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Review

Halophile, an essential platform for bioproduction

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ABSTRACT

Industrial biotechnology aims to compete as a stronger alternative ensuring environmental friendly microbial-based production that seeks to curb the predicament of pollution. However, the high cost of bioprocessing is a severe drawback, and therefore, new approaches must be developed to overcome this challenge. Halophiles have shown potentials of overcoming this challenge and are of much preference for unsterile and continuous contamination-free bioprocess due to their unique ability to grow under harsh environmental conditions. Recent advances in genetic manipulations have been established to better the performance of halophiles for industrial applications. Many researchers produced various products such as polyhydroxyalkanoates (PHA), ectoines, biosurfactants, and antioxidants using halophiles, and further efforts have been established to develop halophiles as the foundation for low-cost bioprocess. This paper provides a useful reference for researchers on the merits, drawbacks, achievements, and application of halophiles for bioproduction.

1. Introduction

Industrial biotechnology has significantly developed in the past years aiming to produce chemicals, materials, and biofuels on a large scale using sustainable resources for partially replacing petroleum-based chemical industry. However, bio-based products by industrial biotechnology processes are too expensive as compared to the industrial chemical products, due to the high cost of production. Intensive sterilization process, heavy consumption of freshwater, batch fermentation, high consumption of raw chemical materials, and requirement of stainless steel fermentors and piping systems, etc., all contribute to the high cost of production, therefore, making it not competitive (Wang et al., 2014; Yue et al., 2014; Yin et al., 2015). In order to make industrial biotechnology as competitive as chemical industry, there is a need to develop a competitive method of approaching bio-based production which will ensure energy and time saving, low freshwater consumption, low cost of substrates and contamination-free continuous fermentation process.

Halophiles are capable of the aforementioned desirable properties (Wang et al., 2014). Many Halophiles are alkaliphilic and can grow in high NaCl. Combination of these alkaliphilic and halophilic properties provide natural contamination-free, allowing a possible unsterile (open) and continuous fermentation process to occur (Yue et al., 2014). Also, several response mechanisms of halophiles under high-salinity conditions produce various valuable biomolecules, and over the past few decades, halophiles have been considered for biotechnological applications (Waditee-Sirisattha et al., 2016). Recently, Halophiles have undergone genetic manipulations to allow the production of a wide range of products (Fu et al., 2014). Halophiles have been recognized as significant sources of stable enzymes that function in very high salinity, an extreme condition that results in denaturation and aggregation of most proteins (DasSarma and DasSarma, 2015). Also, many halophiles are found to be able to accumulate polyhydroxyalkanoates (PHA), a family of biodegradable plastics.

Furthermore, halophiles are capable of producing bioactive compounds, chemicals, and different enzymes for biotechnological use. Some of the bioactive compounds show different activities and have been used as antioxidant, sunscreen, and antibiotics (Hosseini Tafreshi and Shariati, 2009; Chen et al., 2014; Waditee-Sirisattha et al., 2014). Numerous studies on their capabilities to synthesize massive amounts of chemicals such as ectoine, hydroxyectoines, glycine, and betaine have shed light on the production of useful stabilizers of biomolecules and stress-protective agents (Pastor et al., 2010). Moreover, many halophiles can also produce biosurfactants and bio-emulsifiers (Satpute

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Table 1
Specific halophilic microorganisms and their yield of production.

Product	Halophile	Energy source	Yield	Reference
PHA	*Halomonas boliviensis*	Hydrolyzed starch, a mixture of glucose and xylose	61% (wt) PHB, 8.1 g/L CDW	Rivera-Terceros et al. (2015)
	Halomonas sp. TD01	Glucose	Sterile – 80 g/L(80%PHB), Unsterile (1st fermentor) – 40 g/L(60%PHB), Unsterile (2nd fermentor) – 40 g/L(65% –0%PHB)	Tan et al. (2011)
	Haloferax mediterranei	Glucose	85.8 g/L CDW, 48.6% PHB	Don et al. (2006)
Compatible solutes	Ectoine	*Halomonas elongata*	Xylose	333 mmol/kg fresh cell weight of ectoine	Tanimura et al. (2013)
	Ectoine	*Halomonas salina* DSM 5928	Monosodium glutamate	6.9 gl – 1 ectoine conc., 7.9 gl – 1d – 1 productivity	Zhang et al. (2009)
	Hydroxyectoine	*Marinococcus* M52	> 10% dissolved oxygen, Microfiltration bioprocess	1.6 g/L, 3.6 g/L	Schiraldi et al. (2006)
	Glycine betaine	*Aphanothece halophytica*	18 mM $NaNO_3$, CO2	~1.6 folds 29.5 μmol GB/Gfw	Waditee-Sirisattha et al. (2015)
Antioxidants (Carotenoids)	β-Carotene	*Dunaliella salina*	10 mM $NaHCO_3$, Bio-photoreactor	30 mg/L	Zhu and Jiang, 2008
	Phytoene	*Dunaliella bardawil*	10 μg/mL Norflurazon	10.4 g/g Chl	León et al. (2005)
Biosurfactants		*Pseudomonas stutzeri* BK-AB12	Glycerol	53.33% EI_{24}, 48.44 mg/L CMC	Putri and Hertadi (2015)
Hydrolytic enzymes	α-amylase	*Halomonas* sp. AAD2	Peptone, Starch	26.25 U/mL/min	Uzyol et al. (2012)
	Protease	*Bacillus* sp. RRM1	Wheat Bran	2081 U/g	Rajkumar et al. (2011)
	Xylanase	*Chromohalobcter* sp. TPSV 101	Sugarcane bagasse, Wheat bran, Xylan	250 U/mL, 190 U/mL, 61 U/mL	Prakash et al. (2009)

