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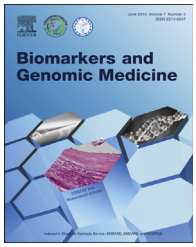

SHORT COMMUNICATION

Antibacterial and antifungal activity of alkylsulfonated chitosan

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Abstract Novel alkylsulfonated chitosan, produced by alkylsulfonation, has become a water-soluble, anionic polymer due to the existence of hydrophilic alkylsulfonic acid group. The alkylsulfonic acid creates specific exchangeable cations, thereby increasing the antimicrobial effectiveness and skin tissue compatibility. Alkylsulfonated chitosan demonstrated outstanding microorganism inhibition against fungal reference strains of *Malassezia furfur* (BCRC 22243), *Malassezia pachydermatis* (BCRC 21676), *Trichophyton rubrum* (BCRC 32805), *Trichophyton mentagrophytes* (BCRC 32066), and *Candida albicans* (BCRC 20518), together with four different bacteria species of *Escherichia coli* (BCRC 11509), *Pseudomonas aeruginosa* (BCRC 11864), *Staphylococcus aureus* (BCRC 10781), and *Propionibacterium acne* (BCRC 10723). Meanwhile, our results indicate that alkylsulfonated chitosan works well in growth inhibition of microorganism strains tested at pH 5–6 and at pH 7.

Introduction

Chitosan is one of the most abundant natural polysaccharide biopolymers and the best known type of amino polysaccharide. It is usually extracted from insects and crustaceans, and from the cellular walls of fungi.[1,2] Chitosan has been reported to be highly biocompatible, to be nontoxic, and to manifest preservative characteristics.[3–5] It is widely applied in a broad range of areas, including medical care, antimicrobial treatment, wastewater management, enzyme stabilization, and cosmetics.[6–12] However, due to its dissolubility, chitosan is difficult to process, resulting in limited function and applicability.

Although chitosan exhibits various valuable biological activities, such as antitumor activity, immunoadjuvant activity, hypolipidemic activity, and homeostatic activity,[13–15] the bactericidal activity of chitosan is particularly interesting.[16,17] Studies on the antibacterial activity of chitosan derivatives have revealed that the

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polymer is effective in inhibiting the growth of bacterial cells.[18,19] Furthermore, the antibacterial property of chitosan can be enhanced by changing the hydrophobic/hydrophilic nature of the polysaccharide backbone. Moreover, the antibacterial activity of chitosan is influenced by its molecular weight, degree of deacetylation, concentration in solution, and pH of the medium.[20,21] The aim of this study was to investigate the known antimicrobial characteristics of alkylsulfonated chitosan against reference strains of microorganism tested.

Materials and methods

Alkylsulfonated chitosan derivatives

Alkylsulfonated chitosan was obtained from Innovation Development Center, Taiwan Hopax Chemical Manufacturing Co. Ltd., Kaohsiung, Taiwan. Alkylsulfonated chitosan was prepared as follows. Chitosan was added to methanol and heated to reflux. Next, 1,3-propane soltone was slowly added to it and the mixture was kept in a state of reflux. After the reactions, the solid and liquid parts (nonreacted PS separation) were separated by exhaustion and ventilation. After washing, the solid part was treated with methanol for several times and then placed in the oven at 85°C until dry. Finally, alkylsulfonated chitosan was baked to yield a yellow solid product (CA Patent number CA2510020, USA). Alkylsulfonated chitosan consists of a chitosan backbone and multiple alkylsulfonic acid functionalities. The average molecular weight of alkylsulfonated chitosan is in the range of 10—55 kDa. Alkylsulfonated chitosan is soluble in ethanol and water, but can be restored only in water at pH = 7. By contrast, the original chitosan copolymer, consisting of a hydrophobic chitin segmental linker, is insoluble in water, whereas alkylsulfonated chitosan is 50% soluble in water.

