



The Prospect of Obtaining Hydrogen Producing Bacteria from Antarctic Environment To Mitigate Global Warming: A Review

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ABSTRACT

The paper reviewed the diversity of psychrophilic bacteria in Antarctica, their physiological and metabolic adaptation to harsh conditions and temperatures below 0°C. The purpose is to look at the possibility of obtaining high hydrogen (H₂) producing bacteria from such environment to control greenhouse gas (GHG) emission and reduce climate change. Thermophiles and mesophilic bacteria have been frequently explored for H₂ productivity over the years. However only 50% of the theoretical biohydrogen yield (4 – 6 mol H₂/ mol_{glucose}) have been achieved by these bacteria so far. Screening cold adapted environments for novel H₂ producers could improve the availability of biohydrogen by tapping their acquired physiological and metabolic characteristics to boost the availability of this clean and cheap fuel. This is because continuous dependence on fossil fuels and other Green House gases producers as the sole future energy sources would be disastrous to generations yet to be born.

Keywords: Fossil fuel, renewable energy, biohydrogen, psychrophiles.

INTRODUCTION

Antarctica is one of the largest continents occupying an enormous kilometer of land. It is located at latitude 90° of the south pole. It is an ice-covered area surrounded by Southern ocean, the meeting point of Atlantic and Indian oceans (Eastman, 2013; Herdendorf, 1990). The continent is naturally occupied by high mountainous valley, high salt concentrated water bodies, permafrost and dry valley (Tedrow and Ugolini, 1966). The origin of Antarctic ice cover can be traced back to early centuries and it constitutes 90 % of the entire water on earth. This characteristic makes the continent the coldest with frequent fluctuations in temperature at different locations and restricts the survival of large terrestrial animals (HEBEL *et al.*, 2012; Petit *et al.*, 1999).

Researches and expeditions into Antarctic continent have reported the existence of microorganisms and lower plants like lichens and mosses in the polar regions. Their survival in such environment maybe because of their ability to adapt swiftly to different environmental conditions although, the diversity varies from one area of the continent to another depending on the prevailing environmental conditions.(HEBEL, *et al.*, 2012; Lange and Kappen, 1972). These

microorganisms are affected by various ecological factors like nutrient availability, salinity and water activity that are peculiar to such an environment (Cowan and Tow, 2004; Yergeau *et al.*, 2007).

The abundance of microbial lives in Antarctica decreases with increased altitude in some areas (Cowan and Tow, 2004). However, their survival in such critical prevailing environmental conditions and involvement in the biogeochemical cycle of compounds have been reported (Montross *et al.*, 2014). This discovery exposes the importance of these organisms and the benefits of their role in global ecology. Furthermore, the advent of metagenomics techniques that can probe the cells to identify their functional characteristics and phylogenetic origin have boosted inquiry into details of microbial lives. In this vein, unique genes, pathways and enzymes of microorganisms from different environments

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have been successfully explored and studied (Bastida *et al.*, 2014; Tseng and Tang, 2014).

Metagenomics have discovered a large number of noncultured bacteria from Antarctica (Handelsman, 2004; Neelakanta and Sultana, 2013). Non-cultivability denotes their viability in temperature below zero degree Celsius without multiplication due to prevailing environmental conditions. For this reason, several potentials of Antarctic microorganisms that remain untapped for a long time have been revealed. Details of their bioactive compounds and enzymes synthesis that can solve health, industrial and environmental problems have been discovered (Bhadury and Wright, 2004; Hentschel *et al.*, 2001).

The discovery of fossil fuel and its long-time use as a source of energy have immensely transformed the lifestyle of mankind over time. It has been the source of powering automobile machines for transportation and industrial activities for the provision of goods and services. Therefore, it has made human endeavours much easier than what it was in the early days. But the emission of heavy radiations and Greenhouse gases to the atmosphere by burning off the fossil fuels has continued to increase over decades. The accumulation of such pollutants in the atmosphere over time have negatively impacted the global climate by significantly raising the temperature of the earth beyond expectation (Alexander *et al.*, 2013; Jamelske *et al.*, 2015).

For example, the initial discovery and usage of fossil fuel witnessed 3×10^6 metric tons of CO₂ emission and the advent of industrialization has increased the emission due to increased consumption of fossil fuel. However, of the 250×10^9 tons of CO₂ emitted between 1751 and 1985, twenty-five percent emission was in 1950, fifty percent in 1973 and seventy-five percent in 1985 (Andres *et al.*, 1999). This trend continued at a geometric progression into the late 20th century and even the 21st century.

