J. Life Science. (1999), 9(1), 69~80

JOURNAL OF
Life Science

Microbial Modification of Extracellular Polysaccharides

Jin Woo Lee*

Faculty of Natural Resources and Life Science, College of Natural Resources and Life Science, Dong-A University, Pusan 604-714, Korea

Received: April 23, 1999

Abstract Some trials to alter the structure of extracellular polysaccharides by means of biotransformation and microbial modification have been reported. Seaweed alginate was acetylated by intact and resting cells of *Pseudomonas syringae* ATCC 19304. Glucose analogs such as 3-*O*-methyl-D-glucose used as sole carbon sources was directly incorporated into curdlan by *Agrobacterium* sp. ATCC 31749. The 2-amino-2-deoxy-D-glucose (glucosamine) and 2-acetamido-2-deoxy -D-glucose (*N*-acetylglucosamine) were incorporated into microbial cellulose by *Acetobacter xylinum* ATCC 10245. The changed monomeric composition in pullulan by *Aureobasidium pullulans* ATCC 42023 as well as zooglan by *Zoogoea ramigera* ATCC 25935 was another effect of glucose analogs used as carbon source. There was no effect of glucose analogs found in polysacharide-7 (PS-7) produced by *Beijerinckia indica*. ATCC 21423.

Key words: biotransformation, microbial modification, extracellular polysaccharides, glucose analog, alginate, curdlan, cellulose, pullulan, zooglan, polysaccharide-7

Introduction

There are a lot of extracellular polysaccharides produced by certain microorganisms such as microbial cellulose [13, 103], curdlan [36,39], pullulan [93,115], gellan [49], zanthan [79], dextran, and zooglan [27,45] (Table 1). Some of them is commercially used as emusifiers, stabilizer, gelling agents, and expanding agents for many industrial applications due to their specific characteristics. Many trials to improve their properties on the demand of industrial uses have been reported. There are basically two ways to modify extracellular polysaccharides; one is chemical method and the other is microbial one. For example, chemically modified cellulose and curdlan exhibited strong antiviral activity *in vitro* [87, 123]. Acetylation of seaweed alginate by *Pseudomonas syringae* was also reported [62]. Acetylation altered the viscosity and calcium induced precipitation of alginates [63].

Glucose-rich extracellular polysaccharides such as cellulose and curdlan have been post-biosynthetically derivatized by nonspecific chemical means to change physical properties [87,123]. Selective chemical modification of polysaccharides under homogeneous conditions also has been reported [97], but disadvantages of this approach include low yields, side reactions, the use of toxic solvents, and purification requirements. Therefore, it was desirable to explore the ability of some microorganisms to modifiy certain parts of polysaccharides or direct, *in vivo*, incorporation of simple sugar analogs as building blocks for polysaccharides. Glucose derivatives may be particularly effective as novel carbon sources since glucose is a main component in many polysaccharides and, therefore, a logical target for direct polymerization by the microorganism.

Biosynthesis of polysaccharides has traditionally been studied using carbon sources such as unmodified simple sugars (glucose, sucrose, and fructose) [50] or microbial mutants [116] to examine effects on the molecular weight and yield of products [109,121], and the composition of main chain or branch [41]. Polysaccharides have not been well studied with respect to the modification by microorganisms and the incorporation of modified or non-native building blocks, unlike the extensive work with proteins for the incorporation of unnatural amino acids [17] and bacterial polyesters with incorporation of a wide range of novel monomers [11,33,112]. Here are some results from the trials to alter the structure of extracellular polysaccharides with means of microorganisms for bio-transformation and use of glucose analogs as the carbon source.

Biotrnaformation: bioacetylation of seaweed alginate

Alginates is one of the few polysaccharides that can be obtained from both eukaryoties and prokaryotes. It is a structual polymer in numerous species of brown seaweed,

*Corresponding author
Phone: 82-51-200-7593, Fax 82-51-200-6993
E-mail: jwlee@seunghak.donga.ac.kr

Table 1. Structure of some extracellular polysaccharides

Microorganism	Polysaccharide	Basic structure
Azotobacter vinelandii	Alginate	1→4 linked ManA and GluA
Pseudomonas aeruginosa	Alginate	1→4 linked ManA and GluA
Acetobacter xylinum	Cellulose	(1→4 glucose)n
Agrobacterium sp.	Curdlan	(1→3 glucose)n
Leuconostoc mesenteroides	Dextran	(1→6 glucose)n
Streptococcus salivarius	Levan	(2→6 fructose)n
Streptococcus mutans	Mutan	(1→3 glucose)n
Aureobasidium pullulans	Pullulan	(1→6 maltotriose)n
Xanthomonas campestris	Xanthan	(-4Glc1-4Glc1-4Glc1-)n

particularly members of the genera *Ascophyllum, Ecklonia, Fusarium, Laminaria, and Macrocystis* [58,90]. Many pseudomonads produce alginate-like exopolysaccharide, bacterial alginate [34]. *Azotobacter vinlandii* [58] and several species of *Pseudomonas* [34,73] including *P. aeruginosa* [7, 29] and *P. syringae* [25] produce bacterial alginates.

Alginates purified from seaweeds consist of two monomer, β-D-mannuronic acid and its C-5 epimer, α-L-guluronic acid [42,71]. The ratio of D-mannuronic acid/ L-guluronic acid (G/M) in alginates is dependent on the algal species as well as the location of the polymer in the plant [42]. Alginates with low M/G ratios produce strong and brittle gels whereas alginates with high M/G ratios produce elastic gels [90]. The most conspicuous difference between seaweed alginate and bacterial alginate is that bacterial polymer is randomly organized and the mannuronic acid residues are acetylated [20]. Most mannuronic acid residues in bacterial alginates are mono-*O*-acetylated at the C-2 or C-3 position. However, some residues amy be 2,3 di-*O*-acetylated [105, 106].

Random acetylation of seaweed alginate by chemical method showed increased viscosity and decrease the affinity of these polymers for calcium ions [107, 108]. The gel made with acetylated alginates also have enhanced swelling ability. Specific acetylation may improve these and other properties, expanding the commercial potential of this polysaccaharide. Seaweed alginate was acetylated by intact and resting cells of *Pseudomonas syringae* ATCC 19304 [21, 22]. Maximum acetylation of this polymer occurred at a pH of 6.0 and a temperature of 25°C. Aeration and gluconic acid were required for an optimal reaction. A reactor which contained activated carbon-immobilized cells was constructed to continuously acetylate seaweed alginate [62]. Growing cells on the surface of activated carbon were observed with scanning electron microscope (SEM). Maximal yield of acetylation was about 90% and the half-life of this system was 6.5 day.

Confirmation of acetylated seaweed alginate and the position of acetylation in seaweed alginate were conducted with a nuclear magnetic resonance (NMR) spectroscopy. Their molecular weights also were compared with a gel permeation chromatography (GPC) (Table 2). Acetylation dramatically effects both the solution properties and the metal induced precipitation of alginates [63]. The presence of acetyl groups on both bacterial and seaweed alginate marginally increased the weight average molecular weight of each polymer by 7% and 11%, respectively. Acetylated bacterial alginate showed a significant increase in solution viscosity compared to its deacetylated countetpart. The presence of acetyl groups decreased the ability of each polymer to bind with calcium but increase their ability to bind with ferric ion (Table 3). By controlling the degree of acetylation on the alginate chains, it was possible to modify solution viscosity and cation induced precipitation of these polymers.

