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Deletion of menaquinone (vitamin-K) biosynthetic gene menA restores ubiquinone (coenzyme Q) biosynthesis in ubiB mutant strain.

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Deletion of Menaquinone (Vitamin-K) biosynthetic gene *menA* restores Ubiquinone (Coenzyme Q) biosynthesis in *ubiB* mutant *strain.*

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Capstone Approval Page

Capstone Title (print or type)

Deletion of Menaquinone (Vitamin-K) biosynthetic gene menA restores Ubiquinone (Coenzyme Q) biosynthesis in ubiB Mutant Strain.

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HONORS THESIS ABSTRACT

Guidelines

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AUTHOR: Garrett Silo Carlson

THESIS TITLE: Deletion of Menaquinone (Vitamin-K) biosynthetic gene *menA* restores Ubiquinone (Coenzyme Q) biosynthesis in *ubiB* mutant *strain*.

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ABSTRACT (100-200 WORDS):

It is well established that for aerobic growth *E.coli* requires either Menaquinone (MK), Ubiquinone (Q) or 2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone (MMQ) as electron carrier to grow on glycerol minimal medium (GMM). Therefore, a double mutant strain blocked in both the MK and Q pathways shouldn't grow on GMM. The following double mutant strains were tested for growth on GMM

(Δ*ubiE* Δ*menA*, Δ*ubiH* Δ*menA*, Δ*ubiF* Δ*menA*, Δ*ubiB* Δ*menA* and Δ*yigP* Δ*menA*). Surprisingly, it was found that the Δ*ubiB* Δ*menA* mutant grew after a long lag and Δ*ubiF* Δ*menA* mutant grew as expected due to the Q pathway intermediate MMQ serving as electron carrier. In GMM Δ*ubiB* Δ*menA* double mutant showed an increase in the rate of growth and growth yield. It is a known fact that all the Q pathway gene mutants including *ubiB* cannot grow on succinate minimal medium (SMM). The *ubiB* mutant has been previously shown to accumulate small amount of Q in stationary phase. To our surprise, the double mutant was also able to grow on succinate and lactate minimal media once adapted to grow in GMM. Quinone analysis of the Δ*ubiB* Δ*menA* mutant using HPLC revealed that it made significant amount of Q. Therefore, the reason behind the growth of double mutant strain is due to increase in the amount of Q.

Introduction

The isoprenoid quinone ubiquinone (coenzyme Q: Q) is widespread amongst living organisms. Aerobic gram-negative bacteria and eukaryotes contain Q as the sole quinone. The facultative anaerobic Gram-negative bacterium *E.coli* contains Q, Menaquinone (MK) and Demethylmenaquinone (DMK). The quinonoid nucleus of Q and MK are derived from shikimate pathway via chorismate (Figure 1). The prenyl side chain of both Q and MK is derived from prenyl diphosphate. It is well established in bacteria and eukaryotes that Q is a component of the respiratory chain and plays a critical role in respiration and oxidative phosphorylation (Meganathan, 2001). It functions as an electron carrier serving as a link between the dehydrogenases and electron transport chain of *E.coli,* when oxygen and nitrate are used as electron acceptors under aerobic and anaerobic conditions respectively (Meganathan, 2001). In eukaryotes, in addition to the respiratory chain, Q is present in all cellular membranes in a reduced form, and in human and animal tissues it serves as an important antioxidant (Ernester and Forsmark-Andree, 1993). The reduced form of Q-10, QH_2-10, is an efficient scavenger of free radicals and effectively prevents peroxidative damage to lipids (Frei, Kim, and Ames, 1990).

It is known that for aerobic growth *E.coli* requires either (MK), (Q) or 2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone (MMQ) as electron carrier to grow on glycerol minimal medium (Gly.M.M) (Meganathan, 2010). The pathway for Q biosynthesis, the intermediates and the genes associated are shown in figure 1. It was previously shown that MMQ, an intermediate, accumulated by the *ubiF* mutant enables it to grow on Gly.M.M. In the current study, we are trying to identify whether any other Q biosynthetic intermediates can substitute for Q in the respiratory chain.