Over the years, increase in energy demand and population explosion, industrialization, and advances in technology have consequently increased mining and burning of fossil fuel leading to accumulation of radiations and GHG in the atmosphere. Climatic changes have been studied through decades of studies and observations on the effects of GHG on the

earth's temperature and the environment in general. These situations have unraveled the need for urgent attention to stop further effects and future problems (Abas *et al.*, 2015).

Researchers have also projected that the future energy consumption will continue to increase with fifty-two percent CO₂ emission in 2040 (EIA, 2013). This, in effect, would double the earth's temperature compared to the situation in 2005 (Capellán-Pérez *et al.*, 2014). The implication of such increase in temperature would further increase human health problems, insect-borne diseases, loss of biodiversity, flood and drought that have already been observed in different parts of the world (Greenstone and Looney, 2011). The environmental impact of long-time global energy monopoly and finiteness of fossil fuel have necessitated the interest in diversifying energy source in order to save the earth from future disasters (Abas, *et al.*, 2015; Rai *et al.*, 2014).

“Some of the challenges in meeting future energy needs and addressing climate change will need to be met through the development of bioenergy. The power and diversity of microbial metabolism, coupled with metabolic engineering and synthetic biology, can be used to produce a panoply of different biofuels from a variety of the possible substrate.” (Zannoni and De Philippis, 2014)

The movement from subsistence to mechanized farming has increased food production and processing of such products generates enormous wastes and biomass that can serve as microbial substrates for degradation into energy. Therefore, the diversity of substrates that can be acted upon by microbes has increased the chances of obtaining other sources of energy which may reduce dependence on fossil fuel (Anselmo Filho and Badr, 2004; Parfitt *et al.*, 2010). For example, global estimates have shown that agriculture alone produces 10-50 billion tons of lignocellulosic biomass from processing crop plants produced annually (Agbor *et al.*, 2014). This enormous amount of waste can be transformed into wealth by conversion to hydrogen through microbial fermentation.

The energy density of hydrogen is 122kJ/g which is 2.75 times greater than fossil fuels. This characteristic and the release of water as

by product of combustion makes hydrogen the suitable alternative to fossil fuels (Alvarado-Cuevas *et al.*, 2013; Debowski *et al.*, 2014; Laocharoen *et al.*, 2014). In addition, it can be used as fuel for vehicles and aircrafts, electricity generation and upgrading crude oils to reduce GHG emission (Singh *et al.*, 2015; Karapinar and Fikret, 2006). Large proportion of available hydrogen worldwide are products of fossil fuels refining, which are expensive and hazardous processes due to high combustion energy required and emission of heavy radiations at the end of the day (Alvarado-Cuevas *et al.*, 2013; Cheng *et al.*, 2015).

Several microbial fermentation techniques have been used for hydrogen production and these methods include; dark fermentation, photo fermentation, a combination of dark and photo fermentation which employ the use of different species of thermophilic and mesophilic bacteria. Despite the effort, only 50% of the theoretical biohydrogen yield ($4 - 6 \text{ mol H}_2 / \text{mol}_{\text{glucose}}$) have been achieved so far. However, this challenges of low productivity and yield have prevented putting climate changes under control due to the fact that the rate of biohydrogen production cannot commensurate with the existing hazardous sources of energy (Kobayashi *et al.*, 2011; Özgür and Peksel, 2013; Zhao *et al.*, 2011).

The need for the production of sustainable hydrogen as a source of energy can be improved by intensifying research effort into the isolation of bacteria from psychrophilic environments. The acquired functional and physiological characteristics from their harsh environments makes them better candidates for industrial production processes. Such efforts would augment the productivity of thermophiles and mesophiles and increase hydrogen availability. This work is necessitated by the absence of research attention to psychrophiles in terms of hydrogen production in an attempt to bridge global warming (Alvarado-Cuevas *et al.*, 2014).

This paper, therefore, intends to review the diversity of microorganisms in Antarctica, their physiological and metabolic functions and the possibility of getting high hydrogen producing bacteria to mitigate CO₂ emission.

Diversity of Microorganisms in Antarctica

The last two decades have experienced an increase in the exploration of Antarctic habitats to understand the role and importance of the microbial communities inhabiting them. Experimental reports have revealed the abundance of microorganisms in different parts of these habitats with its soil alone containing up to 10^9 cells g⁻¹ (Bajerski and Wagner, 2013).

