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Review Article **Open Access**

Potentials and limitations of cold-adapted hydrogen producing bacteria: a mini review

*[1]Mohammed, A., [2]Abdul-Wahab, M. F., [1]Mohammed, J. N., [1]Mohammed, I, L., [1]Sani, R. A., and [1]Majiya, H.

[1]Department of Microbiology, Faculty of Natural Science, Ibrahim Badamasi Babangida University, P. M.B 11, Lapai, Niger State, Nigeria
[2]Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia
Correspondence to: ibrahimnusaiba2@gmail.com

Abstract:

Low-temperature bacteria have potential to produce biohydrogen and are often considered a potential renewable energy generator for the future. However, the bacteria have presented poor hydrogen yield due to slow metabolic rate and prolonged lag phase often caused by their restricted growth temperature limit. The ineffective search for new biocatalysts from cold environments and the application of modification techniques almost jeopardize the economic viability of these strains in the biohydrogen production research. This article examined cold genetic and enzymatic adaptation potentials that led to the continuous expression of novel biocatalysts of biotechnological importance under the following headings; cold-adapted bacteria, biohydrogen-producing bacteria, strategies for adapting to stress in low temperatures, performance of cold-adapted bacteria in biohydrogen production, challenges of cold-adapted bacteria in biohydrogen production and future prospect. Finding new strains and studying their unique properties can improve the efficiency of hydrogen production by cold-adapted bacteria, as this new area has not yet been extensively studied.

Keywords: low-temperature bacteria; cold-adapted bacteria; temperature; mini-review

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Potentiels et limites des bactéries productrices d'hydrogène adaptées au froid: une mini revue

*[1]Abdullahi, M., [2]Abdul-Wahab, M. F., [1]Mohammed, J. N., [1]Mohammed, I. L., [1]Sani, R. A., et [1]Majiya, H.

[1]Département de Microbiologie, Faculté des Sciences Naturelles, Université Ibrahim Badamasi Babangida, P. M. B. 11, Lapai, État du Niger, Nigéria
[2]Département des Biosciences, Faculté des Sciences, Universiti Teknologi Malaisie, 81310 Johor Bahru, Johor, Malaisie
*Correspondance à: ibrahimnusaiba2@gmail.com

Résumé:

Les bactéries à basse température ont le potentiel de produire du biohydrogène et sont souvent considérées comme un potentiel générateur d'énergie renouvelable pour l'avenir. Cependant, les bactéries ont présenté un faible rendement en hydrogène en raison d'un taux métabolique lent et d'une phase de latence prolongée souvent causée par leur limite de température de croissance restreinte. La recherche inefficace de nouveaux biocatalyseurs à partir d'environnements froids et l'application de techniques de modification compromettent presque la viabilité économique de ces souches dans la recherche sur la production de biohydrogène. Cet article a examiné les potentiels d'adaptation génétique et enzymatique au froid qui ont conduit à l'expression continue de nouveaux biocatalyseurs d'importance biotechnologique sous les rubriques suivantes; bactéries adaptées au froid, bactéries productrices de biohydrogène, stratégies d'adaptation au stress à basse température, performances des bactéries adaptées au froid dans la

production de biohydrogène, défis des bactéries adaptées au froid dans la production de biohydrogène et perspectives d'avenir. Trouver de nouvelles souches et étudier leurs propriétés uniques peut améliorer l'efficacité de la production d'hydrogène par des bactéries adaptées au froid, car ce nouveau domaine n'a pas encore été largement étudié.

Mots clés: bactéries à basse température; bactéries adaptées au froid; température; mini-revue

Introduction:

Due to its high energy density and friendly by-product (water) when burnt, hydrogen has attracted much attention as a desirable fuel (1). Among the renewable energies, hydrogen is more critical due to its sustainability and possession of 2.75 times the energy density of fossil fuels. Hydrogen can be produced using conventional and fermentation strategies. However, production through fermentation has emerged as an excellent strategy to extract hydrogen from the abundant waste for eco-friendly products (2). The biological hydrogen production processes are considered to be cost-effective due to the high production rate, low energy requirements and simple operation (3). Dark fermentation has exceptional advantages, such as the ability to produce hydrogen from the breakdown of organic waste, thus stabilizing waste with the potential for contamination (4).

Several bacterial strains are used to produce biohydrogen, which is the most promising for renewable energy generation. Cold-adapted bacteria can thrive at low temperatures (20°C and below) and stance unique constraints through flexible structural and conformational changes to proteins and lipids to express highly reactive enzymes (5). The ability of the bacteria ability to grow at low temperatures confers adaptive resilience to stresses including osmotic and low nutrients that affect other thermal species (6). Resistive loads may have enabled their use in industrial production at ambient temperatures (7), guaranteeing low-temperature operation, high productivity, and reduced production costs (8). Given this unique potential, understanding their biotechnological relevance in applied and biological research is fundamental. Furthermore, the widespread use of cold-active enzymes in industrial productions calls for evaluation of cold -adapted bacteria and elucidation of their potentials in renewable energies.