The high affinity of seaweed alginate for divalent ions, especially calcium ions, is due to the structural characteristics of polyguluronate residues in seaweed alginate. The hydroxyl groups, carboxyl groups of polyguluronate residues and their net negative charge as well as the molecular size

Table 2. Characterization of seaweed and bacterial alginates

Alginates	M_n[a] (x10^4)	M_w[b] (x10^4)	M_w/M_n[c]	M/G ratio[d]	Acetylation[e]
Seaweed	1.4	4.7	3.36	60: 40	0
Acetylated seaweed	1.4	5.2	3.25	60: 40	0.3
Bacterial	4.3	12.7	2.95	82: 18	1.2
Deacetylated bactrial	3.8	11 9	3.13	82: 18	0

a. Number average molecular weight
b. Weight average molecular weight
c. Polydispersity
d. Molar ratio of mannuronic acid to guluronic acid
e. Ratio of acetyl gruops to monomeric sugar residues

Table 3. Precipitation of alginates by cations

Ions	$P_{1/2}$[a]			
	Seaweed alginate	Acetylatd seweed alginate	Bacterial alginate	Deacetylated bacterial alginate
Ca^{2+}	2.6 ±0.5	11.7 ±1.9	13.5 ±1.7	8.4 ±1.2
Sr^{2+}	1.8 ±0.5	5.2 ±1.0	8.5 ±0.9	3.8 ±0.6
Fe^{3+}	1.8 ±0.6	0.6 ±0.1	0.6 ±0.1	0.8 ±0.2
Co^{2+}	9.6 ±0.4	47.0 ±5.6	No affinity[b]	45.0 ±7.2
Pb^{2+}	0.7 ±0.1	1.7 ±0.6	0.5 ±0.1	0.6 ±0.1
U^{6+}	0.9 ±0.2	1.4 ±0.5	0.7 ±0.1	0.5 ±0.1

a. $P_{1/2}$ is the concentration of metal ions (mM) required to precipitate 50% (w/v) of the alginate from 400 μg/ml (w/v) of alginate solutions

b. No affinity signifies that the ion did not precipitate 50% of the alginate sample up to 100 mM ion concentration

and net charge of the calcium ions are all implicated in this interaction [84,95]. Although the ionic effect of the carboxyl group is unlikely to be dramatically influences, acetylation of seaweed alginate probably disturb the basic relationships between the polymer and calcium ions by modifying the ionization properties of the polymer and sterically hindering the binding of these ions to the polymer [80,81].

Because of a higher viscosity and a lower affinity for calcium ions, acetylated alginates can be favorably substitutes for nonacetylated alginates when used as emulsifiers, stabilizers, and gelling agents in many industrial applications. The lower affinity for calcium ions of acetylated alginate confers a more soluble state on the polymer in aqueous solution. Acetylated alginate thus becomes a more desirable emulsifier and stabilizer. Acetylated alginates have the potential to concentrate some toxic, heavy, and/or valuable metals. They were found to bind ferric, uranium and lead ions more than calcium ions. It means that acetylated alginates can be used for isolation and concentration of some toxic and heavy ions from contaminated environment.

Among several strains known to produce bacterial alginate including *A. vinelandii, P. aeruginosa, P. cepacia, P. fluorescens*, only *P. syringae* ATCC 19034 acetylated seaweed alginate [68]. Physiological studies on this strain and its U. V. induced mutants showed no correlation between biosynthesis and acetylation of bacterial alginate. Specific yields of alginate and degree of acetylation in these polymers varied with strain and culture medium. This was indirect evidence that alginate biosynthesis is separate from polysaccharide acetylation. It indicated that the enzyme system involved in alginate biosynthesis was not directly linked to alginate acetylation and explained why microbial acetylation of seaweed alginates with *P. syringae* ATCC 19034 was possible.

Microbial modification with glucose analogs

To biosynthesize modified extracellular polysaccharides such as microbial cellulose, curdlan, pullulan, zooglan, and polysaccharide-7, glucose analogs including 3-*O*-methyl-D-glucose (3-*O*-methylglucose), 2-amino-2-deoxy-D-glucose (glucosamine), 2-acet-amido-2-deoxy-D-glucose (*N*-acetyl-glucosamine), and 2-deoxy-D-glucose (2-deoxyglucose) were used separately as sole carbon sources. Some of glucose analogs directly incorporated into curdlan and microbial cellulose. Another effect of glucose analogs as carbon sources rersulted in the changed monomeric composition in pullulan as well as zooglan. Production of polysacharide-7 by *Beijerinckia indica*. showed compositional consistency. More detailed results are as follows.

Direct incorpration of glucose analog into curdlan and cellulose

Curdlan has been identified as an unbranched homo-β-(1-3)-glucan produced by a mutant of *A. faecalis* var. *myxogenes* 10C3 [39], which has unusual solubility and gelling properties. Curdlan, with a molecular weight of approximately 74,000, is produced on an industrial scale by fermentation of *A. faecalis* var. *myxogenes* [98]. Curdlan production is associated with the poststationary phase of a nitrogen-depleted, aerobic batch culture [88]. Curdlan yield from glucose is usually 50% if the cultivation pH is maintained at 6 [91] and it forms a firm gel when heated in suspension at or above 54°C [74]. Curdlan is insoluble in water at neutral pH [37,38,52], but is soluble in NaOH at NaOH concentrations greater than 0.23N and in DMSO. The solubility behavior was ascribed to a molecular transition from an ordered helical structure to a random coil conformation [88]. The solubility of curdlan in water is dependent on molecular weight. Thus, curdlan with a degree of polymerization (DP) below approximately 35 is soluble in cold water while higher molecular weight curdlan is insoluble. Typically, commercial curdlan has a DP of 155 to 455 depending on the fermentation conditions employed [98]. Changes in composition of curdlan or other exopolymers is expected to result in changes in solubility and functional properties.

Three different types of exopolymers were purified from

cultures of *Agrobacterium* sp. ATCC 31749 grown in a mineral salts medium containing 2% glucose at 30°C for 5 days under aerobic culture conditions [64,67]. These exopolymers were curdlan (extracellular, homo-β-(1-3)-glucan, water-insoluble at neutral pH), a water-soluble non curdlan type exopolymer-A (WSNCE-A), and a water-soluble non curdlan type exopolymer-B (WSNCE-B). Curdlan, WSNCE-A, and WSNCE-B composed by weight 61%, 27%, and 12%, respectively, of the exopolymer made with glucose by *Agrobacterium* sp. Compositions of all polymers were confirmed by gas chromatography (GC) and gas chromatography/ mass spectrometry (GC/MS). The WSNCE-A is composed of glucose and galactose with lower contents of rhamnose whereas WSNCE-B consists of glucose and mannose with lower contents of rhamnose.

Glucose analogs used as sole carbon sources were incorporated into curdlan and WSNCE-A [64]. The incorporation of 3-*O*-methylglucose into curdlan was 8 ~ 12 mole % based on GC and ^{1}H-NMR spectrometry. To confirm the presence of the methyl group in curdlan purified from the culture grown on 3-*O*-methylglucose as sole carbon source, proton NMR spectra were recorded. The signal at 3.36ppm at Fig. 1C was assigned to the methyl group on the basis of correlation with previously reported proton NMR spectrum of mono-methylated D-glucose [32]. Considering that curdlan is known to have β-1,3 linkages, it is therefore expected that 3-*O*-methylglucose repeat units incorporated into curdlan must be linked to neighboring sugars by bonds other than β-1,3, either in the main chain or as branched, or are incorporated at chain end. Incorporation of *N*-acetylglucosamine into WSNCE-A was also confirmed by GC/MS chromatographic analysis, which showed that 10 mole % of main chain repeat units are *N*-acetylglucosamine. Direct evidence was obtained to show *Agrobacterium* sp.

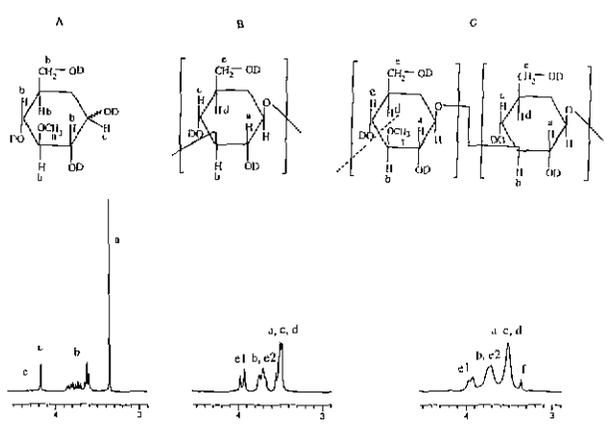

Fig. 1. Proton NMR spectra of (A) 3-*O*-methylglucose, (B) curdlan made with glucose, and (C) curdlan made with 3-*O*-methylglucose. The e1 and e2 represent H-6 (exo) and H-6′ (endo) protons, respectively.

ATCC 31749 synthesized new modified extracellular polysaccharides by feeding glucose analogs as sole carbon sources. This finding bridges an important link between manipulation of polysaccharide structure through biosynthetic feeding strategy and the more traditional selection, mutation, or postsynthetic chemical modification approaches.

