Next Generation Nanochitosan

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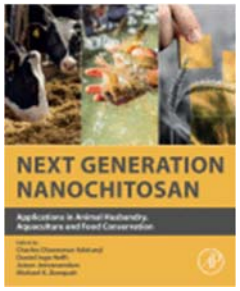

Chapter 12 - Nanochitosan derived from marine bacteria

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Abstract

Nanochitosans are polysaccharides produced by the alkalescent deacetylation of chitin and comprise a series of 2-deoxy-2 (acetylamino) glucose linked by ß-(1-4) glycosidic linkages. These are naturally formed from the deacetylation of shellfish shells and the exoskeleton of aquatic arthropods and crustaceans. Reports of chitosan production from unicellular marine bacteria inhabiting the sea, and possessing distinct animal- and plant-like characteristics abound. This capacity to synthesize chitosan from chitin arises from response to stress under extreme environmental conditions, as a means of survival. Consequently, the microencapsulation of these nanocarriers results in new and improved chitosan nanoparticles, nanochitosan. This nontoxic bioactive material which can serve as an antibacterial agent, gene delivery vector as well as carrier for protein and drug release as compared with chitosan, is limited by its nonspecific molecular weight and higher composition of deacetylated chitin. This chapter highlights the biology and diversity of nanochitosan-producing marine bacteria, including the factors influencing their activities, survival, and distribution. More so, the applications of marine bacterial nanochitosans in transfection and gene delivery; wound healing and drug delivery; feed supplement development and antimicrobial activity are discussed.

- Previous chapter in book
- Next chapter in book

Keywords

Chitosan

Drug delivery

Gene delivery

Marine bacteria

Nanochitosan

Transfection

Biology of marine bacteria

Marine bacteria are single-celled organisms that play vital roles in the marine ecosystem's nutrition cycles, maintenance, and sustainability (Dash et al., 2013). Marine bacteria are predominantly Gram-negative, they can be found in higher loads in bottom sediments, and low concentrations in water. These microorganisms can rapidly adjust towards deterioration and environmental changes. Gulder and Moore (2009) believe that marine bacterial natural products are the sea's treasures, and they consider that it is worth investigating.

Morphology

Marine bacteria are a few microns (0.0005 mm) in cell diameters, and can be morphologically classified into three forms: *Cocci*—spherical; *Bacilli*—elongated rods, and *Spirilla*—helicoidal or spirals (less common). Marine bacteria form a distinct class of organisms, though some, such as the cyanobacteria have animal-like characteristics while acting more like plants (Huber et al., 2007). One of the distinctive plant features of these organisms is the capacity to produce complex compounds such as amino acids with only ammonia as a nitrogen source; even in the absence of chlorophyll and cellulose in their plasma membrane. Marine bacteria mostly constitute spore-forming motile, flexible rods and various types of comma-shaped (vibrios), distinguishing them from their terrestrial counterparts (Buerger et al., 2012).

The minute size of these organisms has a profound bearing on their activities, introducing unique difficulties and problems in the techniques for cumulating, collecting, and approximating actual mass and numbers. The rapid rate of reproduction of marine bacteria is also a feature, and it is achieved by vegetative cell division. However, their reproduction has been that of binary fission in cases where a cluster of organisms occur in sizeable numbers that the coloration of the water is distorted by the aggregation of their bodies. Without perturbation in their biological state, and alongside ideal thriving conditions, the aggregation of individuals can progress geometrically (Davidson and Belbin, 2002), at a rate of bacterial division once every 20 or 30 minutes, leading to a tremendous build-up of extremely large mass.

Physiology

Reports on marine bacteria have been mainly concerned with their biological and physiological activities in relation to the sea and its biotic problems. Marine bacteria play an essential role in sea ecology, and are mainly involved with the specific change of organized materials and not with the build-up or storage of organic content (Wagner-dobler and Biebl, 2006). A clearer insight into their physiology requires that we distinguish between the different modes of nutrition of these organisms.

Autotrophic bacteria

These act like green plants to build proteins and carbohydrates out of simple materials like carbon dioxide and inorganic salts. Autotrophic marine bacteria are either photosynthetic, possessing bacteriochlorin and using radiant energy in protoplasm build-up, or chemosynthetic, deriving energy from the oxidation of different inorganic substances like sulfur or ammonia (Lauro et al., 2009; Das et al., 2006).

Heterotrophic bacteria

These groups of bacteria derive energy from the oxidation of organic substances; hence, they live as parasites or saprophytes. Heterotrophic bacteria are the transforming agents of the sea where they make the continuous return of raw materials possible as a result of equilibrium in the organismal death and birth rates over extended periods. This is accomplished via organic matter mineralization (Day, 2003). Studies have highlighted the presence of a sizeable amount of organic matter in seawater. Although the concentrations per unit volume of water are much smaller than those present in surface soil, the aggregation of organic matter in solution has been estimated to be greater than that occurring in living organisms in the sea. Marine bacteria are the only organisms that can make considerable use of dissolved organic matter and may thus obtain a part of their food through the use of organic matter in solution in the sea. However, some scientists argue that the low concentrations, of dissolved and evenly distributed organic matter in the oceans, might be due to the activities of heterotrophic bacteria (Ziegler et al., 2017; Berthold et al., 2021).