A series of double mutants were constructed as a part of this study. The MK pathway has been blocked in all the double mutants analyzed by deleting the *menA* gene. Different Q pathway gene mutantions were introduced into the *menA* mutant strain individually to obtain the following double mutants. These double mutants do not make either Q or MK and they can grow on glycerol only if the Q pathway intermediate channels electrons to oxygen.

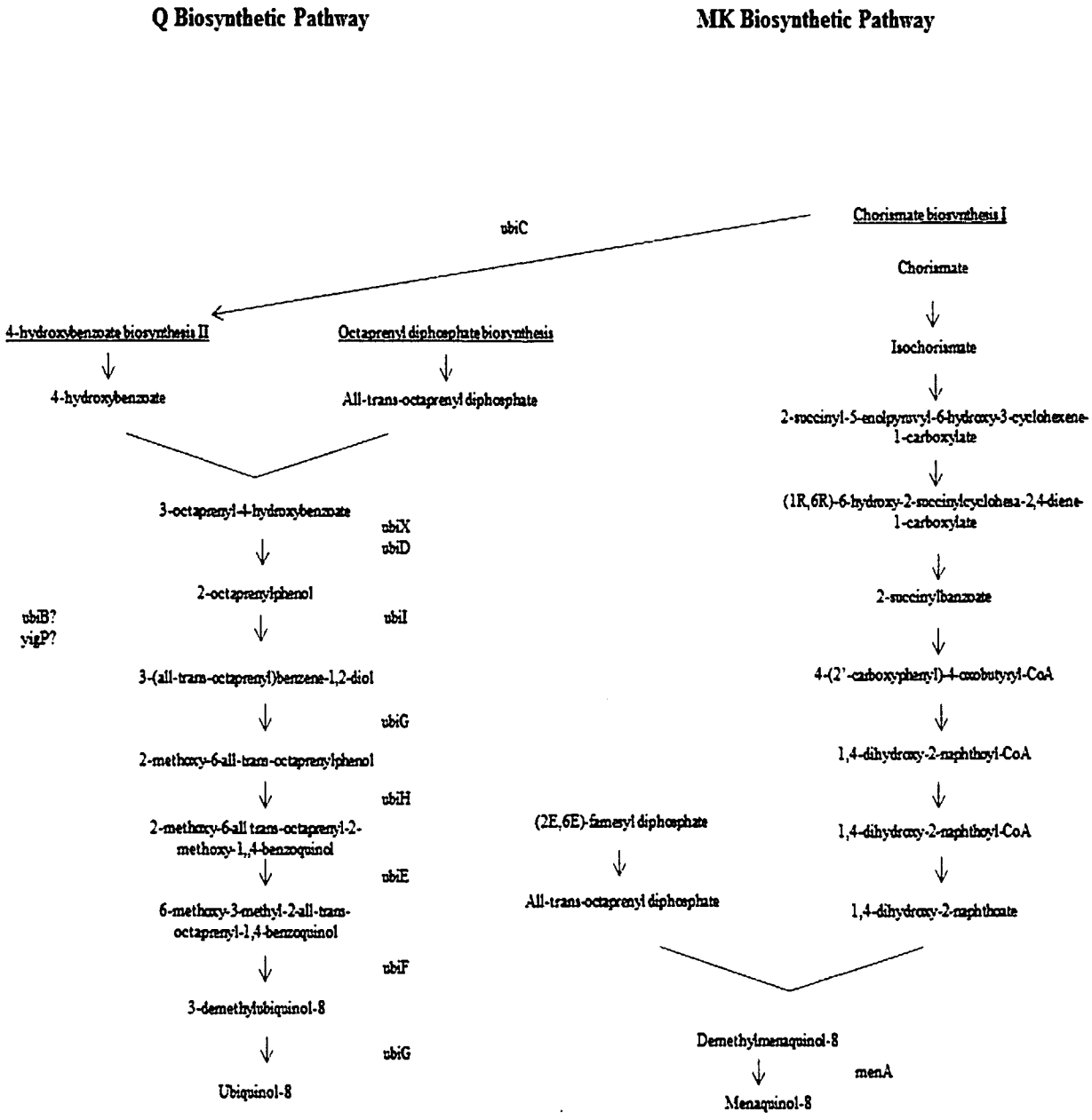

Figure 1: Ubiquinone and Menaquinone Biosynthetic pathways in *E.coli.* The steps catalyzed by *yigP* and *ubiB* genes are currently unknown. The *yigP* and *ubiB* gene mutant strains accumulate octaprenylphenol and hence regulate the Q biosynthesis pathway.