Using cold-adapted bacteria at low temperatures for biohydrogen production saves energy input in bioreactors, which has a significant impact on production costs (7). This new field of research thus underlines the potential of cold-adapted bacteria and, due to their resilience in fermentation, requires a review of their performance in numerous areas. The bacterial strain and its enzymes are currently used as biological tools in anaerobic digestion due to their economic and environmental advantages. However, their capabilities are not fully exploited in fermentative hydrogen production as low hydrogen yield have been recorded in anaerobic fermentation despite vast potential in biotechnological application (9). Therefore, this mini-review is an assessment of the numerous adaptation potentials of cold-active bacteria and their influence on fermentative hydrogen production.

Cold-adapted bacteria:

Cold-adapted bacteria live in cold places and grow well at or below 0-20°C. However, psychrophilic properties allow for rapid growth at temperatures below 15°C than at temperatures above 20°C. Most often, these types of microbiota colonize low-temperature areas like snow, permafrost, sea ice, and glaciers (10). For example, Joshi (6) reported minimal growth below 15°C and optimal growth at 25°C, while Ravi et al., (11) reported an upper growth limit of 37°C. This established the fact that growth temperature range varied between the bacterial species, leading to a division into psychrophilic and psychrotolerant strains (7). In addition, an unquantifiable number of culturable and non-culturable species, including Archaea and Eubacteria, are found in different environments (12).

In this review, cold-adapted bacteria refer to species that originate in a low-temperature environment but have the ability to grow below and above 20°C. These bacterial species adopt unique properties to perform metabolic activities that play important roles in the deep sea, Polar Regions, and frigid Alpines that make up three-quarters of the world. However, the bacteria are ubiquitous and could be found in the sparse cold habitats in temperate regions (13). Their special properties lead to novel catalysts that can improve industrial production techniques (14). Therefore, due to the biodegradability and non-toxicity of their products, they are considered potential reservoirs of biotechnological importance (15).

Biocatalysts are the power of industry due to the demand for dynamism in bioprocessing tools for numerous biochemical processes (8). Therefore, understanding the properties and functions of natural products of cold-adapted bacteria could expand innovative applications to various industries such as food, agriculture, chemicals, and pharmaceuticals (16). In agriculture, their potential has been used to support plant growth and remove waste from

the environment (17). These natural products have shown a wide range of uses in agriculture, medicine, pharmacy, and other fields. The productive abilities are cheap and sustainable sources of products critical to providing basic services to humankind. Therefore, the characteristic splitting of energy into its enthalpy and entropy could be used to increase hydrogen production. Research interests in these bacteria arise from the fact that their enzymes can be used at low temperatures and their thermal stability as well as their rapid and fascinating growth within a short period of time.

Biosynthesis of biocatalyst in psychrophilic and mesophilic states:

Scientists are still studying bacteria mechanisms to gain insight into harvesting biomolecules from cold environments. Cold-adapted bacteria thrive in a broad state (20°C and below) covering cryophilic and psychrophilic temperature ranges and in a finite state in a mesophilic environment (25°C to 37°C). Cold sensations activate histidine kinase to produce aspartate responsible for the transcription of cold genes. It involves proteins transformation in the membrane and the cytoplasm of the cells to create the enzymes needed for the process (18, 19).

Psychrophilic and mesophilic states have opposite influence on the functions of the cold-bacteria and the biosynthesis of biocatalysts. Studying such microbial processes in cold environment will expose salient innovation to improve industrial productivity (20,21). In the psychrophilic state, exposure to interconnected factors such as salinity, alkalinity, high ionic concentration, low nutrient levels, and cryophilic effects drive the reaction to synthesize many adaptable biocatalysts (22,23). This leads to the synthesis of many and new biocatalysts depending on changing environmental stressors. On the other hand, mesophilic state exposes cold-loving bacteria to a harmful state to the cells due to the disruption of cellular processes such as protein synthesis, nucleic acid structure, cold enzymes, and cell division (24). This energy-intensive process redirects the nucleic acids to synthesize more stabilizers to protect the cells instead of synthesizing adaptive biocatalysts. Fig 1 represents the different biocatalysts synthesized by cold-adapted bacteria under psychrophilic and mesophilic conditions.

In the psychrophilic state, more biocatalysts for adaptation to a variety of stressors are expressed than protective proteins. Stressors in the psychrophilic state are intertwined leading to the emergence of new biocatalysts that could be explored for industrial production. Because of the complex interdependent factors that influence the expression many cold genes in the genomes of cold bacteria which trigger the synthesis of unique biocatalysts to adapt to the conditions, a cold environment has become the target of novel biocatalysts of biotechnological importance.

