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Carboxydothermus hydrogenoformans

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Abstract

Carboxydothermus hydrogenoformans is known as a gram positive bacterium. It is thermophilic, and chemolithotrophic in nature. This is an anaerobic bacterium. *C. hydrogenoformans* is also described as a carbon monoxide utilising bacterium. Because this bacterium grows by utilising the carbon monoxide (CO). It acts as a single carbon, source of energy, and this bacterium mainly produces H_2 and CO_2. By cloning and sequencing the *Carboxydothermus hydrogenoformans,* cooF and cooS are derived. These cooF and cooS genes will be present in carbon monoxide dehydrogenase. This carbon monoxide dehydrogenase enzyme plays an important role in the metabolism of carbon monoxide (CO).

Introduction

Carboxydothermus hydrogenoformans is a strictly anaerobic bacteria that live at high temperatures in the range of 60-70° C due to its peculiar property of nucleic acids, lipids and enzymes which allow this organism to develop in such a high temperature environments hence called thermophiles. The reason for their survival at these extreme environments is due to their utilisation of CO (Carbon monoxide) from the atmosphere and releases products like hydrogen(H_2) and traces carbon dioxide and water. This Bacterial thermophile has a marked efficiency in carrying out oxidation of carbon monoxide as a result of the presence of five anaerobic dehydrogenase complexes [1].

C.hydrogenoformans is a eubacterium that utilizes carbon monoxide under chemolithoautotrophic conditions for its growth and is thermophilic living in extreme environments, isolated from hot volcanic springs and is strictly anaerobic, gram positive. 16 S rRNA analysis shows that this bacterium has maximum resemblance to *Thermoterra bacterium* (Svetlitchnyi et al. [2]).

The enzymes secreted by them are stable at certain high temperatures (110°C and above), the catalytic properties being same compared to those with mesophilic bacteria. It is confirmed when genes coding to enzymes in thermophilic bacteria are cloned and made to express in mesophilic hosts where they were made to express the enzymes and are active. This shows that the properties of enzymes are genetically encoded. The mechanisms of their thermostability that involves hydrogen bonds, hydrophobic bonds, disulphide bridges, packing etc. The hyperthermophilic enzymes that denature reversibly compared to mesophilic enzymes and require high Ea for their inactivation and still needs unfolding. Being active at the elevated temperatures, chemical modifications like deamidation, cystein oxidation, peptide bond hydrolysis makes denaturation irreversible (Figure 1) [3].

Importance

Hydrogen gas is noticed as one of the valuable energy carriers in the near future. Various researches on hydrogen generation methods have been carried over the past several decades. The hydrogenogens usually found at volcanic environments. C.*hydrogenoformans* being one of the hydrogenogen is considered as the abnormal, when compared to the remaining hydrogenogens on basis of its stringent carbon monoxide supplements, whereas the remaining were found to have poor growth unless supplemented with other organic substrates [4].

For human beings carbon monoxide is a poisonous gas that binds strongly and irreversibly to the centre of haemoglobin. Apart from its toxic properties it is the source of carbon for many species of organisms and since oxidation of carbon monoxide will convert it to carbon dioxide. In extreme environments these hydrogenogens are able to oxidise in the absence of oxygen using water as an electron acceptor producing hydrogen gas, that is usually dissipated into the environment due to which this organism is seriously engaged and drawn attention by the biotechnologist [5].

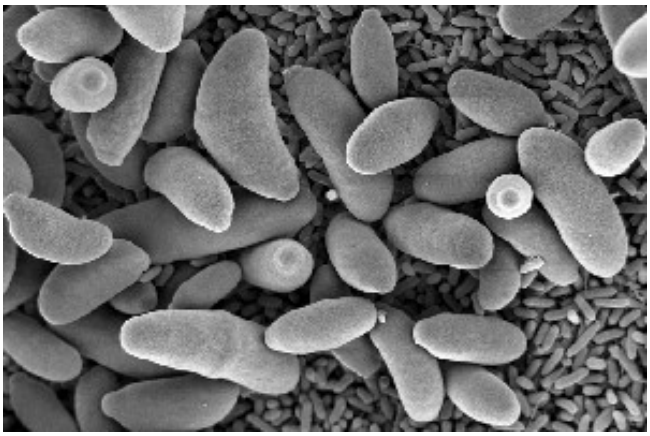

Figure 1: *Carboxydothermus hydrogenoformans.*

Phylogeny Based on Sequence Analysis

The sequence analysis of *C.hydrogenoformans* along with the phylogenetic analysis proposed based on series of markers. This was believed to be included under the order of clostridiales under some of the firmicutes and hence it should show resemblances towards species like clostridium like *Thermoanaerobacter tengcongenesis* for which the genomes are available excluding the remaining taxa. The results of analysis reveals that *C.tengcongenesis* and clostridium species are closely related to each other and not the same case with *C.hydrogenoformans* believing that *C.hydrogenoformans* should be included separately in the order of clostridiales.

