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Application and Future Aspects of Microbial Biosurfactants – Review.

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ABSTRACT

Biosurfactants are microbe-derived compound that is non-toxic and biodegradable. They are amphiphilic in nature and better than synthetic surfactants which are chemically derived compound. According to the chemical structure and molecular weight, biosurfactant can be classified into various forms and each of them having their own characteristics depends on which can be applied in different areas. Due to their versatility of different surface active compounds and one of the most promising future prospect globally and economically biosurfactant are used in many industries. Biosurfactants are produced by several microorganisms which include *Bacillus sp.*, *Candida Antarctica*, *Pseudomonas aeruginosa*, *Acinetobacter sp.* Glycolipid and lipopeptide are most applicable but others are also being used. In this review article, we have discussed the types, soil microorganism from which biosurfactant can be derived and their economic importance and the effects of carbon and nitrogen sources, pH, temperature, aeration and agitation during the production of biosurfactant and the applications in various fields. These molecules have the potential to be used in a variety of industries like humectants, cosmetics, food preservatives, pharmaceuticals and detergents, several other applications are also there like biodegradation, biomedical, agriculture and the petroleum sectors.

Keywords: Microorganisms, Classification, Economic importance, Application.

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INTRODUCTION

Surfactants are usually organic compounds and are amphiphilic in nature, that means they contain both hydrophilic groups (their heads) and hydrophobic groups (their tails). Therefore, a surfactant contains both a water-soluble component and a water-insoluble component. Surfactants diffuse in water and adsorb at interfaces between oil and water the case where water is mixed with oil or at the interface between air and water. The water-insoluble hydrophobic group extends out of the water phase, into the oil phase or into the water phase, while the water-soluble head group remains in the water phase. Biosurfactants are surface-active compounds produced on living surfaces, mostly microbial cell surfaces, or excreted extracellularly. A biosurfactant may have one of the following structures- glycolipids, polysaccharide-phospholipid, lipid complex, mycolic acid, lipoprotein or lipopeptide, phospholipid, or the microbial cell surface itself [1]. Besides having low toxicity and a high degree of biodegradability, they are stable at a wide range of temperatures, pH values and salinities. These molecules have the ability to decrease the interfacial tension, surface tension, and critical micelle concentration [2]. A large population of microorganisms produces potent surface-active agents, (biosurfactants) which vary in their molecular size and chemical properties. The yield of the biosurfactant of a growing organism greatly depends on the nutritional environment. The enormous diversity of biosurfactants makes them a group of biomaterials for an application in many areas such as agriculture, food, health care, public health, environmental pollution control and waste utilization.

The hydrophobic moiety of a biosurfactant molecule brings some changes like surface tension reduction around the organism, reduction of interfacial tension between hydrocarbon molecules and cell wall, membrane modifications like reduction of lipopolysaccharide content of the cell wall due to the increased hydrophobicity, by hydrocarbon encapsulation into micelle's formation thus enhancing the dispersion of hydrocarbons [3]. Thus, the aim of this review is to provide an overview of chemical and physical characteristics of biosurfactant and synthetic surfactants, the effects of biosurfactants on biodegradation of hydrophobic organic contaminants, solubility and sorption. Lastly, some examples of application of biosurfactants for bioremediation and several other are shown.

Classification the biosurfactants

Natural surfactants are classified according to the nature of the charge on individual polar moiety. Anionic surfactants have negative charge usually due to a surfactant of surfactant group. Non-ionic surfactants lack ionic constituents. Characterization of Cationic surfactants is done by a quaternary ammonium group which has both positive and negative charged moieties in the same molecule [4]. Biosurfactant can also be grouped into other categories on the basis of low-molecular-mass-molecules with the lower surface and interfacial tension and high-molecular-mass-polymers which bind tightly to surfaces [5]. Examples of low-molecular-mass-molecules are rhamnolipids [6, 7]. Sophorolipids [8] and bio dispersion [9] are some of the examples of high-molecular-mass-polymers [10]. Different types of biosurfactants are there which are produced by various types of microorganisms. Some of the various kinds of biosurfactants are described below:

Primary group of biosurfactants

Glycolipids are the most common biosurfactants produced microbially. They consist of mono, di, tri and tetra saccharides. The fatty acid components of the glycolipid and microbial phospholipid have same composition [11]. They also contain hydroxyaliphatic acids or long chain aliphatic acids in combination with carbohydrates [12]. Among them, rhamnolipid, trehalolipids and sophorolipids are the most known and studied amongst all glycolipids [12, 13]. These three are disaccharides that are acetylated with long chain fatty acids or hydroxyl fatty acids [5].

