Study Effecting of Hetero Chitosan Mineralization on Structure of *Proteus spp*

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Abstract

DE protection, demineralization, de colorization, and DE acetylation. Protein accesses were used to obtain chitosan from shrimp shell waste. Using FTIR, SEM and XRD. It was characterized. Also analyzed were the physiochemical parameter such as moisture content, hydrogen meter, viscosity, degree of DE acetylation and solubility The age of bacterial culture influenced its susceptibility to chitosan with cells being most prone to chitosan in the late exponential period. Hetero chitosan oligosaccharides were made up of partially DE acetylated chitosan's 90%, 75%, and 50% DE acetylated chitosan's. It was clear that in the presence of the hetero chitosan and their oligosaccharides, the growth of gram –negative bacteria is less inhibited than gram positive bacteria. These results revealed that hetero chitosan's and their oligosaccharide rely on the antibacterial effects degree of DE acetylation, and molecular weight.

Keyword*: Chitosan, Proteus spp, mineral, antimicrobial medicines, Penicillin*

Introduction

It has become great interest not only as an underused resource, but also as anew high potential function material in different fields, and recent progress in chitin chemistry is noteworthy. Antimicrobial medicines have brought about dramatic shift not just in the treatment of infectious disease, but also in the fate of kind. The medicines were synthetic substances in terms of protection and efficacy, andhadlimitations, Fleming had discovered penicillin in 1928. The antibiotic was named penicillin, and in the 1940 it reached clinical use. Penicillin, which is an excellent protection and efficacy agent, led by saving the lives of many wounded soldiers during world war II in the era of antimicrobial chemotherapy[1]. The antimicrobial activities of water soluble chitosan derivatives such as quaternary ammonium chitosan have been identified inseveral studies, hydroxyl propyl chitosan, N-car boxy butyl chitosan, car boxy methylated chitosan and sulfated chitosan. Chitosan copolymer consisting of of â-(1f4)- 2- acetate med-D-glucose, extracted from chitin in the presence of alkali through DE acetylation. This exhibits a broad range of biological activities such as antitumor activity. The possible uses of acetate solution as a food preservative as afresh, natural anti micro bio local agent for antimicrobial packaging films have been studied. The exact mechanism of chitosan's antimicrobial activity and itsderivatives is not yet completely understood but has been suggested to include cell lye sis breakdown of the cytoplasmic membrane barrier and chelation by the chitosan of trace metal cat ions may be required for the growth of the microorganism. A cationic chitosan must communicate with both membranes of the bacterial cell envelope in the killing of gram negative

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bacteria[3]. The genus *Proteus* contain gram negative, possible anaerobic, heterotrophic and proteolysis rods as opportunistic pathogens for human use. Some bacteria have changed the taxonomic classification many times. In the family these throw closely related genera formed the Protease tribe. The genus *Proteus* currently contains *P. mirabilis, P. vulgaris, P. pinner, P. Hauser* and three genome species. Based on molecular studies, *P. Hauser* as well as the genome species were isolated from P. *vulgaris and* from *P. vulgaris* group. Genome species and are labelled with numbers only because there were no metabolic properties indicated to allow their full differentiation[4].

Materials and Methods

Preparation of chitosan solution

A 1% chitosan solution used to be prepared by way of dispersing 150 g of chitosan in 1 l of water, dissolving it and stirring through including 400 ml of 1 M lactic acid and making up to 15 l with water. The pH used to be adjusted to be 5.5 with a saturated NaHCO3 solution.[5]

Enzymatic hydrolysis of chitosan in the UF membrane reactor

The UF membrane reactor (Millipore Minitab" system, Millipore Co., USA). It consisted of a supplement tank, a reservoir tank, a water bath for control- ling reaction temperature, three peristaltic pumps, a membrane cartridge with molecular weight cut off (MWCO) 3000 Da and an enzyme reactor vessel. The quantity of reducing sugar produced from chitosan by means of the UF membran erea ctoratagiven on rate usedtobe incontrast withthe best conditions for the enzyme concentration, incubation time, and reaction temperature determined in the batchreactor. The oligosaccharide sproduced from chitosanat special permeation rates havebeen analyzed by HPL Con the TSK gel NH260 column.

