Electronic Supplementary information for

An unprecedented bacterial cellulosic material for defluoridation of water

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Supporting Information Containing:

Number of pages: 10

Number of tables: 2

Number of figures: 7

Supplementary Note 1:

Isolation of cellulose producing bacteria

Rotten tomatoes were collected from local market and rinsed with distilled water gently. They were cut into small pieces, transferred into 100 mL conical flasks containing Hestrin–Schramm (HS) broth (2.0 % D-glucose, 0.5 % peptone, 0.5 % yeast extract, 0.27 % Na_2HPO_4 and 0.115 % citric acid) (Schramm and Hestrin, 1954) and incubated at 28 °C in for 7 days. The medium was supplemented with 50 mg/l cycloheximide. After the incubation period, 0.1 ml volume of the sample was transferred to the petri dishes containing HS agar (1.5 % agar) by the spread plate technique. The petri dishes were incubated at 28 ℃ for 3 days. Development was checked in spread petri dishes; white- to cream-shaded mucous apparent colonies were observed. All isolates were saved by freezing in glycerol at −20 ℃ and strain stipulation checked on month to month.

Supplementary Note 2:

Screening of cellulose producing bacteria

Screening of cellulose producing bacteria was performed by (I) $CaCO_3$ medium and (II) Pellicle formation in the HS medium.

(I) $CaCO_3$ medium:

Isolates were tested as acetic acid microbes by the visualization of $CaCO_3$ clear zones in plates having $CaCO_3$–ethanol medium (0.05 % D-glucose, 0.3 % peptone, 0.5 % yeast remove, 1.5 % $CaCO_3$, 1.2 % agar, and 1.5 % ethanol).

(II) Pellicle formation in the HS medium:

Pellicle formation was checked in conical flasks containing HS broth. Development of pellicle or biofilm formation in the upper layer of the aqueous medium was acknowledged as an evidence of cellulose production. The pellicle, either stagnant or fragile, was uprooted by centrifugation, washed with distilled water, held in 0.1 M NaOH for 20 min at 80 ℃, and finally neutralized with 0.1 M acetic acid. After perennial washing with distilled water, any remaining disperses was either vacuum dried at 0.1 bar and 40 ℃ or stop dried at −50 ℃ and 0.04 mbar. The resistance of the biofilm to this treatment demonstrated adjustment to cellulose structure.

Supplementary Note 3:

Physiology and phenotypic characterization

A pure culture of the selected isolates was streaked on HS agar petri dish for colony development. For the identification of cellulose producing bacteria different constituents of biochemical tests such as Gram staining, Gelatin liquefaction, Glucose fermentation, Triple sugar iron, Indole production, Methyl Red, Voges-Proskauer, Citrate utilization, Nitrate Reduction, Bile Esculin, Kligner Iron, Oxidase and Catalase were done according to Bergey's Manual for Determinative Bacteriology.[41] Growth on glutamate, mannitol, 30% D-glucose, 0.35% acetic acid was also determined.

Supplementary Note 4:

Genomic DNA isolation

Bacteria grown in the HS broth culture are homogenized using a high concentration of detergents such as SDS, so that the contents are released. Proteins are removed by using strong degradation enzymes such as proteinase K whereas cell wall debris, polysaccharide, and remaining proteins are removed by selective precipitation with CTAB. The high molecular weight DNA is recovered from the resulting supernatant by Isopropanol precipitation.[42] Overnight culture broth (5.0 ml) was centrifuged at 5000 rpm/5 min at 4 ℃. The pellets were resuspended in 567 µL of TE buffer and addition of 30 µL of 10% SDS and 3 µL of proteinase K were done, it was then incubated for 1 h at 37 °C. 100 µL of 5 M NaCl and 80 µL of CTAB/NaCl solution were mixed well; it was then incubated for 10 min at

65 ℃. Equal volumes of Chloroform: Isoamyl alcohol mixture were added and centrifuged at 6500 rpm for 15 min. The aqueous phase was collected into a fresh tube, 3 µL of RNase was added and kept in a water bath at 37 ℃ for 1 h. To the sample 0.6 volumes of isopropanol was added to precipitate nucleic acid. It was kept in −20 ℃ for 15–30 min and it was centrifuged at 10000 rpm for 15–30 min. Pellets were washed with 70% ethanol and centrifuged for 5 mins. The pellet was kept in air dryer. It was then dissolved in 100 µL TE buffer or double distilled water. With the help of 0.8% agarose gel and EtBr visualized the genomic DNA in UV -trans illuminator.

