

REVIEW

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# Production of biodegradable microbial polymers from whey

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## Abstract

Current research in industrial microbiology and biotechnology focuses on the production of biodegradable microbial polymers as an environmentally friendly alternative to still dominant fossil-based plastics. Microbial polymers have an extensive biotechnological potential and are already widely used in a variety of fields ranging from medicine to technology. However, their increase in production and wider use is hampered by the high cost of raw materials and therefore requires a focus on cheaper inputs, including dairy by-products and waste such as cheese whey (CW). This is an environmentally unfriendly by-product of milk processing and reducing it would also reduce the risk of environmental pollution. This review summarises current knowledge on the use of CW and derived products to obtain commercially important microbial polymers, including information about producer cultures, fermentation techniques and methods used, composition of culture medium, cultivation conditions and productivity of bioprocesses. The main methods and applications of cheese whey pre-treatment are also summarised.

**Keywords:** Biopolymers, Bacterial exopolysaccharides, Polyhydroxyalkanoates, Whey

## Introduction

Current topical research in biotechnology is increasingly focused on the development of biodegradable polymers as an alternative to the still dominant petrochemical plastics (Siracusa et al. 2008; Gopi et al. 2016; Narancic and O'Connor 2019). There are several reasons for this, first of all, the urgent need to face up to the challenges posed by global environmental pollution in every possible way (Pandey and Singh 2018; Sadh et al. 2018; Narancic and O'Connor 2019). Therefore, the increased use of renewable resources (i.e. raw materials) and biodegradable polymers of microbial origin obtained therefrom (Rehm 2010; Freitas et al. 2011; Kreyenschulte et al. 2012; Shanmugam et al. 2019) could become particularly important in this context. All the more so, because microbial polymers such as polyhydroxyalkanoates (PHAs) and exopolysaccharides (EPS) may also possess the desirable characteristics of fossil-based plastics (Van de Velde and

Kiekens 2002; Babu et al. 2013). Especially so when used in the form of bio-based microbial polymer composites that could get more versatility and functionality over their components (Chen et al. 2014; Ul-Islam et al. 2015; Sirvio et al. 2018). As a result, microbial polymers also possess extensive biotechnological potential and there are already a great variety of promising applications ranging from medical to technological domains (Rehm 2010; Kreyenschulte et al. 2012; Shanmugam et al. 2019). Focusing on renewable resources as nutrients for microbial growth and polymer synthesis, it is important to expand the use of agricultural by-products and waste (Arancon et al. 2013; Sadh et al. 2018; Tsang et al. 2019), including those from dairy industry such as whey—a main product of cheese and casein production (Prazeres et al. 2012; Lappa et al. 2019), especially since it is not only an environmentally unfriendly product, but also actually a threat to it (Colombo et al. 2016). Thus, the increased use of a renewable feedstock like cheese whey (CW) not only has environmental benefits, but also facilitates the industrial production and application of biopolymers through a significant reduction in the cost of microbial nutrient

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media which is a prerequisite for the overall economic efficiency of their biosynthesis (Gahlavatt and Srivastava 2017). The use of whey or its derivatives for the production of microbial polymers is mentioned in a few specialised reviews on the valorisation potential of CW or dairy waste, including on the properties of individual polymers, but is limited to a narrow range of these compounds such as PHAs (Mollea et al. 2013; Koller et al. 2016; Ryan and Walsh 2016; Amaro et al. 2019; Tsang et al. 2019) and xanthan (Mollea et al. 2013; Özcan and Öner 2015) or bacterial cellulose (BC) (Lappa et al. 2019). Besides, these reviews have employed a relatively limited data volume, and production efficiency, even for such individual polymers, has not been actually compared. This paper provides a brief overview on current approaches to obtain a broader range of commercially viable microbial polymers from whey and whey-derived products.

### Microbial polymers: structural features, properties and applications

Microbial polymers represent a diverse range of macromolecules that include four major classes of which bacterial polysaccharides and polyesters have the widest application and commercial potential compared to polyamides and inorganic anhydrides (polyphosphates) (Rehm 2010). During past decades, microbial polymers, polysaccharides and polyesters have offered a variety of novel applications and retain the potential to replace common, but less favourable materials (Kreyenschulte et al. 2012). In particular, the substitution of non-degradable fossil-based plastics is of considerable and growing interest as it allows for the environmentally and economically beneficial disposal of major waste streams (Luckahan and Pilla 2011; Iram et al. 2019; Narancic and O'Connor 2019). In turn, polysaccharides produced by microorganisms can be classified (Donot et al. 2012) into three main groups based on their cellular location: (a) cytosolic polysaccharides which are the carbon and energy source for cells, (b) polysaccharides such as lipopolysaccharides, peptidoglycans and teichoic acids, which form the cell wall, (c) extracellular polysaccharides exuded out of cells in the form of capsules or biofilms, known as EPS. EPS can also be divided into two groups known as homopolysaccharides (HoPS) which contain only one type of monosaccharide (e.g. glucose or fructose) and heteropolysaccharides (HePS), which are composed of repeating units containing different monosaccharides and non-sugar molecules (Lo et al. 2007). Unlike polysaccharides, microbial polyesters (PHAs) are intracellular polymers and due to their composition (varied (*R*)-3-hydroxy acids) should be qualified as heteropolymers (Koller et al. 2016). Most microbial EPS are linear HePS consisting of three to seven different

monosaccharides arranged in groups to form repeating units. The monomers may be pentoses, hexoses, amino sugars or uronic acids, etc. (Freitas et al. 2011; Rehm 2010; Shanmugam et al. 2019). As a result, microbial EPS may be ionic or non-ionic and represent primarily linear molecules with side chains of varying length and complexity being attached at regular intervals (Shanmugam et al. 2019). In general, EPS biosynthesis can be divided into three main steps: (a) assimilation of carbon substrate, (b) intracellular synthesis of polysaccharide, (c) exudation of polymer out of cell (Becker 2015; Donot et al. 2012). It is carried out on EPS by many cultures of Gram-negative and Gram-positive bacteria, which produce EPS of varying molecular weight and composition, hence of different physico-chemical and functional properties (Lo et al. 2007). Although many producer cultures are available, and therefore, also EPS and PHAs synthesised, only a few of them are characterised by sufficient productivity, and hence commercial potential. The most relevant properties, structural features and applications of such microbial polymers are summarised in Table 1. This demonstrates that the structure and properties of microbial EPS and PHAs are quite diverse and the ability to produce them is relatively widespread amongst bacteria. Accordingly, the actual and even more potential applications of these polymers are also very comprehensive, especially in the food industry, medical and pharmaceutical fields, electronics, etc. (Table 1), which, in turn, stimulate demand and boost their production. However, at the same time, as the annual world production volumes for microbial polymers are growing and already exceed 100,000 metric tonnes, they still represent a very small share of the current polymer market, even compared to some other bio-based plastics (Rehm 2010; Colombo et al. 2016; Revin et al. 2016; Bustamante et al. 2019). Thus, microbial polymers are mainly used in high-value market niches such as biomedicine, pharmaceuticals and cosmetics where they better meet the quality and functional requirements compared to “traditional” ones (Poli et al. 2011). This situation is undesirable as the use of microbial polymers is still inadequate in other important fields; especially with regard to packaging materials where plastics consumption has reached to 40% of the total annual world production (more than 300 million tonnes) (Colombo et al. 2016; Luzi et al. 2019). As these, mainly fossil-based materials, are not biodegradable and currently only partly recycled it leads to the accumulation of wastes that remain in the environment for hundreds of years and pose a serious threat to it (Colombo et al. 2016). Therefore, the use of biodegradable microbial polymers such as PHAs, EPS and their composites with properties that meet the requirements for packaging materials could substantially contribute in this respect