The advent and use of various metagenomic techniques in the search for microorganisms have improved and uplifted understanding of the characteristics of Antarctic bacteria. They have also revealed novel organisms of industrial importance and their biocatalytic products. These techniques have doubled the number of bacteria species through phylogenetic analysis of 16S rRNA of samples collected from oceans, soil, lakes and marine environments (Neelakanta and Sultana, 2013).

Neiderberger *et al.* (2015) investigated microbial communities in the Antarctic soil. Substantial numbers of *Cyanobacteria* species were isolated from moist soil while the dry land gave more of *Acidobacteria*, *Actinobacteria*, *Deinococcus*, *Bacteroides*, *Firmicutes* and *Nitrospora*. The constant wetting of the soil and the abundance of chlorophyll-A were observed to be the reasons behind the presence of photosynthetic bacteria in the wet soil which were more than those in the dry soil. This result demonstrated the importance of water and light to the survival of photosynthetic bacteria.

The research work of Archer *et al.* (2014) examined the bacteria communities in Antarctic ponds. This gave another clue to the diversity of microorganisms in that environment. Distinct bacteria species were found in different levels depending on the depth of the water body. However, the surface samples showed more of *Bacteroides* while *Proteobacteria* were in abundance in the inner depth of the water. The presence of salt tolerant species like *Cyanobacteria* was observed due to high salt concentration in the medium.

Diverse bacterial species in Antarctic terrestrial environment were also reported by the research work of Lars *et al.*, (2014). Though, the soil was deficient in carbon and nitrogen sources, it was reported that NaCl concentration and pH were the factors that determined the distribution of the microorganisms in that habitat. In their report, salt concentrated areas with low pH

values were dominated by *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*.

The study of environmental factors that affect the diversity of microorganisms in the permafrost was investigated by Bakermans *et al.* (2014). Despite the fact that it is hard to sample microorganisms from this area, it was reported that 10^3 viable cells of *Acidobacteria* and *Gemmatomonades* were isolated from one gramme of the soil between the temperature range of -5°C and 15°C in the laboratory. The survival of these psychrophiles at such critical temperature conditions was due to alteration of their genetic make up which transform and confer them with protective abilities.

Furthermore, Bottos *et al.* (2014) focused on the diversity of airborne bacteria in the desert soil of Antarctica. Using the molecular technique, *Bacillus* and *Clostridium* were seen in the aerosol while *Actinobacteria* was found in the ground sample. Few colonies of *Bacteroides*, *Cyanobacteria* and *Acidobacteria* were also found in the soil. The aerosol species was found to have originated from the same environment due to the fact that they did not share a genetic resemblance with other species elsewhere.

In a similar work, Vasileva-Tonkova *et al.* (2014) studied the diversity of bacteria in terrestrial soil of Antarctic environment using both the old culture and the new metagenomic techniques. Two different groups of bacteria with distinct optimum growth temperature were observed. Among the 11 species isolated, the psychrophiles showed optimum growth temperature from 4°C to 30°C whereas psychrotolerant species grew best at optimum range of 15°C and 30°C . The obtained bacteria species were *Pseudomonas*, *Steriohophomomas*, *Brevundimonas*, *Sporosarcina*, *Microbacterium* which were found to be tolerant to high salt (NaCl) concentrations as high as 0.3-3.5 %.

In addition, Ayala-del-Río *et al.* (2010) analyzed the metabolic capacity of *Psychrobacter arcticus* isolated from permafrost as cold as -10°C . Reduced metabolic reactions and transport of nutrients across the cell surfaces were observed. It was also reported that the bacteria re-strategised to survive the low temperature by synthesizing membrane factors that favoured the metabolism

of acetate as the only available food source. It therefore, meant that the bacteria shifted to a different pathway apart from glycolysis to be able to break down the new carbon source for energy. Secondly, fatty acids and waxes were produced by the bacteria for protection against frequent freeze-thaw.

Furthermore, Fan *et al.* (2013) examined bacteria community in three different soil samples of Antarctic island by culturing the isolates using the traditional and metagenomic techniques. The genetic origin of 70 species was traced to their ancestors in the cold environment. However, the species observed were facultative aerobes that included *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Gammaproteobacteria* and *Betaproteobacteria* that had optimum growth between 5°C and 15°C . Microscopic analysis revealed rod and cocci shaped bacteria although the sequence identity of most isolates was not fully achieved; a problem associated with the accuracy of the machine used.

