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# Research on antibacterial activity of various cellulose derivatives and application to food safe field

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## Abstract:

Cellulose derivatives $(C_6H_{10}O_5)_n$ are mainly used in the construction and the engineering field, the ceramics and the paper coating. And they are also used in the fields of demanding the safety, such as medical supplies, cosmetics and toiletries. However, there are a few reports about the antibacterial activities of a cellulose derivative, and it will be considered to be a significant matter to examine the detailed antibacterial activities.

In this research, 7 kinds of cellulose derivatives $(C_6H_{10}O_5)_n$ was used in the antibacterial test. The antibacterial activities test against *Staphylococcus aureus* and *Escherichia coli* was examined by the shake flask method and Minimum Inhibitory Concentration (MIC) measurement method.

Among the cellulose derivatives used for the examination, MIC test was carried out with the samples which soluble in water. On the other hand, the samples that soluble few in water was tested by the shake flask method, respectively.

As a result, although the test result had a little variation in the structure of the cellulose derivatives, some cellulose derivatives tested had possessed the antibacterial effect against test bacteria. MIC values of water-soluble samples (two samples) were 25 and 100 μg/mL against *S. aureus* and 100 and 200 μg/mL against *E. coli*, respectively. In the water insoluble samples (5 samples), 3 samples were effective and 5-log reduction was succeeding within 2 hours.

By preparing the film sheet etc. that are added the cellulose derivative of antibacterial agents, it will be also applicable to the preservation field of several kinds of foods.

**Keywords:** Cellulose derivatives, Antibacterial activity, Shake flask method, MIC

## 1. Introduction

Cellulose derivatives $(C_6H_{10}O_5)_n$ are used in many fields such as the medicines, cosmetics and toiletries that will be requested the safety, construction and engineering works, ceramics, and the paper coating [1]. Cellulose is the recyclable polymer that is synthesized by water, carbon dioxide, and solar energy during all parts of the world (the dry base: about 15.5 billion tons/year). Cellulose derivatives are cheaper than the cheap synthetic polymer such as polyethylene (180-200 Japanese yen/kg), which could be developed as the functionality cellulose [2]. The cellulose derivatives are also valuable material, because it is possible to obtain at low price. Moreover, a lot of preparation methods can be made with the antibiotics such as oxytetracycline and ampicillin etc. [3]. It is also used as a coating agent for dry syrup. Moreover, a part of cellulose derivative can be made with diamines, sustained delivery medicines and the immobilized enzymes [4]. The cellulose derivative is easily processed material. The cellulose derivatives have different character according to the cellulose material such as shape (for instance, the fiber form, the fiber length, the fiber distribution, the

specific surface area, the surface figure, and the shape of cross section, etc) [5]. The cellulose derivatives are widely used in various fields.

In this research, the presence of the antibacterial effect of these materials was investigated for additional functional value.

# 2. Materials and Methods

### 2.1.Test agents

The basic structure and the structural formula of various cellulose derivatives used for experiment were shown in Table 1 and Fig. 1.

***Table 1.*** *Functional group of various cellulose derivatives tested in this experiment.*

| Sample No. | R (substituent) | X | DS (substitution degree) |
|---|---|---|---|
| K-012 | Et | Br | 0.45 |
| K-013 | Pr | Br | 0.41 |
| K-014 | Bu | Br | 0.27 |
| K-015 | Bu | Br | 0.56 |
| K-016 | Bu | Br | 1.16 |
| K-017 | Hex | Br | 0.55 |
| K-019 | Oct | Br | 0.47 |