et al., 2010). Thus, halophiles have broad biotechnological applications and the *Halomonas* sp., for example, have been candidates for the production of various products used in various industries.

This review encapsulates contemporary progress of halophiles and achievements gathered using them for bio-production with the advantage of low energy consumption, less substrates consumption, continuous and unsterile production, and low capital investment yielding in competitive and low-cost production. It also sheds light on recent genetic modifications developed for halophiles to improve their yield of production.

2. Background of halophiles

Halophiles are a group of microscopic organisms that can grow and develop in high salt (NaCl) concentration areas. They are typically categorized as slight, moderate, or extreme according to the extent of their halotolerance (Ollivier et al., 1994). Halophiles can be found in Archaea, Bacteria, and Eukarya (Quillaguamán et al., 2010). The metabolic diversity of halophiles includes oxygenic and anoxygenic phototrophs, aerobic heterotrophs, fermenters, denitrifiers, sulfate reducers, and methanogens (Oren, 2002).

In order to survive high salt concentration, halophiles make use of two distinct adaptive mechanisms. The first mechanism is the accumulation and synthesizing of compatible solutes, which act as stabilizers or shock and stress absorbers in the cell (Mokashe et al., 2018). The compatible solutes stabilize biological structures to help the cells adapt to high salt, desiccation, heat, cold, and even freezing conditions. This Organic-osmolyte mechanism is widespread in bacteria and methanogenic archaea.

Another more radical adaptation mechanism is the controlling of high salt levels by the influx of potassium (K^+) ions into the cytoplasm of the cell to balance osmotic pressure (Williams, 2014). In exchange, sodium (Na^+) ions are freed out of the cytoplasm relatively. This Salt-in-cytoplasm mechanism is employed by a wide variety of extremely halophilic archaea for osmoregulation, and also by fermenting bacteria, acetogenic bacteria, and sulfate reducers. Halophiles which use this mechanism adapt the interior protein chemistry of their cells to high salt concentration by raising the salt concentration in the cytoplasm similar to that of the surrounding environment; this strategy is to achieve a thermodynamic adjustment of the cell.

Properties of halophilic microorganisms (such as the ability to utilize seawater and mixed substrates and grow at high pH) and their negatively charged enzymes make them potentially very useful for industrial biotechnology. Also, biomolecules from halophiles, including enzymes, carotenoid pigments, biopolymers, bacteriorhodopsin, and halocins, can be applied biotechnologically. Halophiles may also be of great worth for bioremediation and biofermentation processes, and other novel applications in medicine and agriculture.

3. Application of halophiles

Halophiles have broad biotechnological applications ranging from agriculture to biomedical, and various studies have shown that halophiles are essential in industrial biotechnology production. Carotenoids such β-carotene produced from green alga *Dunaliella* was applied in food, cosmetic, and pharmaceutical industries for the production of food colorant, cosmetic additives, and multivitamin respectively (Oren, 2010). Biopolymers can be used as emulsifiers and thickeners and have been applied in an industrial process such as exopolysaccharides which are functional in textile, food, pharmaceutical, and petroleum industries (Delgado-García et al., 2015). Bacteriorhodopsin possesses a photochromic property and can potentially be applied in industrial biotechnology for holography, artificial retina, spatial light modulators, computer memories, and optical memories elaboration material (Delgado-García et al., 2015). Halocins are natural antibiotics and have the potential of being used as myocardial protector and control of

infectious bacteria in medicine and pharmaceutical industries, and preserving agents in food and leather industries as well. Furthermore, an evident process such as the production of ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid) from moderately halophilic bacteria was used as a stabilizer for enzymes and applied in cosmetic industries (Oren, 2010).

Some products from halophiles are listed below with a table illustrating specific halophiles and their yield of production (Table 1).

