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Using Different Bioinformatics Software Tools for Determining the Carbon Dioxide (CO_2) Dependence in *Staphhylococcus aureus*

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Abstract

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium that occurs in microscopic bunches similar to grapes. In general, *S. aureus* shows two different colonies; on agar dishes *S. aureus* forms huge golden yellow colonies, while on blood agar dishes it has haemolytic properties. Recently, different major studies have discovered CO_2-dependent *S. aureus* strains that can be found as an inhabitant of small colony variants (SCV) *S. aureus*, which can cause recurrent and persistent infections. Although it has been investigated the mechanism of this CO_2 dependence, this paper aims to use different bioinformatics software tools, such as MUMmer, BLAST, Jalview and ClustalW, in order to compare and search for the differences in the enzymes involved in pathways that can be altered in the CO_2-dependent *S. aureus* strain. That is, the entire genome of CO_2-dependent *S. aureus* strain was compared against another strain, not CO_2-dependent using the software tool MUMmer. Based on this tool, the results show that the plots of the CO_2-dependent strain against the COL and Mu50 strains did not show any gaps, while the connected thick diagonal line reveals strong similarities between the sequences in comparison to the thin diagonal line. Surprisingly, the results of using BLAST alignment tool did not illustrate any obvious mutations. Besides, the comparison between a number of strains and the CO_2-dependent *S. aureus* strain was carried out using the ClustalW alignment tool and Jalview software; consequently it did not show any observable mutations, as well.

Keywords: *Staphylococcus aureus*; CO_2-dependent *S. aureus* strains; MUMmer; BLAST; Jalview; ClustalW

Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive, immobile and spherically shaped (around 1 μm diameter) bacterium that occurs in microscopic bunches similar to grapes, as shown in Figure 1. In general, *S. aureus* shows two different colonies; on agar dishes *S. aureus* forms huge golden yellow colonies, while on blood agar dishes it has haemolytic properties. In addition, *S. aureus* has the ability to produce the lactic acid as it is considered to be an optional anaerobic, which grows either by aerobic respiration or by fermentation [2].

Commonly, *S. aureus* can be found on the skin, like part of the regular flora and in the respiratory tract of the human. Furthermore, this bacterium can be found as skin lesions or impetigo in the adult individual as an abscess in children or as mastitis in cattle [3,4]. It has been recorded that, around 20% of the populations are considered to have a long-term case of *S. aureus*. Moreover, there are different diseases that can be caused by *S. aureus* such as soft tissue infections, life-threatening septicaemia, superficial skin, endocarditis and toxic shock syndrome [5]. In most cases β-lactam antibiotics, clindamycin, tetracycline and sulpha drugs are using as treatments for the *S. aureus* infections. In the past, before introducing penicillin as a treatment in 1949, the major cause of mortality in patients was the hospital-acquired (nosocomial) infections, which are caused by *S. aureus*. After that, the penicillin-resistant *S. aureus* isolates were first discovered in hospitals. Then the first discovery of the Methicillin-resistant nosocomial *S. aureus* isolates was in 1961 [6]. Afterwards, in hospitals and intensive care units the Methicillin-resistant *S. aureus* (MRSA) became common worldwide. Nowadays, more than 30% of the bacterial nosocomial infections occurring in intensive care units are caused by MRSA. Previously, MRSA was confined only to hospitals and then new strains of MRSA arose in the community causing different infections in healthy humans. In the 1990s, an extreme Community-Acquired Methicillin-resistant *S. aureus* (CA-MRSA) strain was discovered in Australia [7]. Normally, CA-MRSA has the ability to cause different infections such as a spontaneous abscess either on the skin or on the soft-tissues. In addition, the CA-MRSA strains can be spread quickly in a community, and can affect children and healthy individuals.

Pinto and Merlino found that the CO2-dependent *S. aureus* was detected for the first time in 1955 [8]. These organisms can be found as an inhabitant of small colony variant (SCV) *S. aureus*, which can cause recurrent and persistent infections. In addition, SCV *S. aureus*

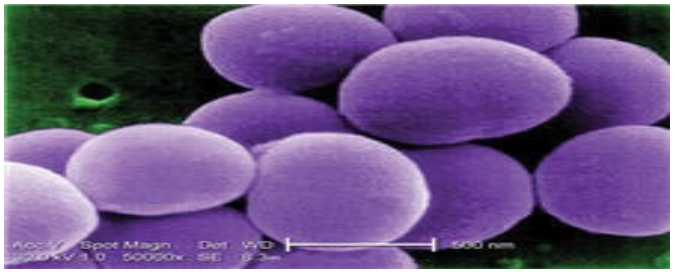

Figure 1: An electronic microscope image of *Staphylococcus aureus* forming grape-like bunches [1].