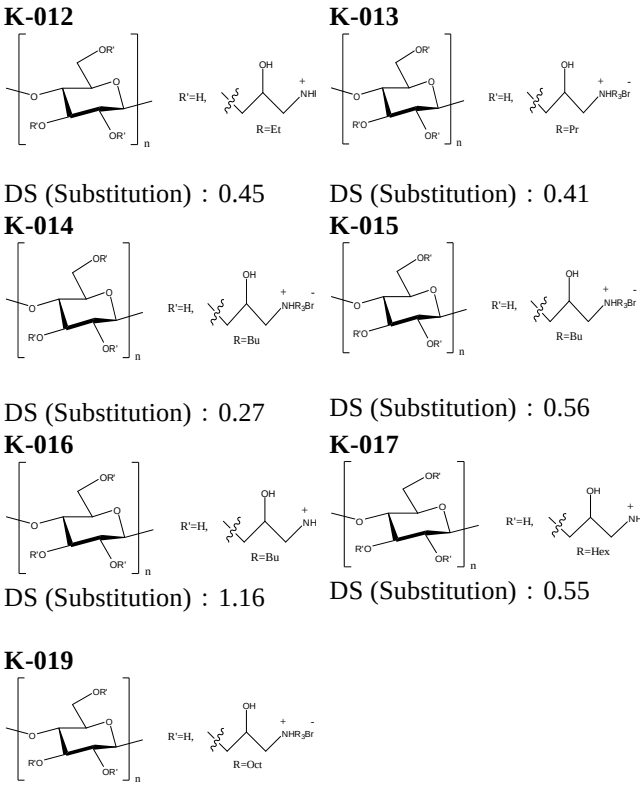


***Fig.1****. The structural formula of various cellulose derivatives used to experiment*

### 2.2. Test bacteria

*Escherichia coli* (*Escherichia coli* NBRC 3972; *E. coli*) and *Staphylococcus aureus* (*Staphylococcus aureus* NBRC 13276; *S. aureus*) were used as tested bacteria.

### 2.3. Shake-flask method

#### *2.3.1. Test agents*

The cellulose derivatives of test agents, K-013, K-014, K-015, K-017 and K-019, were shown in Table 1.

#### *2.3.2. Test medium*

Soybean-Casein Digest Agar（Nihon Pharm. Co., Ltd, Japan : SCD Agar）and Soybean-Casein Digest Broth（Nihon Pharm. Co., Ltd, Japan : SCD broth）was prepared, and was used for the test after the sterilization at 121℃ for 15 min.

#### *2.3.3. Preparation of test bacteria numbers*

Pre-incubation test bacteria was added into 2 mL of SCD broth, and incubated at 37℃ for 16～18 h under shaking condition (rotation speed : 100～120 rpm/min). The incubation bacterial suspension was diluted by SCD broth, and the absorbance at 630 nm ($OD_{630}$) was adjusted to 0.12 ± 0.02 (Bacterial numbers: about $1.0\times10^8$ CFU/mL). The bacterial suspension was 100 hold dilutions by the sterilized physiological saline.

#### *2.3.4. Shake-flask method*

K-013, K-014, K-015, K-017 or K-019 was added to each of flask, respectively (no sample was added to blank flask). Test bacterial suspension (each of 20 mL) was added to each of six flasks, and incubated at 25℃, 100 rpm/min under shaking condition.

### 2.4. Measurement of bacterial numbers

Each of test suspension (0.5 mL) was sampled at 0, 20 min, 40 min, 1 h and 2 h incubation. 10, 100 and 1000 hold dilution was performed by the sterilized physiological saline, and each of diluted sample (0.1 ml) was smeared to each of two SCD Agar by using the bacteria spreader, respectively. After 2 days incubation, the viable bacterial numbers were measured.

### 2.5. MIC（Minimum Inhibitory Concentration）test method

#### *2.5.1. Test agents*

The cellulose derivatives, K-012 and K-016 were used as test agents (refer Table 1).

#### *2.5.2. Test medium*

SCD Agar, SCD broth and Mueller Hinton Broth (Becton Dickinson Microbiology System, USA : MH broth) was used after the sterilization at 121℃ for 15 min.

### *2.5.3. Preparation of test bacterial suspension*

SCD broth was poured into the sterilized test tube, and added one colony (before incubation at 37℃ for 16～18 h). The bacterial suspension was diluted with SCD broth ($OD_{630}$), which was adjusted as 0.12 ± 0.02 (bacterial numbers: about $1.0\times10^8$ CFU/mL), and used as the test bacterial suspension.

### *2.5.4. Preparation of test solution*

K-012 and K-016 were prepared to 800 μg/mL by using MH broth. Two hold dilution was performed, and prepared 800～6.25 μg/mL solution and used as MIC measurement.

### *2.5.5. Measurement of MIC values*

The prepared solutions (each of 100 μL) were poured into 96 well micro plate and added each of 5 μL of test bacterial suspension, and mixture well. Incubation at 37℃ for 16～20 h. When $OD_{630}$ on micro plate reader was below 0.3, its concentration was judged as effectively, and also judged as MIC. At lease, three tests were reaped and calculated each of MIC values.