3.1. Production of PHA by halophiles

Polyhydroxyalkanoates (PHA) are the most versatile bioplastics that have similar properties with petroleum-based plastics (Chen, 2009). As a family of biodegradable and biocompatible polyester, PHA could be developed into an industrial value chain ranging from bioplastics, biofuels, and chemicals to synthetic implants (Chen, 2009; Chen and Patel, 2011). Pötter and Steinbüchel (2005) considered PHA as environmentally friendly substitutes for petroleum-based plastics. However, the cost of producing PHA is still relatively high due to the complexity of the fermentation process and the search for value-added applications such as biomedical and fine chemicals (Chen, 2009; Rehm, 2010). Therefore, PHA could be developed as environmentally friendly bulk plastics, provided the production cost is low and competitive (Chen, 2010; Rehm, 2010).

Halophiles have been exploited and observed to synthesize PHA (Koller et al., 2007). Poly (3-hydroxybutyrate) (PHB) and Poly (3-hydroxybutyrate-*co*-3 hydroxyvalerate) (PHBV) are the two most studied polymers of PHA among > 100 various kinds of PHA and also produced in large scale (Chen, 2009). PHBV is, however, more flexible as compared to PHB which is rigid and brittle, therefore possessed more favorable thermomechanical properties for broader application potentials as medical materials, film products, disposables, and packaging materials (Philip et al., 2007; Chen, 2009). Among the PHA producing halophiles, haloarchaea are the most important group of highly extremophilic PHA producers (Koller, 2019). *Haloferax mediterranei* is so far one of the best-studied PHA producers from the *Halobacteriaceae* family, and it produced 48.6 wt% PHB (Don et al., 2006). Further studies also revealed that *H. mediterranei* synthesized PHA which was a copolymer of PHBV using glucose, extruded starch or hydrolyzed whey as substrates, and several halophilic strains were also reported to synthesize PHBV from non-fatty acid carbohydrates as a carbon source (Han et al., 2013).

Moreover, (Quillaguamán et al., 2008) reported PHA production by a moderate halophilic strain, *Halomonas boliviensis*. This halophile from family *Halomonadaceae* tolerates salt concentration of 0–25% and grows from 0 to 45 °C under pH 6–11. The strain could accumulate PHB from several different carbon sources such as glucose, xylose, sucrose, maltooligosaccharides, sodium acetate, and butyric acid and could also produce PHB with a molecular weight of 1100 kDa under its optimized conditions (Quillaguamán et al., 2008; Van-Thuoc et al., 2008). Halophilic strains *Halomonas* sp. TD01 and *Halomonas* sp. LS21 were recently isolated, and they showed great potential for low-cost PHA production. TD01 was reported to grow optimally at a salt concentration of 5–6% (*w/v*) at pH of 9.0 and the strain also grew to over 80 g/L cell dry weight (CDW) in a lab fermentor and accumulated over 80% PHB on glucose salt (GS) medium. However, TD01 accumulated PHB up 70 wt% CDW with glucose to PHB conversion ratio of over 50% on glucose nitrogen-deficient GS medium, indicating that a nitrogen limitation is beneficial for PHB production by TD01 (Tan et al., 2011). *Halomonas campaniensis* was also isolated, and it was able to grow in artificial seawater and kitchen waste as substrates consisting of cellulose, proteins, fats, fatty acids, and starch. After culturing in kitchen waste simulating (KS) medium, wild-type *H. campaniensis* produced only 26 wt% PHB, while recombinant *H.* campaniensis (with genes of PHB synthesis pathway over-expressed) produced 70 wt% PHB. *Halomonas campaniensis* grew contamination free throughout 65 days under unsterile and continuous conditions at 40 g/L NaCl, 37 °C and pH 10 (Yue et al., 2014). Koller et al. (2007) reported that the cost of PHBV production by *H. mediterranei* was 30% lower than that of recombinant *E. coli*, making halophiles essential for low-cost production of PHA.

In addition, PHA accumulation has been recently identified as a strategy which protects bacterial cells from hypertonic environments (Obruca et al., 2017) and also from fluctuations in osmolarity (Sedlacek et al., 2019) which indicates that PHA accumulation is additional adaptation strategy towards high salinity of the environment. Again, the usage of co-production of high market value products such as compatible solutes, pigments, etc., has been developed to tackle the high cost of producing PHA on a large scale (Kumar and Kim, 2018).

