Carbohydrate Polymers 77 (2009) 754–759

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Influential factors to enhance the moving rate of *Acetobacter xylinum* due to its nanofiber secretion on oriented templates

Yoko Tomita, Tetsuo Kondo *

Bio-Architecture Center & Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

ARTICLE INFO

Article history:
Received 23 October 2008
Received in revised form 3 February 2009
Accepted 23 February 2009
Available online 4 March 2009

Keywords:
Acetobacter xylinum
Cellulose microfibril
Self-assembly
Carboxymethylcellulose
Template
Moving rate

ABSTRACT

Acetobacter xylinum, a bacterium which secretes a cellulose nanofiber, moves due to the inverse force of extrusion of the fiber, which accordingly correlates with the fiber production rate. To improve the production, the moving rate of the bacterium was focused to examine the influential factors on the substrates for culture and additives in the culture medium. From the real-time video analysis, the oriented template having a strong interaction with the secreted cellulose nanofibers proved to be suitable for the bacteria to move faster. Furthermore, addition of carboxymethylcellulose sodium salt (CMC) to the culture medium cause the bacteria to move faster in the culture medium. In this case, secreted cellulose nanofiber formed different from a normal cellulose nanofiber. The above result could provide an understanding how the formation of cellulose nanofibers contributes to the production rate as well as the bacterial moving rate.

1. Introduction

Acetobacter xylinum (=*Gluconacetobacter xylinus*) is a rod-shaped Gram-negative bacterium that is 0.5–1 μm in width and 2–10 μm in length, respectively. The bacterium has an assembled state of cellulose synthesizing catalytic sites (=subunits) linearly arranged in the major axis of the cell (Fig. 1a). The triplet subunits are further termed as a terminal complex (TC). The single subunit extrudes a sub-elementary fibril, which is composed of spontaneously assembled cellulose molecular chains synthesized by each catalytic site to form a stable conformation (Brown, 1996). Then, as shown in Fig. 1b, the arrangement of both TC and its subunits regulates assembling of the sub-elementary fibrils to form a microfibril, and the microfibrils are further assembled to give a nanofiber with ca. 50 nm in width and 10 nm in thickness. In this way, each formation process from synthesized individual molecular chains to the nanofiber is regulated to be spontaneously assembled. The spontaneous assembly is called "self-assembly."

Under a static culture condition, the individual bacterium extrudes a cellulose nanofiber in random directions and thereby a 3D network structure termed "pellicle" is to be formed (Czaja, Romanovicz, & Brown, 2004). Recently, this pellicle has been extensively studied as a most promising material having versatile properties, e.g. biocompatibility, high water absorption capacity, high crystallinity, and high mechanical strength (Brown, 1989; Helenius et al., 2006; Klemm, Heublein, Fink, & Bohn, 2005; Yamanaka et al., 1989). Moreover, new applications have also been widely investigated in the fields of medical, nano-materials, functional foods and so on (Klemm et al., 2006; Lin & Lin, 2004; Shar & Brown, 2005). The remaining issues are still relatively slow production and smaller yield of the pellicle at higher cost when compared with other raw cellulose materials such as wood and cotton.

Brown et al. reported the movement of *A. xylinum* on a glass substrate, which was related to the secretion of a cellulose nanofiber (Brown, Willison, & Richardson, 1976). Namely, the moving behavior of the bacterium was represented by the deposition of the nanofiber on a substrate, which was responsible for both the moving and fiber-producing rate of the bacterium. Recently, Kondo et al. reported that *A. xylinum* moves along the molecular tracks on a nematic ordered cellulose (NOC) template (Kondo, 2007; Kondo et al., 2002; Kondo, Togawa, & Brown, 2001). In the surface structure of NOC as a template, the comprising cellulose molecular chains are oriented uniaxially and the hydroxymethyl groups at the C-6 position, which are equatorial-bonded to the anhydroglucose unit, are vertically orientated to a certain angle (~30° from the vertical axis) against the surface. This indicates that the neighboring anhydroglucose ring planes are tilting and facing each other. Therefore, the hydrophilic and polarized OH groups are totally oriented as molecular tracks only in the stretching direction across the entire NOC surface. Simultaneously, the hydrophobic site due to the anhydroglucose plane was also appeared between the two hydrophilic molecular tracks, resulting in both hydrophilic and hydrophobic tracks next to each other across the NOC surface

* Corresponding author. Tel./fax: +81 092 642 2997.
E-mail address: tekondo@agr.kyushu-u.ac.jp (T. Kondo).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.02.022