Another group of biosurfactants is Rhamnolipids. Rhamnolipids are primarily a crystalline acid and composed of β -hydroxy fatty acid and those are linked by the carboxyl end of a rhamnose sugar molecule. Rhamnolipids are mainly produced by *Pseudomonas aeruginosa*, also classified as, mono and di-rhamnolipids. Other *Pseudomonas* species that have been reported to produce rhamnolipids are *P. putida*, *P. chlororaphis*, *P. fluorescens* and *P. plantarii*. Some produce only mono-rhamnolipids and some of them produce both. The ratio of mono and di-rhamnolipid can also be controlled in the production method. With the help of certain available enzymes, mono-rhamnolipids can be into di-rhamnolipids. In 1984, the first patent for the production

of rhamnolipids was filed by Guerra-Santos and Kaeppli obtained in 1986 for their work with *Pseudomonas aeruginosa* [14]. After that Wagner et al. filed a patent in 1985 for the biotechnical production of rhamnolipids from *Pseudomonas sp.* and obtained the same in 1989 [15, 16]. There has been a great body of research work carried on rhamnolipids in the past few years revealing many of their amazing applications and making them reach the high of popularity among all types of biosurfactants in the global market [17]. The reason behind the current global interest in rhamnolipid production because of their broad range of applications in various industries along with many spectacular environment-friendly properties [18]. They possess the high average emulsifying activity of 10.4-15.5 U m/L filtrate and low average minimum surface tension of 30-32 mN/m, and have high affinity for organic hydrophobic molecules [19].

Sophorolipids a group of biosurfactant belongs to the glycolipids mostly produced by the yeast like *Torulopsis sp.* and *candida sp.* It is composed of a dimeric sugar and a hydroxyl fatty acid. It was found that sophorolipid has low foaming properties and high detergent capacity than surfactin, sodium laurate, and sodium dodecyl sulphate. Sophorolipid has also good biodegrading capacity [20]. There are two types of sophorolipids namely, acidic non-lactonic and lactonic. The surfactant-like properties of these sophorolipids have value as a potential substitute for petroleum-based detergents and emulsifiers. Recently the lactonic sophorolipids have attracted more commercial and scientific attention than their acidic counterparts [21], because of this the acetylated lactonic sophorolipids are being used for the production of cosmetics as anti-dandruff, bacteriostatic agent and deodorants [22].

Trehalolipids are a sub group of glycolipids, the serpentine group saw in many members of the *Mycobacterium* and that is because of the presence of trehalose esters on the cell surface of those microorganisms [23]. The disaccharide of the trehalose is linked at C-6 and C-6' to mycolic acids is associated with most of the species of *Mycobacterium*, *Nocardia* and *Corynebacterium*. *Mycobacterium* cell wall contains an arabinogalactan linked to the wall peptidoglycan and are esterified with mycolic acids. Mycolic acids and their homologs have long chain β -hydroxyl α -branched fatty acids [24]. Trehalolipids isolated from *Rhodococcus*, *erythropolis* and *Arthrobacter* species were found to lower the surface and interfacial tension in culture broth [25].

Lipopeptides called surfactin are mainly produced by *Bacillus sp.* That contains seven amino acids bonded to a carboxyl and hydroxyl groups of a 14-carbon acid. Surfactin like any other biosurfactant reduces surface tension from 72.8-27.9 mN/m with a concentration as low as 0.005% making it one of the most powerful biosurfactant [26]. The cyclic lipopeptide surfactin produced by *Bacillus subtilis* ATCC 21332, is an example of a most powerful biosurfactant. Another important characteristic of surfactin is its ability to lyse mammalian erythrocytes and to form spheroplasts [27]. This characteristic is used for the detection of surfactin production through haemolysis on blood agar. Furthermore, it possesses anti-bacterial, antiviral, anti-fungal, anti-mycoplasma activities.