3.2. Production of compatible solutes from halophiles

Ectoines are the most common compatible solutes which are commercially available as protectants for mammalian cells, DNA, and proteins (Kolp et al., 2006; Pastor et al., 2010). Oren (2010) reported ectoine to have been discovered first in the haloalkaliphilic photosynthetic sulfur bacterium, *Ectothiorhodospira halochloris*, but later a great variety of halophilic and halotolerant bacteria were found to produce this compound, often together with its 5-hydroxy derivative. Many halophiles accumulate these compatible solutes within their cells to maintain osmotic balance under hyperosmotic conditions (Yin et al., 2015). Ectoines can also protect many unstable enzymes and nucleic acids against the harmful action of high salinity, thermal denaturing, desiccation, and freezing, thus, increasing shelf life and activity of enzyme preparation (Kolp et al., 2006). Such compatible solutes have found applications in industrial biotechnology. Recent studies claim that ectoine counteracts effects of ultraviolet UV-A-induced and accelerated aging and therefore added to dermatological cosmetic preparations by cosmetic industries as moisturizers in cosmetics for caring for dry, irritated and aged skin (Oren, 2010). Ectoines stimulate the immune system of the Langerhans cells and the formation of heat shock proteins, thereby reducing the formation of sunburn cells in the skin due to UV radiation (Buenger and Driller, 2004). Ectoines were tested to have the ability to inhibit aggregation and neurotoxicity of Alzheimer's β–amyloid (Kanapathipillai et al., 2005). Various studies have applied technology of osmotic downshock termed as “bacterial milking” for extracting intracellular solutes from several microorganisms (Kunte et al., 2014). Nagata et al. (2008) grew *Halomonas elongata* in a high salt medium in order to accumulate higher amounts of intracellular ectoine; then an osmotic downshock was applied. The bacterium reacts by secreting most of the ectoine to the surrounding medium, followed by crossflow filtration techniques to collect the compound and then purified. Salt was then added to the medium to cause readaptation by the bacterium to the high salinity by producing massive ectoine; therefore, a new cycle for ectoine synthesis (milking procedure) could restart. Fallet et al. (2010) also applied continuous bioprocessing for ectoine production where they developed a two-bioreactor system and optimized the productivity of ectoine in *Chromohalobacter salexigens*. The first bioreactor was used to grow cells and accumulate intracellular ectoine, while the second bioreactor was used for osmotic downshock to excrete ectoine. The culture broth of the first bioreactor was pumped continuous into the second one, and the intracellular ectoine content reached up to 540 mg.

Another compatible solute which has attracted commercial interest is hydroxyectoine due to its better protection abilities than ectoine (Pastor et al., 2010). *Marinococcus* M52, a Gram-positive halophilic eubacterium showed a more rapid accumulation of hydroxyectoine (with hydroxyectoine up to 1.6 g/L) as a result of dissolved oxygen content higher than 10% during cultivation. Also, a microfiltration bioprocess was employed to improve biomass and yield of products (reaching 3.6 g/L of hydroxyectoine), and a novel extraction method based on osmotic down-shock coupled with thermal permeabilization was developed to recover the desired products from the biomass

(Schiraldi et al., 2006). However, it is possible to express the genes for ectoine in *E. coli* or other non-halophilic bacteria and use such recombinant bacteria as a source for the compound. For example, a transgenic *E. coli* with the ectoine operon of *Chromohalobacter salexigens* expressed under control of the Tet promoter excreted ectoine, which accumulated in the medium at concentrations up to 6 g/L (Schubert et al., 2007).

Therefore, engineering halophiles have proven favorable for increasing productivity of ectoine and hydroxyectoines.

3.3. Production of antioxidants from halophiles

Halophilic microorganisms possess adaptabilities to survive in habitats with extremely high salt concentrations. One of these adaptabilities is the capability of the halophilic Archaea to produce extraordinary colored pigments known as carotenoid compounds to overcome intense UV radiation (Oren, 2013). These colored pigments were shown to have potent antioxidant, immune-boosting activities, and likely protecting premature aging (Hosseini Tafreshi and Shariati, 2009). Carotenoids are hydrophobic compounds generally consisting of a C40 hydrocarbon, but the Archaea produce a C50 carotenoid such as α - bacterioruberin, found in many archaeal strains (Jehlička et al., 2013). Halophilic alga, *Dunaliella salina* has been used for carotenoids production and its cultivation for the β-carotene production is the major success story of halophile biotechnology (Vachali et al., 2012). Carotenoids are colorful natural products and therefore use extensively as dyes and functional ingredients in food products, including cosmetics (Hosseini Tafreshi and Shariati, 2009). They are also widely applied in pharmaceutical and medical fields such as antitumor and heart disease prevention agents due to their potent antioxidant and immune-boosting properties (Hosseini Tafreshi and Shariati, 2009). Halophiles are unique features for carotenoids production because their extremely high-salt tolerance prevents contamination by other microorganisms, therefore enabling efficient cultivation under non-sterile conditions (Waditee-Sirisattha et al., 2016).

Increased carotenoid production has been established using seawater cultivation and genetic manipulation, including the feasibility of downstream processes of the cells (Papaioannou et al., 2016). Carotenoid production from halophiles, therefore, poses to be potentially advantageous for industrial biotechnology.