Supplementary Note 5:

Amplification of 16S rRNA

16S ribosomal RNA is a component of the 30S small subunit of prokaryotic ribosome. It is around 1.542 kb (or 1542 nucleotides) in length. 16S rRNA is amplified using universal primers 27F (5'-AGAGTTTGATCCTGGTCAGAACGCT-3'), 1492R (5'-TACGGCTACCTTGTTACGACTTCACCCC-3') were designed by Weisburg *et al.* (1991) and Lane (1991). 16S rRNA were extracted and amplified from the all five BC producing organisms. The isolated DNA from each isolate served as a template for PCR reaction. 1.00 µL of template was added to the sterile PCR tube, to which buffer, dNTP, 16S rRNA gene forward primer, 16S rRNA gene reverse primer, Taq polymerase and double distilled water was added in the total volume of 25 µL, respectively. 16S rRNA gene sequences were searched for the similarity using the nucleotide database, BLAST tool. Species defined sequence results of BLAST were taken for further phylogenetic analysis.

Supplementary Note 6:

Phylogenetic Analysis

Phylogenetic tree was constructed by the neighbor-joining method in Maximum likelihood test using MEGA 5.1 software with bootstrapping 100 replicates. All the five bacterial sequences were run in the MEGA software on the best fit model and then the model used for each sequence to construct the tree. By the distance of the tree parental and neighbor clade compared to the isolates and the species were identified.

Cladistics analysis

The sequences of BC producing microorganisms were subjected to evolutionary reflationary studies within the group using cladistic analysis. The evolutionary relationship of BC producing organisms was studied within the group of sequences, which was identified earlier. Five sequences were analyzed through Clustal Omega online alignment tool. Using the JalView tool, the result summary has the option to calculate the distance tree within the provided sequences using neighbor-joining method. *Glucanobacter cerinus* and *Bacillus* spp. distance to each represents the genus divergence clearly, whereas distance of *Acetobacter* spp. from parental clade of *Glucanobacter cerinus* showed the evolutionary relationship. Cladogram tree is shown in Fig.2b.

Table S1. Biochemical test for the BC producing microorganisms.

Characteristics	BIT02	BITO3	BIT04	BIT05	BIT07
Indole Production	–	–	–	–	–
Methyl Red	–	–	–	–	–
V.P	–	–	–	–	–
Citrate Utilization	–	–	–	–	–
TSI	+	–	+	–	–
H_2S production	–	–	–	–	–
Nitrate reduction	–	–	–	–	–
Bile Esculine	–	–	–	–	–
Catalase	–	–	–	–	–
Oxidase	–	–	–	–	–
Urease	–	–	–	–	–
Gelatin liquefaction	–	–	–	–	–
Growth in presence of					
0.35% acetic acid	+	+	–	+	+
30% D-glucose	+	–	+	–	+
Growth on Mannitol Agar	+	+	+	+	+
Growth on Glutamate Agar	+	–	+	+	+
Acid Production from					
Sucrose	–	–	–	–	–
Fructose	+	+	–	+	+
Glucose	–	–	–	+	+
Galactose	+	–	+	–	+
Maltose	–	–	+	–	–
Sorbitol	–	–	–	–	–
Cellulose	–	–	–	–	–
Xylose	+	+	+	+	+

Positive (+); Negative (-)

Table S2. Pseudo kinetics first and second order correlation coefficient and rate constant for BCs.