The research effort of Ganzert *et al.* (2014) in unveiling bacteria diversity in permafrost with pyrosequencing technique found 82 different groups belonging to *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Chloroflexi* and *Chlorosis*. These bacteria were reported to have survived acidic medium of pH 6. The findings were in agreement with those of Archer *et al.*, (2014), Bakermans *et al.*, (2014) and Ganzert *et al.*, (2014). Halophiles were also found and it was suggested that permafrost soil is a potential source of bacteria of industrial importance.

Another research investigated the influence of surface organic matter on the availability of bacteria in an Antarctic Ocean (Jamieson *et al.*, 2013). Culture and molecular methods used in the research exposed one-third of the bacteria species common to the different parts of the ocean. The number of phyla was more in sediments while species diversity was higher in water with *Proteobacteria* being the most in number. The work revealed the importance and role of substrate availability in the distribution of bacteria in a marine habitat.

In general, the above literature review showed bacteria diversity in different parts of Antarctic habitats revealing that prevailing environmental factors faced by microorganisms

differ from one environment to another. This, in turn, determines the available microbial species in them. The survival of these bacteria and their adaptation to harsh conditions suggests their acquisition of some genetic, metabolic and physiological characteristics for survival in their environments.

Since it has been established that cold-adapted microorganisms have continuously been a source of novel materials for drugs development, it could also be inferred that this region could be an important source of hydrogen producing bacteria for sustainable energy production. This is because, the unique physiological properties of their environment (pH, pressure, temperature, osmolarity) and uncommon functional metabolic properties result in the production of bioactive substances different from those of thermophiles and mesophiles. The commitment of such novel microorganisms into H₂ production will further improve the present slow rate of productivity since these unique properties make them more stable in different environments and industrial applications. Although Antarctica has been a host of these novel bacteria and the advances in metagenomic techniques have improved the search for them, it is believed that the

environment still contains relatively large number of untapped reservoir of microorganisms with marvelous bioactivity.

Table 1 displays novel bacteria isolated from different parts of Antarctic terrestrial environment with optimum growth temperature ranges. With the differences in the prevailing atmospheric conditions of the various places, these microorganisms were able to thrive in those environments and take part in the transformation of nutrients and elements within the immediate community. The heterogeneity of the population in the environment was due to the abundance of organic matter. Most of these isolates appear to be psychrotolerant with optimum growth at temperatures between 5 and 30⁰ C which means they are facultative organisms that can grow in mesophilic environments. For this reason, their physiological and biochemical characteristics can be tapped for the benefit of our environment. The abundance of novel bacteria in Antarctica is evident because few among the diverse isolates were identifiable in the laboratory (Kirby, *et al.*, 2011).

Table1: Novel microbial species isolated from Antarctic soil

| Microbial Species | Taxonomic Affiliation | Source | Growth Range (°c) | Year |
|-------------------------------------|-----------------------|--|-------------------|------|
| <i>Brevibacillus levickii</i> | Actinobacteria | Geothermal soil, Mt Melbourne | 15-55 | 2005 |
| <i>Cryobacterium psychrophilum</i> | Actinobacteria | Antarctic soil | < 18 | 1997 |
| <i>Friedmaniella antarctica</i> | Actinobacteria | Sandstone, McMurdo Valleys | Dry 18-25 | 1997 |
| <i>Micromonospora endolithica</i> | Actinobacteria | Sandstone, McMurdo Valleys Mountains | Dry 8-39 | 2004 |
| <i>Modestobacta multiseptatus</i> | Actinobacteria | Soil asgard Range, Antarctic mountains | Trans 0-28 | 2000 |
| <i>Pseudonocardia antarctica</i> | Actinobacteria | Cyanobacterial mat, McMurdo Valleys | 7-38 | 2004 |
| <i>Streptomyces hypolithicus</i> | Actinobacteria | Hypolithic community, Miers Valley | 22-30 | 2009 |
| <i>Aequorivita sublithicola</i> | Bacteroides | Quartz rock | 2-25 | 2002 |
| <i>Flavobacterium antarcticum</i> | Bacteroides | Penguin habitat, King George Island | 5-24 | 2005 |
| <i>Flavobacterium segetis</i> | Bacteroides | Penguin habitat, King George Island | 5-21 | 2006 |
| <i>Flavobacterium weaverense</i> | Bacteroides | Soil, weaver Peninsula | 5-19 | 2006 |
| <i>Sejonia antarctica</i> | Bacteroides | Penguin habitat, King George Island | 4-28 | 2005 |
| <i>Sejonia jeonii</i> | Bacteroides | Moss sample, King George Island | 4-31 | 2005 |
| <i>Alicyclobacillus pohliae</i> | Firmicutes | Geothermal soil, Mt Melbourne | 42-60 | 2008 |
| <i>Areuniliballus terranovensis</i> | Firmicutes | Geothermal soil, Mt Melbourne | 20-55 | 2005 |
| <i>Sporosarcina antarctica</i> | Firmicutes | Soil, King George Island | 0-23 | 2008 |
| <i>Pseudoalteromonas antarctica</i> | γ-Proteobacteria | Mud, Admiralty Bay, King George Island | 4-30 | 1997 |
| <i>Psychrobacter frigidicola</i> | γ-Proteobacteria | South Shetland Island | 4-30 | 2007 |
| <i>Psychrobacter urativorans</i> | γ-Proteobacteria | Penguin colony, Vestfold Hills | 18-22 | 1996 |
| | γ-Proteobacteria | Penguin colony, Vestfold Hills | 10-25 | 1996 |