**Table 1** Commercially important microbial polymers: the most relevant properties and applications<sup>a</sup>

Polymer class	Producer	Monomers	Characteristics of primary structure	Properties	Applications
Xanthan	<i>Xanthomonas</i> spp., mainly <i>X. campestris</i>	Glucose, mannose and glucuronate	$\beta$ -(1,4)-linked repeating extracellular heteropolymer consisting of pentasaccharide units	Hydrocolloid —High viscosity yield at low shear rates even at low concentrations; —Stability over wide temperature, pH and salt concentrations ranges	Foods, petroleum, industry pharmaceuticals, cosmetics and personal care products, agriculture
Alginate	<i>Pseudomonas</i> spp. and <i>Azotobacter</i> spp.	Mannuronic acid and guluronic acid	$\beta$ -(1,4)-linked non-repeating extracellular heteropolymer	Hydrocolloid Gelling capacity Film-forming	Food hydrocolloid Medicine Surgical dressings Wound management Controlled drug release
Gellan	<i>Spingomonas</i> spp.	Glucose, rhamnose and glucuronate	$\beta$ -(1,3)-linked repeating extracellular heteropolymer consisting of tetrasaccharide units	Hydrocolloid —Stability over wide pH range Gelling capacity Thermoreversible gels	Foods Pet food Pharmaceuticals Research: agar substitute and gel electrophoresis
Curdan	<i>Agrobacterium</i> spp., <i>Rhizobium</i> spp. and <i>Cellulomonas</i> spp.	Glucose	$\beta$ -(1,3)-linked extracellular homopolymer	Gel-forming ability Water insolubility Edible and non-toxic Biological activity	Foods Pharmaceutical industry Heavy metal removal Concrete additive
Dextran	<i>Leuconostoc</i> spp. and <i>Streptococcus</i> spp.	Glucose	$\alpha$ -(1,2)/ $\alpha$ -(1,3)/ $\alpha$ -(1,4)-branched $\alpha$ -(1,6)-linked extracellular homopolymer	Non-ionic Good stability Newtonian fluid behaviour	Foods Pharmaceutical industry: Blood volume expander Chromatographic media
Levan	<i>Bacillus</i> spp., <i>Erwinia</i> spp., <i>Halomonas</i> spp., <i>Zymomonas mobilis</i>	Fructose	$\beta$ -(2,6)-linked, $\beta$ -(2,1)-branched extracellular homopolymer	Low viscosity, high water solubility, Anti-tumour activity Anti-inflammatory. Adhesive strength, Film-forming capacity	Food (prebiotic), Feed, Medicines, Cosmetics Industry
Pullulan	<i>Aureobasidium pullulans</i> <i>Cytaria</i> spp.	Glucose	$\alpha$ -(1,6)-linked maltotriose ( $\alpha$ -(1,4)-linked glucose) units extracellular homopolymer	Easily water soluble, high adhesion, sticking, lubrication, and film forming abilities, biocompatible	Food packaging edible coatings (food, pharmacy tablets), Cosmetics, Paper coating, Flocculating agent
Cellulose	<i>Gluconacetobacter</i> spp. (mainly <i>G. xylinus</i> ), <i>Komagataeibacter</i> spp., <i>Agrobacterium</i> spp., <i>Alcaligenes</i> spp., <i>Rhizobium</i> spp., <i>Sarcina</i> spp., <i>Azotobacter</i> spp.	Glucose	$\beta$ -(1,4)-linked extracellular homopolymer	High crystallinity Insolubility in most solvents High tensile strength Moldability	Foods (indigestible fibre) Biomedical Wound healing Tissue engineered blood vessels Audio transducers diaphragm
Polyhydroxyalkanoates (PHA) (polyesters)	<i>Azotobacter</i> spp., <i>Alcaligenes</i> spp., <i>Cupraivaidus</i> spp., <i>Haloferax</i> spp., <i>Pseudomonas</i> spp.;	(R)-3-hydroxy fatty acids	Intracellular heteropolymer	Thermoplastic or elastomeric materials, with melting points ranging from 40 to 180 °C, resistance to moisture, aroma barrier, UV stable, soluble in halogenated solvents	Biodegradable plastics Drug delivery Tissue engineering Food packaging biomaterial and matrices for displaying or binding proteins

<sup>a</sup> Data summarised in several previous reviews (Shanmugam et al. 2019; Freitas et al. 2011; Rehm 2010; Kreyenschulte et al. 2012)

(Siracusa et al. 2008; Azeredo et al. 2016; Popovic et al. 2018; Luzi et al. 2019). However, it should be emphasised that regardless of their excellent features and environmental advantages, more extensive use of microbial polymers in any field is still hampered by high production costs (Rehm 2010; Kreyenschulte et al. 2012; Amaro et al. 2019). In fact, the main factors responsible for the higher production expenses are the high costs of substrate and downstream processing, especially the former, since the cost of raw materials accounts for at least 40–50% of total production costs, wherein a carbon source contributes to 70–80% of total expenditures (Gahlawat and Srivastava 2017). As a result, growing attention is being paid to various by-products and wastes, mainly from the agricultural and food industries (Arancon et al. 2013; Sadh et al. 2018; Tsang et al. 2019) as potential alternative low-cost carbon and/or nitrogen sources that would be suitable for both microbial growth and polymer production. The use of sugar cane and sugar beet molasses, by-products and wastes from oleochemical (glycerol), dairy (cheese whey), distillery (dregs, thin stillage) industries, extracts and hydrolysates of various organic crops, etc. has been proposed and proven to be appropriate for this purpose (Freitas et al. 2011; Özcan and Öner 2015; Revin et al. 2018; Tsang et al. 2019). As a cheap, carbon-rich raw material available in huge, virtually unlimited quantities, cheese whey is of interest in this respect (Prazeres et al. 2012; Koller et al. 2016; Lappa et al. 2019).

### **Whey characteristics and pre-treatment modes to perform microbial fermentations**

Whey is the major by-product of the cheese or casein manufacture accounting for 80% to 90% of the processed milk and containing about 55% of milk nutrients. CW is rich in fermentable nutrients and contains (% w/v) lactose (4.5–5), soluble proteins (0.6–0.8), lipids (0.4–0.5) as well as mineral salts (0.5–0.7) and minor constituents such as lactic and citric acids, B group vitamins, etc. (Pescuma et al. 2015; Macwan et al. 2016; Ryan and Walsh 2016). CW composition may somewhat vary depending on type, sweet (obtained when adding rennet, pH 6–7) or acid (as a result of acidic fermentation, pH < 5) whey. Acid whey, also known as salty, has higher salt and lower protein content than sweet whey and also has less lactose (3.8–4.3% against 4.5–5% in sweet whey) (Pescuma et al. 2015; Macwan et al. 2016). Due to the high salinity, salty whey is more difficult to process as well as has higher disposal costs than sweet whey (Blaschek et al. 2007). Annually the world dairy industry produces more than 140 million tonnes of whey (Niknezhad et al. 2015) characterised by very high Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) levels, ranging from 27 to 60 kg/m<sup>3</sup> and 50 to 102 kg/m<sup>3</sup>, respectively.