Cellulose, (1→4)-linked-β-D-glucan, is a major structural component of the cell walls of higher plants. Some microorganisms also produce unbranched (1→4)-linked-β-D-glucan, named microbial cellulose (MC) [13,103]. Structurally related polysaccharides such as chitin and chitosan are also found in the biosphere. Chitin occurs as a major cuticular or skeletal component in all arthropods, in some invertebrata, and in some fungi. Chitin is a polysaccharide of high molecular weight and consists of unbranched chains of (1→4)-linked 2-acetamino-2-deoxy-β-D-glucose residues [35]. Chitosan is the fully or partially deacetylated form of chitin. It contains β-(1→4)-linkaged 2-amino-2-deoxy-β-D-glucopyranose and 2-acetamido-2-deoxy-β-D-gluco-pyranose residues [43]. Chitosan is found in the cell walls of some fungi such as *Mucor rouxi* [2,8].

MC is physically different from wood-derived cellulose. Viscometric determination of the weight average degree of polymerization (DPw) of MC is 2190 to 3470 [70]. The very fine fiber structure of MC gives the fiber a surface area about 300 times greater than normal wood fiber [51]. A pulp obtained from bacterial cellulose forms a strong paper and is useful for reinforcing conventional pulp paper [124]. Chitin and chitosan are useful as chelating agents [82], drug carriers [83], membranes [9], water treatment additives [3], and wound-healing agents [5,78]. Commercially, chitosan is derived by the chemical deacetylation of chitin from waste crustacean exoskeletons with strong alkali. This harsh conversion process, as well as variability in source material, leads to inconsistent physicochemical characteristics [2]. The purification of chitosan derived from the cell wall of some fungi also requires strong alkaline treatment with heat, which leads to inconsistent material [120].

Incorporation of glucosamine and *N*-acetylglucosamine used as sole carbon sources into cellulose by *Acetobacter xylinum* ATCC 10245 was confirmed by GC and GC/MS. Based on relative peak areas and response factors of corresponding standard sugars, the average molar percentages of glucosamine and *N*-acetylglucosamine repeat units into exopolymers were 19% and 18%, respectively. The yields of exopolymers made with glucosamine and *N*-acetylglucosamine as carbon source after 7 day culture were 0.37 mg/ml and 0.67 mg/ml, respectively, whereas that of glucose was 3.9 mg/ml. The yield of exopolymer made with the mixture of glucose (0.5%, v/v) and glucosamine (1.5%, v/v) as carbon source was 1.60 mg/ml, and the average molar percentage of glucosamine repeat unit into the exoplymer was 17%. Its conversion rate was 8.0% on the basis of carbon source added (Table 4). The exopolymers made with glucosamine

Table 4. Production of exopolymer with mixed carbon of glucose and glucosamine by *A. xylinum* ATCC 10245[a]

Carbon (%) Glucose: Glucosamine	pH[b]	Yield (mg/ml)	Conversion[c] (%)	Incorportion[d] (%)
2.0 : 0.0	4.16	3.80 ±0.55	19.0	0.1 ±0.2
1.5 : 0.5	4.10	3.20 ±0.19	16.0	4.5 ±0.2
1.0 : 1.0	4.10	2.47 ±0.05	12.4	12.9 ±0.4
0.5 : 1.5	4.10	1.60 ±0.21	8.0	17.4 ±1.3
0.0 : 2.0	4.04	0.34 ±0.04	1.7	19.6 ±2.2

a. Results from 7 day cultures at 30℃ and values are the mean of triplicate experiments
b. The final pH
c. The conversion rate of exopolymer based on the carbon source added
d. The incorporation rate of glucosamine repeat unit into exopolymer

and *N*-acetylglucosamine were fractionated by 10% acetic acid and distilled water (DW), respectively. The molar ratio of glucose to glucosamine in the acetic acid (10%, v/v) soluble fractionation of glucosamine incorporated exopolymer was 0.6 : 1.0 (the molar percentage of glucosamine in this fraction was 63%.). The molar ratio of glucose to *N*-acetylglucosamine in the water soluble fraction of *N*-acetylglucosamine incorporated exopolymer was 0.8: 1.0 (the molar percentage of glucosamine was 56%.) (Fig. 2).

Compared with the biosynthetic steps for more complex polysaccharides such as bacterial alginate, xanthan, and zooglan [24,40,117], the polymerization of cellulose is catalyzed in a single step by the enzyme named cellulose synthase [99,122]. Cellulose synthase transfers the donor substrate, α-linked nucleotide diphosphosugars, (eg. UDP-glucose, UDP-*N*-acetylglucosamine, etc.) to acceptor molecules, forming a β-linked product (cellulose, chitin, etc.) [102]. The *acs* operon consists of three genes, the first of which (*acsAB*) codes for cellulose synthase [72]. This AcsAB polypeptide shared sequence similarities with the NodC protein (51.8% similarity, 26.5% identity), the ExoO

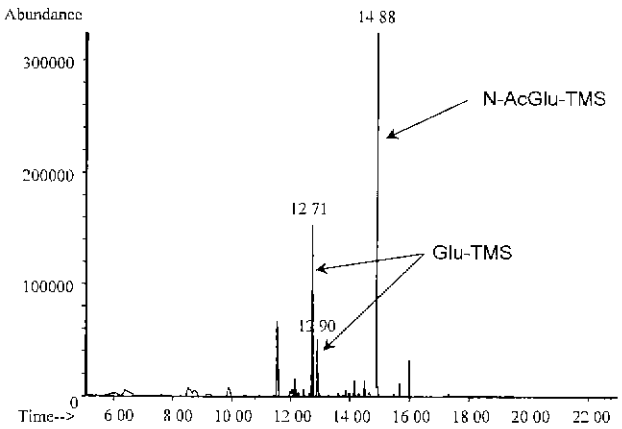

Fig. 2. GC/MS chromatograms of trimethylsilyated (TMS) sugar components of water soluble fraction of extracellular polysaccharide mede with *N*-acetylglucosamine by *A. xylinum* ATCC 10245.

protein (45.4% similarity, 20.0 identity) from *Rhizobium meliloti*, and the HasA protein from group A streptococci (46.1 similarity, 21.8% identity) [101]. The NodC protein showed a 32% identity to chitin synthase III from *Saccharomyces cerevisiae* [12]. The ExoO protein is involved in the synthesis of the exopolysaccharide succinoglycan and catalyzes the formation of a β-1,6 glycosidic linkage [30]. The NodC protein, hyaluronate synthase, is predicted to function in the β-1,4 transfer of *N*-acetylglucosamine to glucuronic acid during hyaluronate biosynthesis [23]. Presumably phosphorylated glucosamine and *N*-acetylglucosamine are substrates for the cellulose synthase due to broad specificity based on homology to other β-glycosyl transferases [101,102]. The polymerization of glucosamine and *N*-acetylglucosamine by *A. xylinum* ATCC 10245 to form glucose-co-glucosamine copolymers like chitosan and glucose-co-*N*-acetylglucosamine copolymers like chitin, respectively, is one possibility based on the broader specificity for substrates of cellulose synthase.

Changed monomeric composition in pullulan and zooglan

Pullulan is one of the few neutral water-soluble microbial polysaccharides that can be produced in large quantities by fermentation [93,115]. Pullulan is an extracellular, unbranched homo-polysaccharide which consists of maltotriose and maltotetraose units with both α-1,6 and α-1,4 linkages [10,16]. This regular alternation of these two bonds imparts distinctive properties of structural flexibility and enhanced solubility [60]. These properties suggest a wide range of both medical and industrial uses [61]. Pullulan produces high-viscosity solutions at relatively low concentrations and can be utilized to form oxygen-impermeable films, thickening or extending agents, or adhesives [76]. Films formed from pullulan are suitable for coating foods and pharmaceuticals especially when exclusion of oxygen is desirable [125]. Important parameters controlling the production of pullulan are temperature [76], initial pH of medium [46,57,86], oxygen supply [96, 119], nitrogen concentration [4,104], and carbon source [6]. The molecular weight of pullulan varies

with culture conditions and strain [93,109,121].

