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Process and Strain Development for Reduction of Broth Viscosity with Improved Yield in Coenzyme Q_{10} Fermentation by *Agrobacterium tumefaciens* ATCC 4452

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Abstract

Viscous nature of the fermentation broth has phenomenal influence on process conditions and parameters in a fermentor. Though broth rheology has attracted significant influence in process research, still there is a challenge to modify fluid dynamics of fermentation broth. During the production of coenzymeQ_{10} (CoQ_{10}) by *Agrobacterium tumefaciens* ATCC 4452, the culture broth becomes highly viscous due to excessive synthesis of exopolysaccharides. This hinders the CoQ_{10} yield and complicates the downstream process. The present study describes how this problem was tackled by media modification and mutation. Induced mutants were generated using UV and EMS as mutagenic agents followed by rational selection based on antibiotic resistance. On screening of these mutants in sucrose based PM-2 medium, UV induced, vancomycin resistant mutant M-6, showed significant reduction (6.29 fold) in viscosity development in the broth. Mutant M-6(S), a natural variant of mutant M-6, resistant to high substrate concentration was further selected for the CoQ_{10} production. Cane molasses as carbon source was found to be best suitable for CoQ_{10} fermentation using mutant M-6(S). Replacing sucrose with cheaper cane molasses significantly reduced the broth viscosity with improved specific CoQ_{10} content, thereby generating cost effective fermentation process. The newly developed mutant strain produced 48.89 mg/L of CoQ_{10} with specific CoQ_{10} content of 1.87 mg/g of DCW at 25°C, 500 rpm agitation and 0.2 vvm aeration using continuous fed batch fermentation and newly formulated cane molasses medium.

Keywords: *Agrobacterium tumefaciens*; CoQ_{10}; Exopolysaccharide; Cane molasses; Viscosity

Introduction

CoQ_{10} is 2,3-dimethoxy-5-methylbenzoquinone with 10 units of isoprenoid chain at the 6-position of the quinone ring. It is distributed widely in the mitochondrial inner membrane, lysosomes, peroxisomes, and microsomes throughout the eukaryotic cells, and is located in the plasma membrane of prokaryotic cells, transferring electrons from complex I/II to the cytochrome bc_1 complex during the oxidative phosphorylation for ATP generation [1,2]. Apart from playing an important role in electron transport chain for ATP synthesis, it also acts as an antioxidant and prooxidant [3]. It is widely used as neutraceuticals and therapeutical supplements in various clinical conditions like cardiovascular disease, neuro-degenerative diseases, and mitochondrial respiratory-chain diseases etc [4-6]. It is also used as a dietary supplement in energy drinks. Hence, there is burgeoning demand for CoQ_{10} in the global market by neutraceutical companies [7].

CoQ_{10} can be produced by chemical, semi chemical and biological methods [8]. Wild type strains and chemical mutants of various microorganisms, including bacteria (e.g., *Agrobacterium*, *Rhodobacter*, *Paracoccus*) and yeasts (e.g. *Candida*, *Rhodotorula*, *Saitoella*) have been reported as CoQ_{10} producers in the patent applications [9]. Among CoQ_{10} producing microorganisms, *Agrobacterium tumefaciens* has been reported to produce higher CoQ_{10} [10]. But during the biosynthesis, the culture broth becomes highly viscous due to formation of exopolysaccharides when cultivated on sucrose-based medium [11]. This markedly changes the rheological nature of the fermentation fluid, thereby affecting oxygen transfer in fermentor [12]. Also during the downstream processing, the highly viscous broth makes the purification process difficult. Many researchers have addressed the issue at the process level. It was reported that sugar to ammonium salt ratio in the medium as well as temperature affects CoQ_{10} production and viscosity [13]. The effect of sucrose concentration on the production of exopolysaccharide, cell growth and specific CoQ_{10} content has been studied. Using pH stat fed batch culture system with regulation of sucrose concentration; there was a significant reduction in exopolysaccharide with concomitant improvement in CoQ_{10} content [14].

In this paper, we describe a combination of mutations method along with process modification to reduce the broth viscosity for improved CoQ_{10} production by *Agrobacterium tumefaciens* ATCC 4452.

