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Short Communication

Strain improvement of *Gluconacetobacter xylinus* NCIM 2526 for bacterial cellulose production

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The present investigation demonstrates the effectiveness of ultraviolet (UV) radiation and ethyl methanesulfonate (EMS) in strain improvement for enhanced cellulose production by *Gluconacetobacter xylinus* NCIM 2526. The mutants were compared with wild type for cellulose production. UV mutants $GHUV_3$, $GHUV_4$, and $GHUV_5$ of *G. xylinus* showed higher cellulose yield than the wild strain. The mutant $GHUV_4$ gave cellulose yield of 3.92 g/l which was 30% more than the wild strain in standard medium. Chemical mutants $GHEM_4$, $GHEM_6$ and $GHEM_7$ of *G. xylinus* showed higher cellulose yield than the parent strain ($GHUV_4$). $GHEM_4$ gave cellulose yield of 5.96 g/l which was 50% more than the parent strain ($GHUV_4$) and 98% more than the wild strain (NCIM 2526). The results indicated that UV and EMS were effective mutagenic agents for strain improvement.

Key words: Bacterial cellulose, *Gluconacetobacter xylinus,* ultraviolet mutagenesis, ethyl methanesulfonate treatment.

INTRODUCTION

Gluconacetobacter xylinus (formerly *Acetobacter xylinum)* has been known to produce pure cellulose for more than 100 years. The production of this bacterial cellulose (BC) is receiving great attention because of its unique properties and wide variety of applications. Due to its high tensile strength and water-holding capacity, BC has been used as raw material for producing high-fidelity acoustic speakers, high quality paper and diet and dessert foods (Ross et al., 1991; Iguchi et al., 2000). Moreover, BC could be used as artificial skin (Joris and Vandamme, 1993) and as a promising material for a potential scaffold for tissue engineering (Svensson et al., 2005). One of the BC application problems in industry is its low productivity. One approach to combat BC productivity is strain improvement of the producer organism. As yet, no total BC synthesis operons have been cloned or no cells transformed with the BC synthesis gene (Romling, 2002). Ishikawa et al. (1995) obtained mutants of *A. xylinum* BPR 2001 resistant to sulfaguanidine by NTG (*N*-methyl-*N*′- nitro-*N*-nitrosoguanidine) treatment. They reported increased cell growth and cellulose production. The aim of the present investigation is to increase cellulose productivity of *G. xylinus* NCIM 2526 by ultraviolet (UV) radiation and ethyl methanesulfonate (EMS) treatment.

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Abbreviations: UV, Ultraviolet; **EMS,** ethyl methanesulfonate; **BC,** bacterial cellulose; **HS,** Hestrin-Schramm; **NTG,** N-methyl-N′-nitro-N-nitrosoguanidine.

MATERIALS AND METHODS

Microorganism and growth media

The organism used was *G. xylinus* NCIM 2526 obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, India. The stock culture was maintained on Hestrin-Schramm (HS) agar slants, transferred monthly and stored at a temperature of 2 - 8°C in the refrigerator. The culture medium used was the standard Hestrin-Schramm (HS) medium (Hestrin and Schramm, 1954) which contains (w/v) 2.0% D-glucose, 0.5% peptone, 0.5% yeast extract, 0.27% disodium phosphate and 0.115% citrate. The cultures were incubated at 30°C in 100 ml quantity of the medium for 14 days. The pellicle formed after 14 days of incubation was removed carefully, boiled in 2.0% NaOH solution for 30 min and thoroughly washed with distilled water. Drying of the pellicle was carried out at 65°C in an oven for 6 h. The dry weight of the cellulose was calculated.

Table 1. UV mutants and their cellulose yield.

S/N	Description	BC yield (g/l)
1	$GHUV_1$	NIL
2	$GHUV_2$	2.21
3	$GHUV_3$	3.45
4	$GHUV_4$	3.92
5	$GHUV_5$	3.61
6	$GHUV_6$	2.01
7	$GHUV_7$	1.25
8	$GHUV_8$	2.98
9	$GHUV_9$	NIL
10	$GHUV_{10}$	1.25
11	$GHUV_{11}$	2.98
12	$GHUV_{12}$	2.54
13	$GHUV_{13}$	2.12
14	$GHUV_{14}$	2.84
15	$GHUV_{15}$	1.23
16	*G. xylinus* (wild strain)	3.04

Organisms were cultured for 14 days in 250 ml Erlenmeyer flasks containing 100 ml of standard HS medium.

Physical mutagenesis and mutant selection

Organism was grown in 50 ml of HS broth at 30°C for 24 h. Such transfers were made thrice to get good growth within 24 h. 20 ml of 24 h old culture broth was harvested by centrifugation at 5000 rpm for 10 min. The supernatant was discarded and the pellet was re-suspended in 10 ml of sterile phosphate buffer pH 7. The total viable count of the sample was found to be 10^7/ml. The suspension was further diluted to have viable count of 10^5/ml. 10 ml of the culture suspension (viable count 10^5/ml) was taken into a Petri plate of 100 mm diameter. The sample was irradiated with a germicidal lamp (UV light source) from a distance of 20 cm. Samples were withdrawn after 10, 20, 30, 45 and 60 s. These irradiated samples were serially diluted in phosphate buffer. The survivors were determined by spreading 0.1 ml of the sample on screening medium (HS agar with 0.02% fluorescent brighter). Fluorescent brightener is a fluorescent dye that avidly binds to β, D-glucans and cellulose producing colony fluoresces when observed under UV-light. A total of 15 colonies (designated as $GHUV_1$ to $GHUV_{15}$) were selected from the plates and tested for cellulose production ability. The results were compared with that of the wild strain.