3.4. Hydrolytic enzymes from halophiles

Many halophiles are capable of secreting extracellular hydrolytic enzymes such as amylases, lipases, proteases, xylanases, and cellulases (Govender et al., 2009; Enache and Kamekura, 2010; Delgado-García et al., 2015). These enzymes are capable of catalyzing hydrolytic reactions under high salt concentrations, and they are referred to as halophilic hydrolases (Delgado-García et al., 2012).

Halophilic amylases were reported to be produced by Halobacteria including *Chromohalobacter* sp., *Halobacillus* sp., *Halothermothrix orenii, Micrococcus halobitus* and *Streptomyces* sp. (Amoozegar et al., 2003; Tan et al., 2008; Chakraborty et al., 2009). Halophilic amylases have higher stability under adverse conditions and therefore attractive for industrial applications such as the treatment of wastewater containing high salts and starch residues, additives in laundry detergents and starch hydrolysis (Gupta et al., 2003; Chakraborty et al., 2009). Halophilic proteases were isolated from *Bacillus* sp., *Chromahalobacter* sp., *Filobacillus* sp., *Halobacillus* sp., *Nesterenkonia* sp., *Pseudoaltermonas* sp., *Salinivobrio* sp., and *Virgibacillus* sp. (Bakhtiar et al., 2005; Karbalaei-Heidari et al., 2009; Shivanand and Jayaraman, 2009; Vidyasagar et al., 2009). These enzymes are active in the presence of NaCl and can thrive in pH of 5–10 and temperature of 40–75 °C, and they are therefore widely applied for laundry additives, pharmaceuticals, waste management, and food processing (Vidyasagar et al., 2009). Researchers also obtained Xylanases from *Bacillus pumilus* GESF-1, *Chromohalobacter* sp., *Glaciecola mesophila,* and *Nesterenkonia* sp. (Govender et al., 2009; Guo et al., 2009; Menon et al., 2010). Xylanases are stable at pH 6–11 and temperature above 60 °C (Ren et al., 2013). They are alkalostable and thermostable, and they possess the potential to be used for bleaching of paper and pulp (Mamo et al., 2009). Cellulases were isolated from *Bacillus* sp., *Halomonas* sp., *Salinivibrio* sp., and from metagenomics library of some soil microbial consortia. They were reported to be thermostable, halostable, and alkalostable, thereby making them favorable for textile, laundry, and food industries (Aygan and Arikan, 2008; Wang et al., 2009).

Halophilic hydrolases are essential for biotechnological application under adverse conditions (Delgado-García et al., 2012), and this is due to their thermostable ability and adaptability to a wide range of pH (Enache and Kamekura, 2010).

3.5. Biosurfactants (BS) and bioemulsifiers (BE)

Biosurfactants (BS) and bioemulsifiers (BE) are amphiphilic compounds from bio-sources (Satpute et al., 2010). They are made up of both hydrophobic and hydrophilic groups and therefore possess both water-soluble component and water-insoluble component. Dastgheib et al. (2008) investigated BS/BE as potential replacements for chemically synthetic surfactants; they have the potential to be applied as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Halophiles such as *Bacillus* sp. BS3 from hypersaline environments other than marine sites produced BS, which showed potential pharmacological importance by suppressing the replication of shrimp white spot syndrome virus (Donio et al., 2013).

Interestingly, immobilized cells of *Natrialba* sp. strain E21 was used for BS production in continuous fermentation. Cell recovery and recycling was eliminated during the bioprocessing and thus offered an economical way for BS/BE production. The maximum emulsification index (EI_{24}) of the biosurfactant produced from the immobilized cells was 62.3%, and the crude biosurfactants produced by the halophilic bacteria E21 were likely to be glycoproteins, glycolipids or lipopeptides according to the thin-layer chromatography (TLC) results (Kebbouche-Gana et al., 2013). Also, halophilic bacteria *Pseudomonas stutzeri* BK-AB12 was used to produce biosurfactant with glycerol as a carbon source. The biosurfactant gave emulsification index (EI_{24}) of 53.33% and critical micelle concentration (CMC) at 48.44 mg/L. A Blue Plate agar-CTAB assay and Fourier-transmission infrared spectroscopy (FTIR) analysis showed that the biosurfactant was an anionic type and most likely a rhamnolipid (Putri and Hertadi, 2015).

Halophilic microorganisms can, therefore, serve as a platform for producing endogenous/heterogeneous Biosurfactants and Bioemulsifiers at low cost.

4. Other potential application of halophiles

Although halophiles are capable of producing various diversified products, some of these products are of minimum yield and needs further improvement. Previous researchers have produced little or no amounts of such products due to the complex nature and high cost of production of these products. For example, although biosynthesis and accumulation of MAAs were predominantly reported in microorganisms that thrive in hypersaline environments such as marine cyanobacteria and eukaryotic algae as an adaptation from the adverse effect of solar UV radiation (Rastogi et al., 2014), large scale production of MAAs is yet to be achieved. Mycosporine-like amino acids (MAAs) represent a suite of small, low-molecular-weight and water-soluble molecules which have unique ultraviolet-absorbing capacities, based on their common cyclohexenone or cyclohexenimine conjugated arrangements (Shang et al., 2018). Ryu et al. (2014) showed that different types of MAAs such as the shinorine, Porphyra-334, and mycosporine-glycine could protect the human fibroblast cells from UV-induced cell death. MAAs are therefore promising candidates for use in cosmetic and

pharmaceutical applications, and they could be exploited biotechnologically in diverse ways.