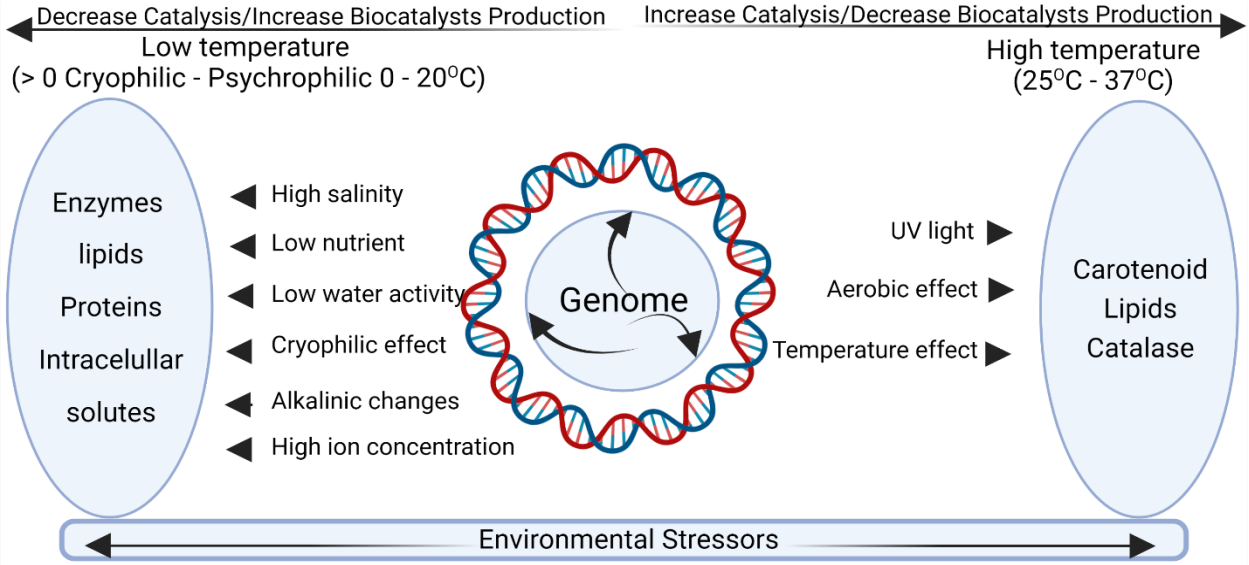

Fig 1: Biocatalysts production by cold adapted bacterial at opposite temperature conditions

Biohydrogen-producing bacteria:

Current routes to hydrogen production rely heavily on fossil fuel conversion and are energy-intensive and expensive. The various methods for hydrogen production are as outlined and classified in Fig 2. In order to reduce the atmospheric impact of the emitted greenhouse gas, more importance is attached to biological pathways with microorganisms as catalysts. Microbial processes are considered safe and inexpensive for hydrogen production because they break down carbohydrates with less energy (25). Several bacteria have been used for biohydrogen production and their catalytic efficiency depends on the temperature limits they can tolerate.

The fermentation processes are the most cost-effective and rely on the use of microorganisms as biocatalysts in the production process (26). However, most bacteria cannot produce hydrogen at temperatures of 20°C due to the inactivation of catalytic activity. This led to exploring production by using cold-active bacteria as a new field of research to boost hydrogen production. An improvement in hydrogen productivity was observed due to the equivalence of the hydrogen produced by some cold-adapted bacteria and that of other thermolabile strains (27,28). However, the efficiency of hydrogen production is challenged by the slow catalytic activity and increased lag time. Therefore, attention is currently focused on fermentative and genetic strategies that can improve their activity to maximize their potential for hydrogen yield.