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strains were found to be an antibiotic-resistant strain as it has a novel mechanism.

Furthermore, a study into SCVs found that they are naturally occurring and are considered to be a slow growing subpopulation of bacteria. Some of these SCVs are occasionally found as auxotrophs for CO_2, whereas most of them are supposed to be menadione, hemin or thymidine auxotrophy [9]. In terms of the CO_2-dependent phenotype the same study found that the micro-colony phenotype is dependent on the gene expression level instead of the existence or absence of one or more particular genes. The reason for this might be because they demonstrated a significant decrease in the proportion of the microcolony phenotype stability. In addition, when multiple subcultures have been done, the micro-colony phenotype returned to normal colony morphology.

Another study by Rahman examined the CO_2 dependent strain both aerobically and anaerobically [10]. The results showed an obvious growth in colony size under both conditions, but with smaller sized colonies when produced anaerobically. Furthermore, after further tests, this study found several important characteristics of the CO_2 dependent strain. For example, this strain was found to be positive for coagulase, DNAse and phosphatase. Besides, it had a fermentative ability during fermentation/oxidation reactions and the organism was sensitive to all other antibiotics but not to tetracycline or penicillin.

Since there has been a little consideration given to the genetic and molecular basis for CO_2 dependence in *S. aureus*, this paper aims to examine the contribution of genomic re-arrangements to the CO_2 dependent phenotype by using different bioinformatics software tools. In particular, these tools will be utilised in order to compare and search for the differences in the enzymes involved in pathways that can be altered in the CO_2-dependent *S. aureus* strain. That is, the entire genome of CO_2-dependent *S. aureus* strain was compared against another strain, not CO_2-dependent using the software tool MUMmer. By using this tool, it has been shown that the plots of the CO2-dependent strain against the COL and Mu50 strains did not show any gaps, while the connected thick diagonal line reveals strong similarities between the sequences in comparison to the thin diagonal line. Surprisingly, the results of using BLAST alignment tool did not illustrate any obvious mutations. Besides, the comparison between a number of strains and the CO_2-dependent *S. aureus* strain was carried out using the ClustalW alignment tool and Jalview software; consequently it did not show any observable mutations, as well.

The rest of this paper is organized as follows, section 4 discusses the related work, methods are explained in section 5, section 6 shows the results; The results are discussed in section 7; section 8 concludes the paper with future works.

Related Work

One of the major studies in CO_2-dependent *S. aureus* field was in (Gómez-González et al.) that investigated about 14 conditions of CO_2-dependent *S. aureus* over a time of three years [9]. The clinical isolates in this study were confirmed to be Small Colony Variants (SCVs) of *S. aureus* as they displayed all the requisite characteristics. This study was aimed at verifying the importance of CO_2 dependent *S. aureus* as a significant pathogen, as well as illustrating the clinical characterisation of the 14 cases and the genetic and phenotypic characterisation of the SCVs isolated. The results of this study showed that 14 out of 14 (100%) of the clinical isolates were found to be CO_2 dependent.

Persson et al. discussed how CO_2 affects the growth of *S. aureus* at body temperature [11]. The *S. aureus* in this study was inoculated on both blood agar and brain–heart infusion broth. These plates were then exposed to either 100% CO_2 or to air at body temperature (37°C) for a time of 8 to 24 hours. Their results showed that 100% CO_2 significantly reduced the growth of *S. aureus* at body temperature. Furthermore, they deduced that increasing the exposure duration considerably increased the inhibiting effect of CO_2.

A study carried out by Arioli et al. focused on the genetic and proteomic analysis approaches to study the CO_2-dependent metabolism of Streptococcus thermophilus, which was grown in either a CO_2 enriched atmosphere or in a N_2 rich atmosphere. The results of this study highlighted the importance of the CO_2-dependent metabolism in the *S. thermophilus* physiology [12].

Different study by Bringel and Hubert has considered the range of genetic lesions in the pathways of both pyrimidine and arginine biosynthesis [13]. This was done on different strains of gram-positive *lactobacilli*, namely 32 strains of *Lactobacillus pentosus*, 150 strains of *L. plantarum*, 10 strains of *L. casei* and 15 strains of *L. paraplantarum*. This study aimed to explain the arginine auxotrophs in *L. plantarum* and the important gene mutations. The results of this study showed that all *L. casei* strains, two of the *L. pentosus* strains and seven of the *L. plantarum* strains were arginine auxotrophs.

