Biological nitrogen fixation: Potential biotechnological applications beyond biofertilizers

In 1838, when Boussingault first reported that legume plants were able to increase their nitrogen content by fixation from air, his results met with disbelief in the scientific community. Fifty years after Boussingault's experiments, Hellriegel and Wilfarth[1] found an increase in nitrogen content of peas carrying root nodules, but not if the plants were free of them. Since then, biological nitrogen fixation (BNF) researches have focused on the understanding of the process, formulations of biofertilizers using N_2-fixing microbes and their applications in agriculture and forestry. Now, the potential utilization of N_2-fixing microbes for formulating biofertilizers for legumes as well as non-legumes has been shown clearly[2,3]. The technology is being practised for crop production in many countries.

Existence of N_2-fixing bacteria has been reported in several biological systems other than plants. *Paenibacillus*, a N_2-fixing genus was observed in lichen-associated bacterial communities[4]. BNF by bacteria belonging to the group gamma-Proteobacteria, isolated from cyanobacteria-deprived lichens was reported recently[5]. N_2-fixing alpha-Proteobacteria were observed to be predominant in bacterial communities in truffles, a type of mushroom[6]. It was also found that mushrooms can fix N_2 only in the presence of N_2-fixing bacteria in association with the mycelium, thus forming biofilms[7]. Interestingly, a recent study on the diversity of bacteria contaminating papermaking machines revealed that N_2-fixing root-nodule bacteria inhabit their microbial biofilms[8]. BNF by intestinal microorganisms of termites and some other invertebrates has been studied[9]. Using multi-isotope imaging mass spectrometry, it has been shown that N_2 fixed by a bacterium in the gills of shipworm was transferred to the host tissues[10]. Thus, it appears that BNF by N_2-fixing bacteria in microbial communities or biofilms is ubiquitous in nature. However, the need for BNF in biofilms has not been adequately explained so far.

In the absence of N_2-fixing bacteria, naturally occurring biofilms are N-deficient for optimal action, as was demonstrated by the increased microbial efficiency of phosphorus solubilization when a N-source was added to a microbial system[11]. Thus, the existence of a N_2 fixer in a biofilm overcomes the N-deficiency, as revealed in the study of Seneviratne and Jayasinghearachchi[12]. This feature can be exploited for improved efficiency of microbial biotechnologies.

A developed fungal-rhizobial biofilm (FRB) was tested recently for its potential to generate bioactive compounds[13]. The biofilm was observed to increase the number of compounds produced by about 12-fold in comparison to its bacterial or fungal monocultures. This technology can be manipulated using different microbes and culture conditions to produce diverse compounds for the discovery of novel drugs. Cellulosic ethanol production system is generally N-deficient. It may be possible that ethanol production would increase, if a N_2 fixer is introduced to the fermentation process. A microbial fuel cell containing a mixed bacterial biofilm showed a five-fold higher rate and efficiency in converting glucose to electricity[14]. This power output may be further increased if substituted with a FRB as it has higher metabolic efficiency than bacterial biofilms. Therefore, the potential applications of BNF for improved 'green' energy production as well as drug discovery demand immediate research efforts to put them into practice. These are only a handful of the avenues for the applications, with many more to be discovered; for example, food technology, enzyme technology, etc. The use of BNF in various microbial biotechnologies will help address the inefficiency of N-deficient microbial processes in the industry. Therefore, there is great scope for developing a BNF-mediated biotechnology for improved efficiency of industrial microbial applications.

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