Materials and Methods

Strains and morphology

The bacterial strain *Agrobacterium tumefaciens* ATCC 4452 and its induced mutants were maintained on Nutrient Agar (NA) slants. The mutants were streaked on NA plates and incubated at 30°C for 2-3 days in order to get the pure clone.

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UV mutagenesis

The culture was grown overnight in 50 ml of nutrient broth (NB) medium in 500 ml flask to get the exponential phase growth culture having viable count of around 10^9-10^{11} cfu/ml. Five ml of the suspension was placed in sterile petri dish and exposed to UV rays (235 nm) at a distance of 10 cm. At regular intervals, the samples were taken out and different dilutions were plated on NA plate to determine viable count. The percent reduction of viability was calculated by comparing viable count with that of unexposed suspension.

EMS mutagenesis

10 ml suspension of exponential phase growth culture having viable count of around 10^9-10^{11} cfu/ml was centrifuged to get a pellet. It was then washed with saline and resuspended in 10 ml of phosphate buffer (pH 7.0). The suspension was treated with 80 µM of EMS with constant shaking. At different time intervals samples were withdrawn and to it 5% sodium thiosulphate was added to stop the mutagenesis. Next the cells were washed and plated on NA plate to determine the viable count. The reduction in viable count was determined as compared to untreated suspension.

Selection of mutants

The mutant showing resistance to antibiotic was selected. Initially antibiotic sensitivity test was performed using different antibiotic discs (Octadisc, Hi-media) by agar diffusion method.

Vancomycin to which the strain was most sensitive was used for selection of resistant mutants by gradient plate technique using vancomycin from 0-60 µg/ml. The plates were incubated at 30°C for 96 h. The mutant colonies appearing towards vancomycin concentration above MIC (30 µg/ml) were picked up and transferred onto NA slants.

Screening of mutants and media modification in shake flask

The wild type strain and mutants were inoculated in 50 ml seed medium containing (per L) 20 g sucrose, 10 g yeast extract, 10 g peptone, 5 g NaCl, pH 7.2 in a 500 ml Erlenmeyer flask and incubated at 30°C with shaking at 220 rpm. After 48 h, 10% of the seed was transferred to 50 ml of different production media in 500 ml Erlenmeyer flask [15]. The production media used [15-18] were PM-2 [consists of (per L) 25 g sucrose, 10 g $(NH_4)_2SO_4$, 2.5 g K_2HPO_4, 2 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 20 g corn steep liquor (CSL), 20 g $CaCO_3$, 1 ml /L trace element solution, pH 7.0], MPM-1 [consists of (per L) 50 g sucrose, 10 g $(NH_4)_2SO_4$, 2.5 g K_2HPO_4, 2 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 40 g CSL, 20 g $CaCO_3$, pH 7.0], MPM-2 [consists of (per L) 30 g glucose, 1 g $(NH_4)_2SO_4$, 2.5 g K_2HPO_4, 2.5 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, pH 7.0], MPM-3 [consists of (per L) 80 g sucrose, 100 g cane molasses, 13 g $(NH_4)_2SO_4$, 2.5 g K_2HPO_4, 2 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 40 g CSL, 20 g $CaCO_3$, pH 7.2], MPM-4 [consists of (per L) 50 g dextrose, 10 g soyabean meal, 5 g yeast extract, 4 g soya peptone, 2.5 g NaCl, 50 g $CaCO_3$, pH 7.2], MPM-5 [consists of (per L) 80 g cane molasses, 13 g $(NH_4)_2SO_4$, 2.5 g K_2HPO_4, 2.5 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 40 g CSL, 20 g $CaCO_3$, pH 7.2], MPM-6 [consists of (per L) 50 g cane molasses, 10 g $(NH_4)_2SO_4$, 2.5 g K_2HPO_4, 2.5 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 20 g CSL, 20 g $CaCO_3$, pH 7.0] and MPM-7 [consists of (per L) 80 g sucrose, 13 g $(NH_4)_2SO_4$, 2.5 g K_2HPO_4, 2 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 40 g CSL, 20 g $CaCO_3$, 1 ml/L trace element solution, pH 7.0]. The production flasks were incubated at 30°C with shaking at 220 rpm for 90-96 h.