Methods

Construction of *ubiB* and *yigP* deletion mutants

The *yigP* and *ubiB* gene mutants were previously constructed in Meganathan's lab. The single gene deletion mutant strains of *ubiH, ubiF, menA* and *ubiE* were obtained from *Coli* Genetic Stock Center (CGSC). All these mutant strains have Kanamycin cassette flanked by FRT sites (FLP recognition targets) instead of the actual gene (Datsenko and Wanner, 2000). Expression of the FLP-recombinase removes the central part of the disruption cassette, leaving behind a 81 bp "scar" sequence which is in the correct reading frame without stop codons. This allows the generation of non-polar, unmarked in-frame deletions and repeated use of the same resistance marker for making multiple knock-outs in the same strain (Baba et al, 2006). The plasmid pCP20 containing the FLP recombinase was transformed into the *menA* mutant strain to remove the cassette (Fig. 3). FLP synthesis and loss of the plasmid are induced at 42°C.

Construction of Double Mutants using P1 transduction

Double mutant strains lacking Q and MK pathways are constructed as follows: Phage P1 was grown individually on the donor mutant strains of *ubiH, ubiF, ubiB, yigP, ubiE* and *ubiB* having a kanamycin casette and the lysate was used to transduce the recipient mutant strain *menA* whose kanamycin cassette was previously deleted (Fig. 3). Transductants containing the desired double mutation in the chromosome were verified by screening using colonyPCR (Datsenko and Wanner, 2000).

Growth Curves of Various Mutants

The mutant strains were grown overnight in LB medium at 37°C with continuous shaking. The tubes containing the cultures were centrifuged and the supernatant was then discarded. The pellet was re-suspended in minimal salt solution and shaken at room temperature for 24 hours to deplete the nutrient pond. The starved cells were used as the inoculum for the growth curves in side arm flasks containing the medium. The carbon source used in side arm flasks for growth curves are succinate (SMM), lactate (LMM), glycerol (Gly.M.M), and glucose (GMM). The growth was monitored using a klett meter equipped with a red filter and readings were taken every 4 hours.

Estimating the growth yield

The cultures were grown in two one liter flasks each containing 250ml of Gly.M.M and were pelleted when the growth reached early stationary phase. The wet weight of the pellet was then determined to get the growth yield.

Results

The Double mutant Δ*ubiB* Δ*menA* can grow on Glycerol Minimal Media

Out of all the double mutant strains analyzed for growth on Gly.M.M and minimal media with casamino acids (CAA). The Δ*ubiB* Δ*menA* and Δ*ubiF* Δ*menA* strains were able to grow (Figure 3). The growth of Δ*ubiF* Δ*menA* was expected because of MMQ. The Δ*ubiB* Δ*menA* strain grew on Gly.M.M after 4 days. When the media was supplemented with casamino acids (CAA) the lag period was shortened by 2 days (Figure 4). When the Δ*ubiB* Δ*menA* strain adapted

to grown on Gly.M.M was re-inoculated in fresh Gly.M.M without CAA, the lag period was just one day. CAA contains 18 of the 20 established amino acids so we decided to analyze what specific amino acids aided in the growth of the double mutant. The amino acids contained in CAA are methionine, glutamine, tryptophan, glycine, arginine, lysine, serine, histidine, threonine, phenylalanine, isoleucine, tyrosine, alanine, cysteine, proline, leucine, aspartic acid, and valine. Comparison of growth in Gly.M.M with each amino acid was conducted in test tubes. The amino acids that aided Δ*ubiB* Δ*menA* to grow were methionine, glutamine, tryptophan, glycine, and arginine. All the double mutant strains were able to grow on GMM (Figure 5).