Source: Kirby *et al.* (2011)

Metabolic Mechanisms of Bacteria Adapted to Cold Temperature

It is discovered that the exposure of microorganisms to cold-temperature environments has transformed their cytoplasmic membranes as well as enzymatic functions because of the prevailing unfavorable conditions. This condition changes the growth status of the individual species to a static stage

in which the bacteria respire without multiplication (De Maayer *et al.*, 2014). The consequences of such growth are a blockage of fluids and waste passages coupled with abnormal biochemical functions (Goodchild *et al.*, 2004; Ratkowsky *et al.*, 2005). Therefore, long time exposure of both psychrophilic and psychrotolerant bacteria to cold environments confers on them certain acquired characteristics and adaptations. However, some of the

challenges faced by these microorganisms in these environments and the mechanisms they use to overcome them are discussed below

Disruption of normal fluid flow across the membrane

Cold temperature habitat triggers the formation of saturated fatty acid in the cell membranes in the bit to protect the organelles from freeze-thaw effects. This development coagulates the fatty acids against the membrane pores thereby blocking and slowing down the rate of movement of dissolved food substances and fluid across the membrane. This factor also lowers metabolic activity of the organism. However, to cushion such effects in order to enhance the free flow of fluids and food substances, psychrophiles synthesize saturated fatty acids with short and branched acyl chains which insulate the cell organelles from the effects of freeze-thaw hence, creating an enabling environment for the cellular exchange of materials (Chattopadhyay, 2006; D'Amico *et al.*, 2006).

Disruption of transcription and translation processes

From the basic knowledge of enzymology, it is known that enzyme activity is lowered at low temperature due to lowering of the activation energy which consequently affects the metabolic functions of the cells. This effect interferes with normal transcription and translation mechanisms which serve as means for exchange of ions and molecules within the cells and between cells and their environment. However, these bacteria by-pass such hindrances by increasing the secretion of RNA polymerase, elongation factors and peptidyl proteins to maintain their catalytic activities (Chattopadhyay, 2006).

Changing their nutritional requirements is another mechanism used by the organisms to cushion such effects in order to survive adverse conditions. The reason is that low-temperature limits the availability and the rate at which organisms can access food substances (Bakermans *et al.*, 2012). They also change metabolic pathways used in degrading substrates to be able to metabolize the available nutrient. Since the prevailing environmental factors makes it difficult to degrade carbohydrate at low temperature values, microorganisms in these environments tend to

modify synthesis mechanisms from the expression of genes for a glycolytic pathway to that of the available substrate. For example, *Pseudomonas articus*, a psychrophile that is isolated from permafrost soil survives the temperature of -10°C by metabolizing acetate that is easily accessible in that environment. Other substrates that could be metabolised at low temperatures include proline, tryptophan and methionine which can easily move across the membrane (Ayala-del-Río, *et al.*, 2010; Bergholz *et al.*, 2009). These substrates also help to energize the cellular mechanism to produce cold-shock proteins that prepares and familiarize the microbial cells against the harsh environment. In addition, Bergholz, *et al.* (2009) and Ponder *et al.* (2005) reported that acetate as a microbial nutrient at low temperatures enhances unsaturation of membrane fatty acids and exopolysaccharides which invariably clears the friction in membrane pores and allow free movement of fluids.

