# Analysis of bacterial carbonic anhydrase

**PROJECT REPORT**

***Submitted in partial fulfilment of the requirements for the award of the Degree of***

**BACHELOR OF TECHNOLOGY**

**in**

**BIOINFORMATICS**

***Under the supervision of***

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**(Assistant Professor)**

**(GRADE-II)**

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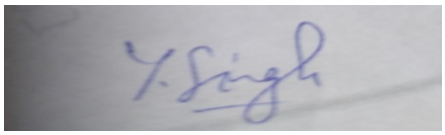
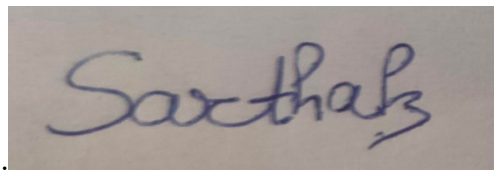
## Candidate's Declaration

I hereby declare that the work presented in this report entitled "Analysis of Bacterial Carbonic Anhydrase" in fulfilment of the requirements for the award of the degree of Bachelor of Technology in Bioinformatics submitted in the department of Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of the work carried out over a period from July 2019 to April 2020. The matter embodied in the report has not been submitted for the award of any other degree or diploma.

Yashaswi Singh Chauhan(161513) .... 

SarthakUpadhyay (161509)..... 

# CERTIFICATE

This is to certify that the work which is being presented in the project title **–ANALYSIS OF BACTERIAL CARBONIC ANHYDRASE** in partial fulfilment of the requirements for the award of the degree of Bachelor of technology and submitted in BIOINFORMATICS Department, Jaypee University of Information Technology, Waknaghat is an authentic record of work carried out by **YASHASWI SINGH CHAUHAN [161513] and SARTHAK UPADHYAY [161509]** during a period from JULY 2019 to APRIL 2020 under the supervision of **Dr. NARENDRA KUMAR**.

The above statement made is correct to the best of our knowledge.

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# ABSTRACT

In the context of bioinformatics, sequence analysis is the task of subjecting DNA ,RNA, Peptide sequences for approaching to know its features , functions , structure and its evolutionary studies. Techniques used to include sequence alignment researches against its biological databases. However, comparing these new sequences with its known functions is the key for understanding the biology of an organism from which the new sequence comes into play.

Thus, the analysis of sequences is used to assign functioning of the gene and proteins by the study of sequence similarities by comparison method. In this bacterial sequences of carbonic anhydrase are retrieved from the ENSEMBL database or NR database in the fasta format. The database shows all the genes entry for bacterial carbonic anhydrase.

# CONTENT

# CHAPTER

# INTRODUCTION

## 1.1CARBONIC ANHYDRASE

Carbonic anhydrase is an important enzyme found in the RBC's and other tubular parts(renal tubules). Carbonic anhydrase catalyzes the inter conversion between CO2 and H2O and dissociated ions such as HCO3-. Zinc ions contain the active site of Carbonic anhydrase. They are known as metalloenzymes as well.

Carbonic anhydrase is used to balance the pH level in blood, it also allows to breathe out CO2 from renal tubules and helps in the respiration process. Its applications consist of the role in anticancer and anti-glaucoma agents and also Carbonic anhydrase affects in the role of treatment of Alzheimer's disease.

It consist of three classes as:

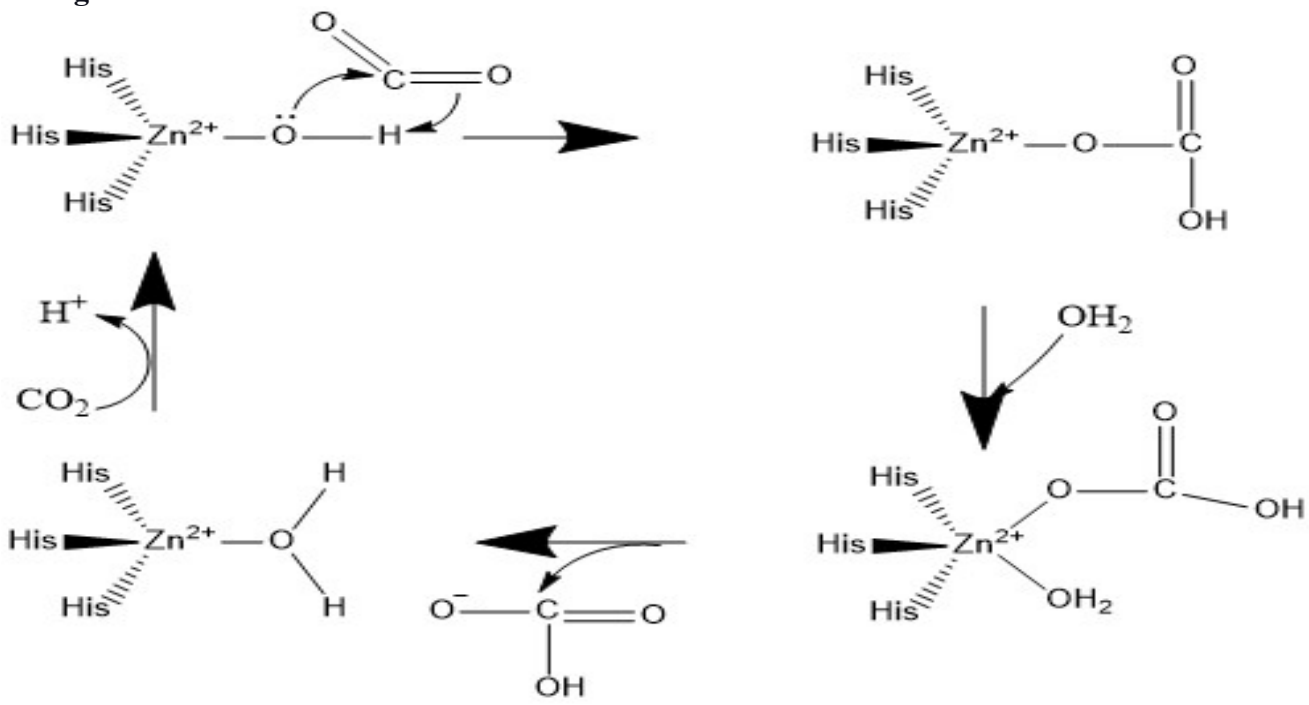
1. Class1: α CA hinge in mammals.
2. Class2: ß CA hinge in bacillus and plants.
3. Class 3: ¥ CA hinge in bacillus in hot springs.