Fatty acids produced from alkanes as a result of oxidation have been considered as surfactants [28]. In addition to the long straight chain acids, microorganisms produce complex fatty acids containing OH group and alkyl branches. Examples of such complex acids include Corynomucolic acids that are also surfactants [29]. The hydrophilic and lipophilic balance of fatty acids is strictly related to the length of the hydrocarbon chain. The most active saturated fatty acids are in the range of C12-C14 for lowering surface and interfacial tensions [5]. A fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Most naturally occurring fatty acids have an even number of carbon atoms 12 to 28. Fatty acids are important sources of fuel because, when metabolized, they yield large quantities of ATP. Long-chain fatty acids cannot cross the blood-brain-barrier and so cannot be used as fuel but medium-chain fatty acids can be used, in addition to glucose and ketone bodies.

Phospholipids are one of the major components of most of the microbial cell wall. The level of phospholipid increases when certain hydrocarbon-degrading bacteria are cultured in alkaline growth media. When hexadecane was used *Acenatobacter sp.* HO1-N, was grown and phospholipid (phosphatidylethylamine) rich vesicles were produced [30]. Phospholipid produced from *Thiobacillus thioxidans* that is responsible for wetting necessity of elemental sulphur for growth [31]. Phosphatidylethylamine produced by *Rhodococcus erythropolis* grown on n-alkaline resulted in the lowering of interfacial tension between hexadecaneto and water less than 1 mN/m and CMC of 30 mg/L [29].

Polymeric types of microbial surfactants are compiled from several components. Emulsan, extracted from *Acinetobacter calcoaceticus*, is the best studied one. It consists of a heteropolysaccharide backbone to which fatty acids are covalently linked [32]. Another example is liposan, a carbohydrate-protein complex synthesized by the yeast *Yarrowia lipolytica* [33]. The best-studied polymeric biosurfactants are emulsan, liposan and mannoprotein and polysaccharide protein. Emulsan is a polyanionic amphipathic heteropolysaccharide bio emulsifier, even they were activated at a very low concentration as 0.001-0.01% for hydrocarbons in water and it has been considered as the most powerful emulsion stabilizer as they can resist inversion even at the water to oil ratio of 1:4 [34]. Ciriglian and Carman (1984) synthesized an extracellular water-soluble emulsifier named liposan using *C. lipolytica*. It is composed of 83% and 17% carbohydrate and protein respectively with the carbohydrate-protein being the heteropolysaccharide consisting of galactose, glucose, galactosamine and galacturonic acid [35]. Some other groups of biosurfactants are also there like biodispersan, alasan, food emulsifier, insecticides and protein complex emulsifiers.

Microorganism producing biosurfactant

Biosurfactants produced extracellularly by a variety of microorganisms mainly by bacteria, fungi and yeasts. Those are diverse in not only their chemical composition (**Table 1**) but also in their nature and the effective amount depends on the type of microorganism producing a particular type of biosurfactant. Many microorganisms for industrial utilization for waste products have been isolated from effluents, wastewater sources and contaminated soils. Thus, these have an ability to grow on substrates considered potentially toxic or unfavourable for other non-producing microorganisms.

Table 1: List of biosurfactant producing organisms.