	Pseudo-first order reaction		Pseudo-second order reaction	
	R^2 value	k_1	R^2 value	k_2
BC1	0.7320	0.00043	0.9996	0.00022188
BC2	0.9418	0.00111	0.9981	0.00024353
BC3	0.9410	0.00105	0.9988	0.00024175
BC4	0.8309	0.00052	0.9996	0.00022581
BC5	0.8143	0.00073	0.9963	0.00023460

The results are of a representative experiment out of three independent repeats.

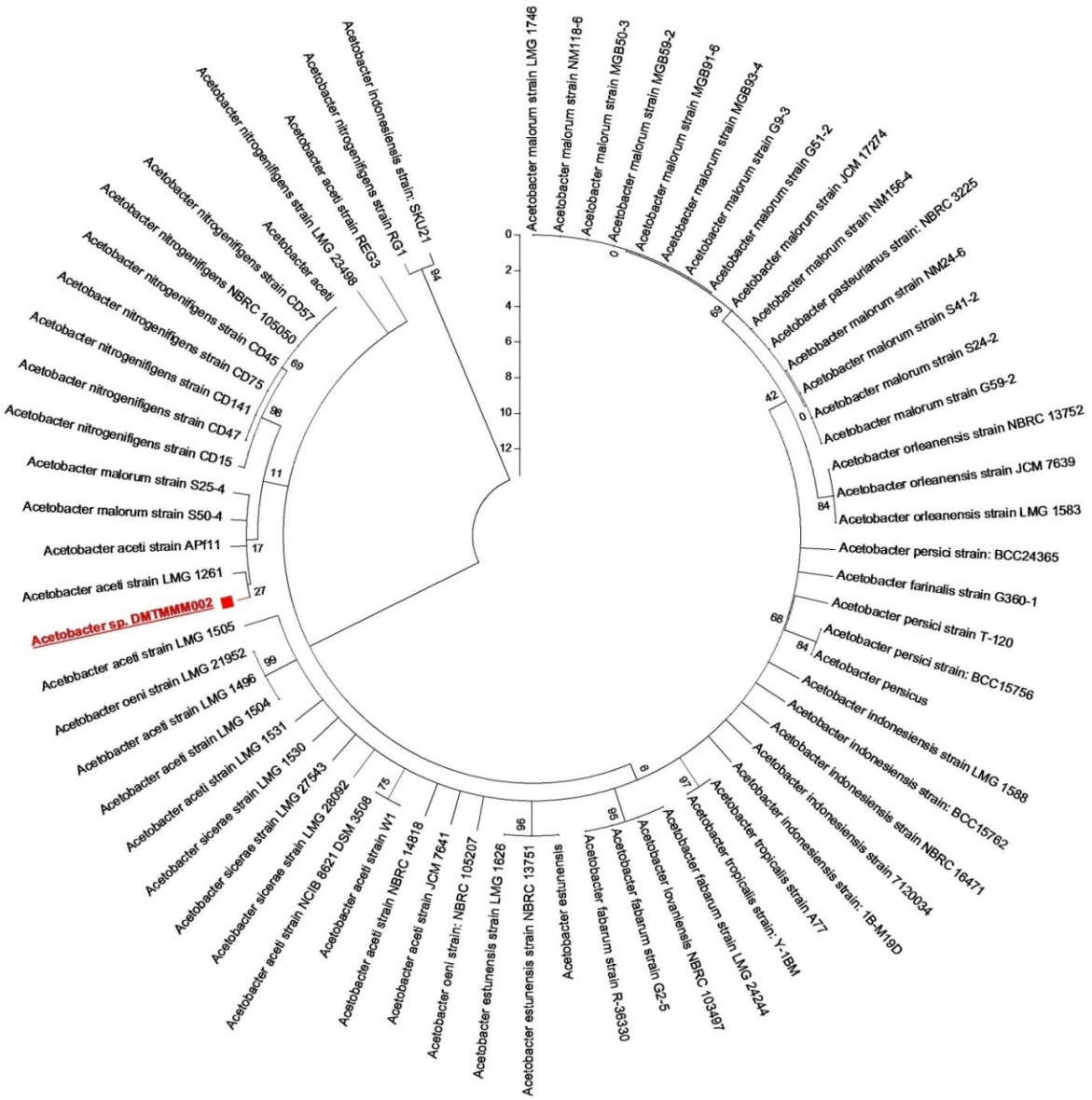

Fig. S1. Phylogenetic analysis of 16S rRNA from strain BIT02

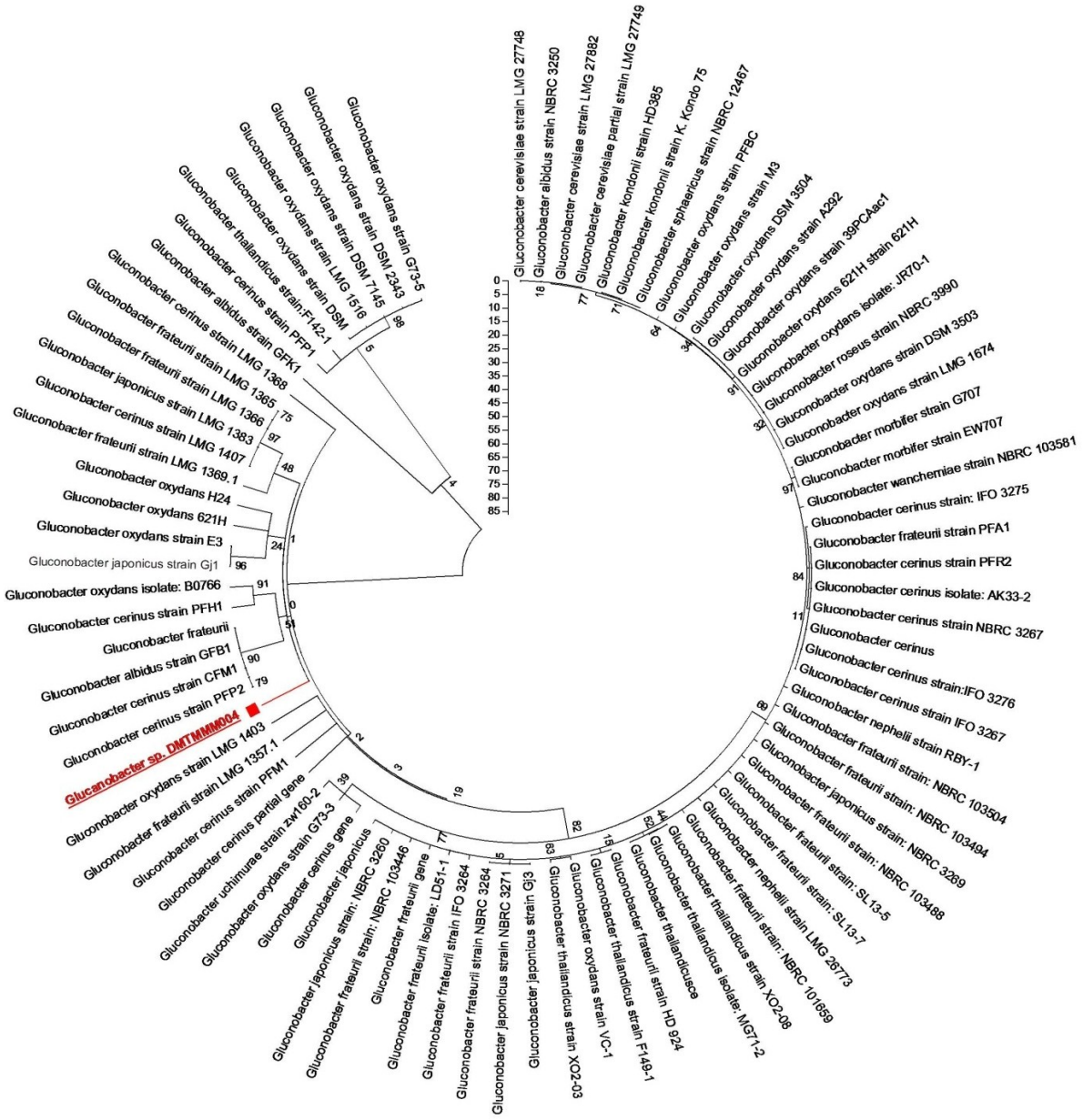