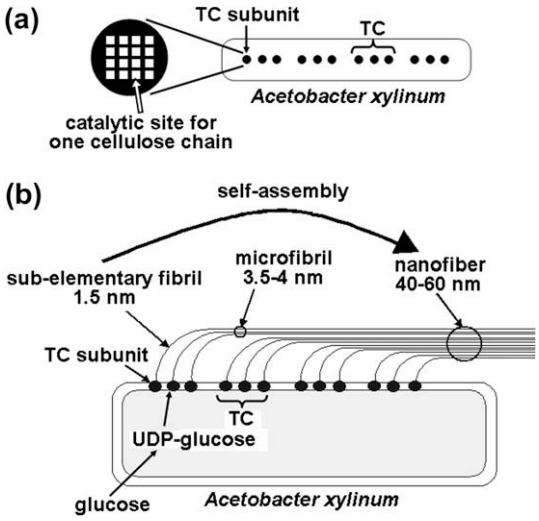

Fig. 1. Schematic figures of the geometry of the TCs and its subunits in relation to the formation of cellulose nanofiber: (a) the top view and (b) the side view of *A. xylinum*.

(Kondo, 2007). These amphiphilic molecular tracks cause the surface of NOC to have a strong interaction with other molecules and substances in the direction of the orientation. In cultivation of *A. xylinum* on the NOC template, the linear movement was induced by the strong interaction between the secreted nanofiber and the template. In this case, the moving rate on the NOC was faster by more than doubled when compared with that on a glass substrate (Hesse & Kondo, 2005; Kondo, 2007; Kondo et al., 2002). In the NOC template, self-assembly of cellulose microfibrils after the secretion was inhibited, resulting in disrupting the formation of a normal cellulose nanofiber. Thus, we have also hypothesized that the degree of self-assembly of cellulose microfibrils may be a rate-controlling factor for the movement of the bacterium.

In this article, prevention of self-assembly of secreted cellulose microfibrils was studied in correlation of the moving rate for the bacterium with the culture conditions such as an additive in the medium and orientation of the template. Carboxymethylcellulose sodium salt (CMC), which is known as a water soluble polysaccharide, was employed to add to the Schramm–Hestrin (SH) medium (Hestrin & Schramm, 1954) used for the cultivation of *A. xylinum*. The CMC could be considered as an inhibitor for self-assembly of secreted cellulose microfibrils by the surface electrostatic repulsion due to the carboxymethyl groups of attached CMC (Ben-Hayyim & Ohad, 1965; Watanabe, Gondo, & Kitao, 2004). Concerning the condition of the templates, the surface properties of NOC were modified by changing the draw ratio, and also addition of chitin as an additive. The obtained results on the influential factors could be a significant information to enhance the moving rate of the bacteria as well as the nanofiber production rate.

2. Experimental

2.1. Materials

Bleached cotton linters with a nominal degree of polymerization (DP) of 1300 were used as the starting material. *N,N*-Dimethylacetamide (99+%) and lithium chloride (LiCl) powder with a special grade were purchased from Sigma Aldrich Japan Co. Ltd. Chitin as another starting material, osmium tetroxide (OsO_4) for electron microscopy and cadmium oxide with the first grade for the viscosity measurements were purchased from Wako Chemicals Co. Ltd. The additive, CMC, with a degree of substitution (DS) of 0.7 was purchased from Aldrich Co. Ltd., and other CMC samples with DSs of 0.74 and 0.89 were kindly gifted by Daicel Chemicals Co. Ltd.

2.2. DP measurements of CMC

The measurement of DP of CMC was according to the previous report (Henley, 1961; Kurata & Tsunashima, 1989). The viscosity-average molecular weight of CMC samples was determined by viscometry in cadoxen to calculate DP. Table 1 lists the both values of DP and DS of the CMC samples, respectively. The DS values of all samples were close to each other, though the values of DP were different.