It is determined by the high organic load of CW, especially of lactose (39–60 kg/m<sup>3</sup>) which is hard to dispose of and thus poses serious problems for the dairy industry and the environment (Güven et al. 2008; Prazeres et al. 2012; Niknezhad et al. 2015). To prevent and overcome these problems, various technologies for CW valorisation have been developed and applied (Arancon et al. 2013; Mollea et al. 2013; Macwan et al. 2016; Chanfrau et al. 2017; Lappa et al. 2019; Tsang et al. 2019). Two different approaches are currently used for this purpose (Mollea et al. 2012). The first one is based on the application of technologies to recover valuable compounds of whey such as proteins (individual or concentrate) and lactose or produce powdered CW. The second approach relies on the application of bioconversion, i.e. microbial fermentation to obtain a variety of value-added products, including single-cell proteins and oils, organic acids (e.g. lactic, succinic, propionic), bacteriocins and biopolymers (enzymes, PHAs, EPS) (Mollea et al. 2013; Chanfrau et al. 2017; Lappa et al. 2019). Although, in principle, CW is a suitable raw material for microbial fermentations, the main disadvantage is that its sole carbon source, lactose, cannot be fermented by certain industrially relevant microorganisms such as non-lactic bacteria, etc. (Pescuma et al. 2015). In addition, a whole whey can be characterised by relatively low carbon-to-nitrogen ratio (C/N) which adversely affects the production of biopolymers (Bosco and Champio 2010; Koller et al. 2019). Therefore, some pre-treatment of CW is required to overcome such limitations, especially with regard to the production of PHAs and EPS (Amaro et al. 2019; Koller et al. 2019). Moreover, whey is a complex, unsterile and often variable by-product, and thus, its direct application in both lab-scale and industrial fermentations might result in some difficulties and uncertainties. Therefore, several whey derivatives that have undergone a series of pre-treatments are often used instead of whey itself. One of these derivatives is whey powder, normally produced by spray-drying, which is diluted in water prior to fermentation and makes it possible to obtain a specific lactose concentration, longer storage of whey as well as avoiding seasonal variations of this by-product (Pisecky 2005; Chegini and Tacheri 2013; Amaro et al. 2019). Another type of pre-treatment regardless of whether CW is a liquid or powder is the removal of the majority of its proteins and other solids by ultrafiltration to create a whey permeate, that retains most of the whey lactose (Koller et al. 2007; Mollea et al. 2013). This protein removal is also commonly obtained by the heat treatment of acidified (pH close to 4) CW followed by filtration and centrifugation to get a so-called whey supernatant (Pantazaki et al. 2009). CW permeates or supernatants are easier to work as they represent sterile, homogenous and clear



solutions which may also be obtained and stored in the form of spray-dried powders (Pisecky 2005). In cases where the producer strains are unable or only partially able to use lactose as the sole carbon source, transformation needs to be performed either using  $\beta$ -galactosidase enzymes (Marangoni et al. 2002; Torres et al. 2010; Semjonovs et al. 2015) or by much less expensive lactose hydrolysis to the equimolar glucose/galactose mixture using mineral acids (Lin and Nickerson 1977; Kucera et al. 2018). Besides, protease-hydrolysed whey or its (hydrolysed/non-hydrolysed) protein fraction (so-called retentate), retained after permeate separation, can be used as an efficient complex nitrogen source (alone or in combination with permeate) to perform microbial fermentations (Obruca et al. 2014; Koller et al. 2019).

## Production of microbial polymers from whey and whey-derived products

### Polyhydroxyalkanoates

PHAs are polyesters of microbial origin synthesised by a wide range of bacteria (Table 1) from a variety of substrates (sugars, lipids, renewable substrates of various origins, etc.). They represent an energy-rich storage material that accumulates in cells in the form of granules under stressful conditions, nutrient (such as oxygen, nitrogen or phosphorus) limitations, etc. in the presence of excess carbon source and the biosynthesis proceeds by PHA synthase (PhaC, EC 2.3.1) from (*R*)-3-hydroxyacyl-CoA as precursors (<https://www.brenda-enzymes.org/enzyme.php?ecno=2.3.1.B2>), whereby the different PhaC types (B2–B4) are determined by the *R*-chain length (Rehm 2010; Amaro et al. 2019; Koller et al. 2016). PHAs are considered to be a particularly attractive and effective alternative to conventional plastics due to the mechanical properties similar to fossil-derived polymers (such as polypropylene), biocompatibility and complete biodegradability (Nath et al. 2008; Koller et al. 2016; Gahlawat and Srivastava 2017). The valuable properties of PHAs determine their widespread application in a wide variety of fields (Table 1), as well as promote their more cost effective production, including the extensive use of renewable resources (Arancon et al. 2013; Özcan and Öner 2015; Sadh et al. 2018; Koller and Brauneegg 2018; Tsang et al. 2019). In this context, relatively extensive research has been also conducted on the use of CW and its derivatives for microbial production of PHAs, including a number of EU-funded projects (WHEYPOL, LIFE + WHEYPACK, etc.) carried out by collaborative consortia of academic and industrial partners (Koller et al. 2007; Koller et al. 2012; Koller and Brauneegg 2018; Alborch 2014). Although many attempts to find more efficient producer strains, optimum nutrient combinations, and cultivation conditions have only resulted in moderate or fairly low

productivity, a number of results have also been obtained which demonstrate that the use of CW or derivatives for the production of PHAs can be both industrially and commercially viable. These data are summarised and represented in Table 2. This demonstrates that higher productivity of PHAs synthesis compared to pure cultures can be achieved using mixed microbial cultures (MMCs) and especially when engineered, i.e. recombinant cultures are used as producers. In addition, good productivity is achieved both when lactose-degrading genes are introduced into a culture that is able to synthesise polyhydroxybutyrate (PHB) by *Cupravidus necator* (Povolo et al. 2010) and when genes synthesising PHB are introduced into the *Escherichia coli* strain (Ahn et al. 2000) that is already able to hydrolyse lactose. This second possibility has proved to be particularly effective since such a recombinant (*Escherichia coli* CGSC 4401) has achieved very high (1.35–4.60 g/L/h) volumetric productivity, which also depends on the type of fermentation and culture conditions (Ahn et al. 2000; Ahn et al. 2001; Park et al. 2002). The data indicate (Table 2) that the most efficient way to produce PHAs is by the fed-batch fermentations in bioreactor, irrespective of the culture method used (pure cultures, MMC or engineered strains). If the fed-batch process is improved, an excellent, still unbeatable, level of volumetric productivity (4.60 g/L/h) is also achieved by the employed cell-recycle system to overcome the problems arising from the continuous addition of feedstock to fed-batch cultures, i.e. to prevent the effect of a rapidly growing fermentation broth volume in the bioreactor (Ahn et al. 2001; Koller et al. 2016). Reasonably good PHA productivity is also achieved by Mixed Microbial Cultures (MMC) using a three-stage process comprising (1) acidogenic fermentation of carbon source to produce volatile fatty acids (VFAs) as the precursors for PHA biosynthesis, (2) culture selection stage, where microorganisms are selected based on PHA storage ability, (3) PHA production stage where the selected microorganisms are fed with the VFA produced in the 1st stage (Duque et al. 2014; Colombo et al. 2016). This method has certain advantages as it does not require strict sterile conditions, makes better use of complex substrates like industrial wastes or by-products and makes it possible to modify the structure of PHAs according to the composition of VHAs in the fermentation medium (Table 2).