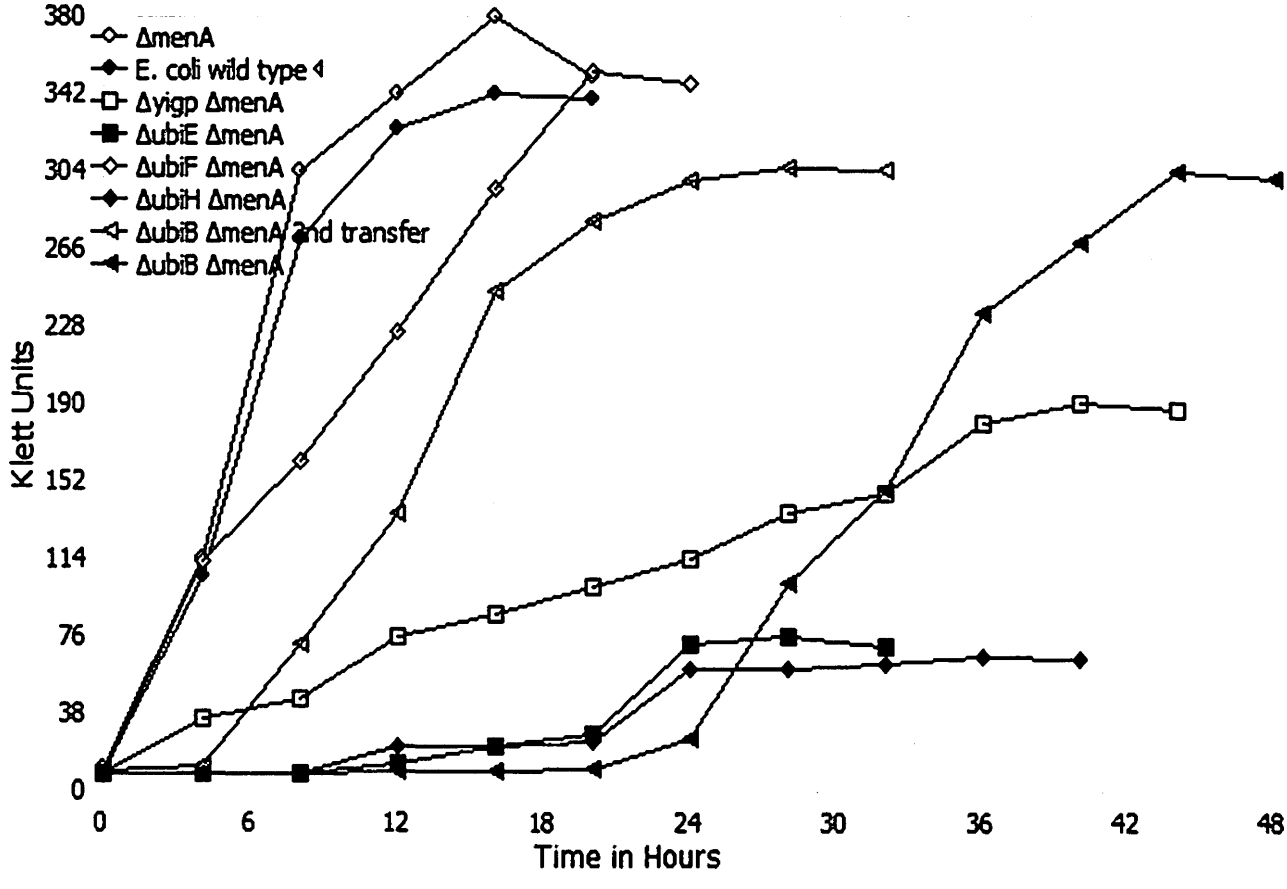

Figure 3: Growth of various double mutants aerobically on GMM.