Although generally obtained from plant and animal sources, the alga, *Dunaliella* could also produce glycerol. However, attempts of mass cultivation of *Dunaliella* for commercial production of glycerol in the past was unfeasible; the high cost of the harvesting of the cells makes production expensive as compared to the low price of glycerol produced by other means (Oren, 2010).

Catalytic pyrolysis of *Dunaliella* cell material at a temperature of 200–240 °C produces an oil-like substance soluble which is soluble in benzene. The overall process proved to be exothermic in order to regain thermal energy needed to initiate the reaction, and up to 75% of the cell material in an algae seawater slurry could be converted to extractable oil (Goldman et al., 1981; Oren, 2010). However, the production cost is high because the harvesting of the microalgae alone is an expensive process (Oren, 2010). Therefore, the biofuel produced may not be able to recompense the cost of harvesting and production.

Hence, there is more room for research and further development on competitive and low-cost commercial production of these products by halophiles.

5. The advantage of halophiles for industrial biotechnology

Halophiles possess several properties that make them unique and preferable for industrial biotechnology. With the advantage of these unique properties, halophiles become a useful tool for competitive and low-cost production that industrial biotechnology cannot overlook (Yin et al., 2015). Many halophiles are alkaliphilic and can grow and survive in hostile environments, especially high salt salinities. As a typical cell will undergo plasmolysis upon exposure to high salt concentration, halophiles to their advantage, require the high salt concentration for growth and survival. They are capable of employing adaptive mechanisms to survive in hostile environments and to overcome salt and water stress.

The ability of halophiles, especially extreme halophiles to flourish in brines, provide ideal conditions for carrying out many biotechnological transformations, due to their great abundance and exclusion of non-halophilic contaminants. Halophiles also can use a variety of energy sources. They can use less expensive and sustainable substrates such as agricultural waste and kitchen waste instead of expensive raw materials as substrates. Their halophilic nature (salt-loving) also enables them to use seawater, which can be recycled during bioprocessing, thereby reducing the high consumption of freshwater. Due to high salt concentration medium during fermentation, equipment made of low-cost materials such as ceramics, plastics, or carbon steels instead of the expensive stainless steel can be used to reduce the high cost of maintenance of equipment (stainless steel) (Hezayen et al., 2000). Halophiles can also undergo unsterile and continuous bioprocessing, which saves energy for intensive sterilization and increases process efficiency. Halophilic production contributes to less environmental pollution as production is bio-based, and bio-based products are biodegradable (Philip et al., 2007; Yin et al., 2015). Table 2 illustrates the advantages of halophiles over non-halophiles.

Table 2
The advantage of halophiles over non-halophiles.

Halophiles	Non-halophiles
Seawater consumption (less dependent on freshwater)	Freshwater consumption
Less energy consumption (no intensive sterilization)	Heavy energy consumption due to intense sterilization
Less microbial contamination	Frequent microbial contamination
Low capital investment	High capital investment

6. Drawbacks of halophilic production and possible ways to overcome them

Although halophiles can carry out the production of valuable chemicals in hypersaline media, too much salt in the media limits the efficiency of fermentation equipment. Salt concentration, therefore, requires rigorous monitoring, and equipment requires frequent and costly maintenance. Also, PHA recovery and purification, extraction of PHA from the biomass is complicated and expensive. Therefore, mass production of PHA by halophiles becomes uneconomical. Again, the cost of harvesting cells of halophiles for production is high; for example, harvesting cells of *Dunaliella* for the production of biofuel and glycerol is expensive and therefore, the yield of production may not be able to recompense the cost of harvesting of cells and the final production cost (Goldman et al., 1981; Oren, 2010). In addition, the cultivation medium contains a high amount of salt, which can be problematic during wastewater treatment.

In spite of the above drawbacks, we have devised a possible solution in Table 3 that can help improve productivity by halophiles.