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Genome Organization

The genome of *Carboxydothermus hydrogenoformans* is organised in to single circular chromosome with 2,401,892 base pairs and with more or less 42.05 % G+C content. The genes that are responsible for the production of CO-dehydrogenase (CODH) for the oxidation of carbon monoxide are cooF and cooS. The genome studies of *C.hydrogenoformans* shows five highly differentiable anaerobic carbon monoxide dehydrogenase complexes. The sequence analysis reveals that 2,646 out of whole genome is set to code for proteins(CDSs) and only 1,512 out of the above protein coding genes are known to have function. The repetitive DNA sequence comprises of 3% and this includes two large clusters of 3.9 and 5.6kb, which in turn each cluster has 59 and 84 partial palindromic repeats of up to 30 base pairs. The enzyme complex that catalyse the carbon monoxide(CO) oxidation to Hydrogen(H_2) and Carbon dioxide (CO_2) was purified and is known to have six hydrophilic and two hydrophobic polypeptides. These hydrophilic subunits are already determined which allows the identification of genes that encode in the whole genome sequence of *C.hydrogenoformans* (Figure 2) [6].

The whole genome sequence of the *C.hydrogenoformans* was completed in the year 2006, having circular chromosome with the total number of all DNA molecules and total size of all DNA molecules are found to be 2401520 bp out of the whole genomic DNA the number of primary annotation coding bases 2185480 bp. The study also reveals the presences of commonly believed total primary annotation of protein coding genes are 2645 proportional to 97.70% of the whole genome. The genes that are presumed to functional are 1847 approximately of 69.82%, and an overall only 3% of the whole genome is made of introns that have repeated sequences all over the length of DNA. These repeated units are divided into two largely clustered (3.9and 5.6Kb) regularly spaced in between forming short palindromic repeats. The clusters are again subdivided containing 59 and 84 partial repeats of palindrome of about 30 bp; (GTTTCAATCCCAGA[A/T]TGGTTCGATTAAAAC) respectively. These repeats are almost identical inside the clusters may sometimes differ in one nucleotide at the centre of the cluster. The repeats at the end of the clusters are found to perverted to most extent, and are widely spreaded among the various groups of bacteria and archae. There is no change in the initial part of the repeated sequence and is totally conserved, even though the presumed function of such repeats is relatively unknown, are now believed to be in associated in portioning of chromosome. The primary annotations of the above conserved hypothetical genes are 355 approximately to be 13.42% when compared to the hypothetical genes comprising of 443 equal to 16.74%. The statistical genome analysis of this organism shows the presence of the t-RNA genes and r-RNA genes and are about 50 (1.84%) and 12(0.44%) respectively. The most astonishing feature of the genome studies is the G+C content that makes the DNA stable at high temperatures making it difficult for the denaturation, comprising of about 42.04% with 1009832bp. The origin site and terminus are clearly seen as a curve on the chromosome along with this a prophage with about 50 CDS adjoining t-RNA on one side proposing to serve as the insertion site. The phylogenetic analysis confirms the resemblance of this organism with prophage lambda to others in firmicutes especially the SPP1 phage which infects *Bacillus subtilis*. In case of firmicutes specific DNA polymerase polC synthesises in the directions of leading strand as 87% of the genes are concentrated in the direction of leading strand, this when compared to *B.subtilis* where polC synthesizes in the direction of leading strand but at the same time another DNA polymerase DNAe start replicating the lagging strand. Similarly in case of non-firmicutes the bacterial DNA polymerase DNAe replicates both the strands. In case of *C.hydrogenoformans* one copy of DNA Pol and two copies of DNAe has been identified and indicates that there is a lack of conserved genes and genome rearrangements will occur at high rates [5].

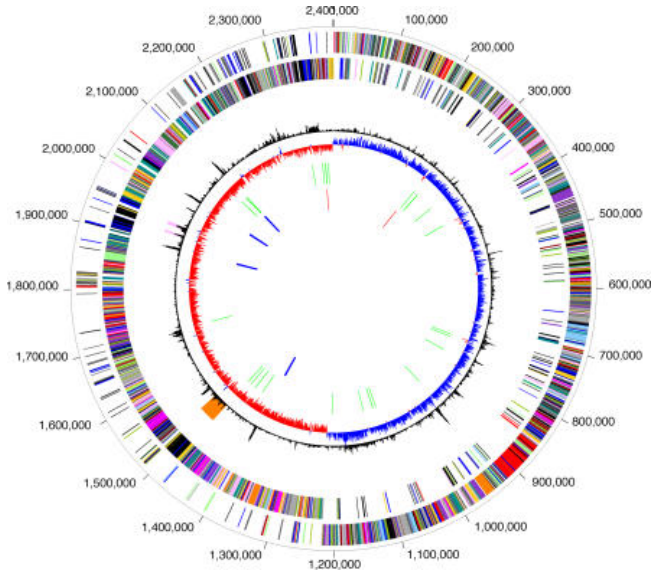

Figure 2: Genome Organization of C.Hydrogenoformans.