The reactor system for semicontinuous production of oliosacharides was additionally operated beneath the choicest conditions. A new substrate used tobe brought to the reactor tankafter sufficient incubation and recycling time had been allowed to hydrolyze the chitosan solution[5].

Assay for antibacterial activity

Antibacterial things to do of chitosan and chi to oligosaccharides were examined as the inhibitory effects against the increase of E.coli. A 0.5 ml of 1% sample solution in 0.05 M acetate buffer (pH 6.0) was introduced to the mixture of 0.5 ml of the cultured microorganism solution and 49 ml of try optic soy broth medium, and incubated with shaking at 37°C. The inhibitory results have been estimated periodically through measuring the turbidity of the cultured medium at 640 nm. In a manage group, 0.5 ml of 0.05 M acetate buffer in region of the oligosaccharide- ides used to be brought to the mixture[5].

Results and Discussion

Binding of chitosan with mineral

The development of chitosan based materials as useful adsorbent polymeric matrices. In particular is a growing area in the area of adsorption science[6]. Chitosan composites have recently been developed to adsorb heavy metals and environmentally friendly dyes. The development of chitosan it has been proved that chitosan composites have better adsorption capacity and resistance to acidic conditions. Chitosan has a high affinity to the surface of silica based minerals due tothe interaction between a part of the polymer protonated amino groups and dissociated silica hydroxyl groups produced in aqueous solution. [7]. The ability of chitosan to organize heavy metal ions Zn(II), Cu(II), Cd(II) and Fe(III), is less than equal to the ability of mineral pores to hold ions of these metals without the formation of chemical bonds. Even though these minerals possess high capacity for adsorption, their structural modification will successfully booster their capabilities, indicates multilayer adsorption on composites for Cr(III) and Fe (III). Kinetic studies have shown that composites give fast kinetics to adsorb Cr(III) and Fe(III) . The

isomorphs substitutions of Al3+ for Si4+ in the tetrahedral layer and Mg2+ for Al3+ in the octahedral layer resulted in Mont morally nit having a negative charged surface. Generated Mont morale nit coated with chitosan for removal of Cr (VI) [8]

Deficiency of chitosan effect on structure of bacteria

Gram positive bacteria structure for Chitosan treated exhibited a biofilm –like structure formed on the surface and bottom of the tube. *B. cereus bears* more negative surface load than *Escherichia. Coli* despite having weak antimicrobial activity in the direction of gram positive, while *B. cereus* chitosan interaction was found to induce the formation of a biofilm like structure Antibiotic9. *B. cereus*produced polysaccharides secreted may some barriers and may increase the bacterial survival rate, that has been found to contain a higher exo polysaccharide content compared to *E. coli* in its colony, *B. cereus* developed red and blue color. By contrast E. coli *was* shown to be transparent and deemed not to produce a biofilm. The chitosan has been found to have lower gram positive B. cereus *inhibition*. Finally the widespread overuse of antibiotic in many natural habitats, such as waste water and river, lakes, drinking water and livestock has been reported to cause the existence of sub inhibitory concentration . [10]

TABLE 1. Effect of various levels of chitosan on the outgrowth of E. coli at pH 6.5 or 5.5.[7] Wang, 1992.