2.3. Templates in the Schramm–Hestrin medium containing CMC

Preparation of templates was followed by the previous method reported by Kondo and co-workers (Kondo, Kasai, & Brown, 2004; Kondo et al., 2001): Bleached cotton linter and chitin were dissolved in *N,N*-dimethylacetamide/LiCl solution with the concentration of 7% (w/w), respectively. After dissolution, the cellulose, chitin and cellulose/chitin (50/50) mixed solutions were separately poured into a flat-bottomed Petri dish, and put under a water vapor atmosphere for 3 days to obtain a coagulated gel-like film. Then, the solvent in the films was exchanged into ultra pure water by putting the film in the fresh water five times per day for 1 week to provide a water-swollen film. The film was cut into a small sheet with 5 mm × 30 mm in size before elongated uniaxially with a manual stretch device to reach 1.0, 1.5 and 2.0 of the each draw ratio at room temperature. The whole stretch procedure was performed under wet state. The obtained stretched films were sterilized with a 70% ethanol aqueous solution for 15 min. After the sterilization, the solution in the films was once exchanged to water by putting the film in the fresh water three times, and finally exchanged into the medium by putting the film in the fresh medium three times. An agar substrate, which is a 3D molecular network structure without orientation, was employed as a control of the template.

As an additive, CMC was used for the Schramm–Hestrin (SH) medium containing 2.0% D-glucose, 0.5% yeast extract, 0.5% peptone, 0.51% di-sodium hydrogenphosphate heptahydrate, 0.115% citric acid (Hestrin & Schramm, 1954). Concentration of CMC added was altered in the range of 0–2.5%. This SH medium containing CMC was employed for the experimental medium in this study.

2.4. Preparation of Acetobacter xylinum for observation

Acetobacter xylinum (NQ-5: ATCC53582) was cultured under shaking with 120 rpm at 30 °C in the SH medium with addition of 0.1% of cellulase ("ONOZUKA" R-10, Yakult Co. Ltd.) for 2.5 days to obtain active bacteria for the observation. Cellulase used here was for hydrolyzing secreted cellulose nanofibers during this activation process not to interrupt bacterial movements. After the activation, cultivated mixture was centrifuged with 1500*g* for 10 min to obtain the bacterial cells. The precipitates including cells were dispersed gently in sterilized pure water. The treatment was repeated three times to dilute the cellulase and other residues. Fol-

Table 1
DP and DS of the CMC samples. The listed samples a, b and c with different DPs correspond with those used in Figs. 3–5.

CMC	DP	DS
a	67	0.74
b	154	0.89
c	328	0.70

lowing the bacterial cells were again collected by centrifugation, they were dispersed gently into the prepared culture medium as described in 2.3 for observation of the bacteria.

2.5. Observation of A. xylinum using real-time video analysis

Light microscopic images were acquired with a light microscope (DMRE, Leica Microsystems) having 50× objective lens of 0.55 in the numerical aperture, coupled with a 1.25× optivar lens. A cooled charge-coupled device camera (Hamamatsu Photonics Co. Shizuoka, Japan) attached with a 0.55× camera lens (HR 055-CMT) from Diagnostic Instruments (Sterling Heights, MI) was equipped with the light microscope. Image frames were captured, digitized, saved, and processed using Image Pro Plus ver. 4.1 software (Media Cybernetics). The rate of the acquisition was three frames per minute. Observed samples were specimens of *A. xylinum* on templates, covered with SH medium containing CMC (0–2.5%, w/w) kept just above the surface at 30 °C, as shown in Fig. 2. The focus of the microscope was set between the bacteria and the surface of the substrate, so that both bacteria and the synthesized cellulose fibers could be observed. The incident light in the system was minimized to prevent drying of the surface by the heat produced. Using Adobe Premier ver. 5.1, each captured frame was sequenced to make a movie at the rate of 30 frames per second. The sequenced images provided the moving rate of *A. xylinum*. Namely, following the captured images of a bacterium moving for 1 min was selected, and the moving distance of the bacterium was measured using Image Pro Plus ver. 4.1.

2.6. Morphologic changes in self-assembly of cellulose microfibrils secreted by A. xylinum

Acetobacter xylinum was cultivated in the medium containing 1% (w/w) of CMC samples. The obtained pellicle having 3D-networks of the secreted nanofiber was washed, dried on a mica sheet, and then provided for observing the morphology of the fibers using Atomic Force Microscopy (AFM) (SPM-9500J3, Shimadzu Co. Ltd.). The width of the fiber of each sample was measured and compared with each other.

2.7. Observation of cellulose nanofibers using field emission scanning electron microscopy (FE-SEM)

After observation of the bacteria using light microscopy, the sample specimens were also observed by FE-SEM (JSM-6301F, JEOL) at 5 or 8 kV. The images were acquired as digitized tif files at 8-bit radiometric resolution. The preparation method was as follows: the specimens were placed into a container with several drops of a 4% OsO_4/H_2O solution, so that osmium vapor could cover the sample surface. The specimens were then dehydrated with an ethanol series (30%, 50%, 70%, 90% and 100%), then dried and mounted on coated with Pt using vacuum deposition equipment (JEE-5B, JEOL).