## 3. Results and Discussion

### 3.1. Shake-flask method

The results by using *S. aureus* and *E. coli* were shown in Fig.2 and Fig.3, respectively.

There are some different results by cellulose derivatives and bacteria tested. K-013 was the most effective activity against *S. aureus* and more than 5 Log reductions were found after 60 minutes reaction. K-015 and K-019 showed the antibacterial effect, and more 5 Log reductions were succeeded at 120 minutes later. Against *E. coli*, K-013 also showed the most effective activity, and 5 Log and/or more reduction was found after 20 minutes later. K-014 showed the effect, and 5 Log and/or more reductions were found after 120 minutes later.

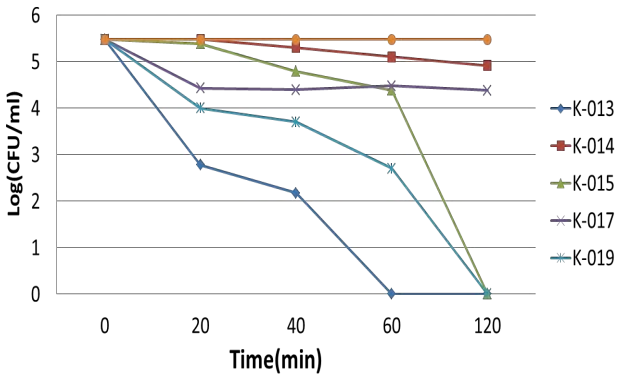


***Fig.2*** *The antibacterial activity against S. aureus tested.*

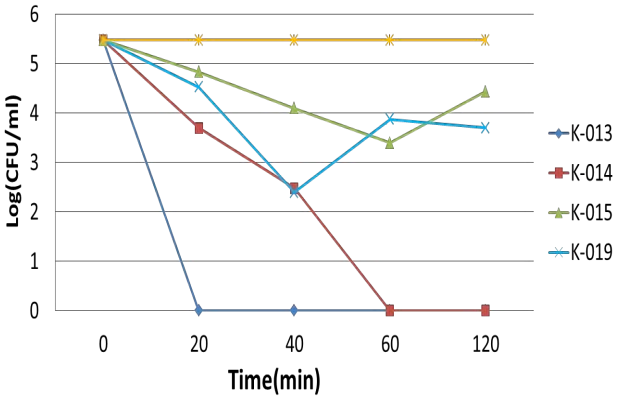


***Fig. 3*** *The antibacterial activity against E. coli tested.*

### 3.2. MIC Measurement

MIC values of K-012 and K-016 against *S. aureus* were 25 and 100μg/mL, respectively. MIC values of K-012 and K-016 against *E. coli* were 400 and 100μg/mL, respectively.

## 4. Conclusion

The antibacterial effect of seven kinds cellulose derivatives against two kinds of bacteria such as *E. coli* and *S. aureus* were evaluated by using MIC measurement or Shake-flask method.

From Shake-flask method, all cellulose derivatives tested possessed the antibacterial activity (Fig. 2 and 3). K-013, 15, and 19 killed the test bacteria finally. Especially, K-013 possessed stronger antibacterial activity, and killed the test bacteria at 60 minutes. On the other hand, K-014 and K-17 possessed the weak antibacterial activity. The result was greatly different depending on the kinds of the test cellulose derivatives.

There was occasionally only the temporary control effect in K-015 and K-019 for *E. coli* (Fig. 3). In K-013 and K-014, the tested bacteria were killed finally, and on K-013, the tested bacteria were killed within 20 minutes. Therefore, the antibacterial activity against *E. coli* was recognized. These results suggest that K-013 was the strongest and possessed the speedy bactericidal rate.

In the result of MIC measurement, the antimicrobial effect by K-012 against *S. aureus* at 25 μg/mL and 400 μg/mL in *E. coli*. On the case of K-016, 100 μg/mL was effective against *E. coli* and *S. aureus*.

In these results, all cellulose derivatives tested possessed the antibacterial activity; its activity level would depend on the difference of structural formula. Further research will be necessary to examine the influence of the functional group.

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