The physiology of marine bacteria can also be classified based on different oxygen tolerances. Some forms utilize free oxygen and are referred to as obligate aerobes, some maintain their function in the absence of free oxygen (obligate anaerobes), and some may thrive in any of the environment (facultative forms), as well as those that require a slight depletion of free oxygen (microaerophiles). Marine bacteria play key functions in the chemical and physiological cycling of many compounds in the sea (Das et al., 2006), depending on their metabolism and thus can be classified as nitrifying, denitrifying, nitrogen-fixing, sulfur, and iron bacteria.

Origin

Unicellular microorganisms are the ancestors of modern bacteria, being the primary type of existence to appear on the earth billions of years ago. Despite the presence of bacterial fossils, the absence of exceptional morpho-types militates against recorded bacterial evolution tracking as a hint of specific bacterial species origin (DeLong and Pace, 2001). Nonetheless, bacterial phylogeny can be reconstructed using gene sequences and those reviews report the first bacterial divergence from the eukaryotic genealogy (Newton et al., 2010). Furthermore, bacteria were integral in the 2nd evolution divergence of archae and eukaryotes through endosymbiotic relationships with predecessors of eukaryotic cells; where proto-eukaryotic cells engulfed alphaproteobacterial symbionts which form mitochondria, found in the eukarya (Brown and Doolittle, 1997). Eventually, cyanobacterial-like organisms were enveloped by a few eukaryotes that previously had mitochondria, as a result of this, chloroplasts appeared in plants and algae. Further endosymbiotic relationships often referred to as derived endosymbiosis also resulted in the origination of some algae (Dyall et al., 2004).

Distribution

Marine bacteria have been found in great abundance in shoreline waters, where the production of plant and animal life is in abundance (Lauro et al., 2009). The vertical distribution of marine bacteria is found in two significant centers: first, in the benthic zone, below the mud-water interface; and second, in the pelagic area attached to floating plants, animals, and other particulate matter. It is worthy of note that, though one center of distribution is in the pelagic zone, bacterial existence is evidently not exactly planktonic but immobile upon organisms, and hence, must closely correspond horizontally and vertically with the maximal distribution of these organisms. Some researchers have also indicated that bacteria occur in relatively small quantities in the water to a depth of 5000 m, and should be regarded as planktonic (Amaral-Zettler et al., 2010). However, since their preferred habitat is that of attachment, it is presently impossible to determine their true planktonic existence. Some may have arrived there accidentally and can thrive and reproduce to the degree that they can utilize the dilute organic matter in the water.

The largest population of marine bacteria is found on the bottom, where huge volumes of wet mud may occur (Zinger et al., 2011). Such dense populations are viable because a considerable part of organic waste, dead carcasses of plants and animals, is concentrated in a thin layer indicating an interface between mud and water, which is continually sinking and forming an abundant rich food supply for bacteria as well as bottom-dwelling competitors. The majority of bacteria are found in the first few millimeters of bottom ooze, and their numbers decrease as the thickness of the bottom deposits increases. Generally, huge numbers are present in fine sediments than in coarse ones (LoveJoy et al., 2006). Many bacteria have a periphytic habit, which makes them attach to plankton organisms for favorable multiplication in the euphotic zone (Day, 2003). A substantial periphytic bacterial population in the plankton causes a rapid bacterial breakdown of significant volumes of dead organisms before they sink to considerable depths, resulting in the generation of a substantial portion of mineralized plant nutrients within or just below the euphotic zone.

Diversity of marine bacteria

The bacterial diversity of the marine environment is high compared with other domains. There are vast numbers of bacterial diversity in marine ecosystems (Babić et al., 2018; Sanz-Sáez et al., 2020).

For instance, prokaryotic cells in the oceans have been estimated to be 1029. Marine environments possess substrates rich in a nutrient that support diverse bacterial growth (Whitman et al., 1998; Egan et al., 2008). Consequently, marine bacterial communities play important roles in different biochemical processes. More so, their uncommon ability to adapt to certain climatic conditions of extreme salinity and pressure affords them some novel characteristics in the marine ecosystem (Hatosy et al., 2013; Gajigan et al., 2018).

The functioning of the marine habitat is largely dependent on diversity and community structure of which bacterial diversity is of great influence (Babić et al., 2017). Marine bacteria diversity can be characterized by both culturing and molecular techniques. Although molecular methods provide an adequate description of bacteria that cannot be cultured, it accounts for the complex bacterial community of the marine ecosystem (Li and Qin, 2005; de Cárcer, 2020). About 90% of the marine bacteria are Gram-negative and over 80% belong to the phyla: firmicutes, proteobacteria, actinobacteria, and bacteroidetes (Sobhana, 2015). Cyanobacteria are the most abundant photosynthetic bacteria in marine environments (Babić et al., 2017; Rajaneesh et al., 2020); the diversity and distribution of marine bacteria indicate *Alteromonas* and *Erythrobacter* as the most abundant culturable genera present in over 90% of the studied samples. Members of the genera: *Halomonas*, *Marinobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Idiomarina*, and *Sulfitobacter* were less abundant but present in almost all the oceanographic regions (Sanz-Sáez et al., 2020). Other identified genera include *Psychrobacter*, *Zunongwangia Leeuwenhoekiella*. More so, molecular techniques identified *Pelagibacteraceae* (SAR11) with *Candidatus pelagibacter* ubique as the most abundant species (Sobhana, 2015). Nonetheless, bacterial species found in the marine ecosystem are often associated with genera that are found on land (Jensen and Fenical, 1996; Ghate et al., 2021).