3. Results and discussion

3.1. Effect of the oriented templates to the moving rate of A. xylinum

Uniaxial drawn films of NOC, chitin and cellulose/chitin were used as a template having an orientation, respectively. The series of the templates were prepared in the same manner; however, NOC was highly non-crystalline (Kondo et al., 2002), but chitin and cellulose/chitin films had some ordered chitin crystallites parallel to the drawing axis (Kondo et al., 2004). The occurrence of molecular association depended on components of the template. Therefore, the above templates have different scales of hierarchical molecular/nano association. From the previous results, the surfaces of the templates are considered to have different magnitudes of interaction with cellulose microfibrils secreted by *A. xylinum*. In any template thus prepared, such an interaction is also supposed to inhibit self-assembly of cellulose microfibrils not to form a cellulose nanofiber. In this connection, the patterns of the movement of the bacteria on the templates could be related evidence.

Fig. 3 shows the changing moving rates of the bacteria on the various templates. The two moving rates were considered; (i) the initial movement that directly moves on the template, and (ii) the scanning movements that move on the previously secreted fiber already deposited on the template. In both cases, there was a similar trend of the change in the moving rates that depend on increasing of the draw ratio. This phenomenon has the potential to control the force of the interaction between cellulose microfibrils and the templates. Here, in the midway to the maximum draw ratio, the moving rate may depend on the constituents of the template. However, at the maximum draw ratio, a significant difference of the rate was not seen among the templates. It was noted that the rates of the movement on the drawn templates were faster than that on non-oriented templates such as an agar substrate and a non-stretched template. It may be because increasing in the contact points by improvement of the surface orientation and flatness that caused a strong force of the interaction with the secreted cellulose microfibrils. Further, the rates of the movement of the bacteria at the first touch on the template were faster than that for the bacteria moving on the already deposited cellulose fiber on the template. Therefore, it is considered that the rate-enhancing effect of the template would diminish when cellulose microfibrils continue to be accumulated.

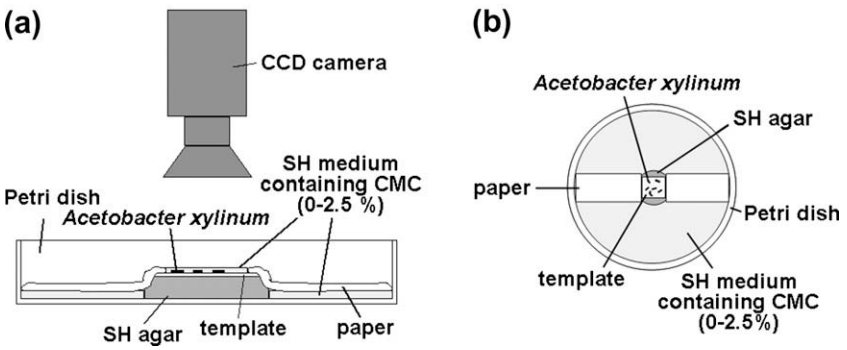

Fig. 2. Schematic figures of the system for observation of *A. xylinum*: (a) the side view, (b) the top view. Two pieces of paper soaked by SH medium containing CMC (0–2.5%) were used to allow templates and the bacteria in the medium to be kept wet.

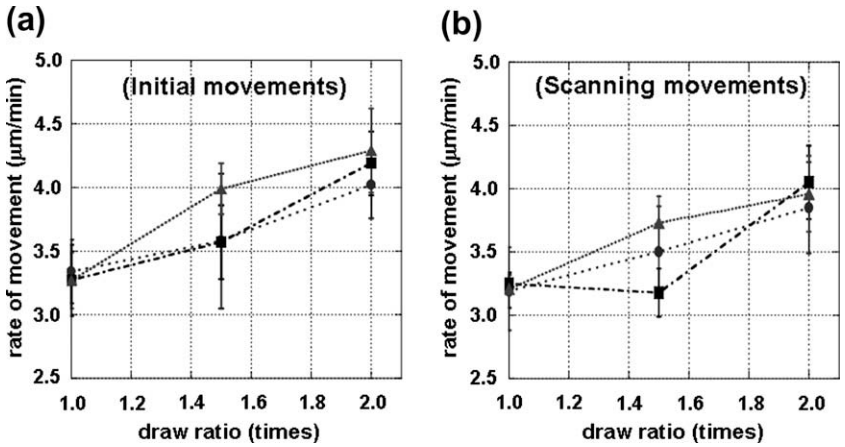

Fig. 3. Rate of the movement of *A. xylinum* on templates (a) when a cellulose fiber was secreted initially on templates (initial movements), (b) when a cellulose fiber was secreted on the previously deposited fiber once on templates (scanning movements) ●: NOC, ■: cellulose/chitin, ▲: chitin.