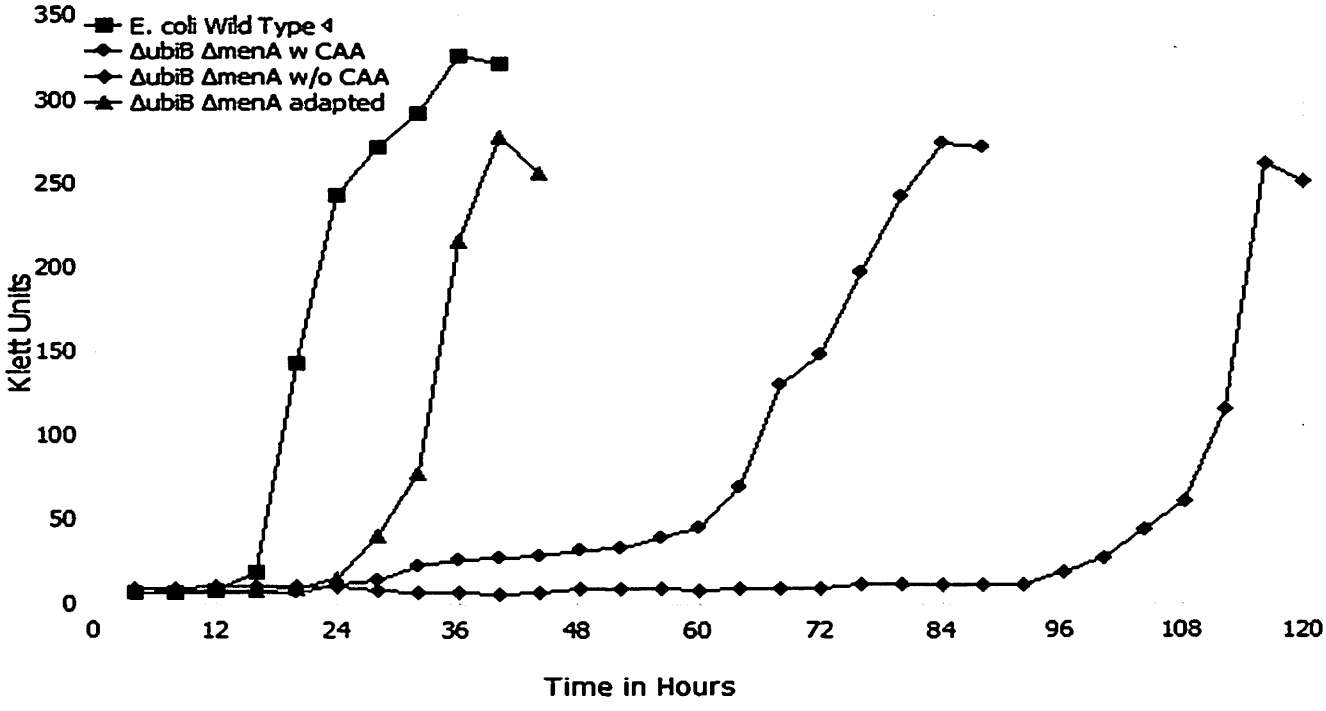

Figure 4: Comparison of growth rates between wild type, Δ*ubiB* Δ*menA:* with CAA, without CAA, and adapted.