### Xanthan

The industrially most important biopolymers also include xanthan (Table 1), which is produced by the phytopathogenic, Gram-negative bacterium of the genus *Xanthomonas* (mainly *X. campestris* pv. *campestris*) and, unlike PHAs, represents an extracellular polysaccharide. It is composed of polymerised

pentasaccharide repeating units which are assembled by the sequential addition of glucose-1-phosphate, glucose, mannose, glucuronic acid, and mannose on a polyprenol phosphate carrier provided by a set of enzyme reactions involving multiple monosaccharide-specific glycosyltransferases, acetyltransferases and ketal pyruvate transferase from nucleotidyl (UDP, GDP) derivative precursors (Katzen et al. 1998; Rehm 2010; Freitas et al. 2011). Xanthan gum is rightly considered to be the first industrially produced biopolymer and also as the most extensively studied and widely accepted commercial EPS (Rossalam and England 2006; Rottava et al. 2009; Freitas et al. 2011). It has a very broad range of applications in various fields (Table 1), largely due to its excellent rheological properties (Rottava et al. 2009). CW and derivatives have also been mentioned as potential carbon sources (Mollea et al. 2013; Özcan and Öner 2015) in the relatively large number of studies focusing on the use of various renewable resources for microbial production of xanthan. In comparative terms, such studies are not very numerous, but they do confirm that these carbon sources are also quite promising. Thus, relatively high productivity was achieved using the lactose-utilising *X. campestris* C7L isolate and different whey-based media (Nitsche et al. 2001). A two-stage fermentation strategy, combining unfiltered whey (0.35% protein) and filtered whey (0.18% protein), was proposed. The first stage, using unfiltered whey medium, showed a xanthan production of 12 g/L (0.40 g/L/h) and a 45% yield, following by the second stage, with filtered whey addition, which produced a final xanthan concentration of 28 g/L (0.58 g/L/h) and a 75% yield.

The final broth viscosity reached 18,000 cP and the polymer produced in this combining system showed typical pseudoplastic and thixotropic behaviour (Nitsche et al. 2001). Slightly lower productivity was achieved using two different strains of *X. campestris* pv *mangiferaeindicae* 1230 and *X. campestris* pv *manihotis* 1182 (Silva et al. 2009). Maximum xanthan production was approximately 25 g/L (0.35 g/L/h), for both strains using Mozzarella cheese whey as the sole carbon source at the shake flask cultivation for 72 h. Noticeably higher productivity (35 g/L, 0.49 g/L/h) was obtained (Mesomo et al. 2009) using a related producer strain *X. campestris* pv *mangiferaeindicae* IBSBF 1230 also using the Mozzarella CW (lactose 44.8 g/kg) as the sole carbon source at the batch cultivation in bioreactor. In turn, cultivation of *X. campestris* ATCC 13,951 in shake flasks using deproteinised CW as the sole carbon source showed relatively lower productivity (20.3 g/L, 0.28 g/L/h) (Sobenes and Alegre 2015). Optimisation experiments were performed in shake flasks using *X. campestris* PTCC1473 and *X. pelargonii*

PTCC1474 producer strains for concentrations of carbon (deproteinised CW), phosphorus ( $\text{KH}_2\text{SO}_4$ ) and magnesium ( $\text{MgSO}_4$ ) in the culture medium (Niknezhad et al. 2015). *X. campestris* PTCC1473 was found to be characterised by higher xanthan formation productivity (16.65 g/L, 0.35 g/L/h) compared to *X. campestris* PTCC1474 (12.28 g/L, 0.26 g/L/h), and with higher polymer yields (0.42 g/g lactose and 0.27 g/g lactose, respectively). In addition, in the first case, the optimal CW concentration (62.5 g/L, 39.1 g/L lactose, respectively) was lower than the required 79 g/L (42.4 g/L lactose) for *X. pelargonii*. Relatively good productivity was achieved using deproteinised whey, i.e. CW permeate hydrolysed to an equimolar glucose/galactose mixture. Thus, using *X. campestris* ATCC13951 and hydrolysed CW permeate medium (glucose/galactose 43 g/L for both), a xanthan concentration of 28 g/L was achieved with a volumetric productivity of 0.29 g/L/h (Savvides et al. 2012). Older studies show relatively modest results. For example, cultivation of *X. campestris* NRRL B-1459 in deproteinised and hydrolysed CW (El-Sawah and Ashour 1999) gave 12.99 g/L xanthan (0.18 g/L/h). In addition, when non-hydrolysed whey is used, the concentration of xanthan is very low (0.4 g/L), indicating low  $\beta$ -galactosidase activity in this producer. Not only CW but also hydrolysed milk permeate (Abd El-Gawad et al. 2001) can be used as a carbon source for the production of xanthan by *X. campestris* LIS3, yielding 26.8 g/L (0.28 g/L/h). Furthermore, enrichment of the medium with lactose (e.g. an initial concentration of 10%) can yield xanthan up to 43.9 g/L (0.46 g/L/h). To ensure an efficient hydrolysis of lactose, the expression of  $\beta$ -galactosidase in *X. campestris* was promoted when the *lac* genes from *E. coli* were inserted into *X. campestris* to get the recombinant strain *X. campestris* 17 (pKM $\phi$ LT), which was able to reach 3.60 g/L and 4.24 g/L of xanthan in the medium with 0.4% lactose and 10% whey, respectively. For the same purpose, several strains of *X. campestris* were genetically modified to get a number of transconjugants (plasmid SUP5011 from *E. coli* to *X. campestris*) and UV mutants with significantly higher  $\beta$ -galactosidase expression and abilities to produce xanthan about 10 g/L (Ghazal et al. 2011). Similar approaches have been used and comparable results obtained in previous studies of genetically modified producer strains *X. campestris* B1459 (Pollock and Thorne 1994) and *X. campestris* ATCC55096 with the ability to produce xanthan up to 20 g/L (San Bias et al. 1991).

### Alginate

Alginates also represent (Table 1) extracellular heteropolysaccharides (HePS) which are composed of mannuronic and guluronic acids that form block structures of poly-mannuronic acid sequences, poly-guluronic acid

**Table 2 Comparatively efficient microbial production of polyhydroxyalkanoates (PHAs) from whey and whey-derived products<sup>a)</sup>**

Substrate	Type of cultures/ producing strain	Culture method	Type of PHA	Volumetric productivity, g/L/h	References
Lactose (Equimolar mixtures of glucose and galactose)	Pure culture/ <i>Pseudomonas cepacia</i> ATCC 17759	Shaking flask	PHB	0.18	Young et al. 1994
Lactose whey supernatant	Pure culture/ <i>Methylobacterium</i> sp. ZP24	Shaking flask, Bioreactor, Fed-Batch	PHB	0.08 0.09	Yellore and Desai 1998; Nath et al. 2008
Dairy waste	Pure culture/ <i>Bacillus megaterium</i> SRKP-3	Bioreactor, Fed-Batch	PHB	0.31	Ram Kumar Pandian et al. 2010
Enzymatically hydrolysed whey permeate + 3HV <sup>b</sup> and 4HB <sup>c</sup> precursors	Pure culture/ <i>Haloferax mediterranei</i> DSM 1411	Bioreactor, Fed-Batch	P-(3HB-co-3HV-co-4HB)	0.14	Koller et al. 2007, Koller et al. 2012
Whey powder supernatant + additives	Pure culture/ <i>Alcaligenes latus</i> ATCC 29714	Shaking flask, Bioreactor, Batch	PHB	0.11	Berwig et al. 2016
Hydrolysed whey permeate + additives	Pure culture/ <i>Cupriavidus necator</i> ( <i>Ralstonia eutropha</i> DSM 545)	Bioreactor, Fed-Batch	P-(3HB-co-3HV)	0.17	Marangoni et al. 2002
Hydrolysed whey powder supernatant + additives	Pure culture/ <i>Haloferax mediterranei</i> ATCC 33500	Bioreactor, Batch	P-(3HB-co-3HV)	0.16	Pais et al. 2016
Fermented whey powder permeate	MMC <sup>d</sup> /Undefined	Bioreactor, Fed-Batch	P-3(HB-co-HV)	0.56	Duque et al. 2014
Fermented whey supernatant	MMC/Undefined	Bioreactor, Fed-Batch	PHB/P-3(HB-co-HV)	0.45	Colombo et al. 2016
Processed whey powder solution	Engineered culture/ <i>rec. Escherichia coli</i> CGSC 4401, harbouring pJC4 ( <i>Alcaligenes latus</i> PHA biosynthesis genes)	Bioreactor, Fed-Batch	PHB	1.42 2.57 4.60 1.35	Ahn et al. 2000a, Ahn et al. 2001, Park et al. 2002
Hydrolysed whey permeate + additives	Engineered culture/ <i>rec. Cupriavidus necator</i> mRePT ( <i>E. coli</i> lactose degradation genes)	Bioreactor, Batch	PHB	0.70	Povolo et al. 2010

