Role of Plasmid in Production of Acetobacter Xylinum Biofilms

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Abstract: Acetobacter xylinum has the ability to produce cellulotic biofilms. Bacterial cellulose is expected to be used in many industrial or biomedical materials for its unique characteristics. A. xylinum contains a complex system of plasmid DNA molecules. A 44 kilobases (kb) plasmid was isolated in wild type of A. xylinum. To improve the cellulose producing ability of A. xylinum, role of the plasmid in production of cellulose was studied. The comparisons between wild type and cured cells of A. xylinum showed that there is considerably difference in cellulose production. In order to study the relationship between plasmid and the rate of cellulose production, bacteria were screened for plasmid profile by a modified method for preparation of plasmid. This method yields high levels of pure plasmid DNA that can be used for common molecular techniques, such as digestion and transformation, with high efficiency.

Key words: Acetobacter xylinum, plasmid, biofilm, cellulose, curing

INTRODUCTION

Cellulose is the most abundant biopolymer on earth, recognized as the major component of plant biomass, but also a representative of microbial extracellular polymers[1]. Bacterial Cellulose (BC) belongs to specific products of primary metabolism and is mainly a protective coating, whereas plant cellulose plays a structural role[2]. Cellulose is synthesized by bacteria belonging to the genera Acetobacter, Rhizobium, Agrobacterium and Sarcina. Its most efficient producers are Gram-negative, acetic acid bacteria Acetobacter xylinum (reclassified as Gluconacetobacter xilinus), which have been applied as model microorganisms for basic and applied studies on cellulose[3]. Acetobacter xylinum produce an extracellular gel- like material or pellicle, which comprises of a random assembly of cellulose ribbons composed of a number of microfibrils. Extracellularly synthesized microbial cellulose differs from plant cellulose with respect to its high crystallinity and purity (free of lignin and other biogenic products), high water-absorption capacity and mechanical strength in the wet state[4]. Several application have been proposed for this cellulotic layer. Because of the unique properties, resulting from the ultrafine reticulated structure, BC has found a multitude of applications in paper, textile and food industries and as a biomaterial in cosmetics and medicine[5]. One of applications of bacterial cellulose is use as burn infection and heal lesions. Modern medical biotechnology has accepted artificial skins as a valid prospect. Several properties advantageous for its use as a temporary skin substitute were recognized for the biofilms[3,5]. Its successful application by dermatologists and plastic surgeons included human second and third degree skin burns, skin grafts, face peelin, infectious enhanced proportions of fibroblasts, collagen, blood vessels and granulation tissue as a result of the cellulotic pellicle use were seen in the healing wound. Wider application of this polysaccharide is obviously dependent on the scale of production and its cost. Therefore, basic studies run together with intensive research on strain improvement and production process development. In the research was studied, about role of plasmid on production of cellulose by elimination of plasmid DNA ,curing,. Curing is an important step in identifying the phenotypic traits encoded by a given plasmid. Plasmid curing studies have been focused on Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, to a lesser extent, strains of degradative Pseudomonads. A multitude of different chemicals including inhibitors of DNA replication, intercalating drugs, bacterial surface agents and Sodium Dodecyl Sulphate (SDS) at elevated temperature has been used as curing agents. However, apart from its spontaneous loss, very little is known about how to cure the plasmids of A. xylinum. The plasmids may prove biotechnologically important as it has been implicated in the production of microbial cellulose[6]. In this study, Acridin orange properties were exploited for curing of the A. xylinum plasmid.

MATERIALS AND METHODS

Bacterium: A freeze-dried culture of A. xylinum was obtained from the Persian Culture Type Collection of
Microorganisms. Following recultured in Schramm Hestrin (SH) medium, the culture was found to be pure and biochemical and morphological tests revealed that the organism conforms to the generic description listed for A. xylinum in Bergey’s Manual of Systematic Bacteriology[7].

Culture conditions: The bacteria was inoculated with 10⁸ to 10⁹ cell per mL in SH medium at 28°C for a week under static conditions to produce cellulose pellicles. For plasmid isolation, Acetobacter xylinum was grown in SH agar.

Determination of wet and dry weights of pellicles: The pellicle was lifted from the plate with a bent glass rod and allowed to drip for 30 min. The pellicle was then placed in a pre weighed plastic weighing plate. The weighing plate and pellicle were then placed in a drying oven for 6 h at 80°C. After being dried and removed from the oven, the weighing boat and pellicle were weighed immediately. Pellicles were also washed with running tap water prior to the wet and dry weight determinations. There were no percentage differences between the wet and dry weight comparisons of pellicles that had not been washed before being allowed to drip for 30 min[8].

Rapid screening for plasmids: A small number of cells were picked by touching a colony with a toothpick. They were then inoculated into a microfuge tube containing 300 µL of SH broth. After overnight growth, the cells were pelleted in a microfuge (10,000 rpm, 2 min). The cells were then suspended by vortexing at full speed for 1 min followed by centrifugation for 10 min at 12,000 rpm. The supernatant and pellicle were weighed immediately. Pellicles were also washed with running tap water prior to the wet and dry weight determinations. There were no percentage differences between the wet and dry weight comparisons of pellicles that had not been washed before being allowed to drip for 30 min[8].