Biosurfactants	Microorganisms	Commercial importance	References
Cellobiose lipids	<i>Ustilago maydis</i>	Antifungal Compounds	[36]
Serrawettin	<i>Serratia marcescens</i>	Emulsification of Hydrocarbons	[37]
Polyol lipids	<i>Rhodotorula glutinis</i> , <i>R. Graminis</i>	Anti-proliferative Activity	[38]
Trehalose lipids	<i>Corynebacterium sp.</i> , <i>Mycobacterium sp Rhodococcus erythropolis</i> , <i>Arthrobacter sp.</i> , <i>Nocardia erythropolis</i> ,	Dissolution of Hydrocarbons	[39]
Ornithine lipids	<i>Thiobacillus thiooxidans</i> , <i>Agrobacterium sp.</i> <i>Pseudomonas sp.</i>	Bio-emulsifiers	[40]
Viscosin	<i>Pseudomonas fluorescens</i> , <i>Leuconostoc mesenteriods</i>	Surface active Lipopeptides	[41]
Rhamnolipids	<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas chlororaphis</i> , <i>Serratia Rubidea</i>	Bioremediation, Antimicrobial and biocontrol properties	[42]
Carbohydrate-lipid	<i>P.fluorescens</i> , <i>Debaryomyces Polmorphus</i>	Bio-emulsifiers	[43]
Protein PA	<i>P.aeruginosa</i>	Bio-emulsifiers	[44]
Diglycosyl diglycerides	<i>Lactobacillus fermentum</i>	Bio-remediation	[45]
Whole cell	<i>Cyanobacteria</i>	Bio-flocculent	[46]
Fatty acids /neutral Lipids	<i>Clavibacter michiganensis</i> subsp. <i>Insidiosus</i>	Bio-emulsifiers	[47]
Sophorolipids	<i>Candida bombicola</i> , <i>C. antartica</i> , <i>Torulopsis petrophilum C. botistae</i> , <i>C. apicola</i> , <i>C. riocensis</i> , <i>C. stellata</i> , <i>C. bogoriensis</i>	Spermicidal, Antimicrobial, Antiviral.	[48]

Liposan	<i>C. tropicalis</i>	Bio-emulsan	[49]
Monnosylerythritol Lipids	<i>C. antartica</i> , <i>Kurtzmanomyces</i> sp., <i>Pseudozyma siamensis</i>	Antifungal compounds	[50]
Surfactin/Iturin	<i>B. subtilis</i> , <i>B. Amyloliquefaciens</i>	Antimicrobial properties	[51]
Subtilisin	<i>B. subtilis</i>	Antimicrobial properties	[52]
Aminoacids lipids	<i>Bacillus</i> sp.	Antimicrobial properties	[53]
Lichenysin	<i>Bacillus licheniformis</i> , <i>B. Subtilis</i>	Microbial enhanced oil recovery (MEOR)	[54]
Peptide lipids	<i>B. licheniformis</i>	Antimicrobial properties	[40]
Phospholipids	<i>Acinetobacter</i> sp.	Bioremediation	[55]
Vesicles & fimbriae	<i>P. marginilis</i> , <i>P. maltophila</i> <i>Acinetobacter calcoaceticus</i>	Bioremediation	[56]
Emulsan	<i>A. calcoaceticus</i>	Microbially enhanced oil recovery (MEOR)	[57]
Alasan	<i>A. radioresistens</i>	Biodegradation of PAH compounds	[58]

Effect of different factors on biosurfactant production from microorganism

There are a number of microorganisms that produce biosurfactant using different types of growth promoting factors. Carbon sources, nitrogen sources and other minerals are used in different parameters to promote the growth of microorganisms in various conditions. Nitrogen sources influence the chemical structure and characteristics of biosurfactant in the presence of iron, manganese, phosphorus, sulphur and magnesium.

Sources of Carbon: a huge number of carbon sources have been used for biosurfactant production. To reduce the surface tension level at higher value sucrose, glucose, sodium pyruvate, yeast extract and beef extract are used as a carbon source. The yield of sophorolipid produced by *C. bombicola* ATCC 22214 increases with the n-alkane chain length and indicated that the different microbes respond differently to the carbon sources [57]. Glycerol is known to be one of the best potential carbon sources in industrial biotechnology, microbiology and biodiesel industries. [59]. The carbon sources that are used in biosurfactant production like glucose, glycerol, sucrose, organic acids and other alkanes are quite expensive and not so much worthy for biosurfactant production. It will be a good and well system with a very low cost if we use industrial/agricultural waste mixtures that would be helpful in both way environmentally and production wise.

Sources of Nitrogen: nitrogen is one of the most important growth factor for any kind of microorganism because it serves the building blocks for the proteins. Various types of nitrogen sources are used in the production of biosurfactants like such as urea, peptone, ammonium sulphate [60], ammonium nitrate [61], sodium nitrate [62] and malt extract. Yeast extract is the most widely used nitrogen substrate for biosurfactant production. But the amount or concentration of nitrogen source used in the culture composition medium depends on the type and nature of microorganism culture and the production occurs during the stationary phase of cell growth. In one experiment it was shown that the concentration of ammonium and the amount biomass produced are proportional to each other in biosurfactant production [63, 64].