3.2. Inhibition effect of CMC to prevent self-assembly of secreted cellulose microfibrils

The self-assembly of cellulose microfibrils secreted by *A. xylinum* in the SH medium containing various CMC were examined using AFM as shown in Fig. 4. From the AFM images, the presence of each CMC sample seems to apparently allow the secreted cellulose fibers to assemble differently. When compared with (i) in Fig. 4, the two figures of (ii) and (iii) displayed large bundles of the individually separated fine cellulose microfibrils after the secretion. Such separated microfibrils could not be seen in Fig. 4(iv), instead, the fibers were much finer than the control shown in Fig. 4(i). This may attribute to the attached long molecular chain of CMC to the bacterial cells or to the small bundles of microfibrils, which in both cases disturbed formation of cellulose fibers. It may be considered to inhibit the small bundles of cellulose microfibrils to be merged by the electrostatic repulsion, resulting in forming some fine fibers. The black continuous lines in the inserted of Fig. 4 show the width of the fibers or bundles of cellulose microfibrils. In order to determine a degree of the inhibition effect depending on the DP of CMC in the self-assembly of cellulose microfibrils, the width of the bundles in the presence of CMC for the SH medium were compared with the control that was cultured in the normal SH medium. Namely, the wider the bundle was, the higher the inhibition effect was. The ratios of the width of the bundles were as follows: the control: sample a: sample b: sample c = 1:

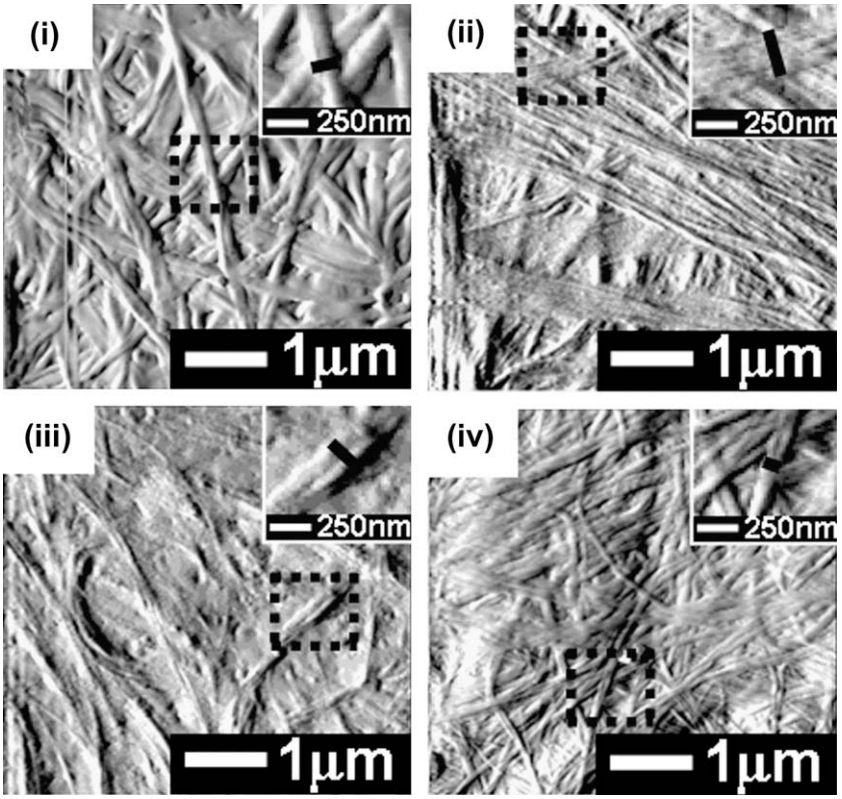

Fig. 4. AFM images of the cellulose nanofibers secreted into the SH medium containing CMC: (i) control, (ii) sample a, (iii) sample b and (iv) sample c. The images inserted in upper right are magnified in the square. Black continuous lines in the inserted images indicate the width of the bundles of cellulose microfibrils. Samples a, b and c with different DPs correspond with those listed in Table 1.