7. Genetic manipulation of halophiles

Genetic manipulation is crucial to improving the performance of halophiles for industrial applications. Researchers have made efforts in modifying halophiles to express foreign genes by developing various genetic tools for them (see Fig. 1). However, further development of these available genetic tools is needed as they suffer some disadvantages. Many researchers have used synthetic biology and genetic modification technologies including recombineering, gene knockout, gene overexpression, etc., to improve accelerated cell growth, high cell density, simplification of downstream separation, enlarged space for more inclusion body accumulation (increased cell size for PHA granules), and to improve production yield and reduce the cost of bioproduction. Fu et al. (2014) developed a genetic manipulation method for *Halomonas* TD01 based on efficient markerless gene knockout procedure. They improved PHB and PHBV production via deleting *prpC* gene encoding 2-methylcitrate synthase and PHA depolymerase gene *phaZ* and also overexpressing *udhA* gene encoding soluble pyridine nucleotide transhydrogenase. The deletion of *prpC* gene in *Halomonas* TD01 significantly increased the conversion efficiency of propionic acid to 3-hydroxyvalerate (3HV) monomer fraction in random PHBV copolymers of 3-hydroxybutyrate (3HB) and 3HV from 10% to almost 100%, and this resulted in the growth of cells to accumulate 70% PHBV in dry weight (CDW) consisting of 12 mol% 3HV from 0.5 g/L propionic acid in glucose mineral medium. Also, it is perceptible that the manipulation of PHA granule-associated proteins leads to an increase in PHA granule size, allowing for more natural separation (Pfeiffer and Jendrossek, 2012). Yue et al. (2014) also improved the PHA synthesis ability of *Halomonas campaniensis* LS21, constructing an overexpression plasmid (pBBR1MCS1-oriC-Pporin-phaCABLS) of PHA synthesis genes containing genes of PHA synthase phaC, β-ketothiolase phaA, NADPH-dependent acetoacetyl-CoA reductase phaB, consisting of a native porin promoter and a native OriC from the host chromosome. In a more understandable context, Yue et al. (2014) constructed *Halomonas campaniensis* LS21 into a recombinant strain that could overexpress its native PHA synthesis genes with its own strong native porin promoter, resulting in a remarkable improvement of PHB accumulation from 26% PHB in the wild-type to 70% PHB in the recombinant strain.

Recently, production of free fatty acids in *Escherichia coli* and cyanobacteria has increased following overexpression of recombinant, leaderless thioesterase I (TesA) from *E. coli*. A homolog of *TesA* from the moderately halophilic bacterium *Chromohalobacter salexigens* was identified, cloned, and recombinantly expressed in *E. coli* strain BL21 and M15 for biofuel production (Schreck et al., 2013). Also, the *alsS* gene from *Bacillus subtilis* together with *ilvC* and *ilvD* genes from *Corynebacterium glutamicum* were overexpressed to generate KIV (2-

Table 3
Possibilities to overcome the disadvantages of utilizing halophiles for production.

Drawback	How to improve	Reference
Hard separation of cells from the medium	Reducing separation difficulty through morphology engineering for large cell sizes	Zhao et al. (2017)
Saline wastewater containing high salt	Treatment by marine bacteria	Huang et al. (2018)
Low conversion of the substrate to product	Conversion of more substrate to the product by weakening competing pathways	Wernick et al. (2016) and Straub et al. (2017)
Difficulty in obtaining intracellular products	Weakening the cell walls by engineering cell synthesis mechanism	Li et al. (2016)
Damage of fermentation equipment due to high salt medium	Saline-akali bioreactors such as plastics, ceramics, or carbon steels can be used instead of the stainless steel fermentors and piping systems	Yin et al. (2015)

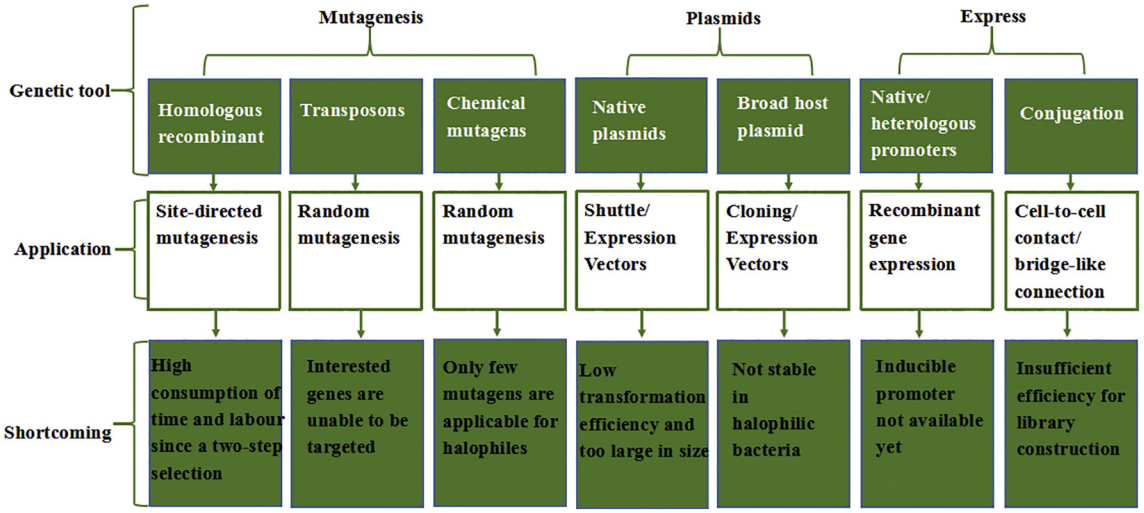

Fig. 1. Currently available genetic tools for Halophiles and some of their disadvantages.