The sucrose based media namely PM-2, MPM-1, MPM-3 and MPM-7 were dosed intermittently at 48 h and 72 h with 5 ml of 30% sucrose solution [12]. The cane molasses based media namely MPM-5 and MPM-6 were dosed intermittently at 48 h and 72 h with 5 ml of 60% cane molasses solution. The glucose based media namely MPM-2 and MPM-4 were dosed intermittently at 48 h and 72 h with 5 ml of 60% glucose solution.

Lab scale fermentation

The performance of mutant M-6(S) in four different media namely PM-2, MPM-3, MPM-5 and MPM-7 was evaluated in 10 L NBS fermentor. The fermentation was carried out at 30°C with 300 rpm and 0.3 vvm aeration. The sucrose based media namely PM-2, MPM-3 and MPM-7 were dosed intermittently at 48 h and 72 h with 500 ml of 30% sucrose solution. Dosing was carried out as per the published protocol [12,15]. The cane molasses based media namely MPM-5 was dosed intermittently at 48 h and 72 h with 500 ml of 60% cane molasses solution. The fermentor was harvested at 96 h and broth viscosity, specific CoQ_{10} content and DCW was estimated.

Viscosity measurement

The viscosity of 35 ml of each of the broth samples was estimated using Vibro Viscometer SV-10 and expressed as cps unit.

Dry cell weight (DCW) measurement

10 ml of broth was centrifuged at 12000 rpm for 20 min in a pre-weighed centrifuge tube. The cell mass was quantified by drying at 60°C until a constant mass.

CoQ_{10} extraction method

CoQ_{10} extraction was carried out at 50°C for 3 h using ethanol-hexane mixture (1:1) from the cell pellet obtained after centrifuging 10 ml of broth. After the extraction phase, separation was carried out using 5 ml of water. The CoQ_{10} containing hexane layer was further concentrated and analyzed by HPLC.

High performance liquid chromatography (HPLC)

The CoQ_{10} extracted from cell biomass was quantified on High Performance Liquid Chromatography (Agilent 1100) using normal phase Kromasil silica column (250 mm×4.6 mm, 5 µ particle size) and hexane: isopropyl alcohol (95:5) as mobile phase with a flow rate of 1ml/min. Detection was carried out at 273 nm.

Estimation of total sugar

Total sugar of the fermentor broth has been estimated by Anthrone method [19].

Fermentation optimization

The fermentation process was optimized for mutant M-6(S) using MPM-5 medium in 10 L NBS fermentor. A 10% (v/v) seed culture was inoculated into a 10 L fermentor with a working volume of 7 L. The fermentation was done by altering the parameters like temperature (25°C, 30°C and 30°C), agitation (300, 500 and 600 rpm) and aeration (0.2 and 0.6 vvm) [20-22]. The continuous feeding was started after 24 h of growth with 20.5% cane molasses solution at the flow rate of 10 ml/h. The batch was harvested at 96 h. The broth samples were analyzed for DCW and specific CoQ_{10} content.

To study the effect of sucrose on viscosity development in the broth, a fermentor batch was carried out using the optimized parameters and replacing the cane molasses in the medium (MPM-5) by equivalent amount of sucrose (4%). The dosing was started after 24 h of growth with 10.25% sucrose solution at the flow rate of 10 ml/h. The batch

was harvested at 96 h. The broth samples were analyzed for DCW and specific CoQ_{10} content.

Statistical analysis

For analyzing differences between two groups, student's t-test was used based on PRISM-5 software. P values below 0.05 were considered statistically significant. The values in all graphs are an average of 3 trials. Unless stated otherwise, all error bars represent standard error of mean.

Results and Discussion

The wild type strain of *Agrobacterium tumefaciens* ATCC 4452 exploited for production of CoQ_{10}, produced excessive amounts of viscous exopolysaccharide on sucrose based medium, which complicated its downstream processing an affected CoQ_{10} yield. To tackle the issue of exopolysaccharide production, type strain was subjected to genetic manipulation by physical and chemical mutagenesis followed by rational selection of mutants, probably lacking polysaccharide biosynthetic pathway resulting in reduced broth viscosity.