Concentrations		Incubation (d)								
E. coli cells/ml. pH of chitosan (%)		0	1	2	3	4	5	6	7	8
6.5	I	6.8 x 10'•	4.5 x 106	5.1 x 10'	9.8 x 10'	6.4 x 10'	4.7 x 10'	3.6 x 10'	2.7 x 10'	1.3 x 10'
	0.5	6.8 x 10'	1.0 x 10'	1.4 x 106	2.3 x 10'	3.4 x 10'	7.3 x 10'	6.5 x 10'	7.7 x 10'	5.3 x 107
	1.0	6.8 x 10'	4.4 x 10'	3.9 x 10‘	8.7 x 106	9.3 x 10‘	1.2 x 10'	6.7 x 10'	4.2 x 10'	8.3 x 10'
	1.5	6.8 x 10"	4.9 x 10'	8.1 x 10‘	1.1 x 106	1.0 x l0^	4.2 x l0^	5.0 x l0^	6.1 x l0^	1.6 x 106
	2.0	6.8 x 10'	2.3 x 10'	4.1 x 10'	1.3 x 10'	9.4 x 10'	9.8 x 10'	7.8 x 106	3.0 x 10'	3.2 x 10'
	2.5	6.8 x 10'	4.4 x 10'	8.0 x 102	6.0 x 10°	4.0 x 10°	2.4 x 10'	3.3 x l0•	7.9 x 10"	1.4 x 10'
5.5	0	6.8 x 10'	5.7 x l0•	1.3 x 10'	3.2 x 107	6.4 x 107	1.5 x 10'	1.2 x 10'	6.1 x 10'	6.3 x 10'
	0.5	6.8 x 10°	3.6 x 10°	<102	<102	<10°	<102	<102	<10°	<102
	1.0	6.8 x 10'	1.4 x 10'	<10°	<10°	<102	<10°	<10°	<102	<10°
	1.5	6.8 x 10'	<10°	<10°	<10°	<10°	<10°	<10°	<10°	<102
	2.0	6.8 x 10°	<102	<102	<10°	<10°	<10°	<102	<10°	<10°
	2 5	6.8 x 10'	<10°	<10°	<10°	<10'	<10°	<102	<10°	<10°

Mineralization with structure of bacteria.

In the biological environment bio mineralization is common and is transmitted by bacteria, protests', fungi plants and animals. [11]. And other referred as biologically passive mineralization BPM. The difference between these modes is that the organism does not regulate and enforce morphology. Although three –mode classification is useful, in the context of non-cell driven mineralization or organic template driven mineralization. First quickly turn into a more stable calcite, calcite precipitation during metabolism has been well exemplified in more recent efforts by arrange of urease active bacteria. Where the breakdown of urea into ammonia and CO2 satisfies both the increase in hydrogen number and the production ofcarbonate The variety of metabolic processes leading mineralization of bacteria Carbonates represent the most common bacterial induced minerals that precipitate during the metabolism of organic matter in response to CO2. Bacterial metal sorption and subsequent precipitation can be necessary and useful for metal and radionuclide removal during metal and radionuclide contaminated waste bioremediation. Biogenic manganese oxide showed a greater metal binding ability than well crystallized synthetic manganese oxide a higher catalytic activity for organic compound degradation [12].

Conclusion

Much work on chitosan and its derivatives has been done for tissue engineering drug delivery, wound healing, water treatment, antitumor and antimicrobial activity, as well as increasing the surface area for cell attachment, migration. The best antibacterial behavior was demonstrated by crab polymer chitosan, while squid polymer chitosan showed the best efficiency to inhibit *S. aurous, B. ceres, and B. subtitles*. The minimum inhibition concentration and minimum bactericidal concentration values of chitosan sources can be applied to different purpose in the food industry, such as natural food preservatives which extend food shelf life. Quarter noised chitosan ,which introduces the hydroxyl group or amino group of polymers to permanently positive charged quaternary groups enhances antimicrobial activity over aside hydrogen number range. Chitosan is extremely effective in extracting mercury from dilute solution. The poor diffusion properties of chitosan in the solid state, in terms of kinetic behavior ,cause a control of the absorption performance by sorbent particle size. However, the characteristic of chitosan makes it possible to use ultrafiltration in liquid form by coupling the chelation mechanism for mercury recovery and anionic dyes coagulation-flocculation. The use of liquid formed chitosan increases the accessibility and /or availability of reactive sites. During polymer dissolution ,the breaking of hydrogen bonds between amino groups and between hydroxyl groups (inter-chain or intra-chain bonds) is far more interact with metal ions and anionic dyes. This can explain the much more efficient use of amino chitosan groups when the polymer is used for color removal in the dissolved –state This effect is less important when considering mercury, in this case the positive effect of using chitosan in the dissolved state (ultrafiltration coupled with chelation)is only important for binding because binding capacities at saturation tend to be values of the same magnitude order. *Escherichia coli* no viable cells were detected after 1 hour experimental groups (those with chitosan) in the presence of 0-25 mm sodium chloride. However except for the 102 colony forming unit /ml inoculum ,the cell counts for chitosan supplemented cell suspensions with 100mM sodium chloride were similar to those of the control. Just 100 mM sodium chloride had an inhibitory effect on *E. coli*. we suspect that 100mM from sodium reversed the impact chitosan on *E. coli* is not dueto competition between chitosan and sodium for binding to the cell surface with negative residues, but because sodium and chitosan form a complex that reduces binding to the cell surface. The amount of free (uncompleted) chitosan at low sodium concentration or with fewer cells (10^2 CFU/ml) would be sufficient to destroy all the cells.In the presence of 100mM