Environmental factors: These factors are important as well as others and have a great effect on the production procedure of biosurfactant. It is always important to optimize the bioprocess to obtain large quantities of bio surf as the product may be affected by changes in aeration or agitation speed, pH, temperature. In most of the cases, biosurfactant productions are reported to be performed in a temperature range of 25-300°C [65]. The best production of biosurfactant was observed at pH 8.0 which is the natural pH of sea water [66].

Applications of biosurfactants

The huge number of applications of biosurfactants is increase day by day. In future, it has been assumed that the growth rate of biosurfactants will be 3-4% per year [67]. Synthetic surfactants are very harmful to the environment and mankind. Microbial cell derived surfactants are less harmful, biodegradable,

low toxicity and economically important. Biosurfactants have several applications in cosmetics and pharmaceuticals, detergent and cleaners, food industries, auxiliaries for textiles, leather and paper, agriculture, petroleum and industry.

Agricultural application

Microbial surfactants are capable of heavy metal absorption. In agricultural field, various types of hazardous chemicals are used like PAH. By the use of biosurfactant in the field harmful chemical substances can be removed from the soil surface area and surfactants also maintain the wettability and texture of the soil. They also reduce the growth of pests and penetrate the toxicants [68].

In recent years one of the most known surfactant rhamnolipid a type of glycolipid has gained some attractions due to its several applications in commercial industries. There is no adverse effect of rhamnolipid on human or environment. Moreover, they are very much useful in the reduction of pathogenic fungi, they used as a fungicide and applied for the control of plant disease [69].

Application in commercial laundry detergent

Due to the amphiphilic nature of surfactant, it is used in all types of detergents. Chemically synthesized surfactants are though harmful for humans, microbial biosurfactant are good enough and used in the detergent production. Fatty acid and lipid group of biosurfactant have a great pH range from 7.0-12.0 [70]. The lipids showed some very good emulsification properties which is compatible with the commercial laundry detergent [71].

Pharmaceutical application

Glycolipid which is one of the most important biosurfactant can be used in several ways. It can be used as antitumor agents, haemolytic agents, antifungal and antiviral agents. Rhamnolipids from *Pseudomonas* and other microorganism are found to be one of the useful biosurfactant that has antifungal, antiviral and haemolytic activity. Sophorolipids, cellobiose lipids and mannosylerythriol lipids known as biosurfactants belonging to glycolipids involved in plant protection through the inhibition of phytopathogenic Fungi growth. They are active against various fungi such as *Saccharomyces sp.*, *Fusarium sp.*, *Penicillium sp.* and *Aspergillus sp.* [72]. Biosurfactants have recently come into view as possible broad-spectrum agents for biotherapy cancer chemotherapy. It has been also studied that their involvement in growth arrest and apoptosis of tumor cells [63].

Biosurfactants have very potent anti-fungal and anti-bacterial activity and also play an important role as an anti-adhesive agent [73], which is helpful to treat the harmful diseases and also use as a therapeutic agent [74].

Glycolipids that extracted from some microbial cell generate cell differentiation rather than cell proliferation in the leukemia cell line. Disclosure of PC12 cell to MEL boost the acetylcholine esterase which obstructed the cell cycle at G1 phase and emerge partial cellular differentiation. Due to this cell differentiation process, it can be suggested that extracellular biosurfactant can be used in the treatment of cancer cell [75].

Application in biodegradation of hydrocarbon compounds

PAH (Polycyclic aromatic hydrocarbon) are hazardous and dangerous to the environment and human which can lead to cancer due to its carcinogenic activity and toxicity. *Actinomyces sp.* have some unique properties which help in the degradation of organic pollutants makes it bio-eliminate. Another most important asset of biosurfactant is the surface activity which is present in almost all types of biosurfactant such as Trehalose lipids, asmycolic lipids that give adherence, hydrophobicity to the microbial cell [76, 77].