The monomeric components of exopolymers produced by *A. pullulans* ATCC 42023 under aerobic conditions with glucose, mannose, and glucose analogs as carbon sources were glucose and mannose (Table 5) [69]. There was no evidence of direct incorporation of glucose analogs into exopolymers. The molar ratio of glucose to mannose and the molecular weight of exopolymers varied with the carbon source and culture time. Exopolymers produced with glucose and mannose as a sole carbon sources showed 87 ± 3 and $89\pm2\%$ of glucose contents, respectively. Its molecular weight decrease from $3.50\sim2.12\times10^6$ to $0.85\sim0.77\times10^6$ with culture time. The molar ratio of glucose to mannose in the exopolymer produced with glucosamine changed from 55 ± 3 : 45 ± 3 to 29 ± 2 : 71 ± 2 and its molecular weight increased from 2.73×10^6 to $4.86\ x10^6$ with culture time. The molar ratio of glucose to mannose in exopolymers ranged from 87 ± 3 : 13 ± 3 to 28 ± 2 : 72 ± 2 and can be controlled by the mixed ratio of glucose to glucosamine as carbon sources (Table 6). On the basis on the results from enzyme hydrolysis of the exopolymers and comparison of ^{1}H- and ^{13}C-NMR chromatograms, the mannose as a monomeric component is substituted for glucose without changing in structure of pullulan. The molecular weight of pullulan decreased late in stationary growth phase due to the dual presence of rare amylase-sensitive maltotetrasoe sites among the predomonantly maltotriose units in pullulan and α-amylase secreted into the medium [14,15,59,]. The substituted of mannose for glucose resulted from the glucose analog used as carbon sources may be the reason not to be attacked by the α-amylase. It also can explain why the molecular weight of exopolymers produced with glucose analogs have higher than that with glucose.

Zoogloea gum (zooglan) is the bacterial exopolysaccharide produced by *Zoogloea ramigera*. It has been reported to consist of glucose, galactose, and pyruvic acid [26,27]. In one study, the relative molar percnetage of these components in the zooglan produced by *Z. ramigera* 115 was 71, 19, and 10, respectively [45]. Another investigation described that of glucose and galactose in the zooglan by *Z. ramigera* ATCC 25935 as 67 and 33, respectively [26]. Zooglan is a long chain polysaccharide consisting of mainly β-1,4-linked glucose residues and β-1,4 and β-1,3-linked galactose residues with branches of glucose residues at the C-3 or C-6 positions of the galactose residues [45, 56].

Z. ramigera is known to play an important role in flocculation during waste water treatment [47]. It was shown that extracellular polysaccharide made by *Z. ramigera* can accumulate certain metal ions such as Cu, Fe, Ni, Co, and Zn [28,55,85]. The weak acidity and structural features of zooglan may offer an explanation for this behavior [45]. The combined functional properties of viscoelasticity, surface-activity, acid stability, and salt compatibility enable this biological macromolecule to function as an effective stabilizer and emulsifier when used in oil-in-water emulsion systems [111].

Variability in the composition of zooglan and the possibility that repeat units other than glucose and galactose might be incorporated were explored with glucose analogs as carbon sources [65]. Specifically, the effects of physiological

Table 5. Production of exopolymer with glucose and its analogs by *A. pullulans* ATCC 42023[a]

Carbon (%)	pH[b]	DCW[c] (mg/ml)	Yield (mg/ml)	Exopolymer (Mol%)[d] Glucose: Mannose
Glucose	6.19	9.79	3.21 ±0.35	87 ±3 : 13 ±3
3-O-Methylglucose	7.47	1.74	0.47 ±0.10	57 ±6 : 43 ±6
Glucosamine	5.75	3.94	0.70 ±0.11	30 ±3 : 70 ±3
N-Acetylglucosamine	7.62	3.15	0.51 ±0.12	43 ±3 : 57 ±3
2-Deoxyglucose	6.82	0.43	0.35 ±0.08	54 ±5 : 46 ±5
Mannose	5.40	7.56	2.63 ±0.07	89 ±4 : 11 ±4

a. Results from 5 day cultures at 30℃ and values are the mean of triplicate experiments
b. The final pH
c. Dry cells weight
d. Mol % of glucose = [(glucose)/{(glucose)+(mannose)}]×100 and
Mol % of mannose = [(mannose)/{(glucose)+(mannose)}]×100

Table 6. Production of exopolymer with mixed carbon of glucose and glucosamine by *A. pullulans* ATCC 42023

Carbon (%) Glucose: Glucosamine	pH	DCW (mg/ml)	Yield (mg/ml)	Exopolymer (Mol%) Glucose: Mannose
2.0 : 0.0	5.80	9.61	3 10 ±0.30	87 ±3 : 13 ±3
1.5 : 0.5	4.86	8.07	2.29 ±0.03	80 ±2 : 20 ±2
1.0 : 1.0	5.02	8.33	1.60 ±0.05	75 ±3 : 25 ±3
0.5 : 1.5	5.07	8.30	1.14 ±0.05	61 ±3 : 39 ±3
0.0 : 2.0	5.52	3.57	0.74 ±0.02	28 ±2 : 72 ±2

parameters such as the glucose concentration and the initial medium pH on the molar ratio of glucose to galactose in products were also studied. Zooglans with altered monomeric compositions were produced by *Z. ramigera* ATCC 25935, by varying glucose concentration and initial medium pH. The relative molar percentage of the monomeric components, glucose and galactose, in the zooglan made with 2% glucose as the carbon source, was 66 and 34 %, respectively. By varying the glucose concentration and initial medium pH, the mol % ratios of glucose to galactose in zooglan ranged from 70 : 30 to 58 : 42 (Table 7). Also, glucose analogs were used as a co-substrate with glucose to produce modified zooglans. The mol % ratios of glucose to galactose in exopolymers produced by co-feeding glucose analogs ranged from 70 : 30 to 9 : 91 (Table 8). However, there was no evidence for the direct incorporation of the above glucose analogs into exopolymers.

The obserbation from production of modified pullulan and zooglan that physiological conditions for microorganisms can lead to modification of exopolysaccharide structures is consistent with other reports [110]. For example, the ratio of mannuronate to guluronate and the degree of *O*-acetylation of bacterial alginate produced *P. aeruginosa* was changed according to the carbon source in nutrient media [75]. The composition as well as the viscosity of xanthan gum produced by *Xanthamonas campestris* was also changed with concentration of mineral salts [113].

Non-metabolizable glucose analogs inhibit the bacterial growth on a wide variety of carbon sources [54]. These compounds probably compete with glucose at the level of transport and/or metabolism [19]. Apparently, the introduction of these glucose analogs into *A. pullulans* and *Z. ramigera* cultivations results in dramatic changes in sugar metabolism due to their competition with glucose [118] and alteration in gene expression involved in biosynthetic pathway [24] so that pathways leading to the formation of mannose or galactose monomer are greatly enhanced at the expense of the formation of polymerizable glucose. Thus, it is concluded that glucose analogs can function as powerful agents for modulating sugar metabolism and, ultimately, extracellular polysaccharide structure.

Compositional consistency of heteropolysaccharide (PS-7)

Heteropolysaccharide-7 (PS-7) is a water-soluble exopolymer produced by *Beijerinckia indica* var. *myxogenes* [48], formerly *Azotobacter indica*. PS-7 consisted of glucose, rhamnose, and uronic acid [89] and is degraded by a sphinganase that cleaves specific members of the gellan-related polysaccharides [77,93]. The molar repeat unit composition of PS-7 produced by *B. indica* was glucose, rhamnose, and galacturonic acid with an acetyl content of about 8.0~10.0% (by weight) [48,100,114]. PS-7 has good pseudoplasticity and the viscosity of PS-7 decreases as the shear rate increases. It has excellent stability and compatibility with high concentrations of salts [89]. It is incompatible with cationic or polyvalent ions at high pH which results in gel formation. These properties indicate that PS-7 is

Table 7. Production of exopolymer with various concentration of glucose as a sole carbon source by Z. *ramigera* ATCC 25935[a]

Glucose (%, w/v)	pH[b]	DCW[c] (mg/ml)	Yield (μg/ml)	Exopolymer (Mol%)[d] Glucose: galactose
0.5	4.7	0.8	71	67 : 33
1.0	4.6	0.9	86	66 : 34
2.0	4.5	1.0	108	66 : 34
3.0	4.5	1.2	132	62 : 38
4.0	4.3	1.6	129	62 : 38
5.0	4.1	1.7	55	58 : 42

a. Results from 5 day cultures at 30℃ and values are the mean of triplicate experiments
b. The final pH
c. Dry cells weight
d. The monomeric composition of glucose to galactose in the exopolymer

Table 8. Production of exopolymer with the mixed carbon of glucose and its analogs by Z. *ramigera* ATCC 25935

Carbon[a]	pH	DCW[c] (mg/ml)	Yield (μg/ml)	Exopolymer (Mol%) Glucose: Mannose
Glucose[b]	4.8	1.2	144	65 . 35
3-O-Methylglucose	5.6	1.6	83	9 : 91
Glucosamine	4.5	1.6	46	33 : 67
N-Acetylglucosamine	6.2	1.4	38	33 : 67

a. Glucose analogs (2%, w/v) as co-substrates were mixed with 1% glucose
b. Carbon source was 3% glucose

suitable as a drilling fluid or additive for a thickened aqueous media for oil recovery, or other potential applications including dripless water-based latex, well-joint cement adhesives, and textile printing [48].