The wild type strain showed more than 95% reduction in viability when exposed to germicidal UV rays for 7 min at a distance of 10 cm. In case of EMS mutagenesis, about 94% reduction in viability was observed when the cells were treated with 80 µM EMS for 30 min. The selections of mutants were carried out based on the resistance to the different antibiotic and finally vancomycin was selected to which strain was found to be most sensitive among all tested antibiotics using octadisc agar diffusion method. The MIC of vancomycin was found to be around 30 µg/ml using gradient plate technique.

Screening of selected mutants from gradient plate which were growing beyond the MIC (30 µg/ml) of vancomycin has been performed in shake flask using sucrose based PM-2 medium. Among the different mutants selected and tested, mutant M-6 showed 6.29 fold decreases in viscosity and 1.27 fold increases in specific CoQ_{10} content as compared to wild type strain, as shown in figure 1. Drop in broth viscosity was significant and it indicated the occurrence of a desirable mutation leading to inhibition of polysaccharide synthesis and improved mass transfer with 2.2 fold increase in DCW of mutant M-6. This mutant was further selected and the cell suspension was subjected to the plate containing agarified MPM-3 medium (containing sucrose and cane molasses) in order to select a natural variant resistant to high osmotic pressure exerted by high concentration of sucrose and cane molasses. It was intended that such mutant could be utilized for CoQ_{10} production on media containing high concentration of sucrose and cane molasses. We observed only a single colony growing on the MPM-3 agarified plate, which was then selected and named as mutant M-6(S).

Attempts were made to formulate new media for CoQ_{10} production using mutant M-6(S). We formulated completely a new media MPM-5 and MPM-6 containing cane molasses as a sole carbon source. In addition to this, MPM-3 containing combination of sucrose and cane molasses was formulated. During shake flask screening, intermittent dosing with the respective carbon source was carried out for each of the medium flasks at log 48 and 72 h to maintain the total sugar concentration above 6%. This feeding helped to achieve extended growth phase and improved biomass yield.

Out of seven media tested in flask, medium MPM-5 containing 8% cane molasses showed maximum specific CoQ_{10} content (1.398 mg/ g of DCW) and least viscosity of broth (3.766 cps) as shown in figure 2. This reduction of broth viscosity and improvement of specific CoQ_{10} content was significant with medium MPM-5 as compared to PM-2 medium. The medium MPM-3 and MPM-6 also showed significant reduction in broth viscosity without any significant change in specific CoQ_{10} content. Since the profile of these two media was identical, only MPM-3 was selected for further studies. In addition to these, medium MPM-7 containing higher sucrose was also selected for further studies in order to compare the performance between two carbon sources i.e., molasses and sucrose.

From the shake flask experiments, we finally selected four media namely initial medium PM-2 (25 g sucrose per L), MPM-3 (combination of 80 g sucrose and 100 g cane molasses per L), MPM-5 (80 g cane molasses per L), and MPM-7 (80 g sucrose per L) to study the

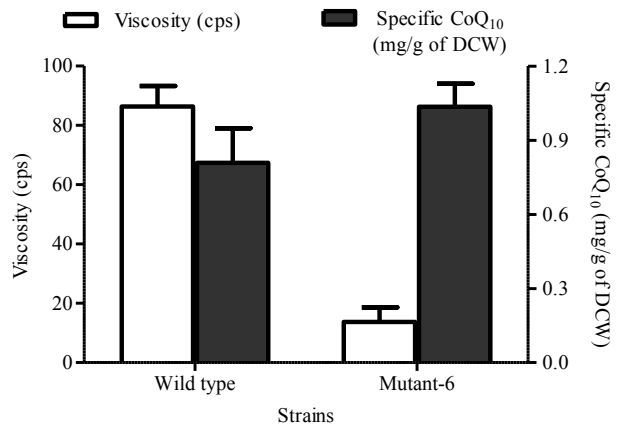


Figure 1: Comparison of viscosity and specific CoQ_{10} content between wild type and mutant M-6 on PM-2 medium at shake flask level. [Mutant M-6 is showing significant reduction in viscosity (p-value 0.0001) and 1.27 fold increase in specific CoQ_{10} content (not significant, p-value 0.0789). For statistical analysis, student's t-test was used using PRISM-5 software, p-value below 0.05 was considered statistically significant. The values in the graph are an average of 3 trials. All error bars represent standard error of mean].