The three classes of CA own alike, sites with a Zn metal center but not structurally close to each other.

## THREE-DIMENSIONAL FORMS:

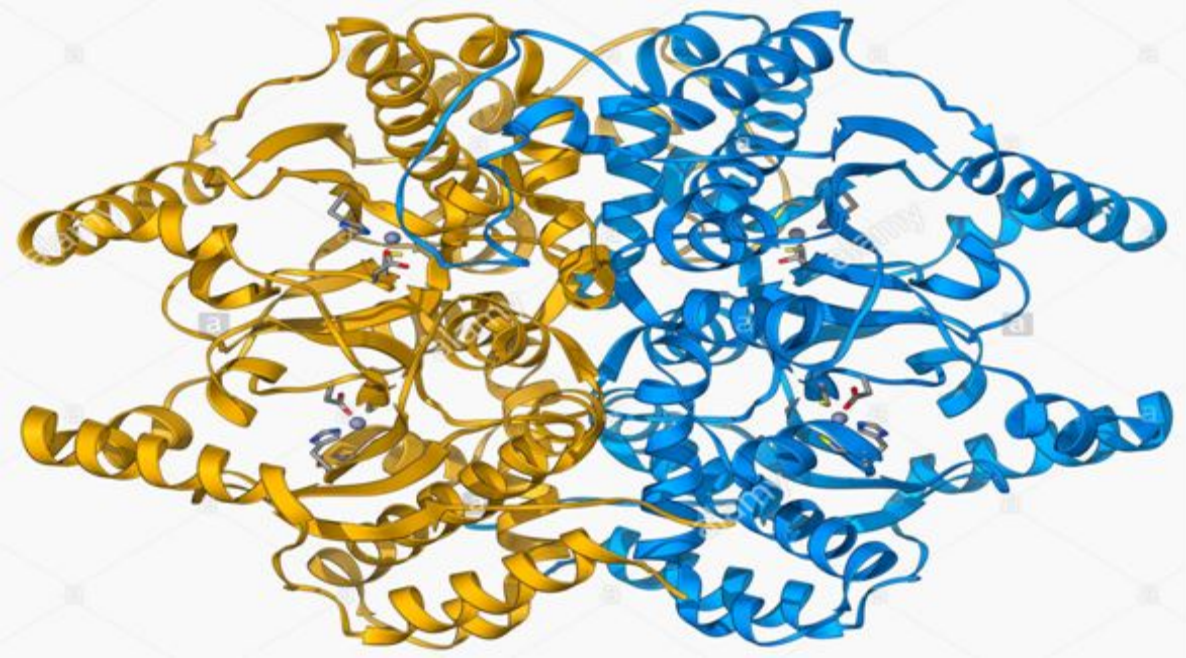
**α-CA:** The human CA I and II crystal type, bovine CA III, and the murine CA V trim form, shown in E.coli, is decided. A structure is available from the Indian buffalo for CA II. The general types of these forms of isozymes are very similar. The dramatic loss of protein stability and enzyme activity of humans as well as bacteria classified into 15 subgroups is almost globular to spherical.

**Figure 1**




**ß-CA:**: The beta-carbonic anhydrase (beta-CAs) are various but fundamentally connected groups of zinc-metalloenzymes set up in eubacterium, plant chloroplasts, red and green algae, and in the Archaea. The enzyme produces rapid interconversion to HCO(3)(-) and H(+) of CO(2) and H(2)O and is suspected of being related to metabolic enzymes that grip or process CO(2) or HCO(3)(-). Beta CA is important for many species to mature when CO(2) is applied atmospherically. Beta CA set up in lower unit eukaryotes and bacteria preserve in the reactions and transformation of bicarbonates and CO2.