The later structural study with the gas chromatographic analysis showed that monomeric components in PS-7 produced by *Beijerinckia indica* ATCC 21423 were rhamnose and glucose and the molar ration of rhamnose to glucose was 4.8 to 1.0 [66]. Galacturonic acid, previously reported as a repeat unit of PS-7, was not found in purified PS-7 in later study. The yield of PS-7 varied with physiological conditions, such as concentration of carbon source and initial pH of medium, but the molar ratio of rhamnose to glucose stayed within 1.0 to 4.6~5.1. Glucose and glucose analogs were fed separately as the carbon source to produce modified PS-7. *B. indica* ATCC 21423 utilized each of these carbon sources and produced exopolymers, although there was no direct incorporation of these sugars into PS-7. The molar ratio of rhamnose to glucose in each exopolymer made with glucose analogs as sole carbon source showed no significant variation (1.0 to 4.5~4.7). Some physiological factors such as source of carbon, concentration of glucose as the carbon source, and initial pH of the medium can affect cell growth and production of exopolymer, but do not effect the composition of the polymer or the molar ratio of glucose to rhamnose (1.0 to 4.4~5.1).

Unlike some other extracellular polysaccharides such as curdlan, microbial cellulose, pullulan, and zooglan, there was neither incorporation of glucose analogs into PS-7 nor chanage in composition and molar ration of components in PS-7. Most of glucose analogs are known to be non-metabolizable and inhibit the bactrial growth on a wide variety of carbon source [54]. Presumably, glucose analogs were metabolized to glucose by exoenzymes and/or intracellular enzymes such as demethylases [18], deaminases [44,53], or deacetylases [1,92]. The difference in growth rate and production of exopolymer may be due to different rates of enzyme specificities for each glucose analog. *B. indica* is a nitrogen fixing organism [31]. Glucosamine may have been the best carbon source for cell growth due to its use as a carbon and nitrogen source after deamination. The compositional consistency in PS-7, even over a wide range of culture conditions, may be an asset in large scale production of polymer with good quality control.

Conclusion

Seaweed alginate was acetylated by *P. syringae* ATCC19304. Acetylation drametically effects both solution properties and metal induced precipitation of alginates. Due to a higher viscosity and a low affinity for calcium ions, acetylated alginate can be favorably substituted nonacetylated alginates in many industrial applications. The ability of *P. syringae* to accetylate seaweed alginate supports the concept which a separation of alginate biosynthsis is separaed from alginate acetylation in this organism.

Direct incoporation of 3-*O*-methylglucose into curdlan by *Agrobacterium* sp. ATCC 31749 was the first demonstration to modify the structure of exracellular polysaccharide with glucose analogs used as sole carbon source. The structure of glucosamine and *N*-acetyl-glucosamine incorporated celluloses resembles to that of chintosan and chitin, respectively. Direct incorporation of glucose analogs into microbial cellulose may be resulted from the broader specificity for substrates of cellulose synthase in *Agrobacterium* sp. ATCC 10245. Comercially, chitosan is derived by the chemical deacetylation of chitin from waste crustacean exoskeletons with string alkali. This harsh conversion process, as well as variability in source material, lead to incosistent physico-chemical characteristics [2]. The formation of chitosan- and chitin-like exopolymers by direct bacterial incorporation of glucosamine and *N*-acetylglucosamine suggest new options in the biosynthesis and purification of consistent materials.

The pullulan and zooglan with various molar ratios of monomeric components were produced with glucose and its analogs used as carbon sources by *A. pullulans* ATCC 4202 and *Z. ramigera* ATCC 25935, respectively. The relative ratio of glucose to mannose in pullulan can be controlled by the ratio of mixed carbon sources. Therefore it is possible to produce the exopolymers with a more defined ratio of their monomeric components. The formation of modified pullulan and zooglan may be resulted from the effect of glucose analogs which modulate sugar metabolism involved in biosynthesis of extracellular polysacharides as well as metabolites for cell growth.

There no effect of glucose analogs found in PS-7 produced by *B. indica* ATCC 21423. It is apparently that this strain has various kinds of enzymes such as the demethylases [18], deaminases [44,53], or deacetylases [1,92], which can convert nonmetabolizable glucose analogs to glucose. The consistency in monomeric composition and molar ratio of monomers in PS-7 may be an asset in large scale production of polymer with good quality control. The microorganis, *B. indica* ATCC 21423, may be a good source for some of valuable enzymes.

Acknowledgements

This paper was supported by the Dong-A University Research Fund, in 1998.

Reference

1. Alfonso, C. O. M. Nuero, F. Santamaria, and F. Reyes. 1995. Purification of a heat-stable chitin deacetylase from *Aspergillus nidulans* and its role in cell degradation. *Cur. Microbiol.* 30, 49-54.
2. Arcidiacono, S. and D. L. Kaplan. 1992. Molecular weight distribution of chitosan isolated from *Mucor rouxii* under