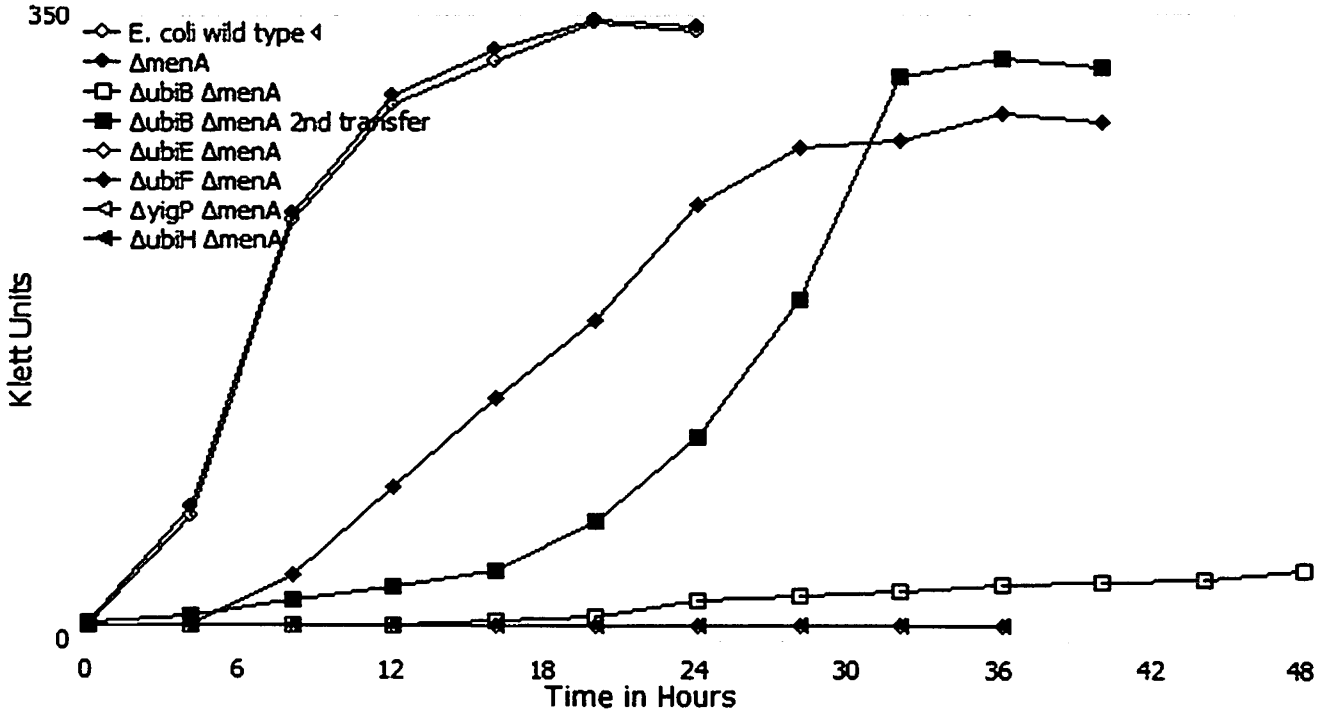

Figure 5: Growth of various double mutants aerobically on Gly.M.M.

The Double mutant Δ*ubiB* Δ*menA* can grow on Succinate and Lactate Minimal Media

Mutants blocked in Q pathway cannot grow on succinate, a characteristic feature seen among all Q pathway mutants. As seen in figure 6, once adapted to Gly.M.M the double mutant Δ*ubiB* Δ*menA* can grow on succinate and lactate, therefore; the double mutant is making enough Q to grow on succinate and lactate. The single mutant *ubiB* cannot grow on succinate even though it makes small amount of Q.