Fig. S2. Phylogenetic analysis of 16S rRNA from strain BIT03

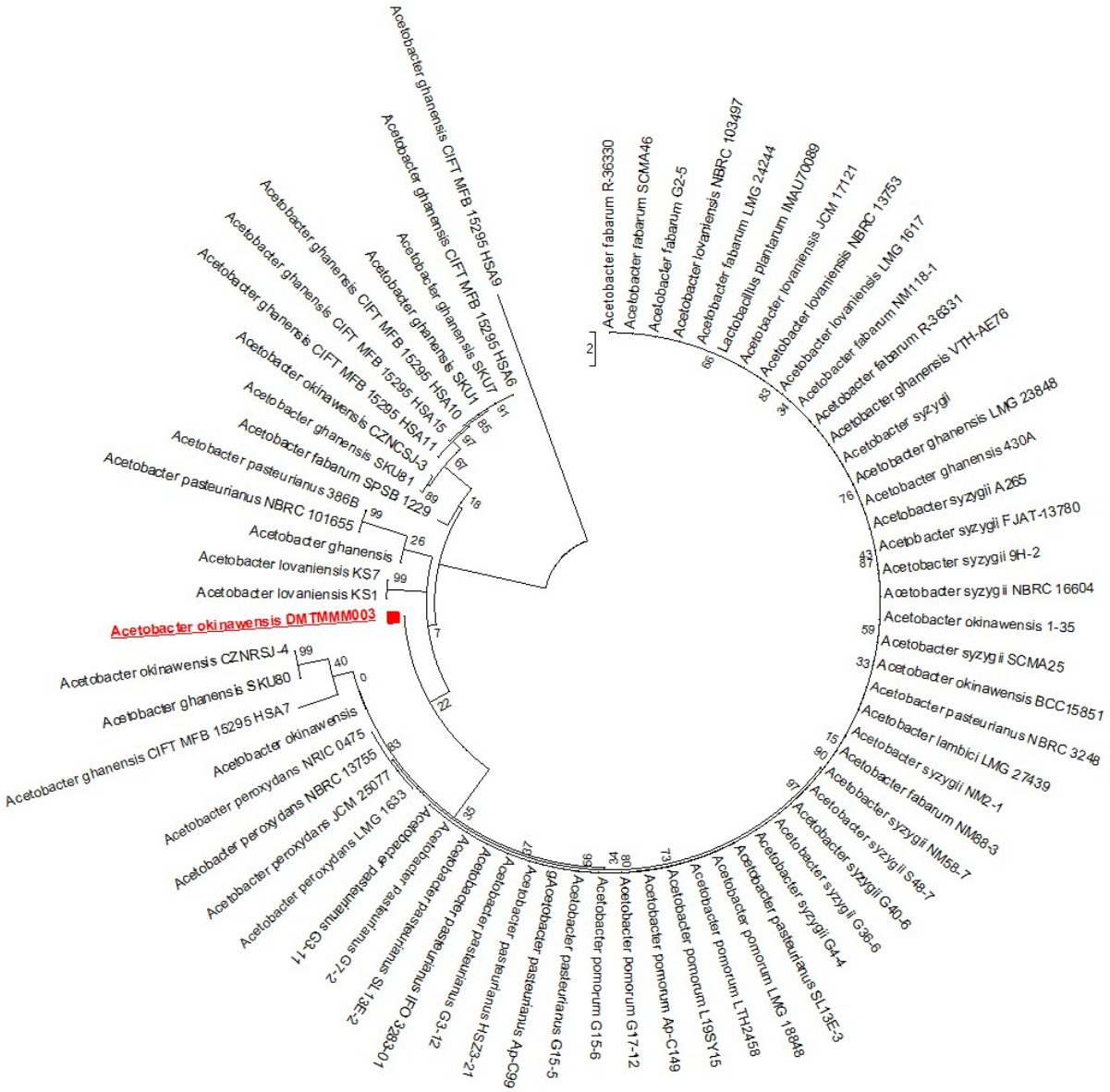

Fig. S3. Phylogenetic analysis of 16S rRNA from strain BIT04

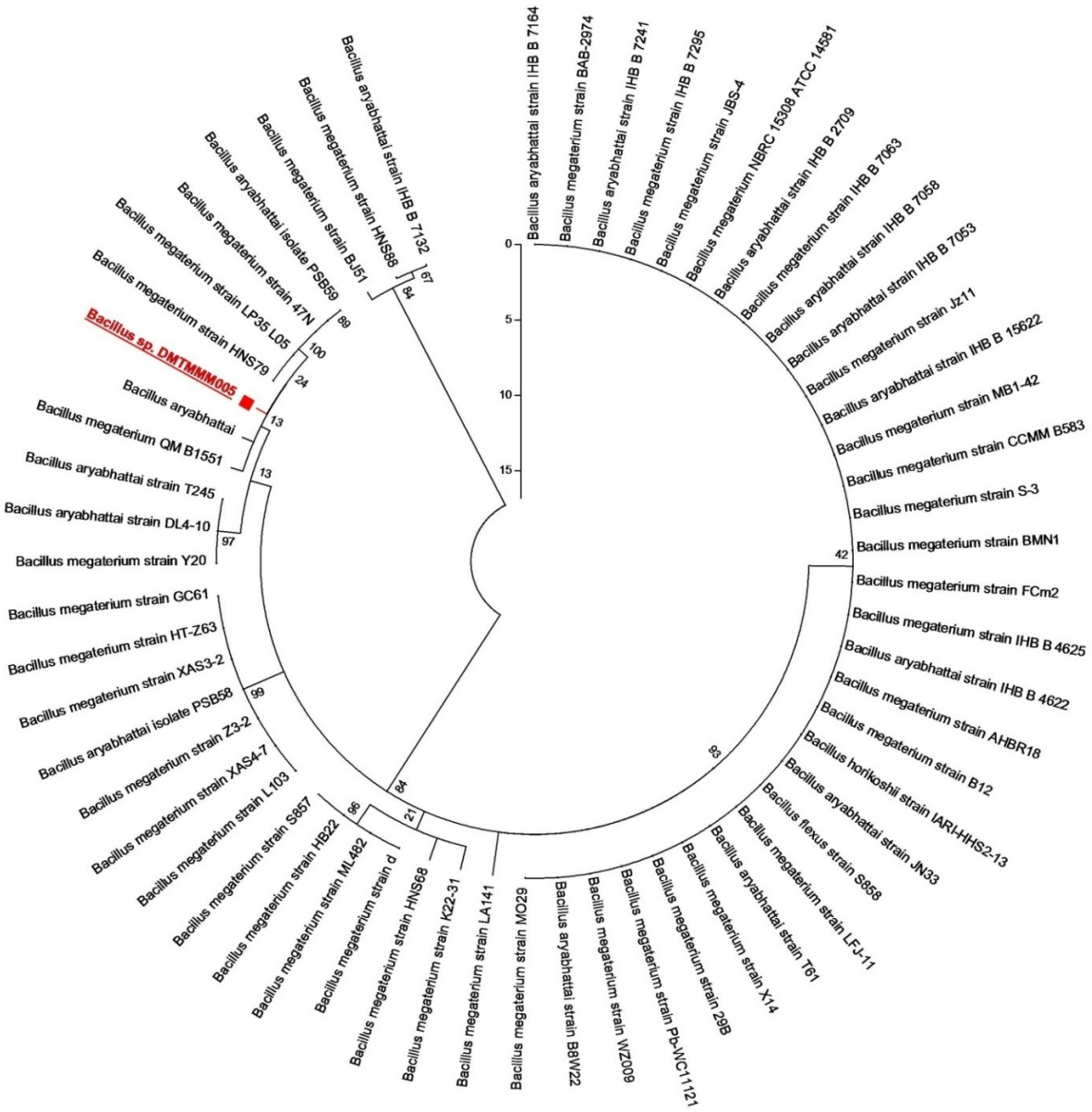