Marine bacteria are found to vary in environments mainly due to their diversity (Rohwer et al., 2002; Ziegler et al., 2017; Berthold et al., 2021). They can be found at the oceanic level, seas, coral reefs, and dark smokers of hot thermal vents at the seafloor as microbial mat or biofilms. They also exhibit symbiotic relationships with corals, sponges, plants, and other marine organisms (Wilson et al., 2010; Kiran et al., 2018). However, the variability of marine bacteria in different niches and microenvironment of the marine ecosystem is essential and dependent on various factors. Mestre et al. (2017), and Bižić-Ionescu et al. (2015) reported significant variation in the bacterial diversity pattern in the marine microhabitats such that diversity changed with the particle composition, depth, and season.

Moreover, Mestre et al. (2020) recently reported annual variations of bacterial communities in the particulate matter continuum owing to the length of days, sea temperature, and fraction size. Though, the coral reefs and the deep-sea marine environs tend to support bacterial activities and diversity as they serve as sources of the organic substrate with unique physicochemical parameters such as extremely high pressure, low temperature, oxygen concentration, fluctuating salinity as well as the absence of light (Piccini and Garcia-Alonso, 2015; Ghosh et al., 2011). The adaptability of bacteria in these unique environments elicits the novelty of most marine bacteria. In general, the adaptability of marine bacteria to the microenvironments influences different processes of the biosynthetic pathways resulting in novel metabolic and bioactive products (Cleary et al., 2017; Poli et al., 2017; Zeaiter et al., 2018). *Pseudomonas*, *Alteromonas*, *Vibrio*, *Bacillus*, and *Streptomyces* genera of marine bacteria are reported to produce novel secondary metabolite (Jensen and Fenical, 1996; Das et al., 2006; Poli et al., 2017).

The metabolic diversity of marine bacteria appropriates them to different roles in the biogeochemical cycles (Wilson et al., 2010; Cevera et al., 2020). For example, sulfate-reducing bacteria (*Desulfovibrio*, *Desulfobulbus*, *Desulfomonas*, *Desulfococcus*, *and Desulfotomaculum*) are involved in the cycling of sulfur and sulfur compounds in marine environments. While *Methanosarcina*, *Methanococcus*, *Methanomicrobium*, *Methanococcoides*, *Methanogenium*, *Methanoplanus*, and *Methanobolus* metabolize trimethylamine to produce methane as an end product. *Nitrosococcus* oxidizes ammonia to nitrite, and *Nitrococcus* oxidizes nitrite to nitrate, before converting nitrogen to a readily accessible form; nitrogen fixation is carried out by *Clostridium* and *Azotobacter*. *Synechococcus* and *Prochlorococcus* (cyanobacteria) are involved in carbon cycling (Gajigan et al., 2018). Similarly, the luminous bacteria (*Photobacterium phosphoreum*, *Photobacterium leiognathi*, *Vibrio harveyi*, and *Vibrio fischeri*) help in nutrient cycling in the sea and produce light when luciferin is in contact with oxygen.

Furthermore, marine bacteria can also degrade natural organic matter such as cellulose (by *Cytophaga*, *Sporocytophaga*), protein (by *Pseudomonas*), chitin (by *Bacillus* and *Vibrio* then pectin (by *Clostridium pectinovorum*) (Ghosh et al., 2011; Liang et al., 2021). The findings by Gómez-Consarnau et al. (2019) and Hassanzadeh et al. (2021) revealed marine bacteria possess rhodopsins that capture solar energy by an energy transducing mechanism reported to contribute rise in sea temperatures. Generally, the marine microbial community has a complex ecosystem, which is yet to be discovered, and new diversities impacts in oceans have not totally been explored. Hence, possible discoveries of marine microbes and their significant impacts on the marine environment and humanity as a whole are inevitable.

Physicochemical factors that influence marine bacteria

The survival and thriving of marine bacteria in their natural habitats are a function of the quality of the environment. Water quality is a dynamic characteristic influenced by a variety of physicochemical parameters that are interdependent and these include nutrients (such as phosphorus and nitrogen), temperature, salinity, light, dissolved oxygen (DO), chemical oxygen demand (COD), and pH (Sun et al., 2021). In the dry season, increased salinity in inversely proportional to water levels falling due to continual evaporation and decreased rainfall. This is particularly seen in desert lakes hosting poly-extremophilic bacteria that possess distinct metabolic capabilities (Salazar et al., 2020).