<sup>a)</sup> Data summarised in recent specialised reviews (Amaro et al. 2019; Koller, 2016)

<sup>b)</sup> <sup>c</sup>P-3-Hydroxyvalerate (HV) and 4-hydroxybutyrate (HB), precursors pentanoic acid and  $\gamma$ -butyrolactone, respectively

<sup>d)</sup> Mixed microbial cultures, including consortia of activated sludge to produce volatile fatty acids, the precursors for PHAs' biosynthesis

sequences and mixed sequences, secreted by bacteria from the genera *Azotobacter* and *Pseudomonas* (Freitas et al. 2010; Rehm 2010). Most important in alginate biosynthesis is alginate synthase (EC 2.4.1.33), i.e. mannuronate transferase (<https://www.brenda-enzymes.org/enzyme.php?ecno=2.4.1.33>), which catalyses the polymerisation of beta-D-mannuronate residues (nucleotide-derived GDP-D-mannuronate) into a mannuronan polymer, an intermediate in the biosynthesis of alginate. The industrial importance of alginates is determined by their ability to modify the rheological properties of various aqueous systems, which, in turn, are highly dependent on polymer composition. They are widely used in the medical, pharmaceutical and food industries as a stabiliser, viscosifier and gelling agent (Khanafari et al.

2007; Rehm 2010; Trujillo-Roldán et al. 2015; Urtuvia et al. 2017). Whilst most alginates are still derived from seaweed and algae, there is an increasing focus on their microbiological production, including the use of renewable resources (Urtuvia et al. 2017). However, currently only a few studies have evaluated the use of CW and its derivatives for microbiological production of alginates (Khanafari et al. 2007 Trujillo-Roldán et al. 2015; Hendawy et al. 2019). Thus, the producer strain *Azotobacter chroococcum* NCBI MH249629 using the culture medium with deproteinised sweet whey (lactose 45 g/L) in shake flasks and under optimised conditions (concentration of lactose and nitrogen sources, pH, T<sup>0</sup>, agitation speed, cultivation time) achieved the alginate concentration of 5.65 g/L (0.08 g/L/h) (Hendawy et al. 2019), which is

commensurate with the capacities of other *Azotobacter* spp. and carbon sources (Urtuvia et al. 2017). Similar results were obtained using a related producer strain *A. chroococcum* DSM1723 and CW containing (lactose 49.1 g/L) medium. Under optimised conditions (pH, salt concentrations,  $T^0$ ) alginate concentrations above 5 g/L (0.05 g/L/h) were achieved, albeit with longer cultivation times and at a higher (35 °C) temperature. The aforementioned results indicate that both *A. chroococcum* producer strains have sufficient  $\beta$ -galactosidase activity, as non-hydrolysed CW is well-utilised for alginate synthesis (Hendawy et al. 2019; Khanafari et al. 2007). In contrast, another producer, *Azotobacter vinelandii* ATCC9046, was able to use only hydrolysed and deproteinised CW as the carbon source (reducing sugars 20 g/L), and only part of the galactose was utilised from hydrolysed lactose (Trujillo-Roldán et al. 2015). The resultant concentrations of alginates were also significantly lower (2.4 g/L, 0.024 g/L/h), but the use of CW significantly affected the properties of the polymer, including increased viscifying power (medium viscosity/g of alginate) already at 40–50 h of cultivation (Trujillo-Roldán et al. 2015). It is noteworthy that *A. vinelandii* is mentioned as an ideal candidate to produce alginates (Urtuvia et al. 2017), which however does not confirm whether or not CW-derived carbon sources are used.

### Gellan

The group of HePS also includes gellan, which is a linear anionic EPS based on a tetrasaccharide repeat unit composed of two molecules of D-glucose, one of D-glucuronic acid and one of L-rhamnose. The native gellan is partially esterified with acyl substituents (1 mol of glycerate and 0.5 mol of acetate) per repeat unit. This multifunctional gelling agent is produced by the non-pathogenic Gram-negative bacteria of *Sphingomonas* genus, most commonly using the strain *S. paucimobilis* ATCC 31461, and its synthesis is a growth-associated process (Fialho et al. 1999; Rehm 2010; Freitas et al. 2011). Moreover, its biosynthetic pathway is quite complex in which the synthesis of the sugar precursors (UDP-glucose, dTDP-rhamnose and UDP-glucuronate) is followed by the formation of the repeat unit by sequential transfer of the sugar donors to an activated lipid carrier by committed glycosyltransferases and by gellan polymerisation and export. This multi-step process is catalysed by a set of enzymes encoded by the gel clusters of at least 22 genes (Fialho et al. 2008). As with xanthan and alginate, a wide range of gellan applications in the food, pharmaceutical, biomedical and other sectors are determined by its valuable rheological properties (Rehm 2010; Fialho et al. 2008). Although there are few studies on the use of lactose or CW in

gellan microbial synthesis, they already confirm the relatively high productivity of this process. Thus, producer *S. paucimobilis* ATCC31461, using 2% lactose as the sole carbon source (yeast extract and peptone as nitrogen sources), in shake flasks (180 rpm), reached gellan concentrations of about 16 g/L (0.33 g/L/h) exceeding those obtained from sucrose or glucose (14 g/L, 0.29 g/L/h), but significantly below that of 4% soluble starch (43.6 g/L, 0.91 g/L) (Bajaj et al. 2006). In an earlier study with the same producer strain (Fialho et al. 1999) in shaking flasks (30 °C, 250 rpm) with 2% lactose, a lower gellan concentration (9 g/L, 0.19 g/L/h) was achieved, but the level of medium viscosity at this concentration was higher as compared to gellan (14 g/L) obtained with 2% glucose. It was also shown that as the sole carbon source, when tested undiluted, sweet CW (lactose 52 g/L) showed a drastic inhibition of gellan synthesis. However, when diluted (1:4 to 1:5), gellan concentrations of 7.2–7.9 g/L (0.15–0.16 g/L/h) are achieved. It was also confirmed that the use of CW significantly affects glycerate and acetate levels in gellan, and its higher viscosity is determined by a higher glycerate content (Fialho et al. 1999). In a recent study, the use of CW for gellan production by *S. paucimobilis* was evaluated in the 3-L bioreactor using various fermentation techniques (batch, fed-batch and continuous) (Gamal et al. 2018). During batch fermentation (28 °C, 250 rpm) of 40% sweet CW (1.84% sugar), a gellan concentration of 7.36 g/L (0.102 g/L/h) was obtained. The continuous feeding at 1.53 g/L/h in the fed-batch fermentation was found to be more favourable than pulsed feeding for gellan production 10.87 g/L (0.226 g/L/h). However, maximum gellan productivity was obtained using a continuous culture technique at 0.055 h<sup>-1</sup> dilution rate producing 8.08 g/L (0.337 g/L/h) (Gamal et al. 2018). Most recently, a new, previously untold, producer culture *Sphingomonas azotofigens* GL-1 (Wang et al. 2020) with a very significant gellan biosynthesis capacity has been revealed. Cultivation in shake flasks (pH 6.5, 30 °C) using a molasses or cheese whey-based medium whose compositions was optimised by response surface methodology. The optimum cheese whey-based medium consisted of cheese whey 68.3 g/L, Na<sub>2</sub>HPO<sub>4</sub> 14.6 g/L and KH<sub>2</sub>PO<sub>4</sub> 7.7 g/L, and maximum gellan production using this medium was 33.75 g/L (0.70 g/L/h). In turn, 14.75 gellan gum was obtained with an optimised molasses medium, which consisted of molasses 50 g/L, Na<sub>2</sub>HPO<sub>4</sub> 9.7 g/L and KH<sub>2</sub>PO<sub>4</sub> 5.9 g/L. The cheese whey-derived gellan showed a higher rhamnose, lower glucuronic acid and higher glycerate content together with a somewhat higher molecular weight compared to the molasses-derived polymer (Wang et al. 2020).