Finally, cold-adapted microorganisms have adopted different physiological and metabolic mechanisms for their survival and these adaptations could be genetic or acquired. These generally transform the movement of metabolites within and outside the organisms, enhance transcription and translation processes, protein synthesis, freeze-thaw protection and fatty acid metabolism which better their chances of survival in harsh environments. These changes brought about by these adaptations diversify and stabilize the functional capabilities of these organisms which make them better candidates for industrial application. This acquired stability, in general, can improve hydrogen production by psychrophiles than mesophiles and thermophiles especially when H_2 producing species are obtained from cold environments.

Hydrogen Production Abilities of Different Bacterial Species

Hydrogen is a renewable energy source that is produced through microbial fermentation/conversion of organic/inorganic substances. For organic materials, different substrates such as lignocellulosic biomass and industrial wastes are degraded by microbes to produce H_2 . While, in the case of inorganic substrates, individual species of microorganisms called photosynthetic bacteria

convert the radiant energy from the sun to H₂ (Nelson and Junge, 2015; Rosales-Colunga *et al.*, 2012). This unique bacterial ability is catalysed by their possession of photosynthetic pigment (bacteriochlorophyll) in the cell membrane.

Hydrogen as a clean energy source can also be produced by other processes such as cracking of natural gas, oxidation of hydrocarbons and coal gasification. However, the fact that the microbial process of production is inexpensive and environmentally friendly makes it much more preferable to the expensive methods of production mentioned earlier (Seelert *et al.*, 2015). In addition, the latter methods lead to production of water rather than CO₂ that has an adverse atmospheric impact (Alvarado-Cuevas *et al.*, 2013). Also, biological fermentation cleans up wastes from the environment thus converting waste to wealth (Ciranna *et al.*, 2014). The high calories of energy (142kJor 61,000Btu/lb) generated from H₂ obtained from these wastes makes them a better energy source than the one obtained from fossil fuel (Alvarado-Cuevas, *et al.*, 2013; Zhao, *et al.*, 2011).

Dark and photo-fermentation, as well as the combination of the two, are the main microbial processes that were used for the production of H₂ and better productivities with an average yield (50%) of the expected yields being obtained from them (Assawamongkholsiri and Reungsang, 2015; Özgür and Peksel, 2013; Singh *et al.*, 2010). The difference in these processes is that dark-fermenters can only perform the process in the absence of light while photo-fermenters are more active in the process when light is available. The latter have dual advantage of conversion of both sunlight and organic substrates to H₂ being an autotrophic organisms. The last method which combines the first two techniques was later introduced to further improve productivity.

The H₂ productivity of different bacterial have been investigated through research efforts to improve hydrogen production. The productivity of those bacteria is summarised in Table 2 below.

Table 2 shows different H₂ producing bacterial species and their optimum temperatures as well as the yields and productivities. The psychrophile among the bacteria was isolated at 4^o C and subjected to H₂ production at 25^o C which shows that the only cold adaptive microbe among the H₂ producing species is a facultative bacteria that can survive the two temperatures and can give the desired product at 25^o C.

The bacteria were isolated from different temperature environments and have shown capabilities in degradation of various carbohydrate and nitrogen sources with better hydrogen yield but not close to the theoretical rate. Although, H₂ productivity and yield of the psychrophile is low due to decrease substrate consumption rate of the bacteria and effects of metabolites, their facultative nature with a growth range between 4-25^o C proves their potential in H₂ productivity (Alvarado-Cuevas, *et al.* (2014).

Since psychrophiles are usually exposed to stress and other harsh environmental challenges, they acquire unique functional characteristics that place them on top of thermophiles and mesophiles as microorganism with high commercial potentials. This, in effect, has led to the development of significant research interest in their biocatalytic properties. In fact, it has been established that such practical advantages have given psychrophilic enzymes high thermal stability and adaptation to low temperature conditions that invariably confer on them better production capabilities than the other species. A typical example of such properties is evident in applications of psychrophilic enzyme in pharmaceutical industries for the production of potential drugs (Van Den Burg, 2003). Zyconotide was the first drug for the treatment of a tumour, viral and inflammatory diseases. It was prepared from a secondary metabolite of cone snail obtained from marine water. However, marine psychrophiles have been reported to have multiple cold adaptive potentials with high productive properties that could be applied in solving the future environmental challenges (Muffler *et al.*, 2015).