General Features of the *C. hydrogenoformans* Genome

The genome study of *C.hydrogenoformans* reveals the expression of five genes that encodes the homologs of cooS, which is known to be the catalytic subunit of the CODH which are supposed to be scattered in the genome and the five distinct complexes of CODH are CODH I-V.

Each of them is supposed to have their function such as:

CODH I – responsible for energy conservation

CODH III – responsible for carbon fixation.

CODH IV – is known for oxidative stress response.

The other two homologs perform the anaerobic function of generating NADPH [5].

The growth is promoted by a process that is initiated by a CO-Dehydrogenase (CODH) that which contains nickel and iron at the centre.(Ferry J.G.1995).Generally the anaerobic bacteria that which employs Acetyl co.A pathway forms a complex with Acetyl co.A synthase (ACS) and carbon monoxide dehydrogenase (CODH). The formed complex then performs catalytic reaction that produces Acetyl co.A from CO, a CH3 group and Co.A. [2].

CODHs

The property of the species that directly or indirectly depends on the carbon monoxide is made possible by using Nickel-iron Co dehydrogenase complexes that catalyses the interconversion of carbon monoxide to carbon dioxide anaerobically. The genomic analysis together with phylogenetical analysis suggesting the presence of five genes that encode coos subunits that catalyse the reaction and is found to be scattered in the genome of *C.hydrogenoformans*. They are proved as the five subunits of CODH complexes that we mention as CODH I-V [5].

Carboxydothermus hydrogenoformans has two specific proteins CODH I and CODH II. The structural studies indicate that CODH I subunit mass is of 62.5 KDa and holoenzyme mass is of 125 KDa and

Feature	Value
Genome size, bp	2,401,892
% G+C	42.0
Predicted protein coding genes (CDSs)	2646
Average CDS length	827
Percent of genome that is coding	91.1
CDSs with assigned function	1512 (57.1%)
Conserved hypothetical CDS[a]	354 (13.4%)
Unknown function CDS[b]	331 (12.5%)
Hypothetical CDS[c]	449 (17.0%)
Transfer RNA	50
Ribosomal RNA	12
Structural RNAs	2
CRISPR regions	2
Prophage	1

[a]Match to genes in other species, but no function known.
[b]Some biochemical function prediction, but cellular role not predictable.
[c]No match to genes in other species.
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Figure 3: General Features of the *C. hydrogenoformans* Genome.

similarly CODH II subunit mass is of 64.5 KDa and its subunit mass is of 129 KDa. Based on the amino terminal sequences it was revealed that the CODH I and CODH II are structurally and immunologically different. As per the TIGR (the institute of genomic research) and national centre for biotechnology information, the N- terminal of the CODH II and 49 KDa chymotryptic peptide is found on DNA from *C.hydrogenoformans* which shows maximum similarity to cooS (the CODH subunit of *R.rubrum.* these regards can be used as evidence to determine the closeness of CODH II with coos [2].

CODHs of *Carboxydothermus hydrogenoformans* consisting of α2 subunit in their structure making them homodimers is also having similarity to the CODH of *R.rubrum* and it differs with acetogenic and methanogenic, where they have α2β2 tetramers or αβγδ pentamers. Various studies suggest that *C.hydrogenoformans* contain Ni in itssubunit occupying 67% in CODH I and 83% in CODH II, where as in *R.rubrum,* Ni occupies 65% (Figure 3) [2].

Crystal Structure of Codh

The crystal structure of carbon monoxide dehydrogenase that which catalyses the oxidation reaction is figured to be 2.2A. Molybdenum being the active site of the enzyme having three oxygen ligands, drives the electrons to electron transport chain containing (2Fe-2S) clusters and FAD. The components of CODH are molybdoprotein (88.7), flavoprotein of 2-KDa and iron sulphur protein of 17.8 KDa framed as dimer resembling to xanthine dehydrogenase (Figure 4) [7].

The regions of heme-binding in CooA is not similar to other heme regions like PAS that are located in globins. Comparative studies revealed the presence of particular residues in the proximity of heme. The interplay of C-helices with the CO-bound heme is required for the proper activation of cooA [8].

