF- κ B, mitogen-activated protein kinases, and some NFAT transcription factors [32].

It has been used in the encapsulation of some drugs such as theophylline, prednisolone, and indomethacin [32]. Chemical modifications of curdlan help in making curdlan more useful by altering its functionality, especially its water solubility [41]. Curdlan can be modified in several ways to make grafted curdlan, hydrogels, and nanocomposites of curdlan. Three-dimensional networks consisting of hydrophilic polymers are called hydrogels. Some of their properties include high flexibility, swelling ability, and biocompatibility. These properties render them useful in pharmaceutical, agriculture, drug delivery, wastewater treatment, and biomedical applications [58]. Curdlan derivatives such as curdlan-sulfate have anti-acquired immunodeficiency syndrome (anti-AIDS) activity [7]. Similarly, other derivatives of curdlan obtained by carboxymethylation and phosphorylation also have great potential [21]. Because of its ability to control and sustain drug release, it has also been utilized as a drug delivery carrier. For example, curdlan hydroxyethyl derivatives have been used for protein drug delivery and it has been used as a carrier of salbutamol sulfate [7]. Curdlan gum has also been successfully used to design the regioselective drug delivery system containing the antiepileptic drug, Lamotrigine [63]. Curdlan and its derivative phosphorylated curdlan have been used to design ionic hydrogels by chemical cross-linking for tetracycline hydrochloride drug delivery studies. The results were promising and could have potential use in pharmaceutical applications [64]. In a recent study, a hybrid of carboxymethyl-curdlan (CMCD) and silica (tetrakis (2-hydroxyethyl) orthosilicate—THEOS) hydrogel was prepared by the sol–gel process. To determine the drug release behavior of this novel hybrid hydrogel, bovine serum albumin (BSA) was employed as the model protein. The hybrid hydrogel allows easy diffusion of BSA through the large, well-connected network of the gel. The THEOS/CMCD hybrid hydrogels show promising results for drug delivery in the biomedical sector [41]. BSA was used as a model protein in another study which aimed to determine whether curdlan gelation through the dialysis method helps in obtaining a protein delivery carrier as well as tap its potential in tissue engineering [65]. Two kinds of hydrogel carriers (Cur/BSA L and Cur/BSA H) were obtained since two different concentrations of the cross-linking agent (CaCl_2) were used. Both the hydrogel carriers showed good swelling ability exhibiting controlled release of BSA up to 4 weeks. However, the Cur/BSA L hydrogel proved to be much better which could be attributed to the lower concentration of CaCl_2 used. Therefore, Cur/BSA L hydrogel seems to be the more promising among the two for tissue engineering applications [65]. Similar to this, to improve the protein delivery through the gastrointestinal tract, the encapsulation and in vitro release of BSA which is within the interpolyelectrolyte complex (PEC) made from xanthan gum and trimethyl chitosan chloride (TMC) were investigated. It was learned that BSA was released without any degradation and was stable [66]. Xanthan-based hydrogels are also being studied for their potential use as scaffolds in tissue engineering and wound healing applications [58]. Agar, a polysaccharide-based gel, is soft but not flexible or tough. On the other hand, curdlan hydrogels are soft, flexible, and tough. Curdlan is the only polysaccharide-based hydrogel that possesses all

these three properties simultaneously [67]. This makes it a potential option to be used as soft tissue in tissue engineering studies as well as regeneration studies.

Curdlan and polyvinyl acid (PVA) have been cross-linked to form scaffold composites. The in vivo study in rats revealed that these scaffold implants were safe to use with no harmful changes. These have great potential to be employed in bone tissue engineering [68]. Apart from these applications, nanocomposite curdlan is of recent interest too. Nanocomposite antibacterial films consisting of glycyrrhiza polysaccharide (GP)-stabilized silver nanoparticles and curdlan have been developed. Curdlan acts as the base matrix for this film. These films could have potential use in wound dressing applications [69]. Silver nanoparticles have also been used in conjunction with trimethyl chitosan chloride (TMC) to form TMC/Ag nanocomposites which inhibited the pathogenic bacteria *Mycobacterium tuberculosis* [70]

Solid lipid nanoparticles (SLN) have been gaining a lot of attention for use as a drug delivery system. SLN has been made wherein the lipid core is cocoa butter whereas curdlan acts as the shell material. A calcium channel blocker, Verapamil, which is ordinarily used for the treatment of high blood pressure, angina, and tachycardia was loaded onto these SLN to study if verapamil helps in overcoming multidrug resistance in cancer cells. It was seen that the drug release rate was remarkably sustained when the drug was loaded onto the SLN [71].

Patents

Patents on curdlan have been either for producing compositions from curdlan or utility-based curdlan patents. USA, China, and Japan are a few of the places which have patents on different compositions and formulations on curdlan. Following are few patents on curdlan over the past 20 years:

Curdlan-containing composition and product comprising curdlan-containing composition (US10561166B2)

The main objective of this invention was to make a curdlan-containing composition to overcome the problem of aggregation of curdlan when dispersed in water and also to provide foodstuffs made of this curdlan-containing composition. In this invention, curdlan was granulated with alkali salt thereby preventing the aggregate formation of curdlan when dispersed in water. This invention helps in forming a curdlan solution very easily which can be used in foodstuffs and other applications. Few potential uses examples using the curdlan-containing composition according to the present embodiment include foodstuffs such as processed meat items like hams and sausages, processed seafood items such as fish paste, desserts such as jellies, puddings, mousses, and yogurts, and confectionaries such as gum and candies. Examples of beverages are soft drinks, fruit juices, jelly-like beverages, and pharmaceutical usage includes capsules, external skin preparation, and ointments [23].