different culture and processing conditions. *Biotechnol. Bioeng.* 39, 281-286.
3. Asano, T. 1978. Chitosan applications in wastewater sludge treatment, pp231-252, *In* Muzzarelli, R. A. A. and E. R. Pariser(ed.), Proceedings of the first international conference on chitin/chitosan, MIT Sea Grant Program, MITSG 78-7, MIT, Cambridge.
4. Auer, D. P. F. and R. J. Seviour. 1990. Influence of varying nitrogen sources on polysaccharide production by *Aureobasidium pullulans* in batch culture. *Appl. Microbiol. Biotechnol.* 32, 637-644.
5. Austin, P. R., C. J. Brine, J. E. Castle, and J. P. Zikakis. 1981. Chitin. new facets of research. *Science* 212, 749-753.
6. Badr-Eldin, S. M., O. M. El-Tayeb, E. G. El-Masry, O. A. Mohamad, and O. A. A. El-Rahman. 1994. Polysaccharide production by *Aureobasidium pullulans*: factors affecting polysaccharide formation. *World J. Microbiol. Biotechnol.* 10, 423-426.
7. Banerjee, P. C., R. I. Vanags, A. M. Chakrobarty, and P. K. Maitra. 1983. Alginic acid synthesis in *Pseudomonas aeruginosa* mutants defective in carbohydrate metabolism. *J. Bacteriol.* 155, 238-245.
8. Bartnicki-Garcia, S. and W. J. Nickerson. 1962. Nutrition, growth, and morphogenesis of *Mucor rouxii*. *J. Bacteriol.* 84, 841-858.
9. Blair, H. S., J. Guthrie, T. Law, and P. Turkington. 1987. Chitosan and modified chitosan membranes. I preparation and characterization. *J. Appl. Polym. Sci.* 33, 641-656.
10. Bouveng, H. O., H. Kiessling, B. Lindberg,, and J. McKay. 1963. Polysaccharides elaborated by *Pullularia pullulans*. *Acta Chem. Scand.* 17, 797-800.
11. Brandl, H., R. A. Gross, R. W. Lenz, and R. C. Fuller. 1990. Plastics from bacteria and for bacteria: poly(β-hydroxyalkanoates) as natural, biocompatible and biodegradable polyesters, pp77, *In* Ghose, T. K. and A. Fiechter(ed.), Advances in biochemical engineering/ biotechnology, vol. 41. Springer, Berlin.
12. Bulawa, C. E. and W. Wasco. 1991. Chitin and nodulation, Nature (London) 353, 710.
13. Canale-Parola, E., and R. S. Wolfe. 1964. Synthesis of cellulose by *Sarcina ventriculi*. *Biochim. Biophys. Acta.* 82, 403-405.
14. Catley, B. J. 1970. Pullulan, a relationship between molecular weight and fine structure. *FEBS Lett.* 10, 190-193.
15. Catley, B. J. 1979. Pullulan synthesis by *Aureobasidium pullulans*. pp69-84, *In* Berkeley, R. C. W., G. W. Gooday, and D. C. Ellwood (ed.), Microbial polysaccharides and polysaccharases, Academic Press Ltd., London.
16. Catley, B. J., A. Ramsay, and C. Servis. 1986. Observations on the structure of fungal extracellular-polysaccharide, pullulan. *Carbohydr. Res.* 153, 79-86.
17. Chung, H., D. R. Benson, and P. G. Schultz. 1993. Probing the structure and mechanism of Ras protein with an expanded genetic code. *Science 259*, 806-809.
18. Chelvarajan, R. L., F. F. Fannin, and L. P. Bush. 1993. Study of nicotine demetylation in *Nicotiana otophora*. *J. Agr. Food Chem.* 41, 858-862.
19. Cloherty, K., D. L. Diamond, K. D. Heard, and A. Carruthers. 1996. Regulation of GLUT1-mediated sugar transport by an antiport/uniport switch mechanism, *Biochem.* 35, 13231-13239.
20. Davidson, J. W., I. W. Sutherland, and C. I. Lawson. 1977. Localization of O-acetylated groups. *J. Gen. Microbiol.* 98, 603-606.
21. Day, D. F and J. W. Lee. 1994. Process for acetylating seaweed alginate with *Pseudomonas syringae* subsp. *phaseolocola*. *US Pat.* 5,308,761.
22. Day, D. F. and J. W. Lee, 1995 Acetylated alginates, and method for producing acetylated alginates. *US Pat.* 5,444, 160.
23. Dougherty, B. A. and I. van de Rijn. 1994. Molecular characterization of *has*A from an operon required for *hyaluronic acid synthesis in group A Streptocicci J. Biol. Chem.* 269, 169-175.
24. Easson, Jr., D. D., A. J. Sinskey, and O. P. Peoples. 1987. Isolation of *Zoogloea remigera* I-16-M exopolysaccharide biosynthetic genes and evidence for instability within this region. *J. Bacteriol.* 169, 4518-4524.
25. Fett, W. F., S. Osman, M. L. Fishman, and T. S. Siebles, III. 1986. Alginate production by plant-pathogenic Pseudomonads. *Appl. Environ. Microbiol.* 52, 466-473.
26. Franzen, L. and A. B. Norberg. 1984. Structural investigation of the acidic polysaccharide secreated by *Zoogloea ramigera* 115. *Carbohydr. Res.* 128, 111-117.
27. Frieman, B. and P. R. Dugan. 1964. Identification of *Zoogloea* species and the relationship to zoogloeal matrix and floc formation. *J. Bacteriol.* 55, 205-207.
28. Friedman, B. A. and P B. Dugan. 1968. Concentration and accumulation of metallic ions by the bacterium *Zoogleoa*. *Develop. Ind. Microbiol.* 9, 381-388.
29. Fyfe, J. A. M., and J. R. W. Govan. 1980. Alginate synthesis in mucoid *Pseudomonas aeruginosa*: a chromosomal locus involved in control. *J. Gen. Microbiol.* 119, 443-450.
30. Glucksmann, M. A. T. L. Reuber, and G. C. Walker. 1993. Family of glycosyl transferases needed for the synthesis of succinoglycan by *Rhizobium melioti*. *J. Bacteriol.* 175, 7033-7044.
31. Gottschalk, G. 1979. *Bacterial metabolism*. pp250-257, Spriner-Verlag, New York
32. Gros, E. G., I. O. Mastronardi, and A. R. Frasca. 1971. P. M. R. spectral assignments for *O*-methyl gruops in mono-methylated D-hexose. *Carbohydr. Res.* 16, 232-234.
33. Gross, R. A. 1994. Bacterial polyesters: structural variability in microbial synthesis, pp173-188, *In* Shalaby, S. W.(ed.), Biomedical polymers: designed-to-degrade systems, Hanser Publishers, New York.
34. Hacking, A. J., I. W. F. Taylor, T. R. Jaramn, and J. R. W. Govan. 1983. Alginate biosynthesis by *Pseudomonas mendocina*. *J. Gen. Microbiol.* 129, 3473-3480.
35. Hackman, R. H. and M. Goldberg. 1974. Light-scattering and infrared-spectrophotometric studies of chitin and chitin derivatives. *Carbohydr. Res.* 38, 35-45.
36. Harada, T., K. Fujimori, S. Hirose, and M. Masada. 1966. Growth and β-glucan 10C3K production by a mutant of *Alcaligenes faecalis* var. *myxogenes* in defined medium. *Agr Biol. Chem.* 30, 764-769.
37. Harada, T., M. Masada, K. Fujimori, and I. Maeda. 1966. Production of a firm, resilient gel-forming polysaccharide by a mutant of *Alcaligenes faecalis* var. *myxogenes* 10C3. *Agr Biol. Chem.* 30, 196-198.
38. Harada, T., M. Masada, H. Hidaka, and M. Takada. 1966. Production of firm, resilient gel-forming polysaccharide in