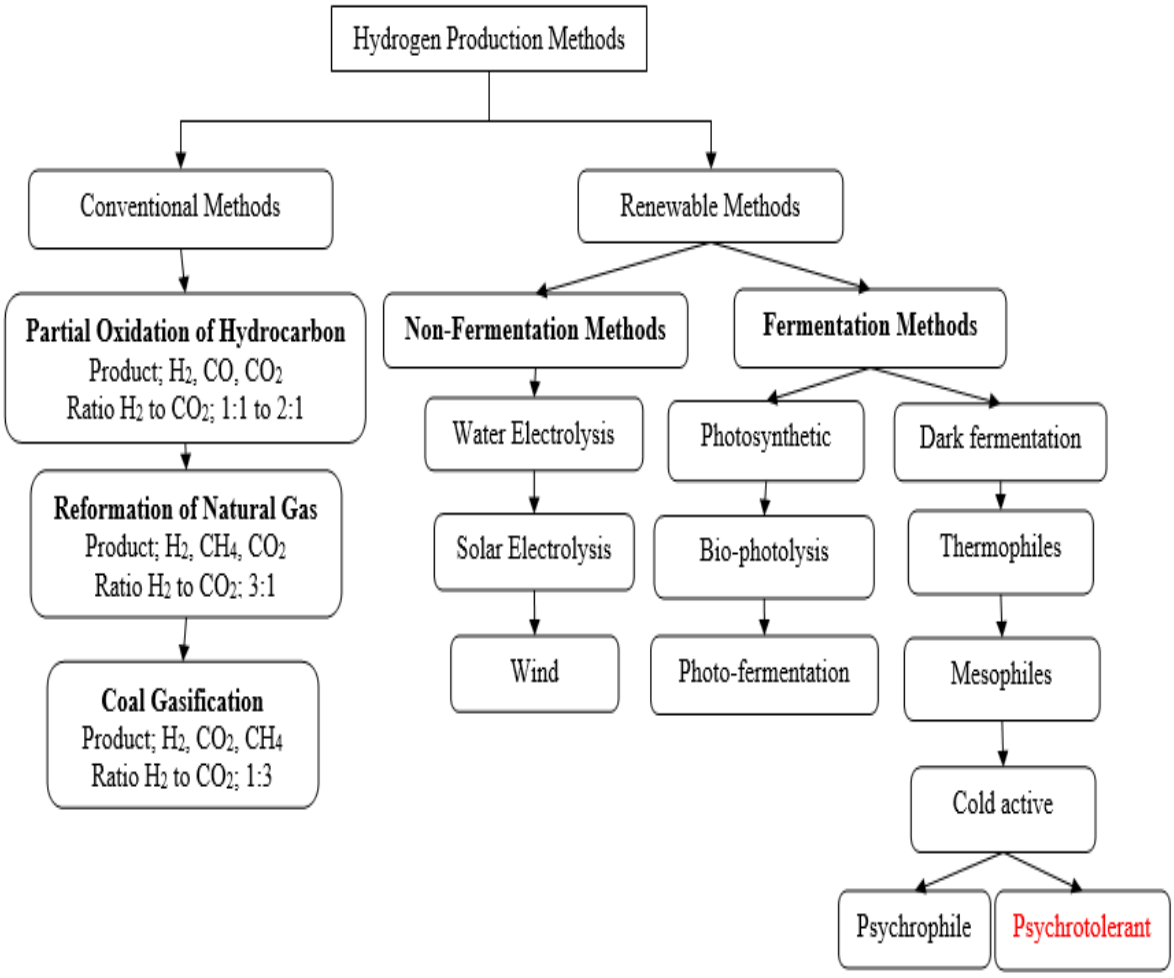

Fig 2: Different hydrogen-producing methods and thermolabile bacteria utilized

Strategies for adapting to stress in low temperatures:

Genetic modification:

Eight independent studies included in this mini-review revealed new genes that encode and are expressed in the cold for new properties at low temperatures. These new biocatalysts with biotechnological potential indicate the existence of limitless properties in these strains for industrial exploration. The genes expressed under cold conditions and the functional proteins and enzymes they encode are summarized in Table 1.

Ijaq et al., (29) examined the genome of *Pseudomonas* sp. Lz4W and discovered genes encoding hypothetical proteins (HPs) whose function has not yet been characterized. The authors discovered that HPs have the function of enhancing membrane stability and the movement of solutes across the membrane in cold areas. The proteins performed this function by distorting the HPs as the temperature rise or fall. It has been observed that at low temperatures, HPs expression increases for the flexibility and survival of the bacteria under cold stress. This finding reveals the hitherto unknown function of HPs in the membrane of cold-adapted bacteria.

Jiang et al., (30) examined the genome of the cold-adapted *Arthrobacter* Z1-20^T, and discovered the abundance of capA genes encoding osmo-protective glycine betaine and cold shock proteins. These novel genes produce clusters and higher copies of lysine as a diamino acid to adapt to cold environments. This study showed that the genes code for essential solutes in addition to proteins for protection in cold environments.

Dai et al., (31) sequenced the genome of *Nesterenkonia* to discover the survival strategies of the bacterium in polar environments. Dissection of the bacterial genome revealed genes encoding for NES-AT protein, which facilitates carbon utilization under nutrient-limited conditions. In addition, the genes that improve glucose metabolism and biofilm production for stress resistance were also found. The study unveils cold-temperature genetic mechanisms for nutrient degradation and how carbon sources are harvested under low-temperature conditions.

Papale et al., (32) studied the genes of cold-adapted *Arthrobacter* sp for making a compound. The authors discovered genes encoding the bphA protein for the production of polychlorinated biophenyls (PCBs) in the cold. This study improved the understanding of PCB secretion by cold-active bacteria and provided the basis for their likely use in cold environments.

Borker et al., (33) reported the detection of genes encoding the production of three enzymes in *Glutamicibacter arilaitensis* LJH19. This bacterium breaks down nocturnal soil compost by secreting amylase, cellulase, and xylanase at low temperatures. Genome analysis revealed 217 unique genes encoding these enzymes and auxin (IAA) in cold environments. The improved germination rate of pea seeds was reported to the IAA, indicating the ability to promote plant growth in cold conditions.

Leng et al., (34) studied the genome of *Planococcus maritimus* XJ11, which produces cold proteases at low temperatures. It was discovered that the bacterium contained genes encoding 21 proteases and 3 serine proteases, adapting the bacterium to low temperature, low salinity, and alkalinity. The potential also allows survival at pH 10 and a temperature of 40°C as the best conditions for catalytic activity. This study showed the production of several enzymes for stability at low temperatures.