sodium, the efficacy of chitosan decreasedwith growing cell counts (10^3 to 10^5 CFU /ml). Better understanding is that chitosan was complexes with sodium. If the sodium and chitosan were varying, the bactericidal effect of chitosan would be independent of the cell counts. Chitosan binds strongly to various metal cat ions, such as Cu. The involvement of –OH and NH2 groups on glucosamine residues as ligands, since the NH2 groups are crucial sites for binding chitosan to cells, it will be predicted that the chitosan –sodium complex will not be able to bind cells, in the presence significant amounts of sodium and cells. The sum of free chitosan would not be adequate to bind all of the cells, so the number of *E. coli* cells will not be significantly reduced .Similarly, the divalent captions decreased the bactericidal effects of chitosan on *E .coli* at concentrations of 10-25 mm *E. coli* the barium, magnesium, sodium at concentrations of 10 and 25*mill micron* also decreased the activity of chitosan. Curiously, the reversal effect seen in 100mM sodium also occurs at 25mM sodium in this higher cell inoculum (10^7 cfu/ml). Chitosan found that enhanced adhesion of *E. coli*, by neutralizing the negative cell charges. The showed that inorganic cat ions (sodium, magnesium)inhibited chitosan –mediate adhesion of *E. coli*, revealed that chitosan induced calcium release the permeability of the membrane improved proved that leakage caused by chitosan was inhibited by divalent cat ions of the order of barium, calcium. Effects of 25 and100 mill micron sodium on activity of chitosan to *E. coli* only occurred athigh densities of the cells. The cat ions bind directly to the chitosan instead of binding on the cell surface, and that it is the formation of these complexes, with the consequent reduction of free chitosan, that leads to a reduction in the activity of chitosan. Magnesium indicated this occurred because inorganic cat ions could substitute the loss of calcium, from the cell surface to form new stabilizing complexes on the cell surfaces that prevent the leakage caused by chitosan. The possible contribution of *Proteus* spp. to intestinal diseases and infections has been somewhat neglected. Research into the virulence of *Proteus* spp. in the urinary tract using the bacteriology of ileac conduits and intestinal segments for bladder augmentation suggests that *Proteus* spp. Should be examined more closely for their potential as gastrointestinal pathogens. There is increasing evidence that *Proteus* species may play a role in inflammatory bowel disease through the direct action of the bacteria, compounded by host immune evasion and perturbation. As Gram- negativeorganisms, *Proteus* species areintrinsicallyproinflammatory result of the production of lipopolysaccharide (LPS) and immune stimulatory flagella proteins. There may be an association between *Proteus* species and inflammatory bowel disease, especially Crown's disease, mainly through population expansion and immune activation. The effect of treating the drug and the degree of mineralization of the chitosan yield load power. The degradation level observed in both chitosan with mineral and chitosan without affecting the absorption enhancing properties of chitosan is still not certain. This could be analyzed using degradation and bio adhesion measurements leakage of *E .coli* cause with chitosan. *E. coli cells* attracted to the medium by glucose. It appears that glucose leakagein the chitosan supplemented cell suspension increased inversely with the log of the viable cell count. We find the chitosan caused protein leakage and U.V absorbent content based on the 260 nm absorbance and protein concentration calculation.

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