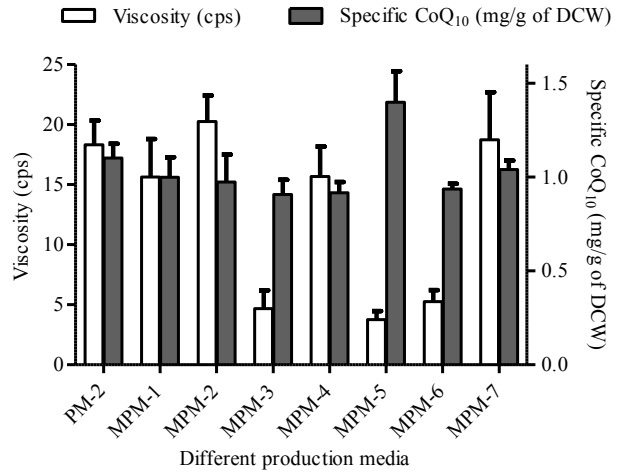


Figure 2: Comparison of viscosity and specific CoQ_{10} content of mutant M-6(S) with different production media at shake flask level. [Significant reduction of viscosity was observed with MPM-3 medium (p-value 0.0007), MPM-5 medium (p-value 0.0003) and MPM-6 medium (p-value 0.0005). Medium MPM-5 shows significant improvement in specific CoQ_{10} content (p-value 0.0475). For statistical analysis, student's t-test was used using PRISM-5 software, p-value below 0.05 was considered statistically significant. The values in the graph are an average of 3 trials. All error bars represent standard error of mean].

performance of mutant M-6(S) in these media at lab scale fermentor level under identical fermentation conditions e.g., temperature 30°C, agitation 300 rpm and aeration 0.3 vvm. The specific CoQ_{10} content and the viscosity obtained with four media in fermentor are shown in figure 3.

Although mutant M-6(S) did not show much viscosity development in the shake flask with any of the tested media, the significant development of broth viscosity was observed in the medium containing sucrose as sole carbon source (PM-2 and MPM-7) at fermentor level. The combination of sucrose and cane molasses did not induce viscosity development in fermentor as seen from medium MPM-3. The significant rise in specific CoQ_{10} content 1.37 mg/g of DCW was achieved by the medium MPM-5 containing cane molasses as sole carbon source in fermentor, however there was no viscosity development. The results indicate that sucrose in the media is a major contributing factor for viscosity development in mutant M-6(S) under accelerated fermentation condition. In addition to this, the cane molasses, a by-product of sugar processing industries, is much cheaper source than sucrose, thus contributing towards cost effective fermentation process.

Hence, it can be concluded that mutant M-6(S) is a promising strain for efficient and cost effective production of CoQ_{10} using MPM-5 medium. Further the optimization of fermentation conditions for mutant M-6(S) were carried out in 10 L laboratory scale fermentor using cane molasses based newly formulated MPM-5 medium.

The optimization was done with respective aeration, agitation and temperature. During fermentation of mutant M-6(S) on cane molasses medium, feeding was carried out using 20.5% of cane molasses solution from 24 h at the flow rate of 10 ml/h. The total sugar concentration dropped from 12% to 8% and then it was maintained at around 8% till end with the help of continuous dosing. The same fed batch strategy was adopted for further optimization in fermentor by altering the parameters. The specific CoQ_{10} content, DCW, and CoQ_{10} titer obtained with different fermentation conditions are expressed in table 1. The maximum specific CoQ_{10} content (1.87 mg/g of DCW) was achieved with 500 rpm agitation, 0.2 vvm aeration and temperature of 25°C. It was observed that lowering the aeration from 0.6 vvm to 0.2 vvm helped in almost 1.36 fold improvement in specific CoQ_{10} content. The temperature of 25°C was found to be optimum for the CoQ_{10} process that showed maximum CoQ_{10} content.

Figure 4 shows the kinetics of the fermentor batch carried out with optimized process parameters using cane molasses medium. The figure 5 shows the HPLC chromatogram of standard CoQ_{10}. The HPLC chromatogram of the crude extract from mutant M-6(S) indicating the presence of CoQ_{10} peak is shown in figure 6.