Another important study was in was in Nicoloff et al. has shown that the wild-type *Lactobacillus plantarum* harbours two different efficient kinds of Carbamoyl-Phosphate Synthetases (CPS) [14]. Carbamoyl phosphate (CP) is considered to be an important intermediary in arginine and pyrimidine biosynthesis and requires ATP and CO_2 to be produced. This study found that the two important CPSs that are arginine-repressed CPS (CPS-A) and pyrimidine-inhibited CPS (CPS-P). Therefore, the results of this study demonstrated that the CP synthesis of the wild-type *L. plantarum* is reliant on higher levels of CO_2.

Methods

This section describes the methods and software tools that have been used.

Comparative genomics using mummer

The MUMmer system was used to align the entire genomes of CO_2-dependent *S. aureus* strain against other closely related strains (non CO_2-dependet). MUMmer was run using a typical command line on the Biolinux terminal, namely: (mummer –mum –b –c genome1 genome2>output-file.mums) (where genome1 and genome2 indicate the sequences to be compared) [15]. This command was used to find all maximal unique matches (-mum) between genome1 and genome2 on both the forward and reverse strands (-b) and reports all the match positions relative to the forward strand (-c). A MUMmer plot program of all the MUMs between two sequences was run to identify the similarity between the different genomes. The MUMmer plot command line is (mummerplot -x '[0, 275287]' -y '[0, 265111]' -postscript -p mummer outputfile.mums). This command was used to plot all of the MUMs in the outputfile.mums file in postscript format (-postscript) between the given ranges for the X and Y-axes. The string specified with the -p option prefixed the output files. These plots are shown in the results section.

The alignment using BLAST

BLAST was used to align the CO_2-dependent *S. aureus* nucleotide sequence against the most closely related sequence that is not CO_2 dependent. This was done by looking at the carbamoyl phosphate

(CP) encoding genes *carA* and *carB* in the closest related sequence and aligning it against the whole genome of the CO_2-dependent *S. aureus* strain. The reasoning behind this alignment was to find the most identical contig from the CO_2-dependent *S. aureus* strain. Then, BLASTP was used to compare the amino acid sequence of this contig against the *carAB* genes amino acid sequences of the closest sequence. In both of the above, the *carAB* genes sequences were used as the query sequence. Furthermore, BLAST was used to align the *carAB* sequences for the large and small subunits from the most identical contig and to find the most closely related organisms that it can be compared to. This will be also detailed in the results section [16].

Multiple sequences alignment using Clustalw and Jalview

ClustalW was used to align the *carAB* large and small subunits for the CO_2-dependent *S. aureus* strain from the most identical contig against the same encoding genes from different protein strains [17-19]. These strains were *S. aureus* COL, *S. aureus* Newman, *S. aureus* Mu50, *S. aureus* N315, *S. aureus* ED98, *S. aureus* ED133, *S. aureus* ST398, *S. epidermidis* ATCC 12228, *S. carnosus* TM300 and *S. saprophyticus* ATCC15305. The *carAB* small and large chain protein sequences for these strains were downloaded using the KEGG database. Additionally, the Jalview software was used to view the similarities and differences between these alignments.

Results

This section presents the results that have been obtained by using different bioinformatics software tools for determining the carbon dioxide (CO_2) dependence in *Staphhylococcus aureus*. This section is divided into different subsections according to the used tools.

Comparative genomics using mummer

The entire genome alignments of CO_2-dependent *S. aureus* strain against non CO_2-dependent strains (Mu50 and COL) were performed using the MUMmer system to examine the similarities and differences occurring at the genomic level. The postscript plot of the whole genome alignment of the CO_2-dependent *S. aureus* strain against that of the *S. aureus* COL strain (Figure 2).

As shown in Figure 2, there are no gaps along the thin diagonal line. This represents a strong similarity between the two sequences. Further, the postscript plot of the entire genome alignment of the CO_2-dependent *S. aureus* strain, against that of the *S. aureus* Mu50 strain (Figure 3).