CODH I gene

CODH I contributes for the energy conservation step having with a catalytic subunit (CooS-I, CHY 1824) along with the electron transfer protein (cooF CHY 1825) are closely located and code for proteins present next to hydrogenase gene cluster (cooMKLXUH CHY 1832-27) similar case was observed in *Rhodospirillum rubrum* and hence these eight proteins forming enzyme complex is known to perform the conversion of carbon monoxide to carbon dioxide and hydrogen. In *R.rubrum* this relates to the energy conservation by proton gradient created across the site of cell membrane. Based on which, the set of genes and protein coding sequences was thought to play a very similar role in *Carboxydothermus hydrogenoformans* in conservation of energy (Figure 5) [5].

CODH III gene

Apart from *Carboxydothermus hydrogenoformans*, many other acetogens and methanogens have ability to fix CO or CO2 by acetyl

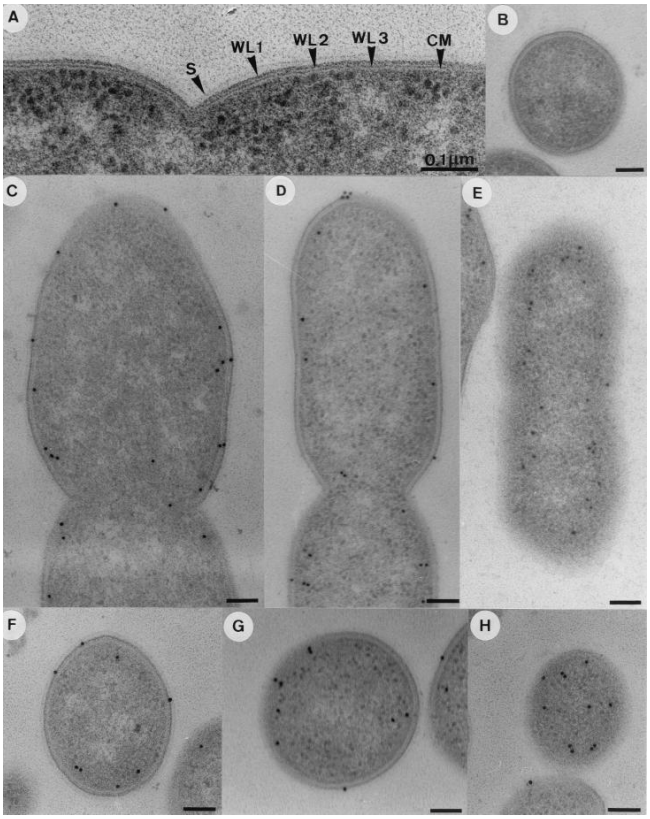


Figure 4: Crystal structure of CODH.

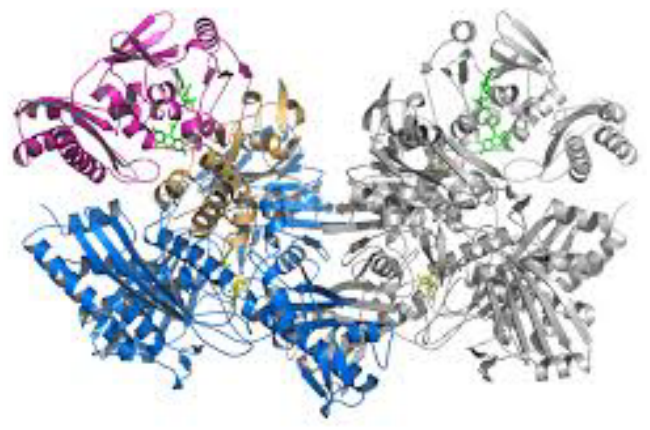
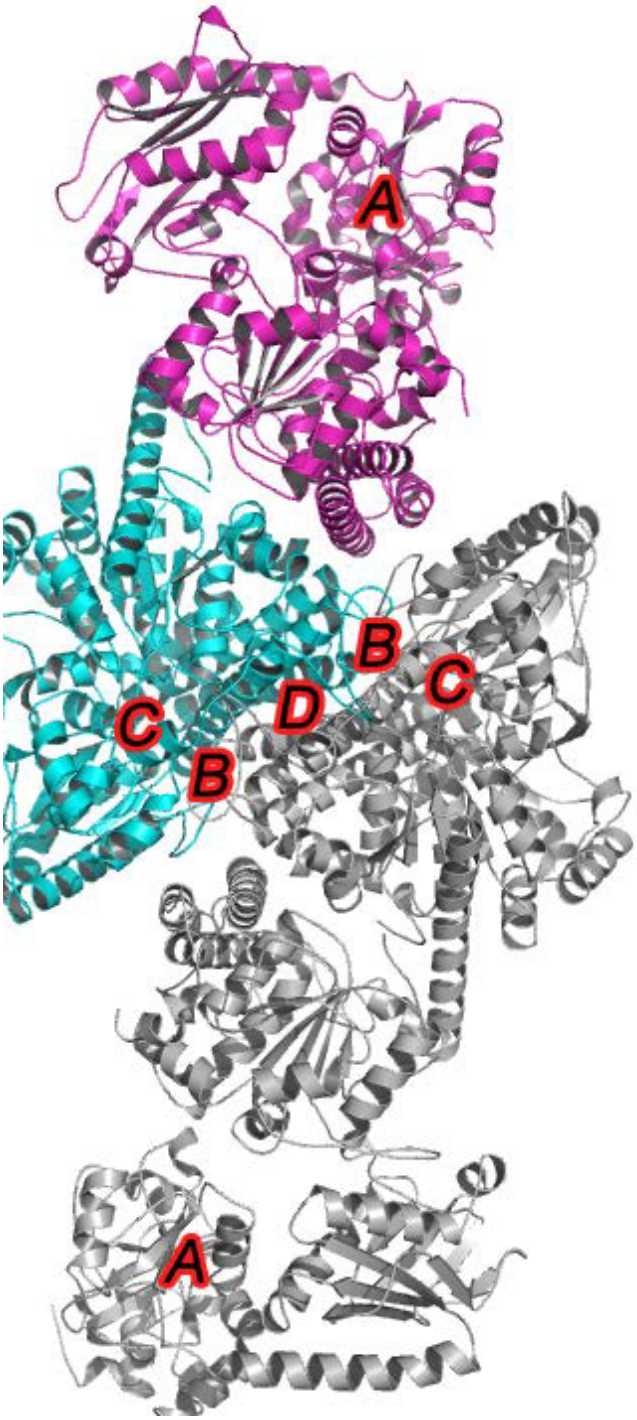