There was not much fluctuation in pH throughout the cycle and it was automatically maintained in the range of 7.2 to 7.7 due to continuous dosing. During the initial 24 h of growth phase, there was rapid built up of biomass and sudden drop in DO. At 24 h, the continuous feeding was started to maintain the total sugar concentration at 8% throughout the cycle. Due to initiation of continuous dosing, DO have started rising and maintained in the range of 50-60% till the end of the cycle. There was a linear rise in CoQ_{10} titer with biomass and reached maximum of 48.89 mg/L at the end of the process. From the process, it is observed that the exponential phase of the culture is maintained till the end with the help of continuous dosing. The optimized process parameters, fermentation medium and continuous dosing has helped to maintain and prolong the exponential growth phase, resulting in higher CoQ_{10} titer and biomass.

It was thought to check the performance of sucrose-based media under optimized fermentation condition with mutant M-6(S). In the fermentor, cane molasses was replaced with equivalent quantity of pure sucrose and sucrose was used as a dosing solution. The comparision of these two carbon source are shown in figure 7, where we found a linear rise in viscosity of the broth reaching up to 128 cps at the end of fermentation for sucrose containing medium. The viscosity was significantly higher, than observed in cane molasses media. It was

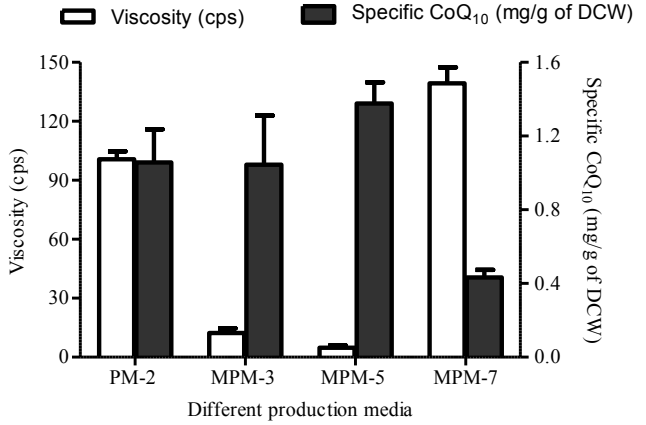


Figure 3: Comparison of viscosity and specific CoQ_{10} content of mutant M-6(S) with different production media at identical fermentor condition. As compared to original medium PM-2, MPM-3 medium showed significant reduction in viscosity (p-value<0.0001), MPM-5 medium showed significant reduction in viscosity (p-value<0.0001) as well as significant improvement in specific CoQ_{10} content (p-value 0.0043). High sucrose containing MPM-7 medium showed significant rise in viscosity (p-value 0.0018) as well as significant drop in specific CoQ_{10} content (p-value 0.0040). For statistical analysis, student's t-test was used using PRISM-5 software, p-value below 0.05 was considered statistically significant. The values in the graph are an average of 3 trials. All error bars represent standard error of mean.

Agitation (rpm)	Aeration (vvm)	Temperature (ºC)	DCW (g/L)	Titer (mg/L)	Specific CoQ_{10} (mg/g DCW)
300	0.6	30	20.5	32.83	1.60146
500	0.6	30	20.94	38.7	1.84814
600	0.6	30	20.89	23.02	1.10196
500	0.2	25	26.1	48.81	1.87011
500	0.2	30	23.22	42.45	1.82817
500	0.2	33	20.64	25.82	1.25097

Table 1: Comparison specific CoQ_{10} content, DCW, and CoQ_{10} titer with different fermentation conditions.