Fig. S4. Phylogenetic analysis of 16S rRNA from strain BIT05

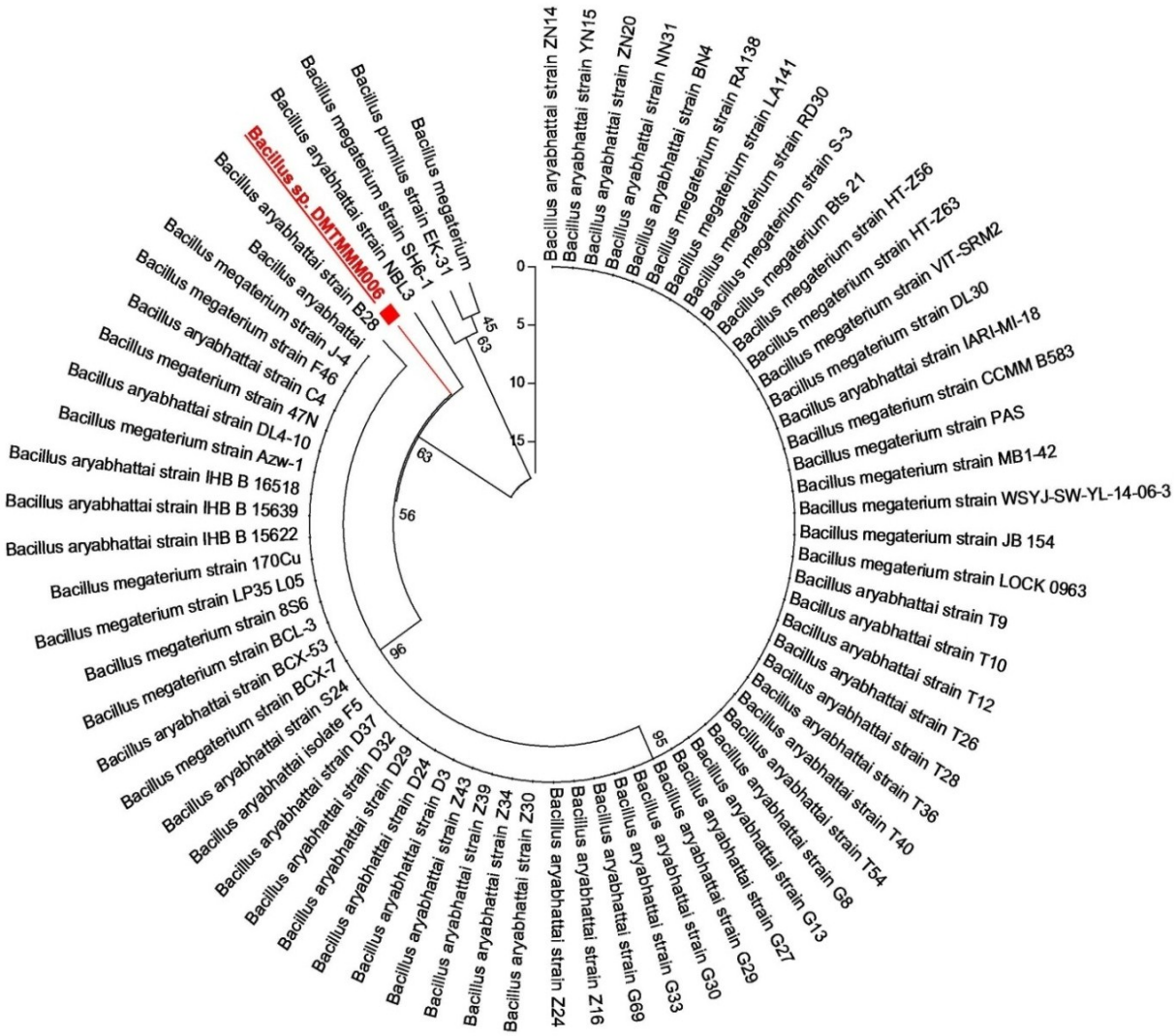

Fig. S5. Phylogenetic analysis of 16S rRNA from strain BIT07

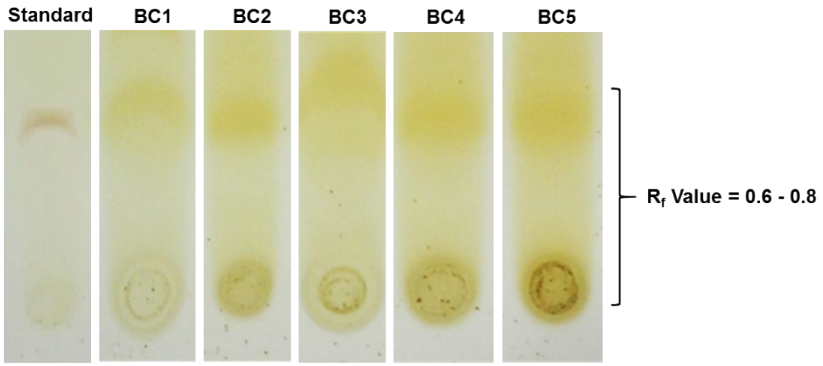

Fig.S6. TLC of hydrolyzed product (glucose) of BCs with standard glucose.