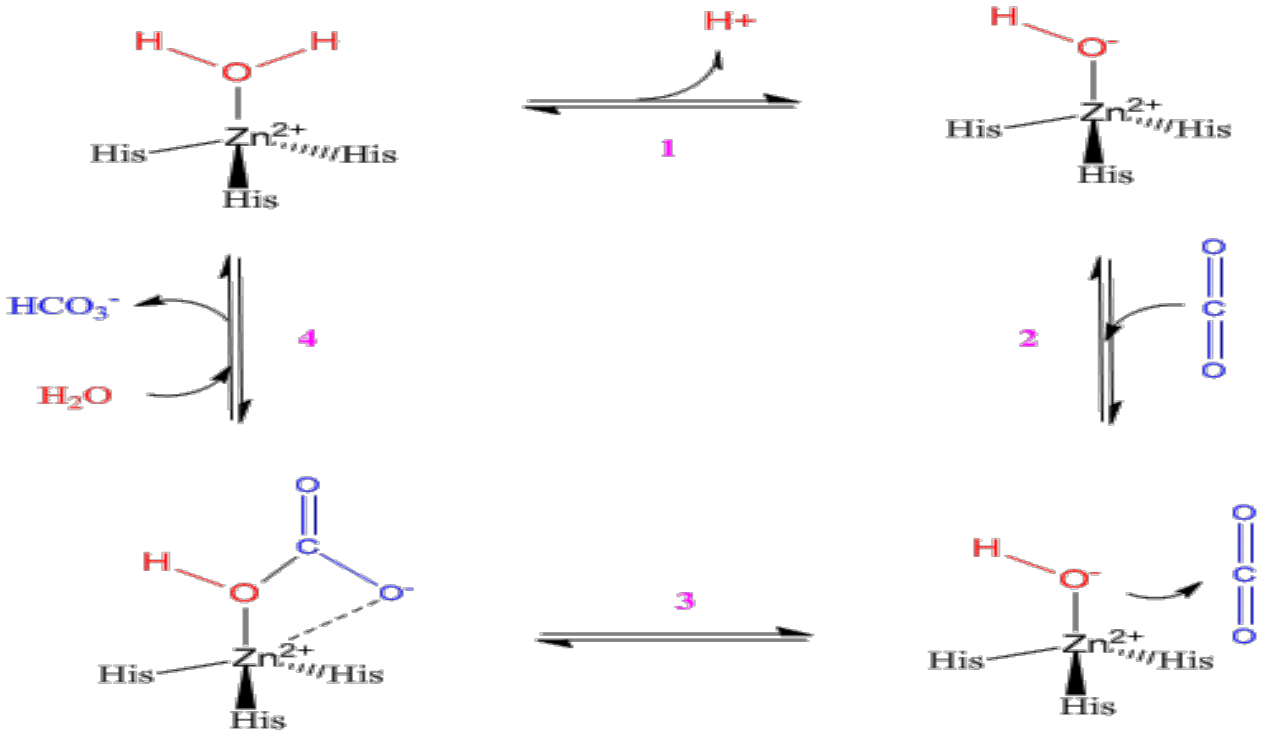
**Figure 2**



**¥CA:** It catalyzes the reversible inter-conversion of $CO_2$ to the HCO3.It is assembled by methanosarcina which is a class of CA. This pleasing tri-meric molecule, highlighting its distinct nature, has different folds than the Alpha-CAs. Seven turns of a left handed Beta-helix are influenced by each sub-unit, with three short strands per turn. There are, therefore, three almost flat Beta-sheets, two with seven strands and one with eight strands aligned. On top of the Beta-helix is a short and long Alpha-helix and a C-terminal or helix.

In the inter-conversion between CO2 and H2CO3 or any of its ionic species, Carbonic Anhydrase is known as a catalyst. Two physiological functions refer to the catalyst. One in red cells, where the hydration of metabolic CO2 in the capillaries of the tissues and its dehydration in the capillaries of the lungs is given. The other is concerned with the transmission of H+ or HCO3 collection to release organs.

**Figure 3**




## Bacterial Carbonic Anhydrases and their hindrance:

Bacterial CAs are important targets for acquiring antibacterial barren from the resistance problems of those agents that are clinically used. For only nine bacterial pathogens, the enzymes that have been characterised so far in detail can be found to have comprehensive data on the CAs present in their genome, and some of them cause serious infections worldwide with major antibiotic resistance problems as well. With key classes of inhibitors, i.e. inorganic anions and sulphonamides/ sulphamates, in vitro inhibition of these enzymes was studied. By catalysing the simple and very important hydration of CO2 to bicarbonate and protons, CAs are involved in crucial steps in the life cycle of many species, including eukaryotes, bacteria and archaea.

In diverse fields such as anti-glaucoma, anti-obesity and diagnostic methods for anti-cancer agents, CA inhibition has pharmacological application.

New drug design techniques are mainly verified by tail approach to the achievement of these CA inhibitor varieties, in addition to metal ion coordination, which leverages more external binding regions within the enzyme site, resulting in an out-sized number of selective isoform compounds.

**CHAPTER 2**

**LITERATURE REVIEW**

# 2.1 About Carbonic Anhydrase

An signicant enzyme found in red blood cells, gastric mucosa, pancreatic cells and even renal tubules is carbonic. In humans, the key function of carbonic anhydrase is to catalyse CO2 conversion to acid and back again. However, it may also help to hold carbon dioxide in the blood, which in turn helps to breathe. Perhaps Carbonic anhydrase commands uniquely wide interest as an enzyme. Since the reaction catalysed in animals is so basic it requires participate in a truly extraordinary collection of physiological processes. Among these are breathing, acid-base balance, bone resorption, calcification, multiple biosynthetic pathways.