Aqueous solution or hydrogel of carboxymethyl-curdlan (WO/2018/014373A1)

This patent has provided a preparation method of aqueous solution or hydrogel of carboxymethyl-curdlan with low concentration and low substitution degree. The preparation method is simple and requires the addition of no other organic solvent or chemical cross-linking agent. When this aqueous solution or hydrogel is used as a thickener, it has excellent thickening and gelling properties at a very low concentration and can provide great aesthetic property [24].

Substitute for fat within the meat and the forming method thereof (US9295277B2)

The fat content of regular meat products is anywhere between 20 and 50%. However, as per the WHO, saturated fatty acid should not be more than 10%, trans fat should not cross 1% and cholesterol should be less than 300 mg. Hence, to solve this problem few technologies have been tried but the problem of complicated forming process, not low enough fat content, and shape change at higher temperatures has been noticed. This invention provides a method for forming a meat fat substitute. The forming composition thereof includes a combination of curdlan and locust bean gum, at least one starch, lactose, and water. The meat substitute is thermo-irreversible, and the fat content is 0–5 weight %. Curdlan can help in dietary calorie control and obesity control [25].

Pet food (US20180343894A1)

This present invention is a method of making solid pet food. Pet food consists of proteins from different sources. A large chunk of these proteins come from animal products with high functionality. Highly functional proteins give a good texture and have good water binding and fat binding properties. However, these proteins are not widely available for use in pet food as they are mainly used for human consumption. Proteins with low functionality do not form solid textures. Therefore, this invention provides a solution to these problems with their formulation of pet food using curdlan. This invention provides a solid food component which is a mixture of curdlan and a protein with a moisture content of about 40%. This solid pet food is in the form of an aggregate, like a chunk [26].

Methods and compositions for conformance control using temperature-triggered polymer gel with magnetic nanoparticles (US20150159079A1)

This invention is a method for enhanced recovery of oil by improving the reservoir volumetric sweep. It consists of injecting the wellbore with a conformance control, high-viscosity solution of polymer that provides a higher flow rate which will distribute into the high-permeability layer than the low-permeability layer.

This conformance control polymer solution will comprise one or more polymers like curdlan, a cross-linking agent, and paramagnetic nanoparticles [27].

The manufacturing method of liquid crystal gel composed of curdlan (JP2007084615)

This patent deals with a method for producing filamentous, columnar, or spherical liquid crystal gel made of curdlan. The objectives of this invention are to provide a method for making liquid crystal gel with curdlan in which curdlan is both the gelling agent and the liquid crystal forming agent, is biodegradable and not toxic to humans, has an optical birefringence gradient, and is capable of elucidating transparency [28].

Agent for stimulating the natural defenses of plants, useful as antiviral, antibacterial, antifungal, and insecticide, comprises curdlan sulfate (FR2865350A1)

This invention is about an agent for activating plants' natural defenses, especially in agronomically beneficial plants and ornamental plants. The stimulation of natural defenses of plants to recognize attacks from pathogens such as bacteria, virus, fungus, or an insect is done by the development of a set of biological modifications that will give a said plant resistance to locate the attacker/ pathogen. Elicitors when brought near a plant will stimulate the same defensiveness as when a plant develops a response when a real pathogen is in contact. Curdlan sulfate is used as an elicitor in aqueous compositions and will serve the purpose of stimulating the resistance defenses in plants [29].

Meat products and processed meat products coated with curdlan gel film (EP1386547A1)

The objective of this invention was to produce safe edible coatings on meat and processed meat products that do not cause any allergies, that is, safe for consumption by humans and do not produce a large amount of waste. This was achieved by applying a thin coat of alkaline curdlan over the weakly acidic surface of meat products. This will form a thin layer of curdlan gel over the surface of meat due to neutralization. Examples of meat wherein this invention can be used are pork, beef, mutton, and poultry meat. Examples of processed meat wherein this invention can be applied are sausages, hams, and salami [30].

Future scope

Most of the studies have used different sugars as carbon sources for the production of curdlan. Therefore, there is a scope of replacing these sugars with low-cost materials such as fruit wastes and crop processing coproducts to lower the cost of manufacturing. Curdlan has also been tried in the biomedical industry, but its applications are curtailed due to its water insolubility. Due to this, functionalized curdlan has started gaining more recognition as discussed in this review. Research in

this particular field of chemical modification of curdlan is the need of the hour to expand its applications. Another area of application for modified curdlan that can be ventured into is wastewater treatment on the similar lines of modified xanthan gum nanocomposites and hydrogels that have been employed for this purpose.

Conclusion

This review aimed to shed light on the structure, production, properties, optimization, and characterization of curdlan. The main focus of this review was to feature curdlan's innumerable applications. Curdlan has a multitude of applications as detailed earlier and is also commercially important. The most reliable way to make the best use of curdlan is through its chemical modification which increases its applications. However, one of the main obstacles is to commercialize the product further as other commercially available polysaccharide gums are comparatively cost-effective.

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Declarations

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