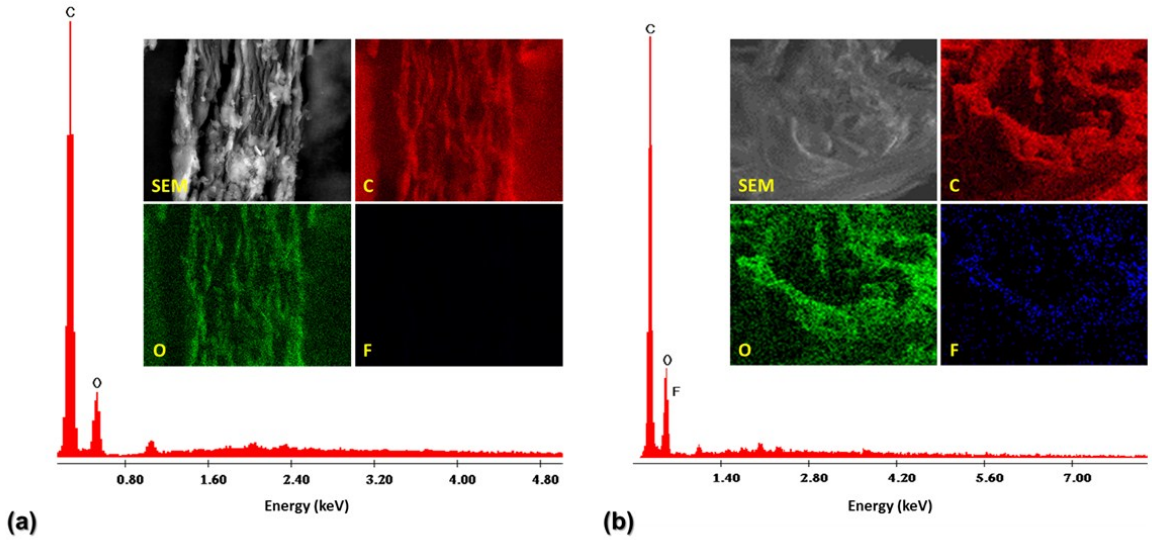

Fig. S7. SEM-EDS of bacterial cellulose and corresponding elemental mapping of fluoride, carbon and oxygen. (a) Parent material (b) after fluoride adsorption.

References

(41) Bergey, D. H.; Buchanan, R. E.; Gibbons, N. E. Manual of determinative bacteriology. *Williams and Wilkins Co., Baltimore,* ***548****,* 1974.

(42) Wilson, K. Preparation of genomic DNA from bacteria. *Current protocols in molecular biology*, 2-4, 1987.