In addition, recent evidences suggests implication in cell growth, with oncogenesis and cancer effects. It appears to facilitate photosynthesis in algae, cyanobacteria and plants, while it is involved in the transport of CO2 or bicarbonate or related processes in other bacteria. In fact, Carbonic anhydrase is not just a single type of enzyme, but it does exist in 3 genetically unrelated isoform families(Alpha, Beta and Gamma ). Practically all different organisms present differently.

Highlights date suggests that only the Alpha genes are found in vertebrate organisms but that they are found in several algae and plants and in some eubacteria are also present.

The Beta genes are present in all the vascular plants studied so far, where they are mainly expressed in the leaf tissue. They can also be present in eubacteria, archaebacteria, and some algae. Both genes are Alpha and Beta in many plants, the eukaryotes and invertebrates are lower together.

And the Gamma Carbonic anhydrase, although first found in an archaeon, anhydrases are missing from some archaebacteria, but are present in some plants and eubacteria.

In the inter-conversion between CO2 and H2CO3, carbonic anhydrase or any of its ionic species is known as a catalyst. There are two physiological functions of the enzyme. One is red cells, where metabolic CO2 hydration in the tissue capillaries and its dehydration in the capillaries of the lungs or gills are used. The second concerns the transfer of the accumulation of H+ or HCO3 to the secretive organs. It is necessary to note that carbonic anhydrase,

through its effect or intracellular CO2 balance, can also play a role in the development of neutral fluid.

The CAs were extensively researched because of their large physiological significance in all kingdoms of life and therapeutic importance as drug targets. The high catalytic potential of HCAII, the relatively simple method of expression and purification, relative stability and extensive biophysical studies have made it an exciting candidate to be incorporated into different biomedical applications, including artificial lungs, biosensors and CO2 sequestration systems. Another way to improve the CO2 transfer of artificial lungs through the hollow fibre membrane has also been shown to be focused on impeller devices that increase the rate of blood mixing. Sadly, this process can not be combined with the CA process. When these two strategies were combined, CA was denatured by the impeller's shear powers, resulting in a loss of enzyme activity. If it is possible to produce such a stable variant of CA, these methods could be combined, leading to a smaller and more effective artificial lungs.

## 2.2 WHY SEQUENCE ANALYSIS IS IMPORTANT :

Sequence analysis is the process of subjecting a sequence of DNA, RNA or peptide to any of a number of analytical methods in bioinformatics to understand its features, functions, structure or evolution. Methodologies used include synchronization of sequences, biological database searches and others. After the advent of high-throughput gene and protein sequence production methods, the speed of adding fresh sequences to databases has increased exponentially. In itself, such a sequence selection does not enhance the understanding of organism biology for the scientist. However, a key way to comprehend the biology of an organism from which the new sequence originates is to equate these new sequences with known functions. So you can use sequence analysis assign genes and proteins work by observing the similarities between the sequences compared. To provide comparison (sequence alignment) and study the alignment product to understand its biology, there are several methods and techniques nowadays.
In molecular biology, sequence analysis covers a very wide range of relevant topics:

1. Sequence comparison to assess similarity, also to deduce whether it is related(homologous).
2. Idenfication of features of the intrinsic sequence such ass active sites, sites of post-translation modification, gene structures, reading frames, distributions of intron and exon and regulatory elements.
**3.** Identification of sequence abnormalities and modifications such as point mutations and single nucleotide polymorphism(SNP) to obtain the gentic marker.
**4.** Showing the genetic diversity of genomes and animals and their evolution.

**5.** Sequence-by-sequence recognition of molecular structure.

## 2.3 ENSEMBL

The aim of the ENSEMBL is to enhance the most Ensembl data base and initiate five auxiliary positions to incorporate genetic data bases.

Ensembl Genetic data is an open project and figures are to the collective. The essential highlight of Ensembl Genetic data's is its avid port, permits coil over a genetic data and witness the corresponding locality of features like the conceptual explanation, succession designs, and test figures.

The latter file models are maintained by Ensembl Genetic data:

1.BED

2.Bedgraph

3.PSL

4.WIG

5.BAM

## 2.4 NR

This database is open access, explained and curated assembly public obtainable nucleotide sequences (DNA, RNA) and their protein results. This database is built by NCBI. A renewal sort of RefSeq protein information which shows non-redundant protein sequences that were presented in mid-2013. This information type was presented to deal with an expanding matter with redundancy within the Prokaryotic RefSeq protein dataset that matched with a big rise in bacterial genome accession from separate spots and strictly connected bacterial strains. For instance, an huge number of high-quality bacterium genome could also give in during a disease outburst. The gave in sequences may review pathogen progression during the outburst but more than half of the encrypted proteins from these genomes could also be similar to each other.

Non-redundant RefSeq protein dataispresently provided for archaeal and bacterium RefSeq genomes, excluding chosen reference genomes, by the NCBI prokaryotic genome observation channel. This scope definition may change later to include extra RefSeq sub-kingdoms or other organism categories.

# CHAPTER 3

## 3.1PURPOSE :

How carbonic anhydrase is used in the reduction of carbon dioxide levels?