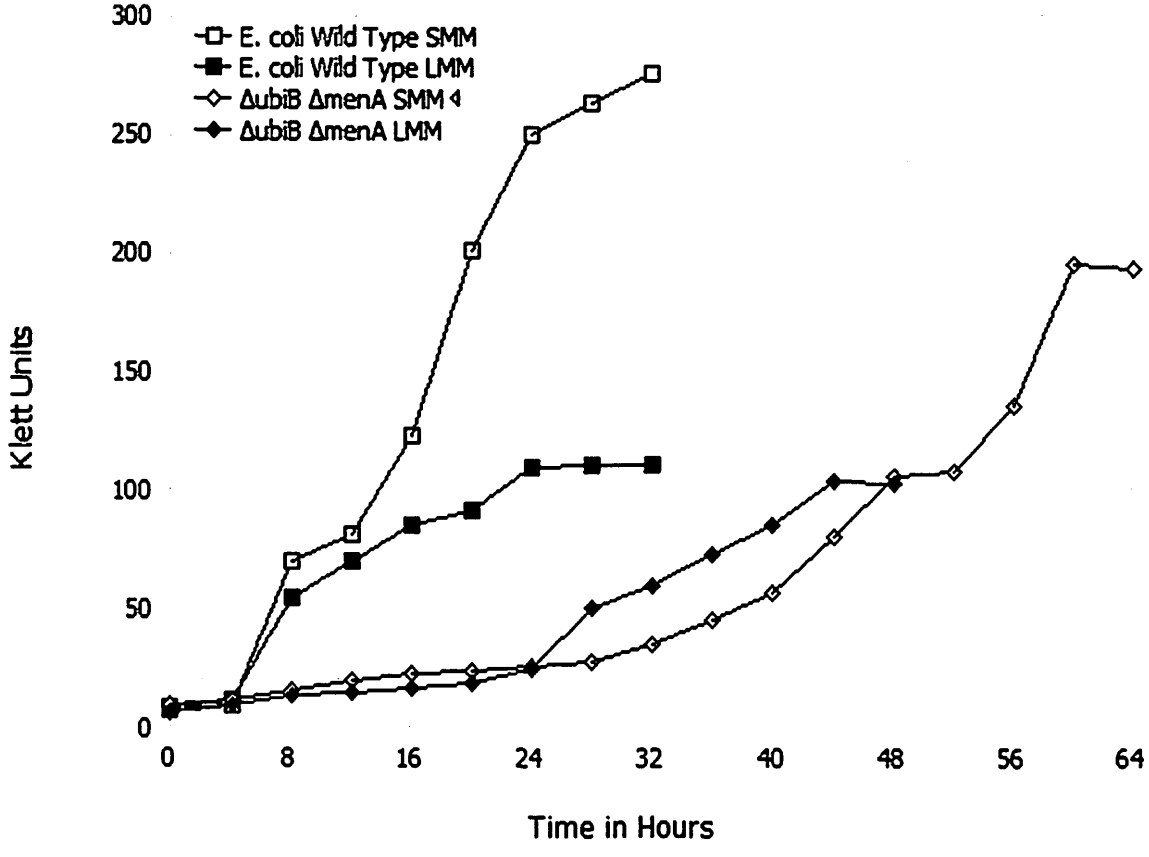

Figure 6. Growth curve of adapted Δ*ubiB* Δ*menA (new)* double mutant in SMM and LMM.

Growth Yield Comparison

The *ΔubiB ΔmenA* strain adapted to grow on glycerol and the wild type strains were grown in 1liter flasks for quantification of wet cell weights. It was observed that for the identical volume of media of 470 ml , the wild type strain yielded 2.06 g whereas the *ΔubiB ΔmenA* strain yielded 3.86 g.

Quinone Analysis using HPLC

The quinone content of *ΔubiB, ΔubiB ΔmenA, ΔyigP and ΔyigP ΔmenA* were analyzed using HPLC. The *ΔyigP* and *ΔubiB* mutants made little Q and lot of octaprenyl phenol (OP). When MK pathway was blocked by introducing *menA* mutation into *ubiB* deletion strain *(ΔubiB ΔmenA)*, the amount of Q increased comparatively (Fig. 7 a,b). This increase in Q is probably responsible for the growth in Gly.M.M.