1.6: 1.3: 0.6. From the result, CMC with a low DP of sample a (DP = 67 having the shortest molecular length) was the most effective for the inhibition in self-assembly of the secreted microfibrils.

3.3. Effect of the CMC concentration to the moving rate of A. xylinum

Fig. 5 shows the moving rate of *A. xylinum* on a SH agar substrate as the reference of a non-ordered template for the culture medium containing CMC. When the concentration of CMC was below 1.5%, the moving rate of the cell was faster than that in the normal SH medium. At more than 1.5% of CMC, the higher the concentration was, the slower the rate of the movement was. This was probably caused by increasing viscosity of the culture medium. In particular, when the concentration was 1%, the average moving rate of the bacteria was the fastest. Furthermore, dependence of the moving rate on DP of the contained CMC molecules exhibited a similar trend to the inhibition effect of self-assembly of cellulose microfibrils as shown in Fig. 4. These results were also agreed with the previous report (Hirai, Tsuji, Yamamoto, & Horii, 1998).

3.4. Movements of A. xylinum on the NOC containing CMC as a template

A nematic ordered cellulose film (NOC) with the draw ratio of 2 was employed as the template with a range of 0–2.5% as the concentration of CMC in the culture medium. Moving rates of the bacterium on the NOC templates containing CMC is shown in Fig. 6. At more than 1% of the concentration, the movement became slow. Similarly to the case of the agar substrate containing CMC, the high viscosity due to this medium also disturbed the bacterial movements. Among them, the template containing 1% of sample c exhibited a positive effect in the rate enhancement of the bacteria, and moreover the rate was the highest among all the conditions. Thus, this is considered as the optimal condition for the bacteria to secrete cellulose fibers at present. However, the CMC attached to both the template and the cellulose microfibrils could be difficult to be removed by washing.

3.5. Moving patterns of A. xylinum

How do the above culture conditions influence on the moving pattern of the bacterium? The light microscopic sequence images

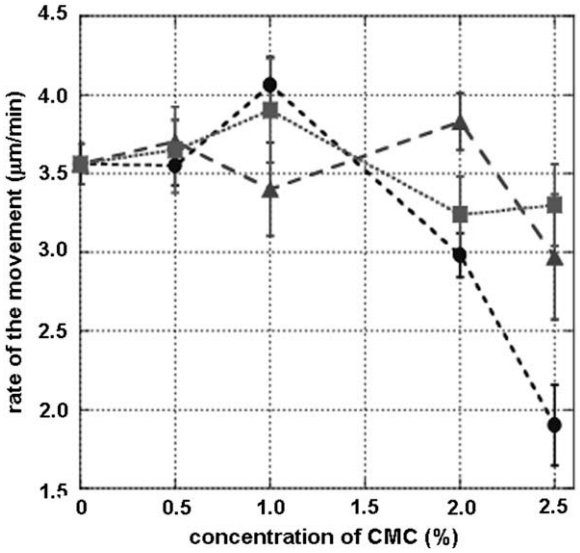

Fig. 5. Rate of the movement of *A. xylinum* on SH agar substrate containing CMC ●: sample a, ■: sample b, ▲: sample c. Samples a, b and c with different DPs correspond with those listed in Table 1.

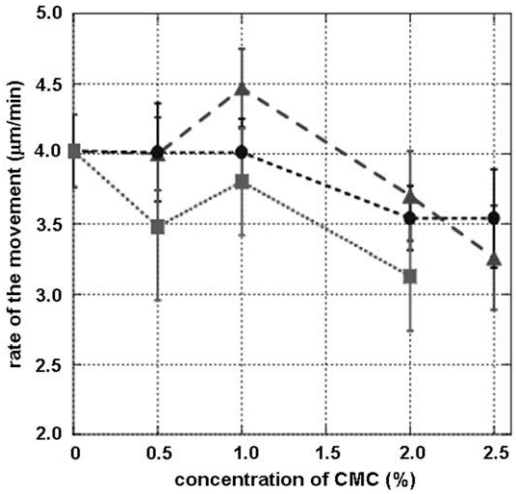

Fig. 6. Rate of the movement of *A. xylinum* on NOC templates containing CMC ●: sample a, ■: sample b, ▲: sample c. Samples a, b and c with different DPs correspond with those listed in Table 1.

in Fig. 7 show the typical three moving patterns on non-oriented agar substrate. The images of FE-SEM in the same figure clearly show that the secreted fiber on the substrate followed the moving pattern. Furthermore, the three patterns of the movements turned to be at the approximately same moving rates. Even though CMC was added to the culture medium, *A. xylinum* moved in either the three patterns which were a circle, a waving and a straight line on the agar substrate having no orientation. This indicates that CMC cannot provide an influence on the moving pattern because the patterns due to the fiber secretion may be influenced directly by the interaction with the surface of the templates.