As a result of eutrophication caused by nutrient pollution, dissolved oxygen, the quantity of oxygen accessible to the varied life-forms in an aquatic ecosystem might drop (Dodds and Whiles, 2017; Bozorg-Haddad et al., 2021). Ocean warming, due to rising global temperatures, causes the upper layer of the sea to reduce in-depth, thus exposing marine microbes to harmful amounts of ultraviolet (UV) rays from the sun (Jin et al., 2021). Harvey et al. (2020) submit that acidification lowers the sea pH while altering the diverse bacterial communities; and causing a symbiotic shift in the relationships existing between the marine bacterial communities, the endosymbiotic photosynthetic dinoflagellates, and corals (O'Brien et al., 2016).

Anthropogenic activities and marine environment

Anthropogenic pollutants such as UV filters in paints, plastics, and sunscreens are toxic to actively growing marine bacterial cells, reducing bacterial growth. Lozano et al. (2020) submit that exposing some of these pollutants to sun light reduces their hazardous impact on marine bacteria in their logarithmic growth phase. Warming and acidification of waters are two issues for marine life that are connected to global warming (Baag and Mandal, 2022). In the same vein, Tetu et al. (2020) highlight leachates discharged into the water due to environmental weathering as having negative impacts on the metabolic activities of certain photosynthetic marine bacteria.

The coast and marshes are interconnected physical and marine ecosystem regions with abundant microbial diversity (Huang et al., 2020; Murray et al., 2019). The interaction of seawater and flowing freshwater results in estuaries which are a unique microbial habitat (Fierer, 2017). Interestingly, microbial communities are affected and respond to the slightest changes in these environments. Coastal wetlands have been gradually degraded and altogether lost in some places with increased human activities and rising sea levels (Tian et al., 2016). Soil salinization is a cause of marshland deterioration and poses a serious environmental issue in coastal areas, potentially affecting ecosystem performance and material recycling in marshes (Li et al., 2020b). Initial reports by Li et al. (2019a) and Li et al. (2019b) argue that environmental condition fluctuations in salinity and organic matter might have a major impact on the composition and functioning of existing microbial associations, as well as biochemical and ecosystem attributes. Guo and colleagues (2017, 2019) affirmed that hydraulic situation and salinity have the ability to change the design and variability of bacterial communities in marshlands. In the coastal region, a little change in nutrients causes a noticeable shift in the dominant microbial species (Li et al., 2019c). As a result, the microbial association and components in diverse marshlands might be affected by soil environmental heterogeneity, which could lead to changes in microbial metabolic activity (Rath et al., 2019).

The distribution of estuarine bacterial species has been found to be influenced by the presence of nitrogen, phosphorus, tidal variations, and variations in vegetation and organic matter (Zhao et al., 2020). Furthermore, there can be the alteration of microbial colonies by the exudates of plant roots, through a change in the substrate provided, and overground litter (Lammel et al., 2015).

Chitin and chitosans

Chitins, precursors for chitosan, are relatively the most abundant polysaccharide in the marine environment, since they form the exoskeleton structure of many of aquatic animals, and are produced by numerous phytoplankton species (Paulsen et al., 2019). The components of chitin include the β-1,4-linked N-acetylglucosamine residues, which are arranged in β (parallel), α (antiparallel), or in γ (mixed strand) nature, and mostly cross-linked with other structural components made of protein or glucan, except for the β-chitin from diatom (Singh et al., 2021). This biopolymer has a high molecular weight with a chemical makeup resembling that of cellulose except for the C2 hydroxyl group being replaced by the acetamido group. A number of chitinolytic bacteria are widely distributed and abundant in the oceans and seashores and are responsible for the recycling of nitrogen and carbon in the chitinous debris, thus, helping to maintain marine life (Kaur et al., 2012). These bacteria can survive stressful environmental conditions in the marine ecosphere, and they play a significant role in converting the most abundant insoluble polysaccharide (chitin) to a more useful biological form (chitosan) that has wider applications, especially in the medical sector.

Chitosans are soluble in almost all aqueous acidic solutions, but insoluble in most organic solvents and water, and is more viscous than chitin (Kumar et al., 2019). They are the simplest and more economical derivatives of chitin. Many marine chitinolytic bacteria have been isolated as free-living in the water bodies and likewise, found in aquatic animals, plants, and sediments. Marine bacteria such as *Vibrio vulnificus*, *Alteromonas* sp., *Salinivibrio costicola*, *Vibrio furnissi*, *Streptomyces* sp., *Moritella marina*, *Pseudoalteromonas piscicida*, *Vibrio alginolyticus*, *Microbulbifer degradans*, *Vibrio cholera*, *Photobacterium galatheae*, *Vibrio proteolyticus*, and *Vibrio anguillarum* have been isolated and screened for their capability to produce chitosan (Souza et al., 2011). Likewise, various chitinolytic genes are often extracted, cloned, characterized, and clarified from marine bacteria. These bacteria also have a broad antimicrobial effect in the presence of chitin, as a result of the expression of chitinase genes they possessed (Paulsen et al., 2016).