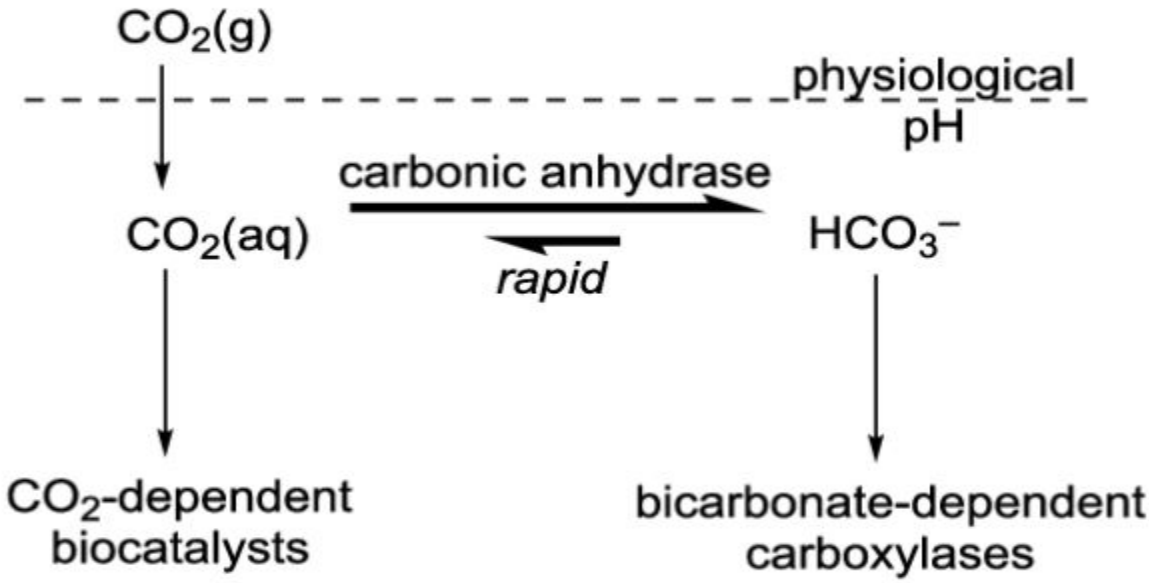
Biological CO2 sequestration in geological formations is one of the methods suggested to rescale the CO2 emitted into the atmosphere. An enzyme is used in this process to improve the hydration and subsequent CO2 precipitation. Recently, CA has been considered a crucial bio-catalyst for CO2 sequestration technology because CO2 building is the primary cause of global change and is important for technology advancement that can reduce atmospheric CO2 levels. Thus, biologically based CO2 capture systems are among the creative ways of enhancing capture performance.

CA catalyzes CO2 to HCO3- hydration and also shows the reverse reaction of HCO3- to CO2 dehydration. The movement of CO2 into a liquid phase helps to separate CO2 from other gases. A liquid membrane for process conversion is contained in the separation process. The effect of CA in hydration ,sometimes converted to precipitated to CaCO3.Through carbonic anhydrase CO2 may be stored in the geological sequestration, or for chemical fixation ,or for storage in 'super critical' . The $CO_2$ conversion by carbonic anhydrase implicit in the formation of H2CO3,and sometimes if mixed with the calcium to form precipitated CaCO3

The addition of CA to the CO2 liquidation transport rapidly improves the process of separation made by the 'scrubbed gas' brought into the module. With the release of aqueous bicarbonate transport liquid containing bacterial carbonic anhydrase during the operation of the reactor at ambient temperature, the effect on CO2 as a mixed gas steam initially shows that it measures 15percent of the release from the atmosphere. Scrubbed gas initiates the release of square of the remaining into different chemical formations with or without the help of the enzymes formatting the derivatives of CO2 as it when reacted with bicarbonates and calcium remaining from the scrubbed gas to derivatives and release of CO2 is approximated.

The hydration of CO2 t bicarbonate is rapidly and selectively catalyzed by carbon anhydrases, often due to the reverse reaction of dehydrating bicarbonate to CO2. By this selective movement of CO2 into the liquid form, CO2 is isolated from other gases. Separation processes using mammalian carbon anhydrase based on packed column absorber reactors have been described. Thus Carbonic anhydrase is the enzyme that can be used for developing technologies for CO2 sequestration. CA is a metalloenzyme that is ubiquitous(mostly zinc containing) and is found in animals, plants and even microbes. They exist in various forms, with various structures and molecular weights, and their operations vary from one to another.