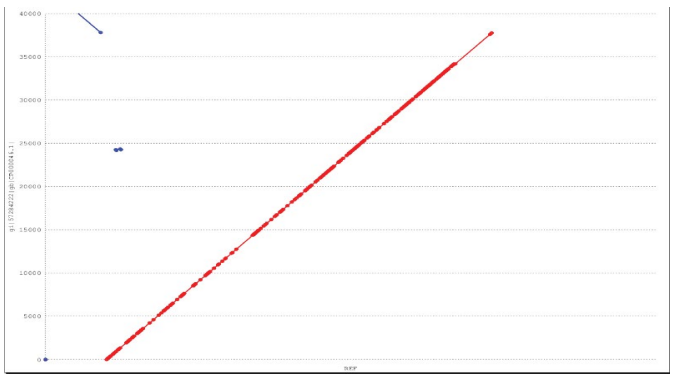

Figure 2: The postscript plot of all MUMs between the CO_2-dependent *S.aureus* strain and *S.aureus* COL. The X-axis demonstrates the reference sequence which is CO_2, and the Y-axis shows the query sequence which is COL.

As shown in Figure 3, the postscript plot of all MUMs between the CO_2-dependent *S.aureus* strain and *S.aureus* Mu50 did not have any obvious gaps. In addition, the thick connected diagonal line reveals strong similarities between the sequences, in comparison to the thin diagonal line.

The alignment using BLAST

The carbamoyl phosphate (CP) requires CO_2 for synthesis and is encoded by two different genes: *carA* for the small subunit and *carB* for the large subunit. Therefore, these two genes from the *S. aureus* Mu50 strain were aligned against the CO_2-dependent *S. aureus* strain to find the closest contig. The results of the alignments using the BLAST alignment tool showed that the nearest to identical contig is 51. Next, this contig was translated into an amino acid sequence to be compared against the amino sequence from the *carAB* gene from the *S. aureus* Mu50 strain using BLASTP.

Multiple sequence alignment using Clustalw and Jalview

The small and large subunits for the CO_2-dependent *S. aureus* *carAB* genes were obtained from the results of aligning the same gene in the *S. aureus* Mu50 strain against the contig 51. In terms of accomplishing the first theory, these *carAB* subunits were aligned against the same gene from different strains. The strains used were *S. aureus* COL, *S. aureus* Newman, *S. aureus* Mu50, *S. aureus* N315, *S. aureus* ED98, *S. aureus* ED133, *S. aureus* ST398, *S. epidermidis* ATCC 12228, *S. carnosus* TM300 and *S. saprohyticus* ATCC15305. The comparison between these strains and the CO2-dependent *S. aureus* strain was carried out using the ClustalW alignment tool and Jalview software. Some of the small and large subunit comparison results are shown in Figure 4.

As illustrated in Figure 4, there was no obvious mutant form of the *carAB* for the CO_2-dependent *S. aureus* strain and this strain was almost identical to the other strains. As a result, the DNA sequence for the *carAB* promoter needs to be compared against a region from a non CO_2-dependent strain. The promoters for the *S. aureus* Mu50 *carA* and *carB* genes were obtained using the microbesonline website. The promoters are Aspartate carbamoyltransferase (*PyrB*) for the carbamoyl phosphate small chain (*carA* or *pyrAA*) and Dihydroorotase (*PyrC*) for the carbamoyl phosphate large chain (*carB*) (Figure 5).

These two promoters for the CO_2-dependent *S. aureus* strain were obtained from the FASTA file. Thus, the sequences for these promoters

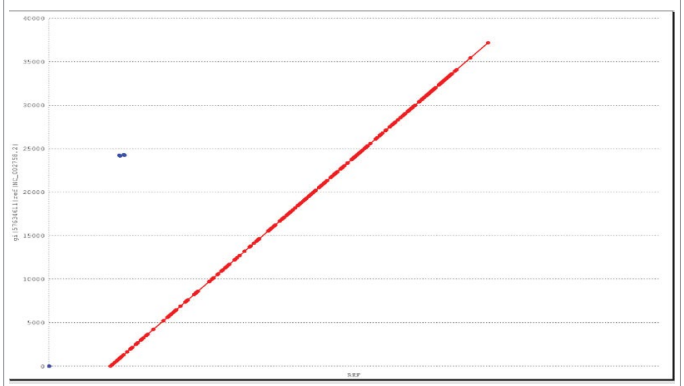

Figure 3: The postscript plot of all MUMs between *S.aureus* CO_2-dependence strain and *S.aureus* Mu50. The X-axis demonstrates the reference sequence, which is CO_2, and the Y-axis shows the query sequence, which is Mu50.