In a similar study, a gene encoding glutathione reductase (GR) from *Psychrobacter* sp. ANT206 was cloned into *Escherichia coli*. The genes provided protection against oxidative stress from peroxide (H_2O_2). The rPsGR is a novel gene that is an antioxidant encoding a cold enzyme with high tolerance to both low temperature and high NaCl concentrations (35). The study showed the production of enzymes that confer resistance to low temperatures and osmotic pressure.

Raymond et al., (36) examined and compared the genomes of permafrost bacteria and mesophilic relatives. Cold shock proteins, RNA helicase, and enzymes involved in oxidative stress and carotenoid production are all present in both genomes. However, the permafrost bacterium contains more genes that express appropriate solutes required for osmoregulation in a frosty environment. In various cryophytes, amino acid (AA) changes promote protein flexibility at freezing temperatures by altering the amount of proline, serine, glycine, and aromaticity. This shows that the >1 cold/warm AA ratios previously used for cold adaptation alone were not sufficient. Cryophytes had a larger amount of serine in their proteins than cold-adapted proteins and fewer proline and acidic residues than mesophiles.

Biosynthesis of enzymes and proteins:

Cold-adapted bacteria overcome several challenges of living in cold habitats through a series of synergistic enzyme modifications from the cell envelope to the creation of cryoprotectants and innovative metabolic abilities. Basic research has provided important insights into

how microorganisms thrive under challenging conditions and the mechanisms of action of the numerous adaptive traits, which form the basis for the knowledge-based development of innovative biotechnological tools (37). There are recent advances in enzyme and protein production and diverse potential in different industries but less improved areas of hydrogen energy production (Table 2).

Flegler and Lipski (38) studied a carotenoid in cold-adapted *Arthrobacter* species and discovered a pink bacteriorubin that is rarely produced in cold environments. The biomolecule offers resistance to freeze-thaw conditions and osmotic pressure due to the high NaCl concentration. The function of bacteriorubin produced under freezing conditions is not fully understood but clarified to be produced at low temperatures. This protein was used in the manufacture of a dye and is used in the manufacture of antioxidants.

A similar study was conducted by Kumar et al., (39) on *Mucilaginibacter* sp under freezing conditions (-80°C). The bacterium was reported to have produced exopolysaccharides (EPS) that conferred viability on mesophilic *Escherichia coli*. The produced EPS effectively removes Cu^{2+}, $Fe^{2+,}$ and Mn^{2+} from the contaminated medium. Therefore, EPS has been used as a cold protector and effectively removes harmful ions.

Rios et al., (40) studied keratinase production by a cold-adapted *Pedobacter* sp 3.14.7. This enzyme produced by the bacterium at low temperature, was shown to be a robust additive that improves the thorough removal of blood stains from cotton towels at temperatures below 20°C. The authors also reported the effective ness of cold enzymes added to detergent when washing stains and dirt.

Herrera et al., (41) explained in their discovery how *Acinetobacter baumannii* alters its membrane lipid composition to maintain protein fluidity, permeability and function under cold conditions. The octanoate (C8:0) fatty acid is the only shortest secondary acyl chain reported from a cold bacterium, replacing the C12:0 fatty acid to confer stability in a cold environment. This acyl chain has been used for resistance to many drugs that are difficult to eradicate in healthcare setting. The study provides new insights into how temperature changes under different conditions affect lipooligosaccharides or lipopolysaccharides.

Rathour et al., (42) worked on alkalophilic amylase enzymes from *Shewanella* sp. The authors discovered that the enzyme effectively breaks down 1,4-glycosidic bonds in starch molecules. This potential makes this cold amylase an important enzyme in biotechnological tools, especially in the food industry due to the breakdown of the complex bond.

Govarthanan et al., (43) studied novel cold proteins that stabilize the cell membrane of mesophilic *Escherichia coli* and *Bacillus subtilis* at low temperatures. The work describes the protein and discovers new, unidentified species that were not clarified by previous *in vivo* studies, suggesting new biocatalysts that could have industrial applications. A new mechanism of cold adaptation in psychrophilic *Pseudomonas helmanticensis* was also discovered by Kumar et al., (44). In their results, they reported upregulation of the production of uncharacterized proteins at low temperatures instead of the usual expression of enzymes for proline, polyamines, unsaturated fatty acid biosynthesis, reactive oxygen species (ROS)-neutralizing pathways, and arginine degradation. From this, they concluded that molecular chaperones and cold shock proteins were proteins expressed by these bacteria against cold stress.

Few cold-adapted acetyl xylan esterases (AcXEs) were also discovered, however the processes that enable them to work are still unclear. This enzyme had maximum activity at 30°C and retained over 70% activity at 0°C. It has the ability to deacetylate xylooligosaccharides and xylan. Esterases are flavoring agents in the food industry, and chemical synthesizers and their degradation potential can eliminate wastes (45).

Biocatalysts that support plant growth are also produced by cold-adapted bacteria. Plant growth promoters are hydrolytic enzymes used in industry and found as important bioactive substances in medicine in all eukaryotic genera. Many researchers have reported using these proteins to increase the productivity of rice, grains, vegetables, and legumes. Although the biosynthesis of these substances in a microbial cell performs specific functions in cold environments, they have the ability to promote plant growth at high altitudes.