Table 2. Hydrogen productivity of different bacteria isolated from various area

| Bacteria | Type | Temp. °C | Substrate | H ₂ Yield/Productivity | Reference |
|--|--------------|----------|-------------------------|--|---|
| <i>Clostridium sp.</i> | Mesophile | 37 | Glucose | 2.61±0.1molH ₂ /3680±ml/L/h | <u>Patel <i>et al.</i> (2015)</u> |
| <i>Rhodobacter sp.</i> | Mesophile | 25 | Malic acid | 3.88 mol H ₂ /mol /1339 ml H ₂ /l | <u>Assawamongk holsiri and Reungsang (2015)</u> |
| <i>Rhodobacter capsulatus</i> | Mesophile | 37 | Lactate | | <u>Merugu <i>et al.</i> (2010)</u> |
| <i>Clostridium amygdalinum</i> | Halophile | | xylan | 40 mmol H ₂ /g | <u>Jayasinghearac hchi <i>et al.</i> (2010)</u> |
| <i>Klebsiella pneumoniae</i> | Mesophile | 37 | Glucose | 482 ml/l/h /2.07 mol/mol | <u>Niu <i>et al.</i> (2010)</u> |
| <i>Clostridium ramosum</i> | Thermophile | 37-55 | Glucose | 1.8 and 1.0 mol H ₂ /mol | <u>(Puhakka <i>et al.</i>, 2012)</u> |
| <i>Thermoanaerobacterium thermosaccharolyticum</i> | Thermophile | 60 | Xylose | 2.09 ± 0.02 mol H ₂ / mol /3020 ± 210mL H ₂ /L | <u>Khamtib and Reungsang (2012)</u> |
| <i>Clostridium sp</i> | Thermophile | 43 | Glucose | 269.3ml/H ₂ /L /2727ml/L | <u>Cai <i>et al.</i> (2013)</u> |
| <i>Rhodopseudomonas pentothentatexigens</i> | Thermophile | 40 | Malic & Glutamic acid | 68.28%/92.22% | <u>Phankhamla <i>et al.</i> (2014)</u> |
| <i>Janthinobacterium agaricidamnorum</i> | Psychrophile | 25* | Tryptophan | 1.57mol H ₂ /mol/253.3ml | <u>Alvarado-Cuevas, <i>et al.</i> (2014)</u> |
| <i>Clostridium butyricum</i> | Mesophile | 37 | Glucose & yeast extract | 2.67molH ₂ /mol/ | <u>Junghare <i>et al.</i> (2012)</u> |
| <i>Clostridium sp</i> | Alkalophile | 37 | Glucose | 2.71 mol H ₂ /mol/0.55Mm-H ₂ /h | <u>Mei <i>et al.</i> (2014)</u> |
| <i>Clostridium sp</i> | Alkalophile | 37 | Wheat straw | 2.61 ± 0.1 mol H ₂ /mol/3270 ± 100ml/L/h | <u>Patel, <i>et al.</i> (2015)</u> |

There are differences in substrate preference between the dark and photo-fermenters. The dark fermenters mostly prefer complex carbohydrates such as cellulose, xylose and glucose. Their efficiency in the degradation of these complex sugars is reported by many researchers (Jiang *et al.*, 2015; Mei *et al.*, 2014; Patel, *et al.*, 2015). In most of these reports, the bacteria widen their substrates for H₂ production ranging from such complex carbon containing substrates like wheat, palm oil sap, rice straw, corn stover to pulp/paper primary sludge (Moreau *et al.*, 2015; Noparat *et al.*,

2011; Pérez-Rangel *et al.*, 2015; Zhang *et al.*, 2015). In terms of H₂ productivity/ yield, 1973 ml/cellulose/140.9 ml/g, 2727ml/L/269.3 mL H₂/L/h and 3020 ± 210 mL H₂/L/3.95 ± 0.20 mmol H₂/L/h were reported for *Clostridium beijerinckii* PS-3, *Clostridium sp* 6A-5 and *Thermoanaerobacterium thermosaccharolyticum* KCU19 respectively (Cai, *et al.*, 2013; Khamtib and Reungsang, 2012; Noparat, *et al.*, 2011). These figures show that the dark fermenters generally are good producers of H₂ with high yield although, their productivity is sometimes hindered by the

metabolites they produce during the process. However, these metabolites happen to be the substrates that are preferred by photo-fermenters.