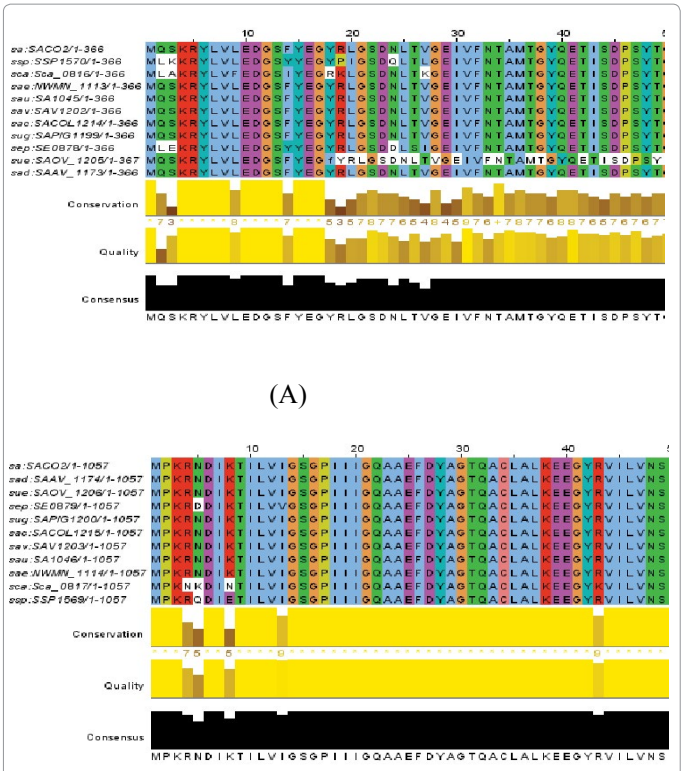

Figure 4: Some parts of the Jalview and ClustalW results comparing the *carAB* genes of the CO_2-dependent *S. aureus* strain against the same gene from different organisms. (A) is the small subunit and (B) is the large subunit.

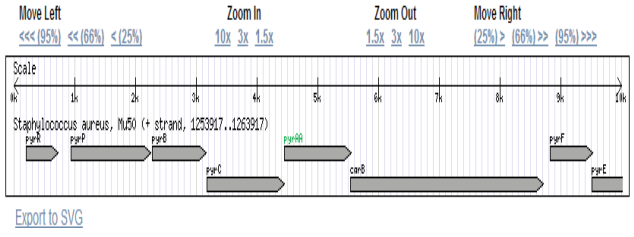

Figure 5: The carAB region of the genome from *S. aureus* Mu50 strain using microbesonline.

from the CO_2-dependent *S. aureus* strain were aligned against the Newman *S. aureus* strain. The alignments were completed using ClustalW and Jalview (Figure 6).

As shown in Figure 6, since the results of comparing the promoters did not show any obvious mutations, the second theory should be investigated. That is, as the results gained from building a model for the wild type strain showed, both pathways for the arginine and proline production were effective. Further comparison, using the two theories, needs to be accomplished to verify the hypothesis for the present study.

Discussion

The results of the present study were clear in terms of detecting the variations in the enzymes in the pathways that can be changed in the CO_2-dependent *S. aureus* strain between the CO_2 dependent strain and other closely related strains, including the wild type Mu50 strain.

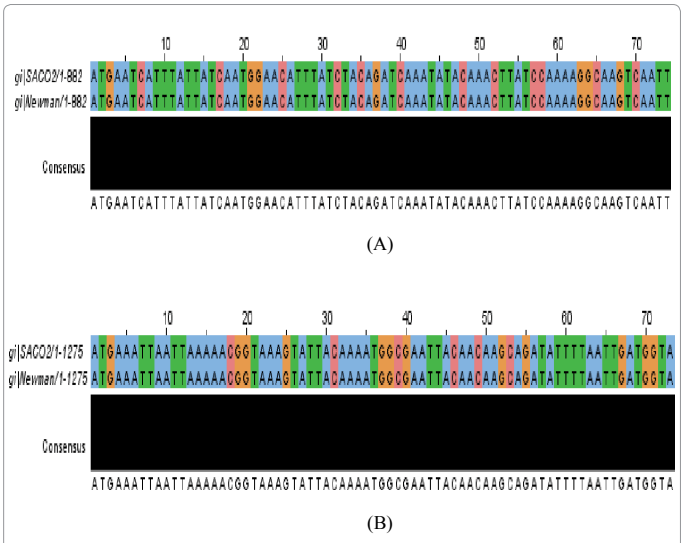

Figure 6: Some parts of the Jalview and ClustalW results for comparing the promoters from the CO_2-dependent *S. aureus* strain against the same promoters from the Newman *S. aureus* strain: (A) *pyrB* promoter and (B) *pyrC* promoter.