In contrast to mesophilic and thermophilic proteins, low-temperature expression techniques are more advantageous. As a result, a wide temperature range can be tolerated since the genes are more strongly expressed at low temperatures than at mesophilic temperatures. Because they produce antifreeze proteins and express stress-induced genes, bacteria susceptible to cold stress are better able to survive. All metagenomes examined had genes encoding functional responses to environmental stress, including exopolysaccharides, cold shock proteins, and membrane changes. At low temperatures, enhanced gene expression can be achieved, which has greater functional characteristics

and biotechnological applications.

Performance of cold-adapted bacteria in biohydrogen production:

The search for sustainable, low-cost and environmentally friendly hydrogen producing sources for large-scale production is still ongoing (19). Cold-active bacteria hold unique properties that will bring great transformation and improvement in hydrogen energy generation. Low-temperature anaerobic digestion is energy-efficient and sustainable for biohydrogen production. However, it is a new area that has only recently received the attention it deserves. Therefore, the current performance of the cold-active bacteria in biohydrogen production and their limitations has been discussed in this section of the review as summarized in Table 3.

Production temperature:

Cold-adapted bacteria have demonstrated biohydrogen production at temperatures as low as 4–9°C, saving energy in the substrate conversion process. This operating temperature inhibited methanogenic activities, preventing consumption of the hydrogen produced in the process (39), which highlights the performance of low temperatures in biohydrogen production. Nevertheless, in this temperature range, the thermophiles lose their ability for the production of biogas during sugar fermentation (3). Conversely, the production of biohydrogen at ambient temperatures (25-30°C) has been demonstrated by the same bacterial strain (4,46,47, 48). This operational temperature range has been chosen for its wide range of applications in industrial processes and upholds great potential for future hydrogen generation. The main limitation, however, is that the operating temperature affects the rate of enzymatic catalysis, resulting in high energy consumption and an increased lag time. A gap of 18-25 hours was observed between the start of substrate consumption and hydrogen production (3). The low-temperature conditions not only affect the individual microbes but also change the microbial community structure.

Because thermodynamics plays a large role in maintaining equilibrium, the rate of chemical and biological reaction processes is reduced compared to higher temperatures. Thus, the process requires more energy to achieve similar efficiency (5). This energy-intensive process uses a significant amount of substrate for the catalytic reaction rather than conversion to hydrogen. The scenario impacted negatively on the hydrogen yield of the bacteria, resulting in a low hydrogen yield compared to thermolabile strains. Therefore, the external energy required by the system is a major disadvantage, prompting the search for systems where hydrogen can be produced with a minimal input of energy.

Substrate utilization:

Cold-adapted bacteria have effectively broken-down various carbon sources for hydrogen production. Accordingly, glucose, xylose, fructose, galactose, sucrose and lactose have been used as substrates since they are available in large quantities from synthetic organic sources, which are high hydrogen producing materials. They are a segment of a range of abundant wastes where the production of cold-adapted biohydrogen can be coupled with the use of hemicellulose and lignocellulosic feedstocks.

The bacteria have also demonstrated the ability to hydrolyze organic wastes such as cheese whey, sewage, industrial sludge, wheat straw hydrolysate, and cane molasses for production (40,42,49). In this way, the susceptibility of the bacterium to the inhibitory compounds that are present in various industrial wastes that lead to hydrogen production is demonstrated. The fermentations simultaneously led to hydrogen production, which suggests further application on an industrial scale under room temperature conditions. This indicates the expression of appropriate enzymes by the bacteria to convert the substrates into hydrogen at low temperatures (3). Therefore, using waste to generate hydrogen energy could reduce production costs and make hydrogen gas more accessible and cheaper.

Biohydrogen production by cold bacteria has not yet been fully explored, indicating a new potential area for hydrogen production. Therefore, the possibility of converting many complex organic wastes, which are abundant in the environment, into the production of hydrogen should be considered. This is intended to demonstrate the competence and cost-effectiveness of the technology as well as its competitiveness on the energy market (50). Meanwhile, the activity of their enzymes at low temperatures comes at the expense of substrate affinity, thereby reducing the state of their physical and chemical properties in affinity for the enzyme. This affects early substrate uptake for conversion to hydrogen.

Potential of hydrogen (pH):

In anaerobic fermenters, the bacteria produce hydrogen simultaneously with acetic and butyric acid and other metabolites as by-products. Meanwhile, it has been shown that the acetic acid pathway is more desirable at low

temperatures for high hydrogen yield by cold bacteria due to its influence on the catalytic activities of the enzymes and conversion of the by-product to hydrogen. The gradual accumulation of volatile fatty acids (VFAs) in the medium becomes toxic to the cells, represents a shift in the metabolic pathway and unnecessarily increases pH, leading to a drop in hydrogen yield (4).

The hydrogen yield of cold bacteria is seen to be at its peak at pH 6-7 ranges with many metabolites produced in the medium. However, a high concentration of acetic acid favors high hydrogen yield at low temperatures while undissociated butyric acid metabolites alter the pH and the hydrogen yield (4). The anaerobic system operated with the strain under cold conditions shows a gradual degradation and an increase in VFAs, leading to an enhanced syntrophic relationship within the existing cells (5).