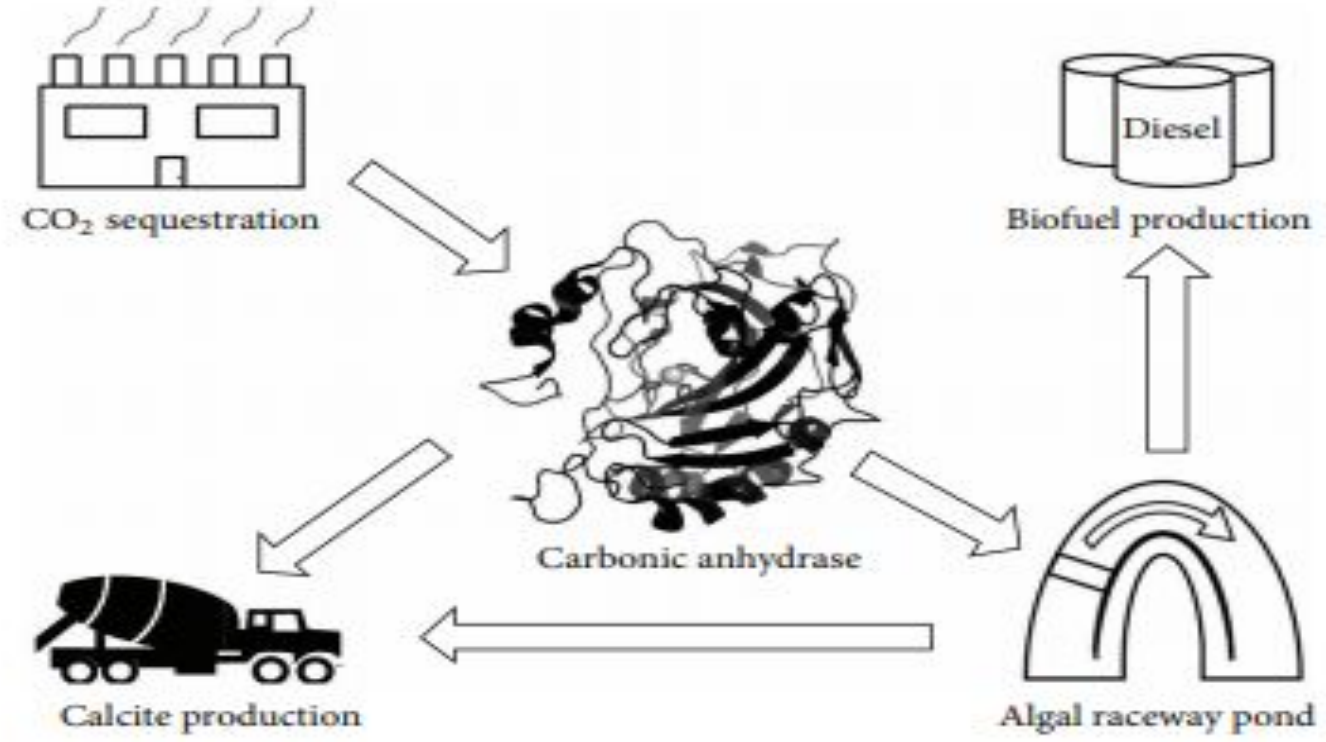
**Figure 4**




Biologically based $CO_2$ improves capture order. The use of kinases involved in such biological CO2 reactions is one of the foremost prominent possibilities; the respiratory system in mammalian cells or photosynthetic systems in plant cells. We may render "bio-mimic" CO2 capture systems based on CO2-catalyzing kinases, which can display high efficiency or performance in CO2 capture and release comparable to biomechanics. Carbonic Anhydrase is a kinesis that can be used for the production of CO2 sequestration technology. CA is metalloenzyme (mostly, a zinc-containing) and ubiquitous, that is, hinge in animals and plants and even in the microbes. They exist in several forms, with different forms and molecular weights, and their activities vary from one to a different. The reversible hydration of carbon dioxide is catalyzed by CA: CO2 + H2O -- H+ + HCO3, including various other reactions. This enzyme is associated with fundamental cell processes, like photosynthesis, respiration, transport of inorganic carbon and ions, calcification, and handling of acid-base equilibrium interestingly**.**

# Atmospheric concentrations of greenhouse gases (GHG)

**Figure 5**

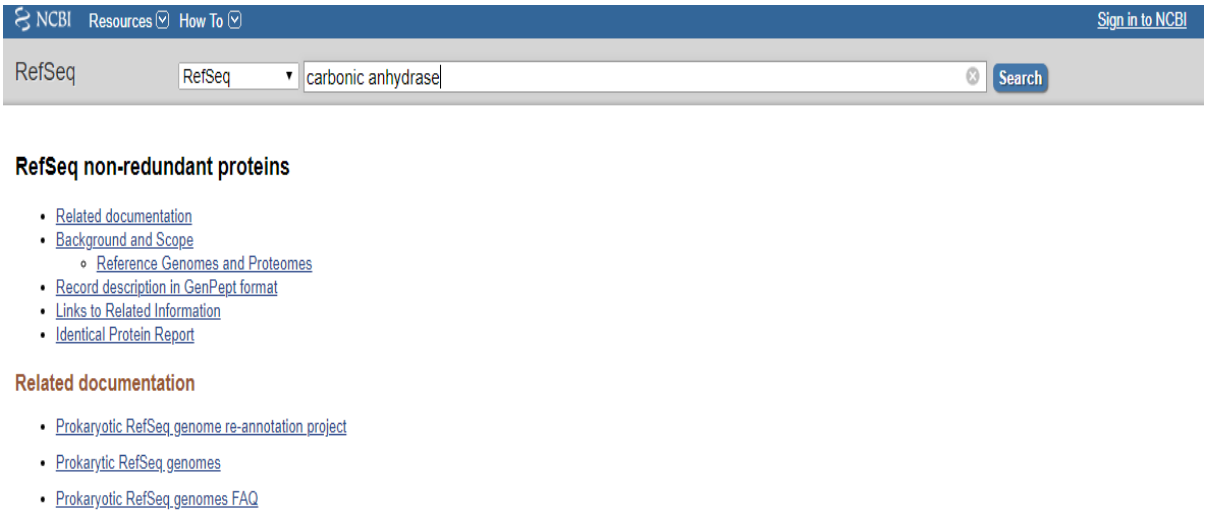



Schematic of CAs centralized ability in the conversion of CO2 into profitable results. A precious source of inorganic carbon for algal cultures generated in raceway ponds is the catalytic transformation of CO2 produced during the oxidation of fossil fuels into bicarbonate (HCO3-) through CA. Algae cultures' lipids and oils are special sources of biofuels, while the production of "waste" yields supplementary beneficial proteins, vitamins, minerals and dietary continuations. The Algal CA may also serve as a great source for production of calcite (CaCO3), which is important in many designs.