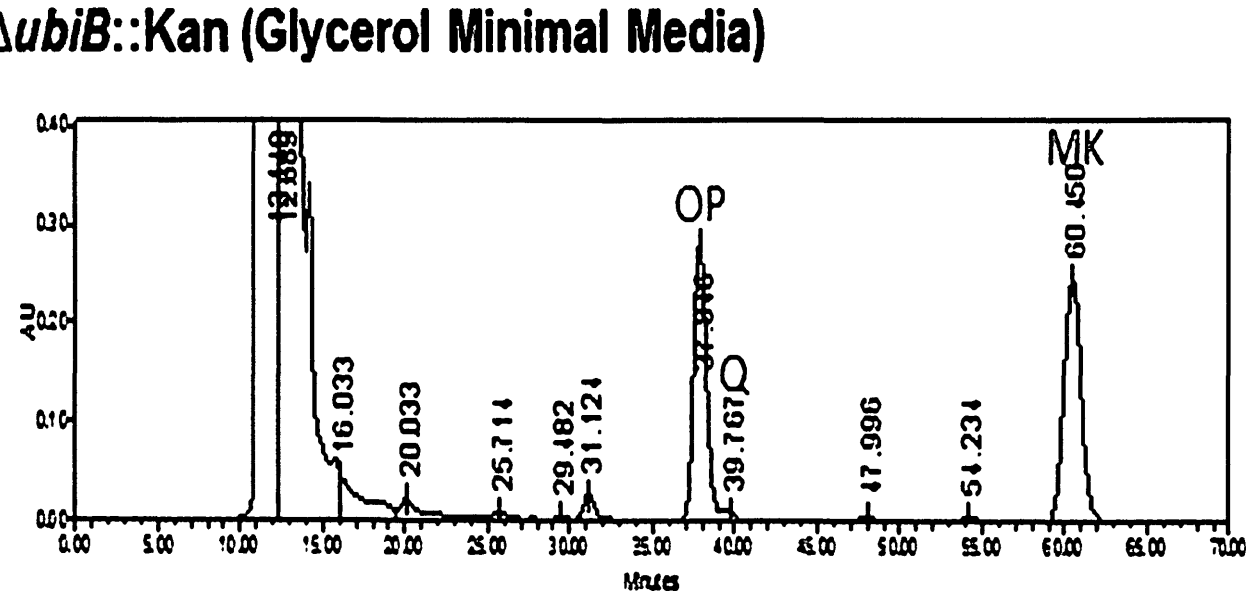

Figure 7 a: HPLC Analysis

ΔubiB ΔmenA (Glycerol Minimal Media)

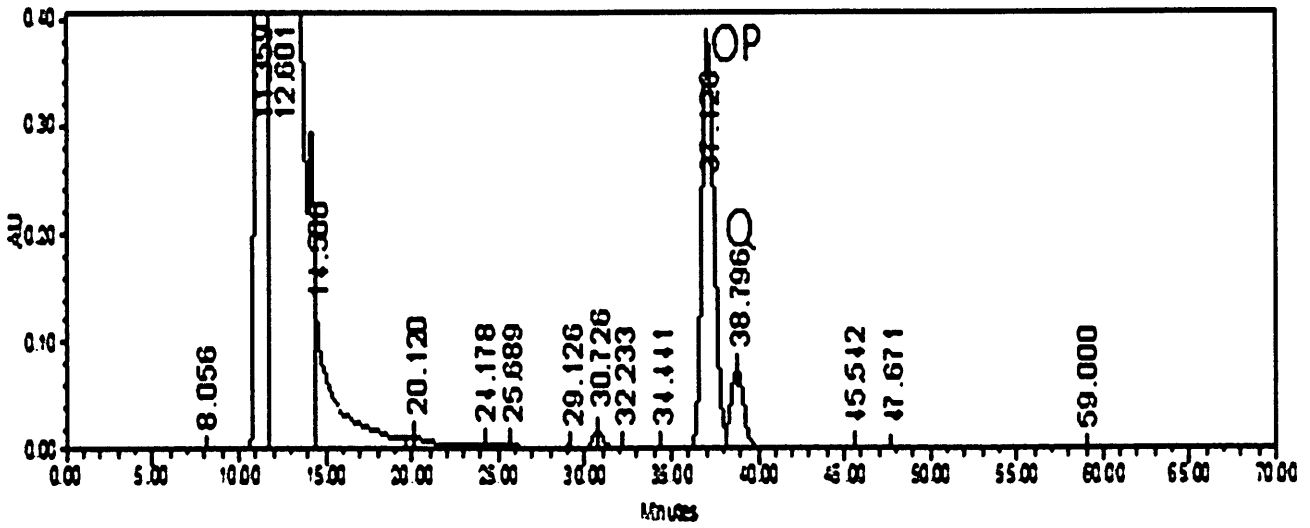

Figure 7 b: HPLC Analysis

Conclusion

The double mutant *ΔubiB ΔmenA* was able to grow in Gly.M.M and even succinate and lactate minimal medias once it was adapted to Gly.M.M. Quinone analysis revealed that *ΔubiB ΔmenA* is able to make a small amount of Q even when Q and MK pathways are blocked. It is possible that the blocking of the MK pathway increases the pool of prenyl side chain inside the cell. The availability of excess prenyl diphosphate which is essential for Q formation may probably be contributing to the increased amount of Qin the *ΔubiB ΔmenA* double mutant strain compared to the individual *ΔubiB* strain. The increase in the amount of Q is probably contributing to its growth in Gly.M.M, SMM, and LMM. Further research must be done to identify the possible reason behind the increased growth yield in *ΔubiB ΔmenA* strain compared to wild type.

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