Genomic insights into the cold adaptation of bacteria from low-temperature ecosystems have unique adaptations to survive and sustain their growth and metabolism in a cold environment. To cope with these environmental stresses, and to survive and thrive in low-temperature environments, these bacteria exhibit several mechanisms of physiological adaptation that are not ubiquitous in other thermolabile bacteria. Therefore, this review revealed a consequent shift in survival strategies, including environmental perception and stress response, linking the increase in abundance of many genes to the adaptation of the bacterial community to the extreme environment. In this way, the cold-adapted bacteria have evolved unique adaptive strategies at the gene and protein level to maintain their metabolic activity to survive in harsh cold conditions.

Recent increased interest in cold environments has led to the identification of numerous new products, mainly from microbes. Further bioprospecting of these environments using modern high-throughput techniques such as metagenomics and metabolic engineering will surely lead to the discovery of other novel tools with diverse bioactivities and applications.

Challenges of cold-adapted bacteria in biohydrogen production:

In fermentative hydrogen production, many ecologically abundant wastes have not been commonly employed or converted to hydrogen production by cold-active bacteria. This is because few reports addressing biohydrogen production by cold-active bacteria are available, indicating a new area of research that has not been fully explored. Thus, the use of these bacteria is yet to be a more viable and cost-effective process for hydrogen production.

The catalytic efficiency of their hydrogenases is a high energy-intensive process due to an extensive increase in lag time with enormous energy consumed and a low hydrogen yield. The limited growth temperature range of the bacteria hampered their catalytic process and enzymes may be denatured when the operating temperature is increased beyond normal. Many metabolites produced during production accumulate and become toxic to the hydrogen-producing cells. The metabolites also determine the pathways for hydrogen production and influence the hydrogen yield.

Conclusion and future perspective:

The review shows that many new proteins and enzymes with unique potential for the common cold and its stressors are produced by cold bacterial strains. This points to the possibility of finding innovative biotechnological potentials for industrial applications by searching for cold environments. Therefore, the efficiency of hydrogen production and the yield of cold-adapted bacteria can be improved by this strategy given that hydrogen production is a function of enzymes and the fact that the field is new and has not yet been extensively explored.

The efficiency of biohydrogen production by a microbial strain depends on several parameters, and temperature is considered to be an important parameter that enhances the catalytic reaction. Studies have shown the resistance of many cold enzymes to mesophilic temperatures down to the 40°C range. These underlines the importance of continuously optimizing the parameters of new strains to increase hydrogen production. The high structural similarity between the crystal structures with that of mesophilic enzymes can be exploited by genetic engineering techniques to confer potentials that can improve biohydrogen production (51). This is easy to change since most of the crystals are on the surface of the enzymes. Similarly, amino acid substitution can be used to map gene clusters and proteins to hydrogen production with high fidelity, and an immobilization technique can be used to increase hydrogen productivity by genetically modified strains.

Cold-adapted bacteria and their biosynthetic products have shown effective biotechnological application and economic benefits in many industries. These unique potentials were acquired through adaptation to low-temperature stressors, resulting in the production of enzymes with high activity and stability. The bacteria have shown that in mesophilic and low temperature ranges they can produce hydrogen, which

inhibits other thermolabile strains. But despite their unique properties, the low hydrogen yield of cold-active bacteria threatens the future of large-scale hydrogen power generation. It is evident that new genes expressed at low temperatures and enzyme modifications can affect hydrogen production in the future. Thus, the cold-active bacteria and their enzymes offer a large reservoir of new biotechnological potential that could improve large-scale hydrogen production and should be explored extensively.

Contributions of authors:

MA conceived the study idea; MA and JMN contributed to the writing of the manuscript; MIL and ARS searched the literature databases for publications used for the review; MFA and HM contributed to proofreading the manuscript. All authors approved the final manu -script submitted.

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Authors declare no conflict of interest

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Table 1: Genes and functions of cold-adapted bacteria encoded under different conditions