Bacterial chitosan

Studies on chitinolytic bacteria show an array of synthesized enzymes hydrolyzing a wide-range of chitin to produce various chitin-derived products at varying proportions (Paulsen et al., 2016), one of which is chitosan. Bacterial chitosan is biopolymers and oligomers produced through the extracellular biotransformation of chitin to chitosan, by the chitin deacetylase (CDA) enzyme synthesized by the fermenting chitinolytic bacteria (Ali et al., 2020a). The enzyme catalyzed the splitting of *N*-acetamido bonds present in chitin to synthesize chitosan and generate acetic acid and glucosamine elements. This chitosan varies from 0% to 100% in deacetylation with different molecular weights and polymerization structures. The presence of this enzyme has been reported in several insect and fungi species. However, the production proportions in these organisms are low, together with the complicated manufacturing procedures (Kaur et al., 2012).

Bacterial chitosan has the improved advantage of higher molecular weight and minimal requirements for extraction and purification procedure of the synthesizing enzymes from the fermenting bacteria. These derived biopolymers also have interesting physicochemical and biological properties, such that they are biorenewable, biofunctional, chelating, biodegradable, anticoagulant, biocompatible, antimicrobial, nonantigenic, polycationic and nontoxic in nature. Likewise, they can chemically be transformed into beads, gels, colloids, fibers, flakes, films, capsules, powders, nanoparticles, nanofibers, etc. For the initial attachment of marine bacteria to chitin substrate, it involves the production and presence of different specific chitin-binding peptides. These peptides group regulate and coordinate the attachment to specific sites and chitin-degradation process in the chitinolytic bacteria, conferring on these bacteria selective privileges over other organisms in the high chitin-abundant water. *Vibrio harveyi*, a marine bacterium, is known to have at least two peptide clusters. The 53 kDa molecular weight peptide is produced constitutively and facilitates the initial adherent to the chitin. Conversely, the second 150 kDa peptide is time dependent peptide, induced and produce only in the presence of chitin (Montgomery and Kirchman, 1993).

Nanochitosans

The microencapsulation of chitosan (as nanocarriers) from marine bacteria is increasingly under investigation, finding applications in the vaccines, drugs, and biologics industries (Elieh-Ali-Komi and Hamblin, 2016). Improved chitosan attracts a lot of attention, especially for its bactericidal properties against a board spectrum of bacteria. Chitosan has a positively charged structural surface that can easily bind and interact with the negative surface of a bacterium to lyse and disrupt the cytoplasmic components of the cell, intercepting the metabolism and growth of the organism (Fadli et al., 2018). Nano-sized chitosan particles thus have enhanced physical property that is projected to be more effective in piercing and disrupting the cell wall when in interaction with pathogenic organisms (Vijayalakshmi et al., 2016). Nanochitosan has a high effective potential in supplying drugs through nasal, gastrointestinal, and pulmonary pathways for the quick recovery of patients (Rashki et al., 2021; Divya and Jisha, 2018).

Various formulating methods have been employed to produce nanochitosan from nanoparticles and nanofibers. These methods focused mainly on interlocking oppositely charged particles together. The preferred method depends on the drug physicochemical properties, the chitosan properties, and acetylation level. The nanoparticle preparation methods include precipitation (coagulate), ionic gelation cross-linking, reverse micellar, spray drying, covalent cross-linking, self-assembling, emulsion droplet coalescence, etc. (Salahuddin and Galal, 2017), while, electrospinning, sol-gel, thermal oxidation, chemical vapor deposition methods, etc. are used for the preparation of chitosan nanofibers (Zhao et al., 2011). Macromolecules and solvents used for preparing nanochitosan include tripolyphosphate (TPP), trifluoroacetic acid (TFA), glutaraldehyde, dichloromethane, trichloromethane, acetic acid, hexafluoro-2-propanol (HFIP), dextran sulfate, polyethylene glycol (PEG), sodium tripolyphosphate (STPP), ligands (Maiti and Jana, 2017). Crosslinking of this polymer with other metals and bioactive ingredients were also reported. Compounds such as $Fe(OH)_2$, lactoglobulin, vitamin C, alginate, sureteric, doxorubicin (DOX) are constantly being incorporated into the nanochitosan to create a synergy that improves the effectiveness of the biopolymer.

Nanochitosan derived from marine bacteria

Nanochitosans are considered **natural bioactive materials** with environmentally beneficial physicochemical constituents. Nanochitosan may be produced by a variety of methods, including ionotropic gelatination of STPP and chitosan (Mesa et al., 2021**;**Morales-Olan et al., 2021). Chitosan has been demonstrated as a possible medication carrier due to its biocompatible ingredients. Presently, chitosan nanoparticles particles are used in pharmaceutics, tissue engineering, wastewater treatment, agriculture, food processing, and medicine, among other applications (Stincone and Brandelli, 2020). Mesa et al. (2021) suggests further chitosan coating of nanoparticles derived from different sources to reduce their unfriendly impacts and boost their bioavailability in the body. Nalini et al. (2018) argue that their deacetylation level and molecular weight can be reconstituted, resulting in a variety of physico-mechanical components.