In the case of NOC templates, *A. xylinum* moves linearly along the molecular orientation of NOC due to the strong interaction between the secreted fiber and the NOC surface (Kondo et al., 2002). On the other hand, the bacterium moved randomly in the present study when the NOC template contained CMC in the medium. This probably attributes to the CMC attached on the oriented molecules in NOC as well as the surface of the secreted cellulose microfibrils, which would prevent cellulose microfibrils from interacting directly with NOC. Namely, the CMC attached on the orientation of NOC worked more effective in prevention of self-assembly of cellulose microfibrils than the free CMC in the medium. Therefore, taking the above results into account, the bacteria move faster when NOC is used as the template in the medium containing CMC.

4. Conclusion

In this article, the optimal condition on the templates containing the additives for the culture medium were studied in order to understand an influential factor in the rate of the movement of *A. xylinum*, which was accompanied with the secretion of a nanofiber. The template having molecular orientation at a higher draw ratio was more effective to enhance the moving rate. Further, in the midway to the maximum draw ratio, the moving rate may depend on the constituents of the template. The additive, CMC, with a lower DP was effective to prevent secreted cellulose microfibrils from self-assembling, which resulted in an increase in the rate of the movement on the agar substrate. In fact, the SH medium containing 1% of CMC with a lower DP enhanced the moving rate of the bacterium cultured on the template without orientation. Furthermore, when NOC with the draw ratio of 2 was employed as a template, the SH medium containing 1% of CMC with a high DP was more effective to the increase in the moving rate. Presumably, alignment of CMC molecules onto the oriented cellulose molecules

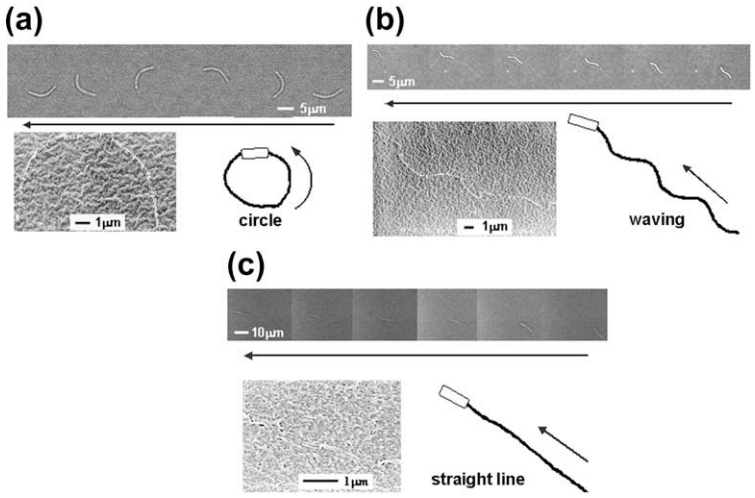

Fig. 7. Moving patterns of *A. xylinum* on the substrates with no orientation and CMC containing in the culture medium: (a) a circle, (b) a waving and (c) a straight line. The upper sequence images are light microscopic ones showing the bacterial movements. The interval between each displayed frame was 100 s. The bottom images are FE-SEM ones showing the secreted fibers under each patterned movement.

in the NOC by a strong interaction caused the increase in the moving rate. From the results, the condition for increasing the moving rate was proposed to employ a flat drawn template with an orientation that can engage with other materials by a strong interaction. Further, the additive to the medium, which prevent self-assembly of secreted cellulose microfibrils was also effective. Thus, understanding how to prevent the self-assembly of the secreted microfibrils on the oriented templates could be at present the easiest way for us to find an optimal condition for enhancing the fiber production. Such a condition could contribute and lead to establishments of the low energy system constructing 3D nano-structure of cellulose nanofibers using this bacterium.

Acknowledgements

The authors thank Dr. Yuzou Sano at Hokkaido University for his technical assistance of FE-SEM observation.

References

Ben-Hayyim, G., & Ohad, I. (1965). Synthesis of cellulose by *Acetobacter xylinum*. VIII. On the formation and orientation of bacterial cellulose fibrils in the presence of acidic polysaccharides. *The Journal of Cell Biology, 25*, 191–207.