# CHAPTER4

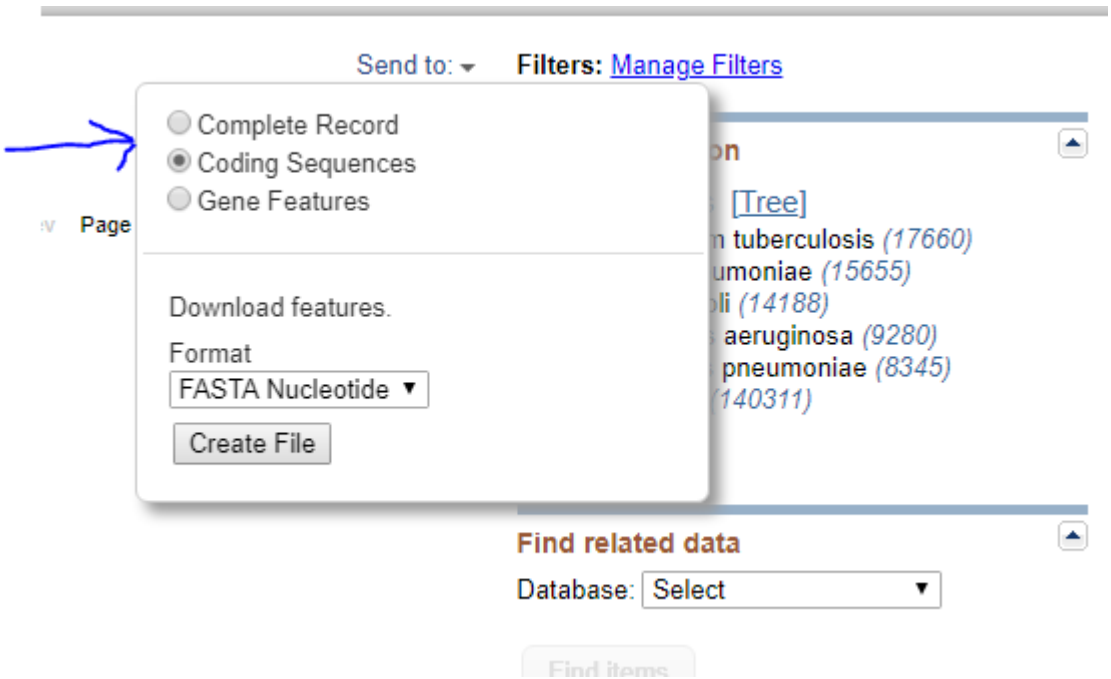
## 4.1 METHODOLOGY

**STEP 1:**Search the bacterium carbonic anhydrase in NR database

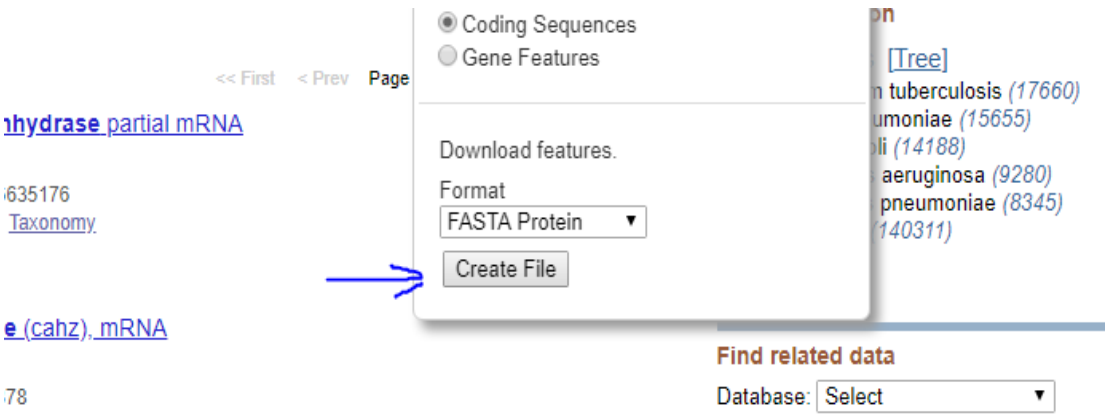



**STEP 2:** We got 205439 nucleotide succession of carbonic anhydrase


**STEP 3:**Now by clicking on 'send to' we select the coding succession.

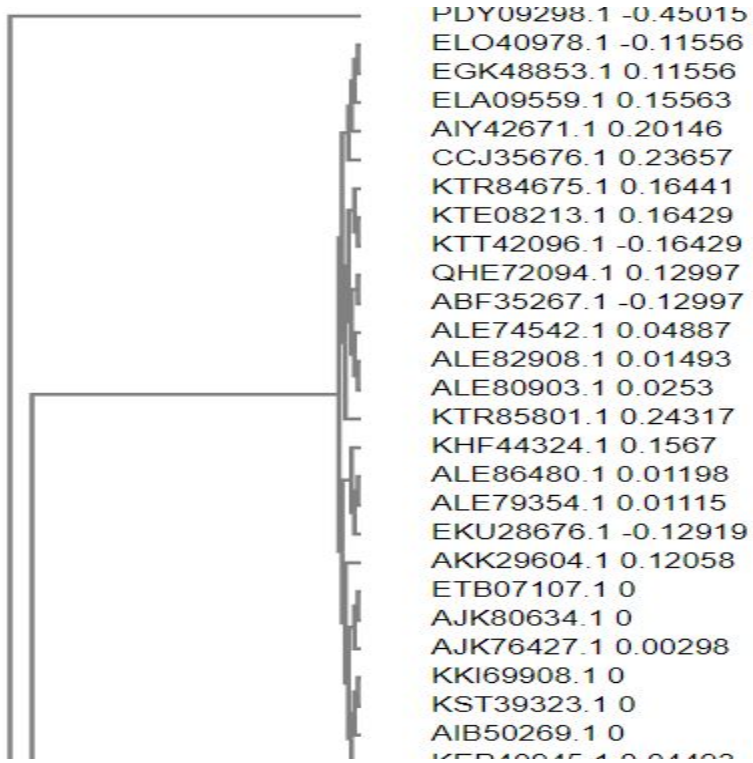


**STEP 4:** Then we will create a file by downloading in FASTA format.



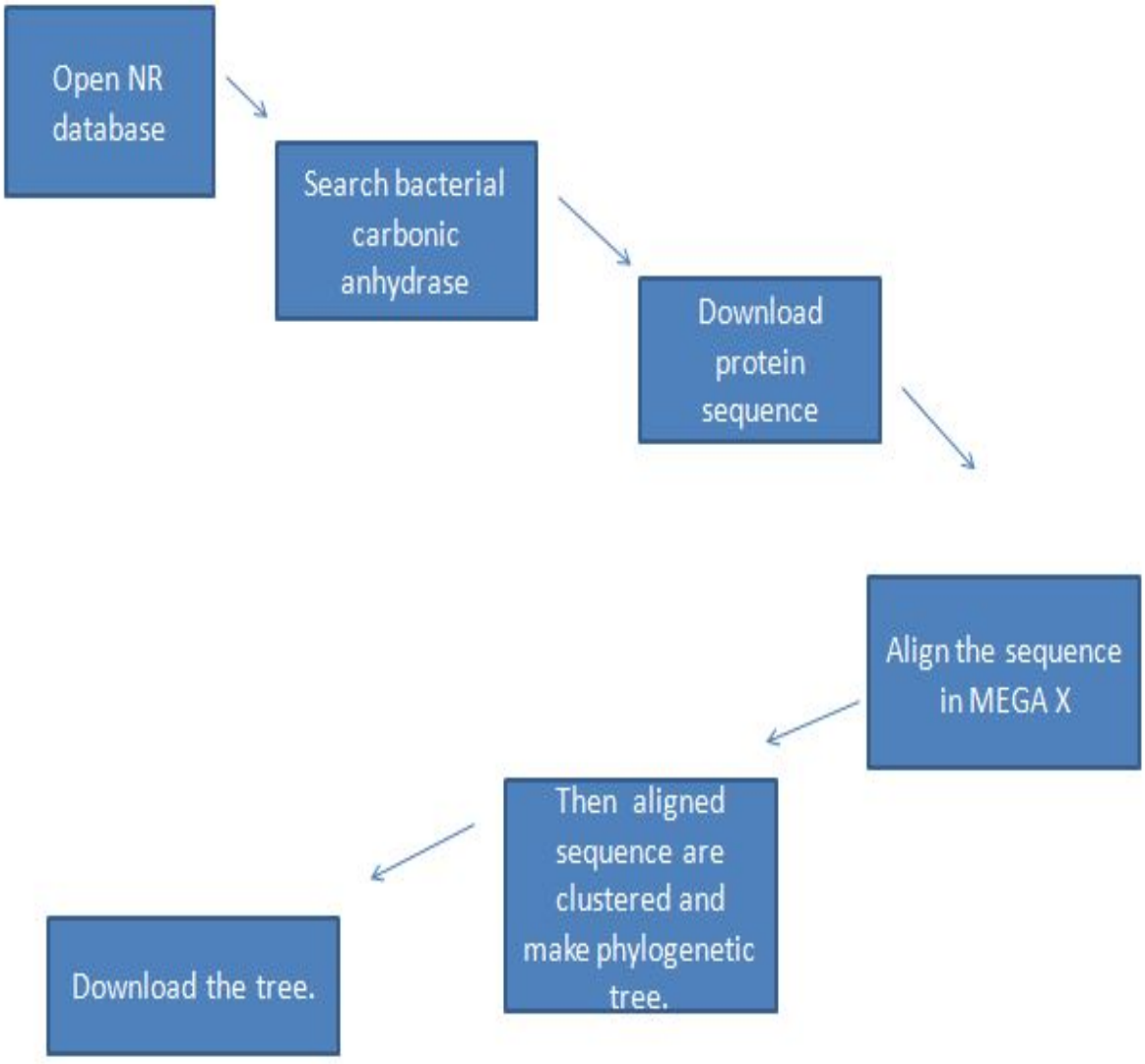

**STEP 5:** Wetook out and downloaded the 3802 bacterium protein succession and aligned them in MEGA X.

**STEP 6:**We aligned the succession and created a phlyogenetic tree with the gap opening and gap extensionpenalty with the score of 1&0.10.



## 3.2FLOW CHART

Flow chart diagram of the methodology used to download fasta succession from the NR Figuresbase.



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