Bacteria	Natural habitat	Methods	Annotated genes	No of coded protein	Functional proteins	Functions in cold	Reference
Pseudomonas sp. Lz4W	Oasis	Molecular and proteomic analysis	4,343	18	Hypothetical protein	Peptidoglycan metabolism, organizing the cell barrier, ATP hydrolysis, enhancing normal passage of fluid through the membrane, and catalysis	(22)
Arthrobacter Z1-20^{T}	Soil	16S rRNA	CapA, glycine betaine	N/A	Protein	Flexibility and stability, substrate binding, Osmoprotectant	(23)
Nesterenkonia sp.	Lake	Genome sequencing &Annotation	NES-AT	N/A	Bacteriorhodopsin	Survival under nutrient-limited, osmotic, and ultraviolet conditions, synthesis of biofilm, metabolism of nutrients	(24)
Arthrobacter antarcticus sp.	Lake sediment	Gene amplification and screening	BphA	N/A	BphA protein	Bioremediation of Polychlorobiphenyls (PCBs)	(25)
Glutamicibacter arilaitensis LJH19	Himalayas Valley	Genomic analysis	N/A	217	Amylase, cellulase, xylanase, IAA	The enzymes enhance the adaptation and breakdown of polysaccharides in a cold environment	(26)
Planococcus maritimus XJ11	Shrimp paste	16S rRNA	21	4	Protease & 3 serine proteases	Increasing activity in a wide temperature range of 10 to 40°C Maximum activity in high salinity	(27)
Psychrobacter sp. ANT206	Sea ice	Gene Cloning, Bioinformatics	PsGR	451	Glutathione reductase	High salt tolerance, high substrate affinity, oxidative stress protectant	(28)
Actinotalea sp. KRMCY2	Permafrost cores	Genomic sequence & annotation	4207	3205	Protein	Interexchange of serine and proline, secretion of cold shock proteins and oxidative enzymes	(29)

IAA= Indole acetic acid; NA = Not available

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Table 2: Enzymes and proteins produced by cold-adapted bacteria and their biotechnological applications

Cold adapted bacteria	Natural habitat	Enzyme/protein	Specific products	Functions of enzymes/proteins in cold	Biotechnological applications	Reference
Arthrobacter agilis DSM 20550^T *Arthrobacter bussei* DSM 109896T	Cheese	Protein	C^{50} carotenoid Bacterioruberin	Cryoprotectants and NaCl stress protectants	Used in the production of antioxidants, dyes	(31)
Mucilaginibacter sp. ERMR7:07	Proglacial water	Protein	Exopolysaccharide	Cryoprotectants	Biosorption of Cu, Fe, Mn and Zn), production of exopolysaccharide	(32)
Pedobacter sp. 3.14.7	Snowy sheathbills	Keratinase	Metalloprotease	Protection in psychrotolerant environment	Degradation of feathers, Bleaching agents, laundry detergent additive	(33)
Acinetobacter baumannii LOS	Indian Shiwalik Himalayas	Acyltransferase	Octanoate (C8:0)/C12:0	Membrane fluidity and permeability	Used to break down complex polymers such as xylan.	(34)
Shewanella sp. ISTPL2	Lake	α-amylase	N/A	Tolerance of metal ions Tolerant to alkaline medium	α-1,4-glycosidic bonds hydrolysis in starch, Ethanol processing, high-fructose corn syrups	(35)
Shewanella BT05	Brackish water	Solutes	IAA, Siderophore hydrogen cyanide	Stability and flexibility	Solubilization of phosphate, Promote plant growth, bioremediation of pesticide	(36)
Pseudomonas helmanticensis	Soil	Protein	Chaperone, cold shock protein	Cold stress protection	Used in the production of essential materials	(37)
Arcticibacterium luteifluviistationis SM1504T	Arctic seawater	Esterase	SGNH-type acetyl xylan-esterases, Tetramers, His 203 and Ser 32	Polysaccharides and Stabilization	Used in CD4 T cell immune responses and vaccine studies	(38)

Note: All bacteria are isolated from the cold environment. The temp. (ºC) Represents specific or dual temperatures at which the undergo activity

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Table 3: Biohydrogen production using different strains of cold-adapted bacteria as inoculum and their hydrogen yield

Cold adapted Bacteria	Natural habitat	Temp. (°C)	pH	Fermentation type	Carbon source	Nitrogen source	Biohydrogen yield	Reference
GA0F bacterium	Glacier sediment	25	7	Anaerobic	CWP, WSH and SCM	Yeast Bacto-tryptone	73.5 ± 10 cm^3 g^{-1}	(44)
Psychrophilic N92	Glacier sediment	29	6.9	Anaerobic	Glucose	$(NH4)_2SO_4$	1.7 mol H_2/mol glucose	(4)
Klebsiella sp. ABZ11	Seawater	30	6.5	Facultative anaerobic	Glucose, sucrose, fructose	Beef extract	3.8 mol/g glucose	(41)
GA0F bacterium	Glacier sediment	26.5	6.2	Anaerobic	Glucose	Tryptone and Yeast	1.93 mol H_2/mol glucose	(39)
Rahnella aquatilis RA9	Demersal lake	20	N/A	Anaerobic	Glucose	Cheese whey	58.1 mL H_2/g CODfed	(42)
Psychrophilic G088	Glacier sediment	20	6.8	Anaerobic	xylose, glucose, fructose, galactose, lactose and sucrose	Tryptone and Yeast	1.7 mol H_2/mol glucose	(3)
Psychrophilic G088	Glacier sediment	25	6.5	Anaerobic	Glucose	Tryptone & yeast extract	1.57 mol H_2/mol glucose	(30)
Sludge strains	Brewery sludge	21	N/A	Anaerobic	Sucrose	N/A	62.6 NmL H_2 g^{-1} sucrose	(37)

Cheese Whey Powder (CWP), Wheat Straw Hydrolysate (WSH), Sugarcane Molasses (SCM)

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