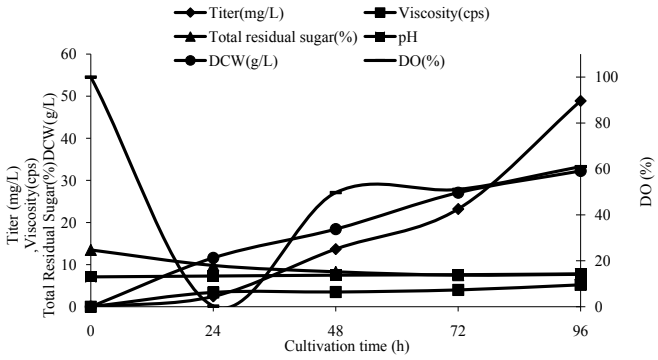
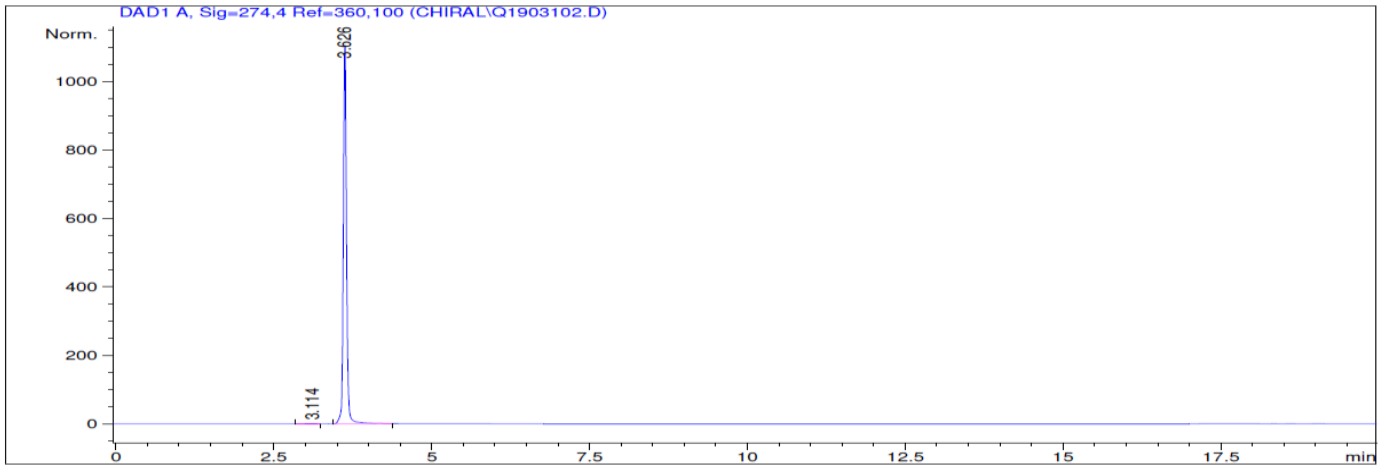


Figure 4: Optimized fermentation profile for CoQ_{10} production using cane molasses based MPM-5 medium for mutant M-6(S).



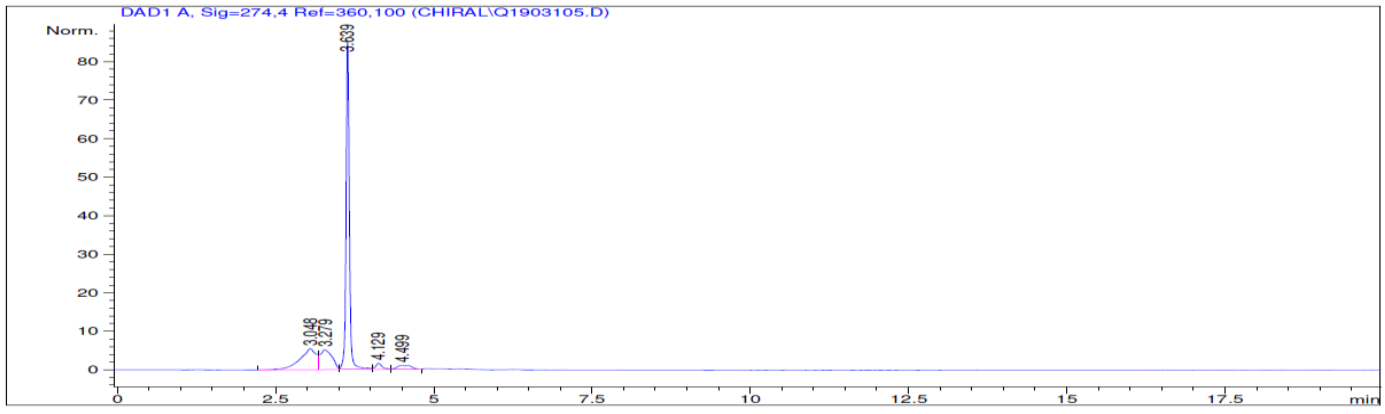
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=274,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.114	BV	0.1853	4.13248	3.64111e-1	0.1047
2	3.626	VB	0.0575	3941.39941	1109.20435	99.8953
Totals :				3945.53189	1109.54076	

Figure 5: HPLC chromatogram of standard CoQ_{10}.