Figure 5: CODH I gene.



Figures 6: CODH III gene.

co-A pathway, in which they convert two molecules of carbon dioxide by condensation into acetyl co-A which acts to be the key and outraging source of ATP production. This enzyme that catalyzes the condensation into acetyl co-A was made to synthesise by *C.hydrogenoformans* in vitro conditions and was successful. The genes that are coding the above complex are concentrated at the CHY 1221-7 location of the genome of *C.hydrogenoformans*. By this analysis it is clear that *C.hydrogenoformans* performs Co fixation by this pathway (Figure 6) [5].

Inhibition of CODH III gene by KCN

CODH III is presumed to be having concerned about the oxidative process, however this oxidation activity of CODH III by potassium cyanide under the suitable catalytic conditions like the abundant presence of carbon monoxide and an electron acceptor like water. As well as in certain conditions of non-turn over where in the absence of carbon monoxide and the electron acceptor. The inhibition rate of potassium cyanide is dependent on the factor like time and concentration of cyanide and appropriate inhibition temperature, since the CODH III is the enzyme produced by the thermophile its stability is obtained at specific temperatures for its activity. The graphical representations are made show in the potassium cyanide activity with respect to the concentration of the cyanide that makes clear understanding about the pattern of inhibition. The process of the inhibition and pattern of inhibition under the non turn over conditions is typically based on the CODH III redox state. This is followed by a principle where the reduced enzyme when incubated with weak reductance. The inhibition occurs very strongly, but at the same time highly oxidized enzyme incubated along with less or weak reductance then the inhibitory effect will be weak. However it is already known that carbon monoxide i*n C.hydrogenoformans* is linked with the reduction of protons finally to hydrogen. Finally in a similar way cyanide also interacts with the reduced cluster in the way that could interact with CODH III and unlike potassium cyanide carbon monoxide is involved in protecting the reduced CODH III over the inhibition done by potassium cyanide. After all the under carbon monoxide unlike the potassium cyanide, there is not much decreased in activity is observed. This method of protection of CODH III by carbon monoxide conveys that both carbon monoxide and potassium cyanide par take the common binding side of CODH III [9].

Reactivation of CODH III gene

It is completely possible to reactivate and recover the reduced CODHIII that is initially inhibited by potassium cyanide by giving the substrates the optimum conditions of high temperature of 70°C and above, and citrate can drive the activation process further, when compare to the process of reactivation under the influence of nitrogen where low temperatures of 23°C to 50°C for incubation is to be maintained. The effect of carbon monoxide at the temperature of 23°C and 50° C is almost nil [9].