### Hyaluronic acid

Extracellular heteropolysaccharides also include hyaluronic acid (HA), which is a linear anionic polymer of repeating disaccharide units that are composed of D-glucuronic acid and N-acetyl-D-glucosamine and produced by several bacterial strains (e.g. *Streptococcus* spp., *Pasteurella multocida*). Reflecting its variety of natural functions in tissues of higher organisms, HA has found several applications in medicine, cosmetics and speciality foods (Chong et al. 2004; Rehm 2010; Freitas et al. 2011), making it a commercially valuable medical biopolymer. Its synthesis from the precursors (UDP-D-glucuronate and UDP-N-acetylglucosamine) is catalysed by the hyaluronan synthase (HasA) (EC 2.4.1.212) (Chong et al. 2004; Rehm 2010). Although HA is increasingly produced by microbial fermentation (Brown et al. 2008), renewable resources, including CW, are still little used and currently limited to a few studies (Amado et al. 2016; Mohan et al. 2016). Thus, the producer strain *Streptococcus equi* subsp. *zooeconomicus* ATCC 35246 was cultivated in several culture media using CW or the concentrated cheese whey protein (WPC) fraction after 10 kDa ultrafiltration of CW. The initial W composition was 5.9 g/L protein and 38.1 g/L reducing sugars; WPC had a protein content of 38.6 g/L and 13.1 g/L reducing sugars. Both CW and WPC were hydrolysed using protease, fractions (WPC) and whey protein hydrolysate (WPH) supplemented with lactose (up to 50 g/L) and fractions W and WH with both lactose and glucose at the same concentration and used for batch fermentations in 0.75-L bioreactors (37 °C, aeration of 1 vvm, agitation of 500 rpm, pH 6.7). Moderate HA concentrations of 2.14 g/L (0.20 g/L/h) and 2.38 g/L (0.43 g/L/h) were achieved in the presence of W and WH fractions, respectively, unlike for WPH with much lower (0.85 g/L, 0.12 g/L/h), even undetectable (WPC) concentration. If a tenfold scale-up in 5-L bioreactors was carried out in W and WH, using glucose instead of lactose as the carbon source due to the rather low its consumption observed in 0.5-L bioreactor cultures, much higher HA concentrations 4.02 g/L (0.58 g/L/h) and 3.19 g/L (0.46 g/L/h) were achieved, respectively. It is noteworthy that significantly cheaper (2.5–3 times) cultivation media were used, as both the W and WH fractions were able to completely replace the more expensive yeast extract and peptone required in standard media (Amado et al. 2016). Another producer strain *Streptococcus thermophilus* NCIM 2904 (Mohan et al. 2016) was also used to obtain HA by including both whey permeate (WP) and whey protein hydrolysate (WPH) in the nutrient medium supplemented with mineral salts. Cultivations were performed in 250-mL shake flasks at 37 °C and 150 rpm for 24 h. Under optimised (WP and WPH concentrations,

initial pH, inoculum size) HA achieved as if low 0.343 g/L (0.014 g/L/h) concentration. However, a further increase in upscaling this process at pilot- or industrial-scale level could be expected. In addition, the observed consistent production of ultralow-molecular weight (9.22–9.46 kDa) HA by such a microbial fermentation would be of high commercial significance over the conventional fragmentation processes for high-molecular weight HA (Mohan et al. 2016).

### Bacterial cellulose

Along with extracellular heteropolysaccharides, glucans, including BC, constitute a significant part amongst other bacterial EPS. This homopolysaccharide is a polymer of D-glucose formed primarily by  $\beta$ -(1,4) glycosidic links from precursors (UDP-D-glucose) by the membrane-embedded glycosyltransferase (cellulose synthase BcsA) (EC 2.4.1.29) (Gorgieva and Trček 2019). BC possesses exclusive structural, mechanical and functional properties and has therefore acquired a wide range of technical, and in particular, biomedical applications (Table 1) (Rehm 2010; Semjonovs et al. 2017; Gorgieva and Trček 2019; Salari et al. 2019). In addition, the extensive use of BC in biocomposites is of particular importance and good prospects (Sirviö et al. 2018). The capacity of BC production is widespread amongst bacteria (Table 1), but the most important and well-known producers are the strains of acetic acid bacteria (AAB) *Komagataeibacter xylinus* (Rehm 2010; Kreyenschulte et al. 2012; Amaro et al. 2019; Gorgieva and Trček 2019). As with other EPS, microbial synthesis of BC is very expensive and alternatives to get cheaper cultivation media formulations are being sought (Rehm 2010; Kreyenschulte et al. 2012; Amaro et al. 2019; Gorgieva and Trček 2019). However, the use of CW as an alternative C and N source remains limited in this respect. Most likely, this is because AAB possesses very low levels, if any, of  $\beta$ -galactosidase activity, with which to obtain glucose from CW lactose for BC synthesis (Pescuma et al. 2015; Lappa et al. 2019). Therefore, either enzymatic (or chemical) hydrolysis (Marangoni et al. 2002; Torres et al. 2010; Kucera et al. 2018) of CW (or CW permeate) or recombinant strains of producers with the inserted gene of  $\beta$ -galactosidase (Battad-Bernardo et al. 2004) are used for this purpose. Thus, the insertion of *lacZ* gene into the wild-type strain of *Acetobacter xylinus* by random transposon mutagenesis, generated the lactose-utilising and cellulose-producing mutant strain *A. xylinum* ITz3. The modified strain produced, on average, a 28-fold increase in cellulose production and a 160-fold increase in  $\beta$ -galactosidase activity when grown in lactose medium.  $\beta$ -Galactosidase activity is present in either lactose or sucrose medium indicating that the gene is constitutively expressed. During static