Determination of the antimicrobial activities of alkylsulfonated chitosan

To determine the antimicrobial activities, alkylsulfonated chitosan was prepared as a 10% solution in water and then sterilized at 121°C for 15 minutes. Reference strains of microorganism used to determine the antimicrobial activities of the chitosan derivatives are as follows: *Malassezia furfur* (BCRC 22243), *Malassezia pachydermatis* (BCRC 21676) *Trichophyton rubrum* (BCRC 32805), *Trichophyton mentagrophytes* (BCRC 32066), and *Candida albicans* (BCRC 20518), together with four different bacteria species of *Escherichia coli* (BCRC 11509), *Pseudomonas aeruginosa* (BCRC 11864), *Staphylococcus aureus* (BCRC 10781), and *Propionibacterium acne* (BCRC 10723). All strains of microorganisms used for antimicrobial activity testing were provided by the Bioresource Collection and Research Center (The Food Industry Research and Development Institute, Hsinchu, Taiwan). Antimicrobial activity assay was performed by the agar dilution method, at alkylsulfonated chitosan final concentrations of 1 mg/mL, 2 mg/mL, 5 mg/mL, and 10 mg/mL, in Mueller—Hinton medium (used for bacterial strains) (BD Diagnostic Systems, BD Biosciences, San Diego, California, USA) or RPMI medium 1640 (used for fungal strains) (GIBCO Laboratories, Life Technologies, Inc., Gaithersburg, Maryland, USA), in accordance with the method of Jorgensen and Turnidge,[22] with minor modifications. Inoculum suspensions were prepared from broth cultures and adjusted to a concentration of approximately 1.0×10^4 colony-forming units (CFU)/mL, and then a 1:100 dilution of the suspension was made. All agar plates (14 mm in diameter) with/without alkylsulfonated chitosan were inoculated with 1 mL of a suspension of microorganism tested by spreading onto the agar plates. All agar plates were incubated at 37°C for 18 hours. Results were obtained from the average of three determinations. Stock cultures were stored in cook meat medium (BD Diagnostic Systems, BD Biosciences, San Diego, California, USA) at −70°C. Working cultures were maintained on agar plates, stored at 4°C, and subcultured fortnightly.

Statistical analysis of antimicrobial effectiveness

The resulting product of chitosan derivatives was used to determine antimicrobial activity against different bacterial and fungal species. Tests for determination of antimicrobial activity were performed by measuring the number of microorganisms growing on the agar plates. The antimicrobial activity of alkylsulfonated chitosan was determined by using the CFU of the control group as the denominator, and the difference of CFU between the control groups and study groups as the numerator. This equation is shown as follows:

$$\text{Antimicrobial activity (\%)} = \frac{\text{CFU of the control group} - \text{CFU of the study group}}{\text{CFU of the control group}} \times 100 \quad (1)$$

Results

Antimicrobial activity of alkylsulfonated chitosan

This study focused on inhibition of antimicrobial activity using a modified chitosan. Antimicrobial inhibition of *E. coli*, *C. albicans*, *S. aureus*, and *M. furfur* could reach as high as 95% after applying even a low concentration (1 mg/mL) of alkylsulfonated chitosan (Table 1). At concentrations of 5 mg/mL and 10 mg/mL, antimicrobial inhibition ability reached 70—100% against most species of bacteria, with the exception of *P. aeruginosa*. By contrast, ineffective inhibition ability against *P. aeruginosa* was discovered at an alkylsulfonated chitosan concentration of 5 mg/mL, whereas a high inhibition ability rate of 91.2% was still achieved at an alkylsulfonated chitosan concentration of 10 mg/mL. Alkylsulfonated chitosan is also effective against anaerobic bacteria such as *P. acne*, with a high inhibition ability rate of 95% being observed at a concentration of 10 mg/mL.

Effect of pH on the antimicrobial activity of alkylsulfonated chitosan

To investigate the effect of pH on the antimicrobial activity of chitosan and alkylsulfonated chitosan, Gram-negative *E.*