CODH IV

As a protection mechanism, the *C.hydrogenoformans* has to face the oxidative stress in the environment which is purely dependent on protein called Rubrerythrin. This protein is found in all of the anaerobes and not the aerobes. Rubrerythrin plays a major role in crashing the reactive oxygen by reduction of Hydrogen peroxide, the whole process undergoing this step is however not explained. *C.hydrogenoformans* has genes that encode for three subunits of rubrerythrin of which one is indulged in operon formation with genes that encode CooS-IV, and a cooF homolog and NAD/FAD-dependent oxido reductase (CHY0735-8). The function of the operon is to encode for a complex, where the electrons are channelled to rubrerythrin that are grabbed from the carbon monoxide by CODH, finally by reduction mechanism to reduce hydrogen peroxide to water. The remaining subunits of rubrerythrin are cooF and NAD/FAD- dependent oxido reductase are supposed to be the electron carriers, hence CODH-IV is enrolled to play a major role in taking up oxidative stress response.

The genes that code for different CODHs are different for aerobes and anaerobes to metabolize the carbon monoxide. The CODHs

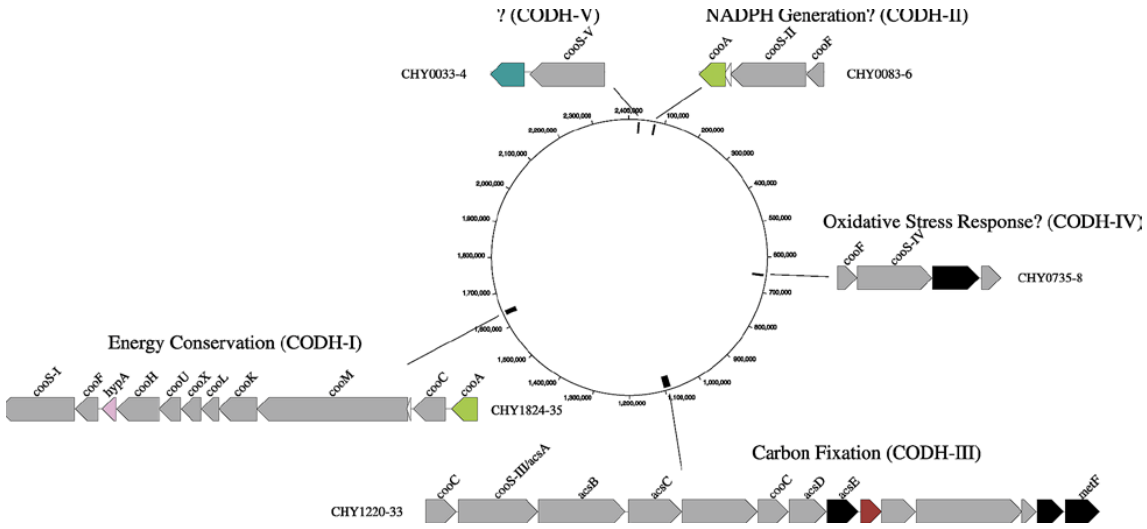


Figure 7: Genome Locations of Genes Predicted to Encode Five CODH Complexes.

obtained from aerobic bacteria contains molybdoprotein (coxL), flavoprotein(coxM), ironsulphur protein(coxS) that are included under the family of molybdenum hydroxylases. The oxidative reduction of carbon monoxide by the complexes processed through reductive pentose phosphate cycle. The gene clusters(CHY 0690-2) is similar and are homologous to the clusters of the *Oligotropha carboxidovorans*, but the phylogenetic analysis suggest that coxL is not grouped in CODH subfamily and so by this it is evident to confirm that this gene cluster inside *C.hydrogenoformans* may not code for CODH. Surprisingly *R.rubrum* is known to have the gene clusters of both anaerobic CODH and also the CODH from the aerobic bacteria *O.carboxidovorans*; by this reason, these photosynthetic bacteria can utilize the carbon monoxide aerobically as well as anaerobically for energy (Figure 7). Genome Locations of Genes Predicted to Encode Five CODH Complexes

Gene Inducing Signal Transduction Pathway

Carboxydothermus hydrogenoformans has ability to act according ly to various environmental intimations by undergoing signal transduction pathways. To process these pathways *C.hydrogenoformans* has 83 single component regulators and 13 twin component systems that includes two chemotaxis systems these count of components regulators are average for the genome size of the *C.hydrogenoformans*. Some of these two component regulator systems are located next to the transporters inside the genome, where as some of them are near to oxidoreductases and are concerned about the regulation activity for solute uptake. *C.hydrogenoformans* also contain regular down rush of the chemotaxis gene and whole set genes for flagella are thought to be located inside a cluster of about 70 genes (CHY 0963-1033). The genes related to chemotaxis make the organism set according to the environment. For example it helps the organism to keep in search of food or more in a direction of food source, and in the same way it transducts the signals to the organism to keep itself away from toxic chemicals. As the gene of *C.hydrogenoformans* is capable of conducting single transduction pathways, there is every chance for this organism to uptake gradients of organic nutrients, and gases like Carbon monoxide. The genome of the *C.hydrogenoformans* lacks genes for transcription factor like Lac1, Pad R, Deo R, that which are present in clostridial family. And also the genome does not code for proteins of Lux R family that are actually present in large numbers in one component and twin component systems [8].