Table 4
Recent technologies used to engineer halophiles and the change in properties after molecular engineering.

Technology	Changes in property	Reference
Genome-based metabolic systems engineering	PTS^{fru} and wild type *Halomonas smyrnensis* $AAD6^{T}$ were restructured with insertional mutagenesis and triparental mating technique to construct a novel strain *Halomonas smyrnensis* BMA14 carrying Ω cassette insertion in HPr region of the PTS^{fru}. This enhanced BMA14's substrate (sucrose) conversion efficiency and resulted in over-production of levan compared to $AAD6^{T}$.	Aydin et al. (2018)
Chromosomal expression system	A $PhaP_{Ah}$ expression strain *Halomonas* TD-ΔCG-4P, which harbors a chromosomal expression system with *phaC* deletion was constructed. This strain exhibited stable expression level of PhaP in the absence of antibiotic. Also, *Halomonas* TD-ΔCG-4P is deficient in PHA synthesis; thus, energy would be saved from PHA production, and less PhaP would be co-precipitated in the insoluble fraction of the cells.	Lan et al. (2016)
Cloning system (Modular vector)	Construction of vector pHsal-C allowed autonomous maintenance in *Halomonas salinarium*, allowing high-level, constitutive expression of heterologous genes in *Halomonas salinarium*, and quantifying promoter activities as well.	Silva-Rocha et al. (2015)
Suicide vector	Construction of suicide vector pHsal-S allowed modification of chromosomal sequences to generate stable and permanent genotypes in *Halomonas salinarium*.	Silva-Rocha et al. (2015)
Gene knockout	After the deletion of prpC gene in *Halomonas* TD01, the conversion efficiency of propionic acid to 3HV monomer fraction in random PHBV copolymers of 3HB and 3HV increased. As a result, cell growth increased, and PHBV accumulation also increased.	Fu et al. (2014)
	Again, deletion of the actin-like protein gene *mreB* combined with weak expression of *mreB* in a plasmid under inducible expression of *sulA* gene (inhibitor of cell division *FtsZ* ring assembly), resulted in a huge cell size of *H.* TD01 with an increase of over 100% PHB accumulation due to the weakened cytoskeleton of the cells.	Jiang et al. (2015)
Overexpression plasmid (Recombinant bacteria)	An overexpression vector pBBR1MCS1-OriC-Pporin-phaCABLS was constructed and applied in *Halomonas campaniensis* LS21. Recombinant *Halomonas campaniensis* LS21 demonstrated a stable and strong expression of the PHB synthesis genes and consumed fatty acid at a slightly slower rate than the wild type.	Yue et al. (2014)
	Additionally, *H.* TD01 cells were also enlarged by overexpression of the cell division inhibitor MinCD, which benefits PHA accumulation as well as simplifying downstream purification.	Jiang et al. (2015)

Ketoisovalerate) for isobutanol production (Rabinovitch-Deere et al., 2013). Tanimura et al. (2013) enhanced ectoine production from glucose by constructing a lysC-overexpressing *Halomonas elongata transformatant* (*H. elongata*/pHS15N-lysC) using a strain carrying an empty vector (*H. elongata*/pHS15N) as a control. After 4 h of cultivation, *H. elongata*/pHS15N-lysC produced 207 mmol/kg FW of ectoine and 0.39 mol of ectoine/mol of glucose whereas *H. elongata*/pHS15N produced 174 mmol/kg FW of ectoine and 0.31 mol of ectoine/mol of glucose. Production of ectoine by *H. elongata*/pHS15N-lysC was 1.2-fold higher than that of the control, and the yield was slightly increased, indicating that overexpression of lysC enhances ectoine production from glucose. Table 4 gives a list of technologies used by researchers to engineer halophiles to improve their productivity.

8. Conclusion

Biotechnological potentials of halophiles have been described and are now well understood. This review described halophiles as producers of diversified products. It also described the advantages and disadvantages of using halophiles for bioproduction and the possible ways to overcome these disadvantages. Recent advances in genetic manipulations and engineering technologies have also been established to improve the performance of halophiles for industrial applications. However, certain drawbacks call for further development of these genetic tools in order to make halophiles more productive and essential for low-cost production. Therefore, our prospects will look at how to generate possible solutions to the challenges which these available genetic tools face and how to develop these genetic tools to increase productivity.

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Declaration of competing interest

This statement is to certify that all the authors have no conflict of interest to disclose and that we all accept to publish our work in the Journal of Microbiological Methods.

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