natural medium by a mutant of *Alcaligenes faecalis* var. *myxogenes* 10C3. *J. Ferment. Technol.* 44, 20-24.
39. Harada, T., A. Misaki, and H. Saito. 1968. Curdlan: a bacterial gel-forming β-1,3-glucan. *Arch. Biochem* 124, 292-298.
40. Harding, N. E., J. M. Cleary, D. K. Cabanas, I. G. Rosen, and K. S. Kang. 1987. Genetic and physical analyses of a cluster of genes essential for xanthan gum biosynthesis in *Zanthomonas campestris*. *J. Bacteriol.* 169, 2854-2861.
41. Hassler, R. A. and D. H. Doherty. 1990. Genetic engineering of polysaccharide structure: production of variants of xanthan gum in *Xanthomonas campestris*. *Biotechnol. Prog.* 6, 182-187.
42. Haug, A., and B. Larsen. 1962. Quantitative determination of the uronic acid composition of alginate. *Acta Chem. Scand.* 16, 1908-1918.
43. Hirano, S., Y. Ohe, and H. Ono. 1976. Selective *N*-acylation of chitosan. *Carbohydr. Res.* 47, 315-320.
44. Horjales, E., M. M. Altamirano, M. L. Calcagno, Z. Dauter, K. Wilson, R. C. Garratt, and G. Oliva. 1992. Crystallization and preliminary crystallograhic studies of glucosamine-6-phosphate deaminase from *Escherichia coli* K12. *J. Mol. Biol.* 226, 1283-1286
45. Ikeda, F., H. Shuto, T. Saito. T. Fuui, and K. Toita. 1982. An extracellular polysaccharide produced by *Zoogloea ramigera* 115. *Eur. J. Biochem.* 123, 437-445.
46. Imshenetskii, A. A., T. F. Kondrat'eva, and A. N. Smut'ko. 1981. Influence of the acidity of the medium, conditions of aeration, and temperature on pullulan biosynthesis by polyploid strains of *Pullularia* (*Aureobasidium*) *pullulans*. *Microbiol.* 50, 330-333.
47. Joyce, G. H. and P. R. Dugan. 1970. The role of floc-forming bacteria in BOD removal from waste water. *Develop. Ind. Microbiol.* 11, 377-386.
48. Kang, K. S. and W. H. McNeely. 1977. PS-7-a new bacterial hetero-polysaccharide, pp220-230, *In* Sandford, P. A. and A. Laskin, (ed.), Extracellular microbial polysaccharides, American Chemical Society, Washington, DC.
49. Kang, K. S., G. T. Veeder, P. J. Mirrassoul, K. Kaneto, and I. W. Cottrell. 1982. Agar-like polysaccharide produced by *Pseudomonas* species. Production and basic properties. *Appl. Environ. Microbiol.* 43, 1086-1091.
50. Kaplan, D. L., B. J Wiley, J. M. Mayer, S. Arcidiacono, J. Keith, S. J, Lombardi, D. Ball, and A. L. Allen. 1994. Biosynthetic polysaccharides, pp189-212, *In* Shalaby, S. W.(ed.), Biomedical polymers: designed-to- degrade systems, Hanser Publishers, New York.
51. Kent, R. A., R. S. Stephens, and J. A. Westland. 1991. Bacterial cellulose fiber provides an alternative for thickening and coating. *Food Technol.* June, 108.
52. Kimura, H., S. Sato, T. Nakagawa, H. Nakatani, A. Matsukura, T. Suzuki, M. Asai, T. Kanamaru, M. Shibata, and S. Yamatodani. 1973. New thermo-gelable polysaccharide-containing food stuffs. *U.S. Pat.* 3,754,925.
53. Kimura, M., A. Takatsuki, and I. Yamaguchi 1994. Blasticidin S deaminase gene from *Aspergillus terreus* (BSD). a new drug resistance gene for transfection of mammalian cells. *Biochim. Biophys. Acta* 1219, 653-659.
54. Kornberg, H. and L. T. M. Lamboure. 1994. The role of phosphoenolpyruvate in the simultaneous uptake of fructose and 2-deoxyglucose by *Escherichia coli*. *Proc. Nat. Acad. Sci. USA*. 91, 11080-11083.
55. Kulm, S. P. and R. M. Pfister. 1990. Accumulation of cadmium by immobilized *Zoogloea ramigera* 115. *J. Ind. Microbiol.* 6, 123-128.
56. Kwon, K. J., K. J. Park, J. D. Kim, J. Y. Kong, and I. S. Kong. 1994. Isolation of two different polysaccharides from halophilic *Zoogloea* sp.. *Biotechnol. Lett.* 16, 783-788.
57. Lacroix, C., A. LeDuy, G. Noel,and L. Choplin. 1985. Effect of pH on the batch fermentation of pullulan from sucrose medium. *Biotechnol. Bioeng.* 27, 202-207.
58. Larsen, B. and A. Haug. 1971. Biosynthesis of alginate. I composition and structure of alginate produced by *Azotobacter vinelandii* (lipman). *Carbohydr. Res.* 17, 287-296.
59. Leathers, T. D. 1987. Host amylase and pullulan production, pp 175-185, *In* Kaplan, D. L.(ed.), Materials Biotechnology Symposium Proceedings, U. S Army Natick Research, Development, & Engineering Center, Natick.
60. Leathers, T. D. 1993. Substrate regulation and specificity of amylases from *Aureobasidium* strain NRRL Y-12,974. *FEMS Microbiol. Lett.* 110, 271-222.
61. LeDuy, A., J. E. Zajic, J. H. T. Luong, and L. Choplin. 1988. Pullulan, pp650-660, *In* Mark, H. F., N. M. Bikales, C. G. Overberger, G. Menges, and J. I. Kroschwitz(ed.), Encyclopedia of polymer science and engineering. Vol. 13, Wiley, Toronto.
62. Lee, J. W., and D. F. Day. 1995. Bioacetylation of seaweed algnate *Appl. Environ. Microbiol.* 61, 650-655.
63. Lee, J. W , R. D. Ashby, and D. F. Day. 1996. Role of acetylation on metal induced precipitation of alginates. *Carbohydr. Polym.* 29, 337-345.
64. Lee, J W., W. G. Yeomans, A. L. Allen, D. L. Kaplan, F. Deng, and R. A. Gross. 1997a. Exopolymers from curdlan production: incorporation of glucose-related sugars by *Agrobacterium* sp. strain ATCC 31749. *Can. J. Microbiol.* 43, 149-156.
65. Lee, J. W , W. G. Yeomans, A. L. Allen, R. A. Gross, and D. L Kaplan. 1997b. Production of zoogloea gum by *Zoogloea ramigera* with glucose analogs. *Biotechnol. Lett.* 19, 799-802.
66. Lee, J. W., W. G. Yeamans, A. L. Allen, R. A. Gross., and D. L Kaplan. 1997c. Compositional consitency of a heteropolysaccharide-7 produced by *Beijerinckia indica*. *Biotechnol. Lett.* 19, 803-807.
67. Lee, J. W , W. G. Yeamans, A. L. Allen, R. A. Gross., and D. L. Kaplan 1997d. Microbial production of water-soluble non curdlan type exopolymer-B with controlled composition by *Agrobacterium* sp.. *Biotechnol. Lett.* 19, 1217-1221.
68 Lee, J W. and D F. Day. 1998. The separation of alginate biosynthesis and acetylation in *Pseudomonas syringae*. *Can. J. Microbiol.* 44, 394-398.
69. Lee, J. W., W. G. Yeamans, A. L Allen, R. A. Gross., and D. L. Kaplan 1999. Biosynthesis of nevel exopolymers by *Aureobasidium pullulans*. submitted to *Appl. Environ. Microbiol.*
70. Legge, R. L. 1990. Microbial cellulose as a speciality chemical. *Biotech. Adv.* 8, 303-319.
71. Lin, T., and W. Z. Hassid. 1966. Pathway of alginic acid synthesis in the marine brown alga, *Fucus gardneri* silva. *J. Biol. Chem.* 241, 5284-5297.
72. Lin, F. C., R. M. Brown, Jr. R. R. Drake, and B. E. Haley. 1990. Identification of the uridine 5'-diphosphoglucose (UDP-