Curing of Acetobacter xylinum: Acetobacter xylinum cured by growth at 28°C in SH medium containing a subinhibitory concentration of Acradin orange (5 to 600 µg). Cultures containing the highest concentration of Acradin orange which growth was clearly visible were diluted and spread into SH plates. Resulting clones were tested for chloramphenicol sensitivity and electrophoresis. A cell lysate of A xylinum and its cured derivative were cultivate as described in culture conditions of materials and methods and the presence or
absence of plasmid DNA was tested using agarose gel electrophoresis. A purified sample of A. xylinum plasmid as well as lambda (digested with HindIII) were also included.

**Restriction analysis of isolated plasmid DNA:** Plasmids isolated from A. xylinum were digested with two restriction enzymes (BamHI and HindIII) at the same time. Aliquots of A. xylinum plasmid DNA, prepared as described above, were digested overnight at 37°C with ten units of various restriction enzymes and the fragments thus generated were fractionated by electrophoresis on 0.7% agarose gel as described above. The bands, which were visualized under ultraviolet light, were compare with standard marker.

**RESULTS AND DISCUSSION**

*A. xylinum* is an acetic acid bacterium which has the ability to produce cellulose extracellularly. To improve the cellulose-producing ability of *A. xylinum*, recombinant DNA techniques seems to be proper method. The genus *A. xylinum* contains several species which are being extensively studied in connection with acetic acid production and cellulose formation\[12\]. There have been several reports of spontaneous mutation in *A. xylinum* strains affecting both morphological and physiological properties, including loss of the ability to produce cellulose\[13,14\]. Bacteria of this genus are not genitically well characterized and genetic investigations of the phenotypic instabilities have not been reported. To improve the cellulose producing ability of *A. xylinum*, we studied role of the plasmid in production of cellulose. Most strains of *A. xylinum* examined contain a rather complex system of plasmids\[15\]. Alterations in the plasmid profile were found in both cellulose negative mutants and cellulose producing revertants of *A. xylinum*. Cells of *A. xylinum* produce large amounts of exopolysaccharide, which interferes with the extraction of plasmid DNA\[16,17\]. To repress capsular polysaccharide production, bacteria were cultured in SH medium containing bismuth nitrate and sodium salicylate. After treatment, bacterial cells were more readily lysed in alkaline detergents. The resulting plasmid preparations contained virtually no capsular polysaccharide and relatively small quantities of lipopolysaccharide and protein, yet they produced yields of nucleic acids similar to those of conventional plasmid preparations. Conventional preparations from encapsulated organisms were largely insoluble and appeared as smears following agarose gel electrophoresis, with indefinite plasmid banding. Plasmids prepared by the new method were highly soluble in conventional buffers and exhibited high-resolution plasmid banding patterns in agarose gels. The method proved effective with encapsulated or mucoid strains of *A. xylinum*. The method for plasmid isolation was not suitable for each bacteria because of the inhibitory effects of bismuth. Thus, removal of contaminating bacterial surface structures enabled the rapid isolation and characterization of plasmids from mucoid isolates, without the use of organic solvents, CsCl gradients, or expensive, disposable columns\[16\]. In this study, a simple and rapid method for screening plasmids of *A. xylinum* has been developed. It is especially useful when sample sizes are large. This method can be achieved in one step simply by phenol/chloroform treatment of the cells pelleted from a volume as small as 300 μL. The experiment from growing cells to the completion of plasmid extraction can be carried out using the same microfuge tube. The whole procedure takes about 40 min from harvesting the bacterial cultures to visualization of the DNA bands in agarose gel. Using this method, detected plasmids in *A. xylinum* have detected.

**In conclusion,** this new method for the isolation of plasmid DNA from lysis-resistant *A. xylinum* has major advantages over the classic method that requires phenol-extraction and cesium chloride gradient centrifugation. This new method does not employ highly toxic reagents, is considerably cheaper and is easy to perform. This method provides high yields of pure DNA, which can be used for restriction analysis, transformation and other molecular techniques. This plasmid isolation technique is also applicable to other Gram-negative bacteria and will improve the efficient engineering of biotechnologically and therapeutically useful *A. xylinum* recombinants for cellulose production. To construct a physical map, isolate plasmid was cut with
different restriction endonucleases and subjected to agarose gel electrophoresis. The results showed that it carried single restriction sites for \textit{Bam}HI and \textit{Hind}III. The molecular size of this plasmid was estimated to be 4.4 kb based on the sum total of the restriction fragment lengths (Fig. 1). Isolation of the plasmid have been performed reproducibly several times, both from stationary and log-phase cells. This result demonstrates that the band pattern observed in agarose gel electrophoresis are stable characteristics. From present data the following conclusions can be drawn: (I) studied wild type \textit{A. xylinum} contains a 4.4 kb plasmid (II) Curing strains have identifiable changes in their cellulose production compared to the wild type. Indicating that these plasmids may be involved in cellulose biosynthesis. Success in isolation of plasmid has shown that allows insertion of foreign genes, demonstrating the potential of \textit{A.xylinum} being developed into a vector for gene cloning.

REFERENCES