Chemical mutagenesis and mutant selection

The best UV mutant ($GHUV_4$) was used for chemical mutagenesis. The culture suspension was prepared in the same manner as described earlier. To 5 ml of cell suspension with viability of 10^5/ml, 5 ml of sterile solution of EMS (200 $\mu g ml^{-1}$) was added. The reaction was allowed to proceed. Samples were withdrawn from the reaction mixture at intervals of 15, 30, 60, 90, 120 and 150 min. Immediately, the samples were resuspended in sterile buffer pH 7. The suspended sample was again centrifuged at 5000 rpm for 10 min. The supernatant was discarded. Cells were washed thrice with sterile distilled water to remove traces of EMS. The samples were serially diluted in the same buffer and plated on screening medium as mentioned earlier. A total of 15 colonies (designated as $GHEM_1$ to $GHEM_{15}$) were selected from the plates and similarly tested for cellulose production ability.

RESULTS AND DISCUSSION

Strain improvement can generally be described as the use of any scientific techniques that allow the isolation of cultures exhibiting a desired phenotype. The technology has been utilized for more than 50 years in conjugation with modern submerged culture fermentations (Victor and Graham, 1999). There are only few reports of mutating *A. xylinum* for increased cellulose production. Ishikawa et al. (1995) subjected the cells of *A. xylinum* subsp. *sucrofermentans* BPR 2001 to chemical mutagenesis by NTG (N-methyl-N′-nitro-N-nitrosoguanidine). The mutant BPR 3001E gave 40% higher cellulose than parent *G. xylinum*. They found that cellulose production was associated with growth.

We tried mutagenesis of the organism both by physical and chemical mutagenic agents. Bapiraju et al. (2004) reported mutation induced enhanced lipase production from *Rhizopus* sp. using UV radiation and NTG. Kadam and associates (Kadam et al., 2006) successfully employed UV mutagenesis to improve strain of *Lactobacillus delbruekii* for lactic acid production. EMS is a well-known mutagenic agent, whose mode of action is attributed to alkylation at nitrogen position 7 of guanosine of the DNA molecule, leading to transversion or transition type of mutations (Freese, 1961). Successful use of EMS in induced mutations has been reported for many bacterial strains (Shanthamma et al., 1972; Haq et al., 2009). The colonies of *G. xylinus* are typically small and convex in appearance, eventually developing uneven edges where the bacterial cells break through the cellulose. Mutant selection was based on growth rate, colony morphology and fluorescence on screening medium.

Wild strain of *G. xylinus* was subjected to UV mutagenesis. A total of 15 mutants were selected and screened for their cellulose production abilities (Table 1). Three UV mutants $GHUV_3$, $GHUV_4$ and $GHUV_5$ produced 3.45, 3.92 and 3.61 g/l cellulose, respectively. The mutant $GHUV_4$ gave maximum cellulose production of 3.92 g/l which was 30% more than the wild strain in standard HS medium. Furthermore, the best UV mutant $GHUV_4$ was subjected to chemical mutagenesis. The mutants $GHEM_4$, $GHEM_6$ and $GHEM_7$ produced 5.96, 5.62 and 4.89 g/l cellulose, respectively (Table 2). The mutant $GHEM_4$ gave cellulose yield of 5.96 g/l which was 50% more than the parent strain ($GHUV_4$) and 98% more than that of wild strain (NCIM 2526). Haq et al. (2009) reported strain improvement of *Bacillus licheniformis* for alpha amylase production using EMS. This is one of the rare reports of mutagenesis for increased cellulose production using UV radiation and EMS. Effectiveness of UV radiation (physical mutagen) and EMS (chemical mutagen) in strain improvement for enhanced cellulose production has been demon-

Table 2. EMS mutants and their cellulose yield.

S/N	Description	BC yield (g/l)
1	$GHEM_1$	3.56
2	$GHEM_2$	3.54
3	$GHEM_3$	2.54
4	$GHEM_4$	5.96
5	$GHEM_5$	NIL
6	$GHEM_6$	5.62
7	$GHEM_7$	4.89
8	$GHEM_8$	2.54
9	$GHEM_9$	1.28
10	$GHEM_{10}$	1.85
11	$GHEM_{11}$	3.79
12	$GHEM_{12}$	2.78
13	$GHEM_{13}$	2.55
14	$GHEM_{14}$	1.98
15	$GHEM_{15}$	3.01
16	$GHUV_4$ (parent strain)	3.90

Organisms were cultured for 14 days in 250 ml Erlenmeyer flasks containing 100 ml of standard HS medium.

strated in this investigation.

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