batch cultivation in the whey (lactose 20 g/L) containing the medium (yeast extract and peptone as the N-sources), 1.82 g/L of BC was obtained (0.019 g/L/h) (Battad-Bernardo et al. 2004). Several studies have confirmed that AABs, which are capable of producing BC, have a very poor ability to use lactose for this purpose. For instance, *Komagataeibacter sucrofermentans* DSM 15973, although it achieved 1.6–1.7 g/L BC, did so over a very long time and with low productivity (0.0044–0.0047 g/L/h) (Tsouko et al. 2015). Likewise, using the producer strains *A. xylinum* ATCC10821 and *A. xylinum* ATCC23770 on diluted CW permeate (lactose 1.3% w/v) (Thomson and Hamilton 2001), where almost negligible BC amounts (0.041 and 0.176 g/L, respectively) were obtained during 12 days of fermentation. Moreover, *Gluconoacetobacter sacchari* isolated from Kombucha tea reached only 0.31 g/L BC (0.0032 g/L/h) on lactose (20 g/L) and negligible (0.08 g/L) or a very small amount (0.15 g/L) on undiluted or diluted (1:50) CW powder-based culture medium (Carreira et al. 2011). Similarly, negligible BC concentrations (0.09–0.10 g/L) were obtained by cultivation of *A. xylinum* ATCC 10821 in the Hestrin–Schramm (HS) medium during 96 h on galactose as the sole carbon source (Mikkelsen et al. 2009). Slightly higher, but nevertheless very modest, results were obtained with *Gluconoacetobacter* sp. isolated from fruit in the HS medium on lactose or galactose (both 2%) as the only carbon sources with BC concentrations of 0.65 g/L (0.0039 g/L/h) and 0.50 g/L (0.0030 g/L/h) (Jahan et al. 2011). It has also been found that *A. xylinum* ATCC10245 can use galactose (20 g/L) relatively well as the sole carbon source in the GAM cultivation medium with the yield of BC 2.56 g/L (0.015 g/L/h) (Abdelhady et al. 2015). Higher BC concentrations and productivity are achieved using CW after hydrolysis with  $\beta$ -galactosidase to an equimolar glucose/galactose mixture. Thus, the producer strain *Gluconoacetobacter xylinus* PTCC 1734 in the medium containing CW hydrolyzate (reducing sugars 30 g/L) without any additional supplementation (150 ml medium in 300-mL flasks, 28 °C, static) reached 3.5 g/L BC (0.0104 g/L/h) within 14 days (Salari et al. 2018). However, significantly higher BC productivity was achieved with the production strain *Komagataeibacter rhaeticus* P 1463 using a modified HS medium with CW hydrolyzate (reducing sugars 20 g/L) as the sole carbon source (Semjonovs et al. 2017). Under similar culture conditions (50 ml medium in 300-ml flasks or 300 ml medium in 3000-ml jars, 28 °C, static, 5 or 14 days, respectively), bacterial cellulose was obtained at 2.90 g/L (0.024 g/L/h) and 6.55 g/L (0.019 g/L/h) concentrations, respectively (Semjonovs et al. 2017). However, very promising results were obtained when non-hydrolysed milk whey was used in combination with other renewable resources such as

rotten fruit extracts (Jozala et al. 2015). Thus, during cultivation of the producer strain *G. xylinus* ATCC 53582, a significant amount of BC (about 50 mg/mL, 0.52 mg/mL/h) was observed in 24-well microplates (30 °C) in the medium consisting of 30% whey and 50% rotten fruit extract (C/N ratio 27.5) (Jozala et al. 2015). Significant amounts of BC (5.45 g/L, 0.076 g/L/h) were also obtained using the producer culture *Gluconoacetobacter sucrofermentans* B-11267 in non-hydrolysed CW medium (250-ml shake flasks, 28 °C, 250 rpm) (Revin et al. 2018). Besides, the use of CW also affects the BC micromorphological properties including the reduction of crystallinity index (Revin et al. 2018). Recently, the very promising use of CW permeate as a supplemental nutritional, probably nitrogen source for BC microbial production, has been reported (Bekatorou et al. 2019), which makes it possible to reduce the amount of yeast extract in the medium. Using the mixture of Corinthian currant grapes (CFS) extract (sugars 20 g/L), CW (50.4%) and yeast extract (1.7%), the producer *Komagataeibacter sucrofermentans* DSM 15973 achieved 8.4 g/L of BC (0.05 g/L/h) in this relatively low-cost cultivation medium (Bekatorou et al. 2019).

#### Curdlan and pullulan

Both curdlan and pullulan are also glucose-derived extracellular homopolysaccharides which, however, have different producers, structural and physico-chemical properties (Table 1). Pullulan is a water-soluble neutral  $\alpha$ -glucan, which consists of linear chains of D-glucopyranosyl units that alternate regularly between one (1,6)- $\alpha$ -D and two (1,4)- $\alpha$ -D linkages, or a linear polymer of maltotriosyl units connected by (1,6)- $\alpha$ -D linkages. The mechanism of its biosynthesis from precursors (UDP-D-glucose) by transglycosylation of oligosaccharides is not completely understood (Cheng et al. 2011). In turn, curdlan represents a water-insoluble  $\beta$ -glucan-type polysaccharide composed exclusively of  $\beta$ -1,3-linked glucose residues that have a complex tertiary structure (Eweda et al. 2015; Liu et al. 2015). It is also formed from UDP-D-glucose as a precursor and polymerised by curdlan (1,3- $\beta$ -glucan) synthase (EC 2.4.1.34) (<https://www.brendaenzymes.org/enzyme.php?ecno=2.4.1.34>). Differences in structure and properties of these both polymers, in particular their water solubility, also influence their applications which are quite versatile and significant (Table 2) (Abdel-Hafez et al. 2007; Liu et al. 2015). Although it is possible to use various renewable resources for microbiological synthesis of curdlan and pullulan, the use of cheese whey and related products has been the subject of very few studies (Roukas 1999; Abdel-Hafez et al. 2007; Eweda et al. 2015; Liu et al. 2015). It has been shown that yeast-like fungi *Aureobasidium pullulans*

ATCC42023 can use media containing hydrolysed sweet CW (5% lactose, 0.8% to 1% protein) to produce (shake flasks, 200 rpm, 28 °C) pullulan. In addition, the concentration of pullulan obtained with acid hydrolysed CW was 7.6 g/L (0.063 g/L/h). In contrast, it increased with enzyme hydrolysed whey (10.16 g/L, 0.084 g/L/h) and could reach 12.4 g/L (0.103 g/L/h) using additives (0.05% glutamic acid, 0.30%  $\text{KH}_2\text{PO}_4$ ) in the medium (Abdel-Hafez et al. 2007). These data are consistent with the previous study (Roukas 1999), using *Aureobasidium pullulans* P56 and deproteinised whey (5% lactose) hydrolysed with acid or enzymatically. In shake flasks (200 rpm, 30 °C), the use of enzyme hydrolysed CW (lactose 25 g/L or 50 g/L) resulted in slightly higher polymer concentrations (6.0 g/L, 0.025 g/L/h) compared to acid hydrolysed (5.0 g/L, 0.021 g/L/h) whey. Maximum concentrations of pullulan were obtained (11.0 g/L, 0.046 g/L/h) when enzymatically hydrolysed lactose (25 g/L) was supplemented with 1% glutamic acid, 0.5%  $\text{K}_2\text{HPO}_4$ , 2.5% olive oil and 0.5% Tween80 (Roukas 1999). Unlike the pullulan production, the use of CW for the microbial synthesis of curdlan has still not been reported, except for a couple of studies on the utilisation of lactose for this purpose as the sole carbon source (Eweda et al. 2015; Liu et al. 2015). Thus, *Agrobacterium* strain HX1126 produced 21.4 g/L of curdlan (0.25 g/L/h) using lactose (50 g/L) in shake flasks (180 rpm, 30 °C) (Liu et al. 2015). This has also been demonstrated by several strains of rhizosphere isolates (Eweda et al. 2015). For example, the isolate K17 in the yeast-peptone (YP) medium (lactose 10%) in shake flasks (150 rpm, 28 °C) achieved the curdlan concentration of 5.2 g/L (0.072 g/L/h). The productivity already achieved on lactose in these studies suggests that cheese whey itself could also be used for a further microbial production of curdlan.