The most fascinating protein involved in signal transduction in the genome of *C.hydrogenoformans* is sigma 45 transcriptional regulator that contains iron-hydrogenase domain acting like a sensory module (CHY 1547), the domain of which has 4Fe-4S clusters which is believed to use molecular forms of hydrogen for substrate reduction. This combination of sigma 54 with DNA binding HTH-8 domain clearly explains that it is the regulator of gene expression specific to *C.hydrogenoformans* with response to availability of hydrogen [8].

Gene CooA Homologs as Co-Sensors

To demonstrate the cooA- homologs as co-sensors, CooA is cloned in one of *E.coli* reporter strain in which system containing promoter that which is located infront of lacZ. Assuming that CO-dependent response affects the binding of specific regions, and results showing the accumulation of β-galactosidase in response to antibiotic conditions provided. This clearly explains us that the variable homologs of cooA is sensible to Carbon monoxide and hence acts as co-sensors (Figure 8) [8].

Inactive Fe(II) CooA structure adapted from that of the strain with PDB identification no. 1FT9. The protein consists of two monomers,

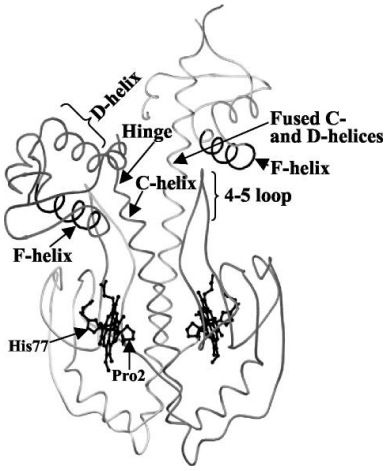


Figure 8: GENE CooA HOMOLOGS AS CO-SENSORS.

shaded differently in this figure, which dimerize along the central C-helices of adjacent effector-binding domains [8].

Presumed function of CODH:

CODH I is concerned in generation of energy by inducing electrons from CO, which are later passed through the ferridoxin- like protein B to hydrogenase, and this is the place where intracellular protons get reduced to H_2. CODH II is concerned over the generation of NADPH and utilizes few of CO_2 for carbon assimilation. Eventhough the bacterium *Carboxydothermus hydrogenoformans*, the genomic sequence of which has 80% homogeneity towards ACS of *C.thermoaceticum* due to which the calvin-benson-bassham is not operated with the absence of ribulose -1,5-bisphosphate carboxylase activity (Figure 9) [2]. Hypothetical scheme showing the function of the two CODHs in C. hydrogenoformans. CODH I is involved in energy generation and CODH II serves anabolic functions. For details refer to the text. The scheme is not intended to give correct stoichiometries. Abbreviations: B, ferredoxin-like protein B; H2ase, membrane-bound [NiFe] hydrogenase.

Some of the photosynthetic bacteria express the proteins needed for the metabolism of the carbon monoxide and the net reaction is

$$CO + H2O \rightarrow CO2 + H2$$

The proteins expressed by the bacterium contains enzymes CODH and CO- tolerant hydrogenase enzyme. The sequence of amino acid from large subunit of COOH, shows maximum similarities as well as identity with that of other Ni-Fe hydrogenases. The analysis shows the possible best similarity of COOH dehydrogenases is with HYC E gene having (58% similarity and 37% identity) from an *E.coli* assumed to be the largest subunit of Ni-Fe hydrogenase. It shows exceptions and resistant to Carbon monoxide inhibition and also as high ratio of hydrogen evolving to uptake of hydrogen in comparison to many other hydrogenases. Non ionic detergents as inhibitory effect on tightly membrane bound Carbon monoxide induced hydrogenases and the presence of Ni was marked out. Analysis on strains UR294 which is having defective in nickel insertion and revealed the Ni insertion mechanism and utilisation by hydrogenase and CODH [10].