On the other hand, photo-fermenters prefer simple substrates like malate, acetate, glutamate and lactate. High tolerance to acidic medium also widens their scope of metabolic substrates to include olive mill waste water, leather industrial effluent and marine organic waste water (Cai and Wang, 2012; Eroglu *et al.*, 2011; Merugu, *et al.*, 2010). Such media were also reported to be rich in nitrogen and metals that support the growth of the bacteria species. The fermentative capabilities of photosynthetic bacteria in terms of degradation of acidic effluent and sludge suggest that they are more efficient than dark fermenters.

It has been observed that the H₂ productivity of bacteria is at its best at late exponential and early stationary growth phases and this has led researchers to immobilize cells to extend these phases in order to improve hydrogen productivity (Junghare, *et al.*, 2012; Phankhamla, *et al.*, 2014). For instance, Liu *et al.* (2011) investigated immobilized cells of photosynthetic bacteria with the aim of improving H₂ productivity. In their work, the cells of *Rhodospseudomonas fecalis* were immobilized and 3.15 mol H₂/mol acetate was reported as the highest yield. This result proved that the technique can enhance H₂ productivity especially when the factors such as pH, substrate and light were optimized.

In a similar work, Seelert, *et al.* (2015) investigated H₂ productivity of immobilized *Clostridium beijerinckii* and observed a reduction in the lag phase which reduced the initial static bacterial growth phase of familiarization and production of metabolic enzymes that led to increased exponential phase thus increasing the rate of production of hydrogen. However, the respective productivity and yield of 0.6 to 3.2 mmol/L/h and 2.1 ± 0.7 mol H₂/ mol glucose were small when compared to the 2.09 to 27.1 mmol/L/h obtained by Rittmann and Herwig (2012). This was probably due to the inhibitory effects of ethanol and acetate that were produced as metabolic waste products.

The challenges associated with immobilization of cells is their inability to be recycled. The

cells cannot be re-collected from the medium and re-used for another production. However, their production potentials can be improved by eliminating pathways for the production of other metabolites by genetic manipulation of the cells. It has also been observed that further research is required in terms of substrate uptake by immobilized cells and conversion efficiency to improve the H₂ productivity of this technique (Seelert, *et al.*, 2015).

Frequent research into H₂ production potential of bacteria has also unraveled the significance of hydrogenase and nitrogenase enzymes in substrates metabolism. Therefore, many research work have been carried out on these enzymes in order to increase their substrate degradation efficiency through genetic manipulation. Some of these research include that of Seol *et al.* (2014) that investigated the production efficiency of genetically altered *Escherichia coli* BW25113. This research work succeeded in knocking out the gene for NADPH which invariably transferred the metabolic mechanism of the bacteria to Entner-Doudoroff pathway thereby increasing the efficiency of pyruvate production. However, 1.03 mol/mol of glucose was reported as the H₂ yield.

Similarly, the research work of Trchounian and Trchounian (2013) examined the efficiency of hydrogenases in genetically altered *Escherichia coli* using mixed and single carbon sources as substrates at different pH values. Specific hydrogenase genes (Hyd-4 and Hyd-2) of the altered *E.coli* using glucose and glycerol as substrate showed H₂ productivity at pH 5.5.

Finally, although the reviewed techniques aimed at the production of H₂ by various microorganisms showed that productivity in one way or the other has been enhanced, the productivity is still not good enough to satisfy the growing population explosion, industrialization and advances in technology due to increased demand. Therefore, to augment and improve the production capability of thermophiles and mesophiles that are frequently investigated for H₂ productivity and improve availability, identification, isolation, characterization and optimization of production parameters of psychrophilic H₂ producing species from cold environments must be upheld by global research community. This, in no small measure, could bring positive changes

into obtaining the quantity and quality of clean energy for the present and future global demand.

CONCLUSION

The possibility of obtaining novel hydrogen producing bacteria from cold adapted environment was investigated in this review. Different bacteria species and their mechanisms of adaptation to harsh psychrophilic environment have been explored. The H₂ productivity of thermophiles and mesophilic bacteria using dark, photo, combine dark and photo and cell immobilization techniques have been investigated for quite long. But the bulk of available H₂ comes from the hazardous fossil sources. The reason being that the H₂ yield of these bacteria still remains at 50 % of the theoretical or expected value. Therefore, investigation of novel H₂ producing psychrophilic bacteria may boost the availability of this clean energy source due to acquired physiological and metabolic characteristics from their harsh environmental conditions. This can serve as a measure for controlling GHG emission and future climate change.

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