Application in food industries

The main properties of biosurfactant are the decrease of surface tension and interfacial tension which helps them to promote the formulation and emulsification. In the food industry, they are used to maintain the

texture, shelf life and aeration system [78]. In the food industry, lipases are used to synthesize emulsifiers such as mono and diglycerides. Through solid state substrate and submerged process lipase can be produced from biosurfactant. The synthesis of lipases and biosurfactants by microorganisms may occur due to the need of microorganisms to metabolize compounds which are insoluble in water [79]. Biosurfactant has a great emulsifying activity which helps in the food preservation. Emulsification of the biosurfactant is derived from different *Bacillus sp.* and few *Pseudomonas sp.* Surfactant from different oils showed a great emulsifying activity especially from gingelly oil and sunflower oil. Due to this emulsifying property of biosurfactant it has been used in several food industries as a food preservatives. (80)

Application in cosmetic industry

Biosurfactants are multifunctional. The Properties of biosurfactant which help the biosurfactant to utilize in the cosmetic product industries over chemical surfactants are emulsification, foaming, water holding capacity and spreading capacity. These biosurfactants are used in the cosmetic industries in various products such as emulsifiers, solubilizers, antimicrobial agents, and bath products like anti-dandruff shampoo, baby products, toothpastes and many more. Biosurfactants are also used to remove the skin roughness [74].

Application in generation of electricity

As a green energy source and energy-saving pollutants treatment process microbial fuel cell (MFC) is pretty much useful with great interest. Exoelectrogenic bacteria play an important role in MFC. Recently some chemical surfactant has been used like tween-80 and EDTA to improve the performance of bacteria and MFC. But chemical surfactant was toxic to the bacteria and reduces the biofilm formation and viability, to overcome this issue microbial surfactants are quite helpful in the improvement of the microbial fuel cell such as rhamnolipids and sophorolipids [81].

Advantages and disadvantages of biosurfactant

Natural surfactants have many advantages when compared with chemical surfactants including low toxicity, low irritancy high biodegradability, and compatibility with human skin [76]. Therefore, they are superior to the synthetic surfactants. The most important advantage of a microbial surfactant over chemical surfactant is its ecological acceptance [82].

Biosurfactants are biodegradable in nature. Biosurfactants are naturally degraded by fungi, bacteria that provide a healthy condition to the environment and suited for bioremediation and dispersion of oil spills. [73]. Microbial surfactant are much less toxic than chemically synthesized surfactants and thus they are used in various industries which is used worldwide commercially and the use of biosurfactants are just increasing every year due to its numerous advantages such as biocompatibility, digestibility, specificity, good economic value and biodegradable.

On the other hand, despite of all the advantages biosurfactants have many disadvantages; one of the problems is related to large-scale and cheap production of biosurfactants. Large quantities of biosurfactant are particularly needed in petroleum and environmental applications, which, may be expensive. To succeed in dealing with these kinds of problem, processes should be combined with the utilization of waste substrates and at the same time their polluting effect, which balances the overall costs. Another problem may occur in accruing pure substances which are of particularly important in pharmaceutical, food and cosmetic applications. Downstream processing is involved with multiple consecutive steps. Therefore, high yields and biosurfactant concentrations in bioreactors are essential for their enhanced recovery and purification.

CONCLUSION

This review provides information about biosurfactant production by microorganisms. The scale-up of biosurfactants for industrial production is still challenging. Since the composition of the final products is affected by the nutrient, environmental factors and micronutrient it is obvious to find a right surfactant for industrial scale up. In this review, we have provided an overview of the characteristics and applications of biosurfactant in various fields. Manufacturers are staking money on biosurfactants because of their promising properties. Using mutants or super active microbial strains with high yielding capacities and cheap renewable

substrates as raw material the production of biosurfactants has been ameliorated at the industrial level. Current market trends show that the demand of biosurfactants such as rhamnolipids, glycolipids, lipopeptides, phospholipids and sulphorolipids is going to increase at a high rate because of their utility in agriculture, food, detergent, paint, textile, cosmetics and pharmaceutical industries. The addition of microbial surfactants enhances the solubility and removal of some major soil contaminants. Biosurfactant (bio emulsifier) production with esterase activity in the culture media shows a two-fold increase in the emulsification of hydrophobic substrates ascertaining that the biosurfactant-esterase complex could also be instrumental in the remediation of polluted sites. Thus, biosurfactants are a boon to Mother Nature, for her cleansing and purification from the contamination caused by human activities. Activity of biosurfactants will lead to a cleaner and safer environment for the younger generations.

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