The United States Food and Drug Administration **(US FDA**) asserts that nanochitosans are a nontoxic biocompatible polymer with remarkable properties as nano-size with a large surface area in relation to volume ratio, leading to possible applications in dietary and wound dressing. They also possess reactive hyroxyl ion (–OH), azanide (–NH2), and cationic-natured ammonia groups (–NH3). Considering the possible applications as gene carriers, drug delivery, plant protection, encapsulation, and plant stimulating agents; further examination of nanochitosan bio-adhesivity, bioactivity, biodegradability, and biocompatibility nature may be necessary, as well as the antibacterial and antifungal agent, through some avenues (Mesa et al., 2021). Industries have recognized the potential utilization of nanotechnology and materials generated from nanotechnology or nanoparticles developed in the fields of consumer end materials like individual care materials, paints, and microelectronics/automotive, and pharmaceuticals. In the food and agricultural industries, nano-sensors can be employed in storage systems and pathogen detection as well as nano-encapsulation/nano-delivery of food ingredients and nano-formulations of agrochemicals (Srinivasan et al., 2021; Seyedmohammadi et al., 2016).

Marine bacterial nanochitosan consists of antibacterial agents, gene delivery vectors, and carriers for protein and drugs release. Stincone and Brandelli (2020) submit that nanochitosan can be used as adjuvants for the nasal administration of vaccines against influenza, piglet paratyphoid, and hepatitis B. Reports have demonstrated enhanced antigen absorption by mucosal lymphoid tissues, which elicit active immune responses to antigens using nanoparticles. Chitosan has been demonstrated in hastening wound healing by aiding skin cell proliferation, while limiting pathogenic infection in wounds. Nanochitosan derived from marine microorganisms can help wounds heal faster by inhibiting the spread of opportunistic infection. When tested on skin cell fibroblasts and keratinocytes in the lab, nanochitosan particles from marine bacteria can restore skin characteristics, resulting in antiaging skincare products (Chawla et al., 2015). Marine bacterial nanochitosan particles are currently employed in food preservation and teeth problems treatment. Nanochitosan is also used as a constituent of antimicrobial preparations, textile manufacturing, and some medicinal applications. Nanochitosan exhibits antibacterial effects against *Escherichia*, *Saprophyticus*, and *Staphylococcus* species (Chawla et al., 2015).

In comparison to the typical response, the application of nano chitosan to influenza B or Panama virus resulted in an active immunogenicity stimulation (Bacon et al., 2015). On bacteria and certain encapsulated viral particles, chitosan exerts a lytic impact (Carroll et al., 2021). Bacteria and other organisms inhabiting marine and freshwater sediments produce an array of unique chemicals or bioactive compounds, such as antimicrobials, in order to resist diseases in their environment and protect their hosts (from which they obtain carbon sources) from other surface-dwelling bacteria (Srinivasan et al., 2021; Alexpandi et al., 2019). Other bioactive compounds of marine bacterial origin include antiquorum-sensing, antibacterial, antifungal, antiviral, and anticancer substances (Carroll et al., 2021). Previous reports by Saurav et al. (2017) record secondary metabolites of marine bacteria and organisms linked to their hosts. These bioactive compounds produced have resulted in the development of novel methods for the production of unique compounds. Reports by Alexpandi et al. (2019) proved marine microorganisms to be a promising source of new therapeutic substances.

Despite its inherent qualities and plausible applications against microbial infections, there are several issues with nanochitosan, that might limit its uses. Chitosan has no specific molecular weight, making molecular weight delivery difficult, and hence a challenge for regulatory bodies to approve the widespread use of chitosan, particularly in medicine. Chitosan is made up of deacetylated chitin, and nanochitosan has a greater degree of deacetylation (DDA), and hence has a more active antibacterial effect (Kravanja et al., 2019). According to research by Younes and Rinaudo (2015), prolonged treatment with NaOH for a long time and incubation at a high temperature might result in chitosan with a high DDA (>90%), indicating that the sample contains fewer than 10% *N*-acetylglucosamine. The purity of chitosan is also a serious issue that prevents its use since a small amount of *N*-acetylglucosamine can inhibit bioactivity against bacteria. Finally, chitosan has limited solubility at alkaline or neutral pH, as it can only dissolve at acidic pH (Varlamov and Mysvakina, 2018). Low pH increases positive charges on chitosan, resulting in a stronger antibacterial activity. However, low pH conditions are generally hostile to human tissues, cells, or organs (Hosseinneiad and Jafari, 2016).