Brown, R. M. Jr., (1989). Bacterial cellulose. In J. F. Kennedy, G. O. Philips, & P. A. Williams (Eds.), *Cellulose: Structural and functional aspects* (pp. 145–151). Chichester: Ellis Horwood Ltd..

Brown, R. M. Jr., (1996). The biosynthesis of cellulose. *Journal of Macromolecular Science – Pure and Applied Chemistry, A33*, 1345–1373.

Brown, R. M., Jr., Willison, J. H. M., & Richardson, C. L. (1976). Cellulose biosynthesis in *Acetobacter xylinum*: Visualization of the site of synthesis and direct measurement of the *in vivo* process. *Proceedings of the National Academy of Sciences of the United States of America, 73*, 4565–4569.

Czaja, W., Romanovicz, D., & Brown, R. M. Jr., (2004). Structural investigation of microbial cellulose produced in stationary and agitated culture. *Cellulose, 11*, 403–411.

Helenius, G., Bäckdahl, H., Bodin, A., Nannmark, U., Gatenholm, P., & Risberg, B. (2006). *In vivo* biocompatibility of bacterial cellulose. *Journal of Biomedical Materials Research, 76A*, 431–438.

Henley, D. (1961). A macromolecular study of cellulose in the solvent *Cadoxen*. *Arkiv För Kemi, 18*, 327–392.

Hesse, S., & Kondo, T. (2005). Behavior of cellulose production of *Acetobacter xylinum* in ^{13}C-enriched cultivation media including movements on nematic ordered cellulose templates. *Carbohydrate Polymers, 60*, 457–465.

Hestrin, S., & Schramm, M. (1954). Synthesis of cellulose by *Acetobacter xylinum*: Preparation of freeze dried cells capable of polymerizing glucose to cellulose. *Biochemical Journal, 58*, 345–352.

Hirai, A., Tsuji, M., Yamamoto, H., & Horii, F. (1998). In situ crystallization of bacterial cellulose III. Influences of different polymeric additives on the formation of microfibrils as revealed by transmission electron microscopy. *Cellulose, 5*, 201–213.

Klemm, D., Heublein, B., Fink, H.-P., & Bohn, A. (2005). Cellulose: Fascinating biopolymer and sustainable raw material. *Angewante Chemie International Edition, 44*, 3358–3393.

Klemm, D., Schumann, D., Kramer, F., Hesler, N., Hornung, M., Schmauder, H.-P., et al. (2006). Nanocelluloses as innovative polymers in research and application. *Advances in Polymer Science, 205*, 49–96.

Kondo, T. (2007). Nematic ordered cellulose: Its structure and properties. In R. M. Brown, Jr. & I. M. Saxena, Jr. (Eds.), *Cellulose: Molecular and structural biology, selected articles on the synthesis, structure, and application of cellulose* (pp. 285–305). Springer.

Kondo, T., Kasai, W., & Brown, R. M. Jr., (2004). Formation of nematic ordered cellulose and chitin. *Cellulose, 11*, 463–474.

Kondo, T., Nojiri, M., Hishikawa, Y., Togawa, E., Romanovicz, D., & Brown, R. M. Jr., (2002). Biodirected epitaxial nanodeposition of polymers on oriented macromolecular templates. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 14008–14013.

Kondo, T., Togawa, E., & Brown, R. M. Jr., (2001). "Nematic Ordered Cellulose": A concept of glucan chain association. *Biomacromolecules, 2*, 1324–1330.

Kurata, M., & Tsunashima, Y. (1989). Section VII solution properties. In J. Brandrup & E. H. Immergut (Eds.), *Polymer handbook* (3rd ed., pp. 1–148). John Wiley Sons, Inc..

Lin, K. W., & Lin, H. Y. (2004). Quality characteristics of Chinese-style meatball containing bacterial cellulose (Nata). *Journal of Food Science, 69*, SNQ107–SNQ111.

Shar, J., & Brown, R. M. Jr., (2005). Towards electronic paper displays made from microbial cellulose. *Applied Microbiology and Biotechnology, 66*, 352–355.

Watanabe, M., Gondo, T., & Kitao, O. (2004). Advanced wet-end system with carboxymetyl-cellulose. *Tappi Journal, 3*, 15–19.

Yamanaka, S., Watanabe, K., Kitamura, N., Iguchi, M., Mitsuhashi, S., Nishi, Y., et al. (1989). The structure and mechanical properties of sheets prepared from bacterial cellulose. *Journal of Materials Science, 24*, 3141–3145.