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=274,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.048	BV	0.2371	99.00759	5.47072	19.6434
2	3.279	VV	0.1702	67.60805	5.18158	13.4137
3	3.639	VV	0.0563	309.81522	85.54632	61.4683
4	4.129	VB	0.1080	11.73013	1.59220	2.3273
5	4.499	BV	0.2158	15.86315	1.01801	3.1473
Totals :				504.02414	98.80883	

Figure 6: HPLC chromatogram of crude extract.

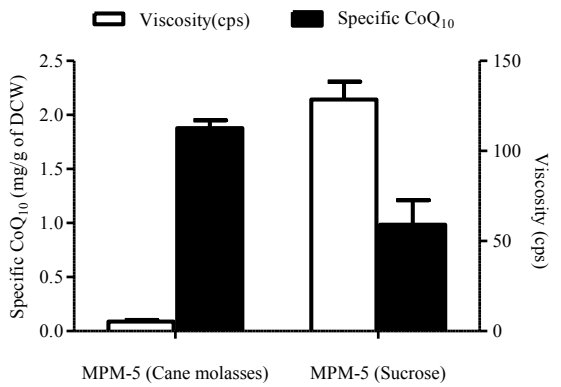


Figure 7: Comparison of viscosity and specific CoQ_{10} content of mutant M-6(S) between medium MPM-5 and medium MPM-5 in which cane molasses was replaced by equivalent quantity of sucrose (using optimized fermentor conditions). By replacing cane molasses with sucrose, there was a significant rise in broth viscosity (p-value<0.0001) with 1.9 fold decrease in specific CoQ_{10} content (significant, p-value<0.0001). For statistical analysis, student's t-test was used using PRISM-5 software, p-value below 0.05 was considered statistically significant. The values in the graph are an average of 3 trials. All error bars represent standard error of mean.

also observed that on sucrose medium after 72 h of cycle, CoQ_{10} titer decreased to 1.9 fold with increasing viscosity, which might be due to poor mass transfer in fermentor. The wild type strain had showed more than 400cps viscosity when grown on this medium in the fermentor. In case of the mutant strain M-6(S), although the viscosity development on sucrose medium was less than that obtained with wild type strain, it was still higher than viscosity observed on the solely molasses medium. Thus it can be concluded that sucrose is a major contributing factor for viscosity development in broth, due to induction of polysaccharide biosynthesis and hence not a suitable carbon source for CoQ_{10} process using *Agrobacterium tumefaciens*. Mutant strain M-6(S) showed substantial improvement over wild type strain in terms of maintaining broth rheology under accelerated fermentation condition, hence it could be exploited further for CoQ_{10} bioprocess. It would be worth investigating the changes occurred at molecular level in mutant M-6(S) especially on exopolysaccharide biosynthesis pathway.

Conclusion

Development of enormous viscosity of the broth, due to exopolysaccharide production, was a major hurdle faced during CoQ10 fermentation process using *Agrobacterium tumefaciens* ATCC 4452 wild type strain, which not only affected the yield, but also made the bio-separation process difficult. Attempts were made to overcome this problem by developing a suitable mutant strain and production medium for fermentation process, which resulted in reduction of broth viscosity and higher content of CoQ10. The newly developed mutant strain mutant-6(S) produced 48.89 mg/L of CoQ10 with specific CoQ_{10} content 1.87 mg/g of DCW at 25C, 500 rpm and 0.2 vvm in the continuous fed batch fermentation using newly formulated cane molasses based medium MPM-5. Replacing sucrose in the fermentation medium with cheaper cane molasses helped in improving CoQ10 content without producing viscous exopolysaccharide and thus made the process simpler and cost effective. The sucrose was found to be the contributing factor in development of viscosity in *Agrobacterium tumefaciens* ATCC 4452. Re-mutagenesis of existing mutant and evaluation of new mutant strains at fermentor level with additional nutrient supplementation may lead to economic CoQ_{10} bioprocess.

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