Applications of marine bacterial nanochitosan

Drug delivery

Biocompatibility, nontoxicity, easy availability, cost-effectiveness, and biodegradability of chitosan (Rizeq et al., 2019) make it widely accepted as a carrier of several hydrophobic, hydrophilic, and unstable drugs. Before the advent of nanoparticles (for instance, chitosan-based NPs), one of the most challenging aspects of medicine administration was delivering active components to required locations in the body while avoiding side effects on healthy organs (Sudhakar and Jayaveera, 2014). The stability of marine-derived nanochitosan for a long time before it reaches the target organ makes it suitable for drug delivery (Dawood et al., 2020) and chitosan-based NP especially ones derived from marine bacteria have shown low toxicity *in-vivo and in-vitro* studies (Mohammed et al., 2017). Removal of nanochitosans is made easy through renal clearance after the delivery (Rizeq et al., 2019), which is made possible because of the ideal molecular weight (1–100 nm) of marine-derived nanochitosan (Singh et al., 2015).

Feed supplement and development

A study on *Liza ramada* (grey mullet) fed with dietary chitosan nanoparticles exhibited growth improvement, proper utilization of feed, and also an improvement in the antioxidative responses and immunity of *L. ramada* when continuously fed with nanochitosan (Dawood et al., 2020). The study also recorded enhanced intestinal histo-morphometry and antibacterial capacity in *L. ramada*, when fed with dietary nanochitosan, confirming the potential of nanochitosan as a potential fed supplement for animals mainly aquatic animals (Abdel-Tawwab et al., 2019).

Antimicrobial activity

Nanochitosan has broad-spectrum antimicrobial activity against diverse disease-causing microorganisms. In a study by Ikono et al. (2019), the antibacterial activity of nanochitosan was exhibited against various biofilm species of *Streptococcus mutans*. The bactericidal activities of nanochitosan have been reported both Gram-positive bacteria, like *Bacillus cereus*, *Listeria monocytogenes Staphylococcus aureus*, *Lactobacillus plantarum*, *Bacillus megaterium*, *Lactobacillus brevis*; and Gram-negative organisms like *Salmonella typhimurium*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Enterobacter aerogenes*, *Escherichia coli*, and *Vibrio cholera* (Badawy et al., 2017; Tabesh et al., 2018). Reports from the study showed pronounced antibacterial activity of a low concentration (15% [v/v]) of nanochitosan on *S. mutans* biofilms with an evidenced decrease in the rate of survival of microbial cells (Ikono et al., 2019), hence, the potential use of nanochitosan in development of an oral-health care product, for example, mouthwash and toothpaste (Carrouel et al., 2020). Nanochitosans also have significant antifungal activity, with actions against fungi like *Alternaria alternata*, *Phomopsis asparagi*, *Rhizopus oryzae*, *Aspergillus niger*, and *Rhizopus stolonifera* (Vilaplana et al., 2020). This potential is bolstered by the nature of chitosan (polycationic) which without any chemical moderation confers a natural antifungal potency on the particle.

Wound-healing

The anti-inflammatory, hemostatic, antimicrobial, film-forming, and analgesic activities of nanochitosan can be used as a healing agent for wounds (Gupta et al., 2019), this activity is expressed in wound dressings as a form of antimicrobial agent, which can be in form of a fiber employed for wound dressings (Madni et al., 2021), as hydrogel, membrane, or sponge form (Li et al., 2020a). Electrospun nanochitosan fibers consisting of cross-linked collagen exhibited a heightened rate of wound healing and regeneration of damaged tissues in comparison to collagen and gauze dressings (Chen et al., 2008). The nanochitosan variant was developed through the use of electrospun chitosan fiber composed of polyethylene oxide for periodontitis (Qasim et al., 2017). A nanochitosan-gelatin sponge showed increased antibacterial activity against *Streptococcus* and enterotoxigenic *E. coli* K88 than conventional drugs like cefradine and penicillin respectively. Also, nanochitosan mesh membrane can help promote the recovery rate of the stratum granulosum and reduce the wound-healing time (Liu et al., 2020, 2021). Moreover, nanochitosan can be used in sutures for patients by taking advantage of its nontoxicity (Bakshi et al., 2020).

Transfection and gene delivery

Nanochitosan have the ability to transport bio-active materials into cells without affecting the components of the compound or target cell (Oh and Park, 2014). Nanochitosan–DNA complexes of 50–100 nm when transfected into cells showed no cellular toxicity compared to the control polyethylenimine–DNA complexes (PEI/pDNA), at which exhibited cytotoxicity equal concentrations (Ashfaq and Ahmed, 2021). This is a crucial development for biopharmaceuticals, as it requires a proper delivery system that can preserve sensitive bio-active compounds, for example, genes and proteins, against chemical and enzymatic counter-action (Loh et al., 2010; Gao et al., 2009). However, the transfection capability of nanochitosan is reduced compared to the common transfection agent—lipofectamine (Raik et al., 2018). During gene delivery, nanochitosan interacts with electronegative DNA and is converted into a polyelectrolyte complex (PEC) (Wang et al., 2018).

All of these deductions show marine bacteria-derived nanochitosan has a broad spectrum of applications as a carrier of drugs and genes in pharmaceuticals (Rizeq et al., 2019).