### Dextran and levan

Like pullulan, dextran is also an extracellular water-soluble neutral  $\alpha$ -glucan, which consists of linear chains of D-glucopyranosyl units with predominantly  $\alpha$ -(1,6) linkages in the main chain and a variable amount of  $\alpha$ -(1,2),  $\alpha$ -(1,3) and  $\alpha$ -(1,4) branched linkages. Dextran is synthesised from sucrose by dextranase (EC 2.4.1.5) (<https://www.brenda-enzymes.org/enzyme.php?ecno=2.4.1.5>) which is an extracellular glucosyltransferase that catalyses the gradual transfer of D-glucopyranosyl residues from sucrose to dextran and the release of fructose residues. As one of the predominant members of lactic acid bacteria (LAB) group, *Leuconostocs* spp. is not only important in food fermentations, but also is a prominent producer culture for commercial production of dextran, where various strains of *L. mesenteroides* are used predominantly (Table 1) (Rehm

2010). As a biodegradable, biocompatible and easily soluble polymer, dextran is widely used in the food and pharmaceutical industries, medicine (blood volume expander) and biochemistry (chromatographic media), etc. (Santos et al. 2005; Lule et al. 2015; Lule et al. 2016). As with other biopolymers, a greater use of renewable and less expensive resources is important for the microbial production of dextran. Sugar beet or sugar cane molasses are most widely used in dextran production, because the main sugar beet or cane molasses are the most widely used renewable sources of sucrose for dextran production. However, there are opportunities to use also other renewable nutrients, including CW. In this regard, the ability of *L. mesenteroides* to produce dextran in combined sucrose–whey culture media has been proven for a long time (Schwarz and Bodie 1984). Thus, the cultivation (shake flasks, 150 rpm, 25–27 °C) of *L. mesenteroides* ATCC14935 in combined medium containing the sweet CW powder (4%) and sucrose (10%) and additives (0.5% yeast extract, 0.1%  $\text{K}_2\text{HPO}_4$ ), resulted in substantially increased medium viscosity (greater than 500 cps within 46 h). The product properties were dextran-compatible and 95% sucrose and 40% lactose were consumed (Schwarz and Bodie 1984). The producer strain *L. mesenteroides* NRRL B512F in the broth consisting of Carob (*Ceratonia siliqua*) pod extract (CPE, sucrose 20 g/L) and percentage of deproteinised CW to obtain 5% of lactose in the medium during batch cultivation (5-L bioreactor, aeration 0.05 vvm, 35 °C, pH 6.7) achieved dextran concentration of 7.23 g/L (0.52 g/L/h) that was somewhat higher than when using lactose (5%) alone instead of CW (6.88 g/L, 0.49 g/L/h) (Santos et al. 2005). In a later study using the same strain *L. mesenteroides* NRRL B512F (PTCC 1591) and the medium containing molasses (Brix 40) as the carbon source and CW powder (2–10%) as the nitrogen source in shaking flasks (150 rpm, 30 °C, pH 6.8), concentrations of dextran reached 8.95 g/L (0.19 g/L/h) and 12.68 g/L (0.26 g/L/h) at 6% and 10% concentrations of CW, respectively (Moosavi-Nasab et al. 2010). Using other producers *L. mesenteroides* NCDC 744 and *L. mesenteroides* NCDC 745, 12.7 g/L (0.79 g/L/h) and 10.51 g/L (0.66 g/L/h) of dextran were obtained respectively in partially deproteinised paneer whey medium with 10% sucrose, 0.1% yeast extract and 0.1%  $\text{K}_2\text{HPO}_4$  (Lule et al. 2015). Increased concentration of dextran (17.25 g/L) was observed under optimised (15% sucrose, 25 °C) conditions using the strain *L. mesenteroides* NCDC 7459 (BA08) (Lule et al. 2016). Recently, quite good results have been achieved with the use of producer strains *L. mesenteroides* NRRL B512F, NCIB 8023 and NRRL B12, to obtain 16.35 g/L (0.68 g/L/h), 15.89 g/L (0.66 g/L/h) and 16.28 g/L (0.68 g/L/h) respectively in batch fermentation (30 °C) and the medium containing a



milk whey permeate (15 g/L), sucrose 20 g/L and yeast extract (15 g/L (Esmailnejad-Moghadam et al. 2019). There are still few, if any, reports on the use of whey for the microbial production of levan which is a  $\beta$ -fructan, a homopolysaccharide of fructose, which is of practical importance and with a widespread application (Table 1). Levan is formed by polymerisation of D-fructosyl units by  $\beta$ -2,6-linkages in linear chains with  $\beta$ -2,1 linkages for branching points which is carried out by levansucrase (EC 2.4.10), an extracellular fructosyltransferase (<https://www.brenda-enzymes.org/enzyme.php?ecno=2.4.1.10>) that catalyses the transfer of fructosyl residues from sucrose to levan and the release of glucose residues. The producer *Azotobacter vinelandii* D-08 has been reported to produce 14 g/L (0.58 g/L/h) in the medium consisting of molasses, distillery dregs and milk whey permeate (in proportion 5:3:2, respectively) in shake flasks (250 rpm, 28 °C) (Revin et al. 2016). It should be noted that dextran from whey can also be produced by culture from another genus. For instance, *Weissella cibaria* NCIHB42196 (Cinti 2015) in the medium containing lactose (10%) and sucrose (5%) reached the levan concentration of 31 g/L. It is noteworthy that the potential for the simultaneous formation of levan and dextran by *L. mesenteroides* KIBGE-IB22 has been strongly confirmed by NMR analysis (Siddiqui et al. 2014), which indicates the presence and function of both dextransucrase and levansucrase for this strain. This is in good agreement with previous data (Kang et al. 2005) and it is expected that the simultaneous formation of dextran and levan by *L. mesenteroides* spp. could also occur in the presence of CW or lactose.

## Conclusions

Cheese whey (CW) and its derivatives can be used as the renewable carbon and/or nitrogen sources for the production of microbial polymers such as exopolysaccharides (EPS) and polyhydroxyalkanoates (PHAs).

In many cases, the use of CW and their derivatives makes it possible to achieve high productivity, in particular for the fed-batch cultivation in bioreactors, which could improve the overall economic efficiency of microbial polymer production as well as promote the development of more environmentally friendly technologies and wider use of biodegradable and biocompatible materials.

The effectiveness of whey product application for microbial fermentation is largely dependent on the ability of the producer cultures to hydrolyse lactose.

In cases where the cultures of the producers are not capable of directly utilising lactose, the enzyme hydrolysis with  $\beta$ -galactosidase is preferred for the pre-treatment of whey.

To provide a higher carbon/nitrogen (C/N) ratio that is more favourable to microbial polymer biosynthesis, CW permeate, i.e. the fraction with wholly or partially separated proteins, has certain benefits.

## Abbreviations

AAB: Acetic acid bacteria; AT: Acetyltransferases; BC: Bacterial cellulose; BOD: Biological oxygen demand; C: Carbon; N: Nitrogen; CW: Cheese whey; COD: Chemical oxygen demand; CFS: Corinthian currant grapes; EPS: Exopolysaccharides; GT: Glycosyltransferases; HePS: Heteropolysaccharides; HoPS: Homopolysaccharides; HasA: Hyaluronan synthase; HA: Hyaluronic acid; KPT: Ketol pyruvate transferase; MMC: Mixed microbial cultures; PHA: Polyhydroxyalkanoates; PHB: Polyhydroxybutyrate; GAM: Glucose-ethanol acetic acid medium; VFAs: Volatile fatty acids; WP: Whey permeate; WPC: Concentrated whey protein; WPH: Whey protein hydrolyzate; YP: Yeast peptone.

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## Authors' contributions

General writing of the manuscript, investigation, data collection and analysis—PZ; data search, editing and design—SK; conceptualisation, critical revision and supervision—PS. All authors contributed to the study conception and design, read and approved the final manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

The datasets used and/or analysed during the current study are available from the first author on reasonable request and/or are freely accessible via the Internet.

## Ethics approval and consent to participate

Not applicable.

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## Competing interests

The authors declare that they have no competing interests.

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