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Extending the limits of *Bacillus* for novel biotechnological applications

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ABSTRACT

Bacillus, generally regarded as safe, has emerged as a robust organism that can withstand adverse environmental conditions and grows easily to very high densities. *Bacillus* has been recognized for its biotechnological applications on an industrial scale. Recent efforts have shown the potential of *Bacillus* to generate biofuels (hydrogen), biopolymers (polyhydroxyalkanoates), and bioactive molecules (acyl-homoserine lactonases). *Bacillus* can be considered the dark horse in the race to generate sustainable energy, ecofriendly non-fossil fuel-based polymers, and bioactive molecules for use as therapeutics.

Contents

Abbreviations: PHA, Polyhydroxyalkanoate; PHB, Polyhydroxybutyrate; HAs, Hydroxyacids; HB, Hydroxybutyrate; HV, Hydroxyvalerate; HHx, Hydroxyhexanoate; HO, Hydroxyoctanoate; HD, Hydroxydecanoate; HDD, Hydroxydodecanoate; DCW, Dry cell weight; M_n, Number average molecular weight; AHL, Acylhomoserine lactone; HSL, Homoserine lactone; C4HSL, *N*-butanoyl HSL; C5HSL, *N*-pentanoyl-HSL; C6HSL, *N*-hexanoyl-HSL; C7HSL, *N*-heptanoyl-HSL; C8HSL, *N*-octanoyl-HSL; C10HSL, *N*-decanoyl-HSL; C12HSL, *N*-dodecanoyl-HSL; 3OC4HSL, 3-oxo-*N*-butanoyl-HSL; 3OC6HSL, 3-oxo-*N*-hexanoyl-HSL; 3OC8HSL, 3-oxo-*N*-octanoyl-HSL; 3OC10HSL, 3-oxo-*N*-decanoyl-HSL; 3OC12HSL, 3-oxo-*N*-dodecanoyl-HSL; 3OHC4HSL, 3-hydroxybutanoyl-HSL; 3OHC12HSL, 3-hydroxydodecanoyl-HSL; BHP, Biological hydrogen production.

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1. Introduction

Identifying and building sustainable energy systems are among the most critical issues at present. They must be addressed systematically (Angenent et al., 2004; Turner, 2004). Many microbial metabolic pathways have been elucidated that can be easily exploited to generate energy and produce valuable chemicals. Non-fossil fuels have gained recognition in view of the fact that fossil fuel reserves will only last for approximately 75 years (Weisz, 2004). At present, we have solar, wind, nuclear, and geothermal energy as major alternative resources for sustainable energy production. On the other hand, conversion of biomass into a number of liquid fuels and hydrogen (H_2) is possible. Using H_2 as an energy carrier could address issues of sustainability, environmental emissions, and energy security. H_2 is a clean alternative to presently available fossil fuels, because of its unique feature: its combustion leads to water as the only by-product (Veziroğlu, 1995). Photobiological and photoelectrochemical approaches to H_2 production seem sustainable but are limited by sunlight-collecting systems (Gräetzel, 2001; Khaselev and Turner, 1998; Lewis, 2001; Melis, 2002). A major drawback of this process is that most H_2-evolving enzymes, especially hydrogenases, are irreversibly inhibited by oxygen, which is an inherent by-product of oxygenic photosynthesis. Until solar-based systems are made sustainable, dark fermentative systems hold promise (Angenent et al., 2004; Levin et al., 2004). In the last 10 years or so, dark fermentative biological H_2 production (BHP) has received significant attention (Park et al., 2005; Patel et al., 2010, 2012b; Rittmann and Herwig, 2012). For sustainable H_2 production, anaerobic digestion is among the most promising methods, because it uses biowastes rich in organic matter as feedstock (Han and Shin, 2004; Yu et al., 2002; Zhang et al., 2003). The highest yields of H_2 (3.3 mol/mol sugar) have been reported with strains of *Clostridia* in pure cultures or mixed cultures (Collet et al., 2004; Kalia and Purohit, 2008; Patel et al., 2012b; Yokoi et al., 1998). However, the major hurdles with *Clostridia* are their demand for strictly anaerobic conditions and the limited information available about their actual contribution to H_2 production (Chang et al., 2006). *Bacillus*, on the other hand, can grow aerobically and produce H_2 fermentatively (Kalia et al., 1994; Patel et al., 2010, 2012a; Porwal et al., 2008; Sonakya et al., 2001). In order to distinguish target H_2 producers from other microorganisms coexisting in the H_2-producing system, a combination of the 16S-rDNA-based method with other techniques, such as immunofluorescence, has been used to analyze bacterial communities (Chang et al., 2006; Fang et al., 2002; Zhang and Fang, 2000). However, these approaches still do not reveal much with respect to a particular metabolic activity. Mining of genomic databases has been employed as a new approach for deducing specific metabolic processes (Kalia et al., 2003a,b). This approach is likely to prove beneficial because it requires much less effort to find microbes that can give better yields, with the ultimate objective of employing waste biomass as raw material (Sonakya et al., 2001). However, as biowastes may have their inherent microbial populations, there is always the concern that the H_2 producers may not be able to perform in the presence of other bacteria. A few studies have been performed to determine whether H_2 producers can in fact perform in a defined consortium (Patel and Kalia, 2013; Patel et al., 2010). These studies show that the success of the defined consortium is likely to depend upon the abilities of the H_2 producers to withstand adverse conditions and compete with other bacteria that may threaten their existence. Here, we propose *Bacillus* as a candidate H_2 producer because 1) it can grow well to high densities under fermentative conditions; 2) it can withstand adverse conditions through sporulation; 3) it can compete with other bacteria by secreting the quorum-quenching enzyme lactonase; 4) it can efficiently hydrolyze biowastes with the help of enzymes such as protease, amylase, lipase, and cellulase; and 5) it can produce biopolymers (polyhydroxyalkanoates (PHA)) from biowaste hydrolysate. In this article, we shall focus largely on the H_2, PHA, and acyl-homoserine lactone (AHL) lactonase production abilities of *Bacillus*.