Future prospects

Since the advent of nanoscience and nanotechnology, there has been an increase in its uses and infiltration into various aspects such as pharmaceutical, medicine, energy, among others (Ramsden, 2018). The science of nanoparticles, which involves the use of small-sized particles (Khan et al., 2019) has been significantly improved by the discovery of nanochitosan, which include chitosan, a biocompatible, readily degradable polymer that has a minimum side effect and safe on humans (Mohammed et al., 2017). The makeup of nanochitosan typically includes mucoadhesive properties and a positive surface charge (Silva et al., 2017). These properties help with adherence to mucus membranes gradual and prolonged release of bioactive particles to target sites (Cao et al., 2019). Marine bacteria-derived nanochitosans have various applications in the delivery of oral, topical, and suppository medications, especially for the therapeutic care of gastrointestinal diseases, cancerous diseases, tumors, pulmonary diseases, and other related disease conditions (Mikušová and Mikuš, 2021). The use of marine bacteria-derived nanochitosans is further encouraged by low toxicity both in some *in vivo* and *in vitro* designs (Mohammed et al., 2017).

Though the prospects of marine bacteria derived, nanochitosan looks promising, there are challenges associated with the application, one of which is the poor understanding of the mechanisms of biosynthesis of nanochitosan using bacteria; as well as the seemingly tedious purification steps (Iravani, 2014). An increase in production to meet sizeable industrial demand should also be considered (Hasan et al., 2018).

Nanochitosan production

There are factors to be put into consideration in future research on marine bacteria-derived nanochitosan. A key factor is the selection of suitable bacteria candidates for nanochitosan production, with regard to their applications (Liu et al., 2020). Selection criteria may include enzyme activities, growth rate, biochemical pathways and also size, shape, and synthesis rate per time (Hasan et al., 2018). Also, biocatalysts are a significant component during nanoparticle synthesis (Ovais et al., 2018); and these catalysts can be obtained in the form of whole cells, crude enzymes, or purified enzymes. Whole-cell biocatalysts are preferred because they are cheaper and can be recycled during nanochitosan synthesis (Singh et al., 2020). Most of the reactions responsible for nanochitosan synthesis seem to be bio-reductions, which include the use of coenzymes such as nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotides (FAD and FADH), however, such routes of synthesis are expensive (Subbaiya et al., 2017).

The optimization of growth conditions which include temperature, light, pH, should also be considered as there is the need to increase the production of enzymes so as to synthesize a significant amount of biomass (Ali et al., 2020). When utilizing crude enzymes and whole cells, harvesting time is critical, necessitating the monitoring of enzymatic activity in relation to growth time (Singh et al., 2018). The yield and the production rate should be considered in the use of bacteria for the production of nanoparticles on an industrial scale (Mukherjee and Patra, 2016). In addition, isolation, purification, and stabilization of the produced nanochitosan, which has not been well investigated (Ovais et al., 2018) are also of importance, and challenges in this regard must be overcome. When challenges of purification and stabilization are resolved, then industrial scale-up and synthesis of nanochitosan using biomass can be attained (Yanat and Schroën, 2021; Balakrishnan et al., 2017). In light of this, stability of produced nanochitosan after storage for long periods at room temperature should be investigated (Saeed et al., 2020). Proteins and enzymes secreted by bacteria that help maintain stability should be considered and further studied to synthesize clean, stable nanochitosan (Singh et al., 2019).

Applications

The antivirulence properties of nanochitosan and its derivatives should also be studied at the biochemical and molecular level of bacteria pathogenesis (Badawy et al., 2020). Suppression of quorum sensing (QS) signaling circuits by chitosan and its derivatives should be considered in future studies as QS signaling circuit targeting in biofilm-forming pathogenic bacteria could minimize its pathogenesis (Nag et al., 2021). In the same vein, *in vivo* and human trials should be conducted to ascertain the antibiofilm activity of nanochitosan (Sivanesan et al., 2021) and also the effect of low molecular weight chitosan on biofilm matrix (Kašparová et al., 2021). A combination of nanochitosan nanoparticle with antibiofilm drugs and conventional antibiotics as treatment of bacterial infections should also be considered (Khan et al., 2020).

Finally, hazard identification, safety evaluation, and classification of risk should be incorporated into research on marine bacteria-derived nanochitosan (Bellich et al., 2016). Investigations should be carried out on each nanochitosan on a case-by-case basis in the requisite ways focusing on their portal of entry (Iravani, 2014), as well as the potentially toxic effects of nanochitosan when applied topically as a wound healing agent (Darwesh et al., 2018).

Conclusion

In conclusion, the synthesis of nanochitosan from marine bacteria has aided in resolving issues around safety, stability, eco-friendliness and toxicity, due to diverse availability of bacteria in marine habitats, and its biological synthesis that does not involve the use of toxic chemicals while reducing the total cost of production. The biosynthesis of nanochitosan extra-cellularly using marine bacteria and their bioactive compounds is a constructive route, considering that size and shape can be controlled. The nanoscience field is an emerging one with a lot of concern about its effects on health and the environment due to the use of chemical synthesis. Therefore, the call for biosynthesis using marine bacteria could be further explored as they contain valuable resources that can provide materials beneficial to human life, drug delivery, and therapeutics among others. There is, however, a huge need to fine-tune these processes, alongside proper ethical and quality control.

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