Sporulation Studies

The property of sporulation was thought to be not the inherited nature of the *C.hydrogenoformans* but when the morphological analysis was carried out for the cells of *C.hydrogenoformans*, it revealed the screening of endospore like structures within the cells and experiments conducted to find the genes that actually code for the spore formation. The analysis revealed that there are a number of homologs of genes that relates to the sporulation connecting all the stages of sporulation. It was found that spoOA gene is the master switch gene in spore formation, and various factors σH, σE, σF, σG, σK. Later the phylogenetic analysis was conducted to find out the possible sporulation genes with the different species of organisms. By this analysis it was found that totally 37 genes are responsible for the endospore formation and that those genes are specific to clostridiales and bacillales. Most of the novel genes are assumed to be membrane proteins leaving some of them as hypothetical proteins; some of these novel gens are responsible for the endospore formation inside the *Bacillus subtilis.* And the remaining genes are found to perform the function of endospore formation. Apart from the sigma factors, a very common sigma factor (CHY 1519) which was earlier thought to have associated to perform sporulation activities is linked with the heat shock [5].

Current and Future Strategies

The genomic studies of C.*hydrogenoformans* will allow us to know in detail the study of hydrogenogens, which relates to an essential industrial process for generating hydrogen. The carbon monoxide present in the environment promotes the formation of several proteins like CO-dehydrogenase, Fe-s protein and also CO-tolerant hydrogenase. The electrons formed in the oxidation of the carbon monoxide are transported through the Fe-s protein finally to the hydrogenase for the hydrogen production [11].

Hyperthermophilic enzymes serve as the models for their scientific studies by biologist, chemist, physicist who are undergoing research in understanding enzyme evolution, molecular mechanism for protein thermostability leading to efficient strategies protein engineering and biotechnological applications. Taq polymerase enzyme from *Thermus acquaticus*.

Although C.*hydrogenoformans* is included in firmicutes which has the ability to sporulate in the stressed environment and commonly it lacks the genes that which are present in remaining archaeons that sporulate. C.*hydrogenoformans* has become a basic model for the sporulation studies [5].

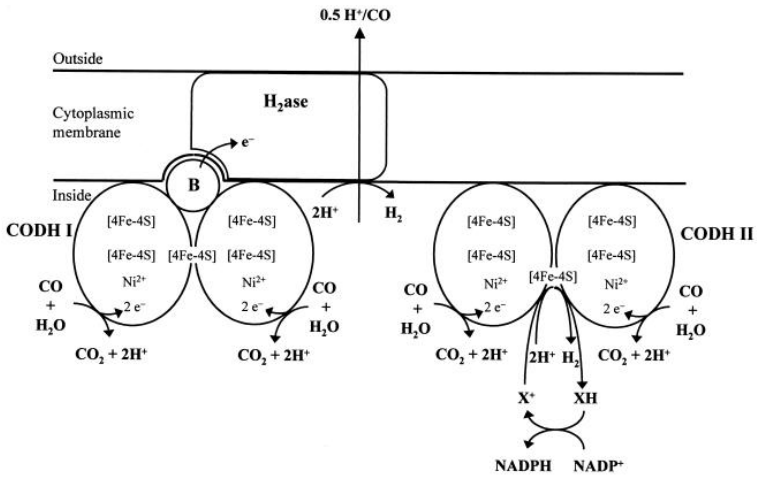


Figure 9: Presumed function of CODH.

Anaerobes like *C.hydrogenoformans* that have adapted themselves living in extreme environments under environmental stresses with their enzymes that have gained importance in terms of applications of biotechnology for production of fuel, which in turn based on the biomass of the substrates, and in the organic waste treatment procedures. These are the qualities examined by the biologist and there is need of more experimental studies to undergo in order to acquire better use of these organisms before they are accepted and appreciated by science and technology [12].

Selenocysteine Containing Protein

The selenocysteine protein and the selenocysteine insertion machinery along with selenocysteine t-rna are present in the genome of *C.hydrogenoformans*. The location of the selenocysteine insertion machinery is located next to the UGA codon and these selenocysteine proteins are found to be redox proteins. Two transporters and one methylated- DNA-protein-cysteine methyl transferase which is thought to function as the transfer of alkyl groups to cysteine that is normally present in every organism but whereas the *C.hydrogenoformans* contains selenocysteine in place of cysteine and studies are ongoing to get a clue that this selenocysteine is evolved from cysteine containing protein. Currently research is ongoing in finding out the new selenocysteine proteins [5].

Conclusion

In the present day, the industries are shifting from their use of non-renewable energy resources and looking for the use of renewable sources and also the market has a marked increase in need of thermostable enzyme production that has importance as the analytical tools and also as biocatalyst gained significance in large scale productions. The bacterium *C.hydrogenoformans* being highlighted in this particular situation as the bacterium is capable of living in not only the extreme environments but also the production of thermostable enzymes that are stable at high temperatures and performing different functions related to the metabolism of the organism by performing actions like utilization of carbon monoxide by oxidative stress reduction and by utilising water as the substrate in production of hydrogen gas, which is the final product that is gaining importance as a bio fuel in the current day industries [13].

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