Glc) binding subunit of cellulose synthase in *Acetobacter xylinum* using the photoaffinity probe 5-azido-UDP-Glc. *J. Biol. Chem.* 265, 4782-4784.
73. Linker, A. and R. S. Jones. 1964. A polysaccharide resembling alginic acid from a *Pseudomonas* microorganism. *Nature* 204, 187-188.
74. Maeda, I., H. Saito, M. Masada, A. Misaki, and T. Harada. 1967. Properties of gels formed by heat treatment of curdlan, a bacterial β-1,3 glucan. *Agr. Biol. Chem.* 31, 1184-1188.
75. Marty, N., J. Dournes, G. Chabanon, and H. Montrozier. 1992. Influence of nutrient media on the chemical composition of the exopolysaccharide from mucoid and non-mucoid *Pseudomonas aeruginosa*. *FEMS Microbiol. Lett.* 98, 35-44.
76. McNeil, B. and B. Kristiansen. 1990. Temperature effects on polysaccharide formation by *Aureobasidium pullulans* in stirred tanks. *Enz. Microb. Technol.* 12, 521-526.
77. Mikolajczak, M. J., L. Thorne, T. J. Pollock, and R. W. Armentrout. 1994. Sphinganase, a new endoglycanase that cleaves specific members of the gellan family of polysaccharides *Appl. Environ. Microbiol.* 60, 402-407.
78. Minami, S., Y. Okamoto, K. Miyatake, A. Matsuhashi, Y. Kitamura, T. Tanigawa, Y. Tanaka, and Y. Shigemasa. 1996. Chitin induces the IV collagen and elastic fiber in implanted non-woven fabric of polyester. *Carbohydr. Polym.* 29, 295-299.
79. Moraine, R. A. and P. Rogovin. 1971. Xanthan biopolymer production at increased concentration by pH control. *Biotechnol. Bioeng.* 13, 381-391.
80. Morris, E. R., D. A. Rees, and D. Thom. 1978. Chiroptical and stoichimetric evidence of a specific primary dimerization process in alginate gelation. *Carbohydr. Res.* 66, 145-154.
81. Morris, E. R. and D. A. Rees. 1980. Competitive inhibition of interchain interactions in polysaccharide system. *J. Mol. Biol.* 138, 363-374.
82. Muzzarelli, R. A. A. 1977. *Chitin.* pp. 207-253, Pergamon Press, New York.
83. Nakatsuka, S. and A. L. Andrady. 1992. Permeability of vitamin B-12 in chitosan membranes. effect of crossing and blending with poly(vinyl alcohol) on permeability. *J. Appl. Polym. Sci.* 44, 17-28.
84. Nilsson, S. 1992. A thermodynamic analysis of calcium-alginate gel formation in the presence of inert electrolyte. *Biopolym.* 32, 1311-1315.
85. Norberg, A. B. and H. Persson. 1984. Accumulation of heavy metal ions by *Zoogloea ramigera*. *Biotechnol. Bioeng.* 26, 239-246.
86 Ono, K., N. Yasuda, and S. Ueda. 1977. Effect of pH on pullulan elaboration by *Aureobasidium pullulans* S-1. *Agr. Biol. Chem.* 41, 2113-2118.
87. Osawa, Z., T. Morota, K. Hatanaka, T. Akaike, K. Matsuzaki, H. Nakashima, N. Yamamoto, E. Suzuki, H. Miyano, T. Mimura, and Y. Kaneko. 1993. Synthesis of sulfated derivatives of curdlan and their anti-HIV activity. *Carbohydr. Polym.* 21, 283-288.
88. Orts, W. J., J. D. Rousseau, and H. G. Lawford. 1987. Improved microbial production of curdlan type-polysaccharide, pp459-469, *In* Stivala, S., V. Crescenzi, and I. C. M. Dea(ed.), Industrial polysaccharides, Gordon and Breach Science, New York.
89. Paul, F., A. Morin, and P. Monsan. 1986. Microbial polysaccharides with actual potential industrial applications. *Biotech. Adv.* 4, 245-259.
90. Penman, A. and G. R. Sanderson. 1972. A method for the determination of uronic acid sequence in alginate. *Carbohydr. Res.* 25, 273-282.
91. Phillips, K. R., J. Pik, H. G. Lawford, B. Lavers, A. Kligerman, and G. R. Lawford. 1983 Production of curdlan-type polysaccharide by *Alcaligenes faecalis* in batch and continuous culture. *Can. J. Microbiol.* 29, 1331-1338.
92. Picard, F. J. and J. R. Dillon. 1989. Cloning and organization of seven arginine biosynthesis genes from *Neisseria gonorrhoeae*. *J. Bacteriol.* 171, 1644-1651.
93. Pollock, T. J., L. Thorne, and R. W. Armentrout. 1992. Isolation of new *Aureobasidium* strains that produce high-molecular-weight pullulan with reduced pigmentation. *Appl. Environ. Microbiol.* 58, 877-883.
94. Pollock, T. J. 1993. Gellan-related polysaccharides and the genus *Sphingomonas*. *J. Gen. Microbiol.* 139, 1939-1945.
95. Rees, D. A. 1972. Shapely polysaccharides. *Biochem. J.* 126, 257-273.
96. Rho, D., A. Mulchandsani, J. H. T. Luong, and A. LeDuy. 1988. Oxygen requirement in pullulan fermentation. *Appl. Microbiol. Biotechnol.* 28, 361-366.
97. Roesser, D. S., S. P. McCarthy, R. A. Gross, and D. L. Kaplan. 1996. Effects of substitution site on acetyl amylose biodegradability by amylase enzymes. *Macromol.* 29, 1-9.
98. Roller, S. and I. C. M. Dea. 1992. Biotechnology in the production and modification of biopolymers for foods. *Crt. Rev. Biotechnol.* 12, 261-277
99. Ross, P., R. Mayer, and M. Benziman. Cellulose biosynthesis and function in bacteria. *Microbiol. Rev.* 55, 35-58.
100. Sandford, P. A. and J. Baird, J. 1983. Industrial utilization of polysaccharides, pp411-490, *In* Aspinall, G. O.(ed.), Polysaccharide II, Academic Press, London.
101. Saxena, I. M., K. Kudlicka, K. Okuda, and R. M. Brown, Jr. 1994. Characterization of genes in the cellulose-synthesizing operon (*acs* operon) of *Acetobacter xylinum*: implications for cellulose crystallization. *J. Bacteriol.* 176, 5735-5752.
102. Saxena, J. M., R. M. Brown, Jr. M. Fevre, R. A. Geremia, and B. Henrissat. 1995. Multidomain architecture of β-glycosyl transferase: implications for mechanism of action. *J. Bacteriol* 177, 1419-1424.
103. Schramn, M., and S. Hestrin. 1954. Synthesis of cellulose by *Acetobacter xylinum*. 1. Micromethod for the determination of celluloses. *Biochem. J.* 56, 163-166.
104. Seviour, R. J. and B. Kristiansen. 1983 Effect of ammonium ion concentration on polysaccharide production by *Aureobasidium pullulans* in batch culture. *Eur. J. Appl. Microbiol. Biotechnol.* 17, 178-181.
105. Sherbroack-Cox, V., N. Russell, and P Gacesa. 1984. The purification and chemical characterization of the alginate present in extracellular material produced by mucoid strains of *Pseudomonas aeruginosa*. *Carbohydr. Res.* 135, 147-154.
106. Skjåk-Bræk, G., B. Larsen, and H. Grasdalen. 1985. The role of *O*-acetyl groups in the biosynthesis of alginate by *Azotobacter vinelandii*. *Carbohydr. Res.* 145, 169-174.
107. Skjåk-Bræk, G., F. Zanetti, and S. Paoletti. 1989. Effect of acetylation on some solution and gelling properties of alginates. *Carbohydr. Res.* 185, 131-138.
108. Skjåk-Bræk, G., S. Paoletti, and T. Gianferrara. 1989.

Selective acetylation of mannuronic acid residues in calcium alginate gels. *Carbohydr. Res.* 185, 119-129.
109. Slodki, M. E., and M. C. Cadmus. 1978. Production of microbial polysaccharides. *Adv. Appl. Microbiol.* 23, 19-54.
110. Slodki, M. E. 1987. New bacterial polysaccharide, pp3-13, *In* Stivala, S., V. Crescenzi, and I C. M. Dea(ed.), Industrial polysaccharide, Gordon and Breach Science Pub. New York.
111. Stauffer, K. R., J. G. Leeder, and S. S. Wang. 1980. Characterization of zooglan-115, an extracellular glycan of *Zoogloea ramigera*-115. *J. Food Sci.* 45, 946-952.
112. SteinbUchel, A. 1991. Polyhydroxyalkanoic acids, pp123, *In* Byrom, D. (ed.), Biomaterials: novel materials from biological sources, Stockton Press, New York.
113. Sutherland, I. W. 1990. Physiology and industrial production, pp70-88, *In* Biotechnology of microbial exopolysaccharides. Cambridge Univ. Press, Cambridge.
114. Symes, K. C. 1982. Lipophylic polysaccharides. *Carbohydr. Polym.* 2, 276-281.
115. Taguchi, R., Y. Kikuchi, Y. Sakano, and T. Kobayashi. 1973. Structural uniformity of pullulan produced by several strains of *Pullularia pullulans*. *Agr. Biol. Chem.* 37, 1583-1588.
116. Thorne, L., L. Tansey, and T. J. Pollock. 1987. Clustering of mutations blocking synthesis of xanthan gum by *Xanthomonas campestris*. *J. Bacteriol.* 169, 3593-3600.
117. Venturi, V., M. Otten, V. Korse, B. Brouwer, J. Leong, and P. Weisbeek. 1995. Alginate regulatory and biosynthetic gene homology in *Pseudomonas putida* WCS358: correlation with the siderophore regulatory gene *pfr*A. *Gene* 155, 83-88.
118. Versa, J. C., A. M. Reyes, J. G. Carcamo, F. V. Velasquez, C. I. Rivas, R. H. Zhang, P. Strobel, R. Iribarren, H. I. Scher, J. C. Slebe, and D. W. Golde. 1996. Genistein is a natural inhibitor of hexose and dehydroascorbic acid transport through the glucose transporter, GLUT1. *J. Bio. Chem.* 271, 8719-8724.
119. Wecker, A., and U. Onken. 1991. Influence of dissolved oxygen concentration and shear rate on the production of pullulan by *Aureobasidium pullulans*. *Biotechnol. Lett.* 13, 155-160.
120. White, S. A., P. R. Farina, and I. Fulton. 1979. Production and isolation of chitosan from *Mucor rouxii*. *Appl. Environ. Microbiol.* 38, 323-328.
121. Wiley, B. J., D. H. Ball, S. M. Arcidiacono, J. M. Mayer, and D. L. Kaplan. 1993. Control of molecular weight distribution of the biopolymer pullulan produced by *Aureobasidium pullulans*. *J. Environ. Polym. Degrad.* 1, 3-9.
122. Wong, H. C., A L. Fear, R. D. Calhoon, G. H. Eichinger, R. Mayer, D. Amikam, M. Benziman, D. H. Gelfand, J. H. Meade, A. W. Emerick, R. Bruner, A. Ben-Bassat, and R. Tal. 1990. Genetic organization of the cellulose synthase operon in *Acetobacter xylinum*. *Proc. Nat. Acad. Sci. USA.* 87, 8130-8134.
123. Yamamoto, I., K. Takayama, K. Homma, T. Gonda, K. Matsuzaki, K. Hatanaka, T. Uryu, O. Yoshida, H. Nakashima, N. Yamamoto, Y. Kaneko, and T. Mimura. 1991. Synthesis, structure and antiviral activity of sulfates of cellulose and its branched derivatives. *Carbohydr. Polym.* 14, 53-63.
124. Yamanaka, S., K. Watanabe, N. Kitamura, M. Iguchi, S. Mituhashi, Y. Nishi, and M. Uryu. 1989. The structure and mechanical properties of sheets prepared from bacterial cellulose. *J. Mat. Sci.* 24, 3141-3143.
125. Yuen, S. 1974. Pullulan and its applications. *Proc. Biochem.* 9, 7-9.