As illustrated in the results section, the entire genome nucleotide sequence of the CO_2-dependent *S. aureus* strain was compared against the Mu50 and COL strains to look for similarities and differences occurring at the genomic level. As demonstrated in Figures 2 and 3, the plots of the CO_2-dependent strain against the COL and Mu50 strains did not show any gaps. This might mean that the similarities between these sequences are very strong. Additionally, the connected thick diagonal line reveals strong similarities between the sequences in comparison to the thin diagonal line. As suggested by Delcher et al. the connected thick diagonal line between the two sequences represents high homologous relationships between the two sequences compared [20].

The BLAST alignment tool was used to align the *S. aureus* Mu50 *carAB* sequences against the whole genome of the CO_2-dependent *S. aureus* strain to find the most identical contig. The alignment results strongly supported contig 51 as being the nearest match. Then the amino acid sequence of this contig was aligned against the amino acid sequence from the *carAB* genes in the wild type strain Mu50. Surprisingly, the results of this comparison did not illustrate any obvious mutations, as shown in Figure 4. The possible explanation for this could be that the strains that were used for comparison against the CO_2-dependent *S. aureus* strain were from the same organism, namely *staphylococci*. Furthermore, these strains were very closely related to the CO_2-dependent *S. aureus* and therefore the group membership and the phylogenetic characteristics would be almost identical.

Due to the failure to find any mutations from the *carAB* comparison, the promoter DNA sequence for the *carAB* chains of the CO_2-dependent *S. aureus* strain needed to be compared against the same promoter sequences from a non CO_2-dependent strain. As demonstrated in the results section, the promoters of the *carAB* for *S. aureus* Mu50 were obtained using the microbesonline website. These promoters are *PyrB* for the carbamoyl phosphate small chain (*carA* or *pyrAA*) and *PyrC* for the carbamoyl phosphate large chain (*carB*). In all organisms, there are two ways of synthesizing the pyrimidine nucleotides, either using the salvage pathways of preformed nucleosides and pyrimidine bases or from bicarbonate and intermediaries of the central metabolism using the de novo pathway (Ghim and Neuhard) [21]. The de novo

pathway is catalysed by ATP, glutamine (ammonia), aspartate and 5-phosphoribosyl-1-pyrophosphate (PRPP) and by bicarbonate and UMP when the exogenous pyrimidines are not present in the culture. This process is performed using six different enzymes that are encoded by the *pyr* operon: aspartate transcarbamoylase (*pyrB*), dihydroorotase (*pyrC*), dihydroorotate dehydrogenase (*pyrD*), carbamoylphosphate synthetase (*carAB* in enteric bacteria and *pyrA* in *Bacillus spp.*), orotate phosphoribosyltransferase (*pyrE*) and orotidine 5'-phosphate decarboxylase (*pyrF*) [21].

In the current study, the *pyrB* and *pyrC* promoters DNA sequences from the CO_2-dependent *S. aureus* strain were compared against the same promoters from a non CO_2-dependent strain, namely the Newman *S. aureus* strain. Because the results of comparing the promoters DNA sequences did not show any clear mutations either, as shown in Figure 6, the second theory needed to be tested.

Conclusion and Future Work

This paper applied numerous bioinformatics software tools in order to compare and search for the differences in the enzymes involved in pathways that can be altered in the CO_2-dependent *S. aureus* strain. Therefore, the entire genome of CO_2-dependent *S. aureus* strain was compared against another strain, not CO_2-dependent using the software tool MUMmer. By this tool, the results that the plots of the CO_2-dependent strain against the COL and Mu50 strains did not show any gaps while the connected thick diagonal line reveals strong similarities between the sequences in comparison to the thin diagonal line. Surprisingly, the results of using BLAST alignment tool did not illustrate any obvious mutations. Furthermore, the comparison between a number of strains and the CO_2-dependent *S. aureus* strain was carried out using the ClustalW alignment tool and Jalview software; therefore it did not show any observable mutations.

Since the results of comparing the *carAB* genes and their promoters of the CO_2-dependent *S. aureus* strain against other closely related strains did not show any observable mutations, an alternative pathway of producing arginine, proline or pyrimidine from glutamate without CO_2 enrichment needs to be achieved as one of our future works.

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