2. Biological hydrogen production

In prokaryotes, H_2 evolution is primarily a means to release excess protons through hydrogenase and nitrogenase enzymes. Hydrogenases vary in size, structure, and electron donor. Hydrogenases are classified as [NiFe]-, [FeFe]-, and [Fe]-containing enzymes, depending on the type of metals involved and their active sites. Among all the hydrogenases, [NiFe]-hydrogenases constitute the largest class. They are classified into 4 groups based on enzyme function: (i) uptake [NiFe]-hydrogenases oxidize H_2 to reduce anaerobic/aerobic electron acceptors; (ii) cytoplasmic H_2 sensors and cyanobacterial uptake [NiFe]-hydrogenases are induced under N_2-fixing conditions; (iii) bidirectional, heteromultimeric, cytoplasmic [NiFe]-hydrogenases reoxidize cofactors and occur in archaea; and (iv) H_2-evolving, energy-conserving, and membrane-associated hydrogenases catalyze H_2 formation from reduced ferredoxin. In nature, [FeFe]-hydrogenases are involved in H_2 production, in contrast to [NiFe]-hydrogenases, which are primarily H_2 consumers. These are found in anaerobic prokaryotes, such as *Clostridia* and sulfate reducers, and in eukaryotes (Atta and Meyer, 2000; Kalia and Purohit, 2008).

BHP has been studied in a wide range of organisms. Anaerobic BHP in *Clostridium* is mediated by 2 enzymes: pyruvate-ferredoxin oxidoreductase (POR) and H_2-POR (Carere et al., 2008). In contrast, the fermentative pathway for H_2 production was initially observed in *Escherichia coli*. In this case, pyruvate is degraded into formate, with the release of acetyl-CoA through the action of pyruvate-formate lyase. Formate is transformed into H_2 with the help of 2 enzymes: formate dehydrogenase and hydrogenase (Kondratieva and Gogotov, 1983). In contrast to these 2 well-studied H_2-producing organisms, *Bacillus* has yet to be shown to have clearly established enzymes involved in BHP, primarily because it has still not gained recognition as a strong contender for BHP. In mixed bacterial cultures, H_2 production from glucose was found to be evolved primarily by nitrogenase under well-defined physiological conditions: 50% of the H_2 evolved was due to nitrogenase (0.46 nmol C_2H_4 produced/mg protein/h). This observation was further supported by the dramatic decrease in H_2 production seen in the presence of nitrates and carbon monoxide, which are known to repress nitrogenase (Kumar et al., 1998). Certain organisms, such as *Bacillus*, *Methanococcus*, and *Pyrococcus* spp., produce H_2 (Kalia et al., 1994; Kondratieva and Gogotov, 1983; Silva et al., 2000), perhaps through