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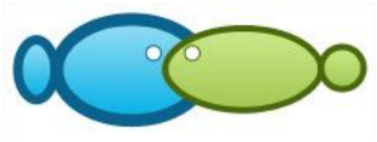

Bioprospection of alginate lyase from bacteria associated with brown algae *Hydroclathrus* sp. as antibiofilm agent: a review

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Abstract. Infection by biofilm-producing microorganisms has been a health threat withdrawing attention around the world. The threat is increasing in developing countries due to poor sanitation conditions, suitable climates for microbial proliferation and limited economic resources. Most cases of chronic infection by pathogenic microorganisms are related to biofilm formation. Thus, biofilm degradation ability by antibiofilm agent is needed to eradicate biofilm-producing pathogenic bacteria. Alginate lyase is an antibiofilm material commonly produced by bacteria associated with alginate-rich algae. The enzyme has a potent ability to depolymerize alginate, the main component of biofilm. The search of alginate lyase enzyme-producing bacteria in the marine environment has resulted in the discovery of beneficial species of alginate-lyase producing bacteria and subsequently new types of alginate lyase enzymes. This paper assesses the bio-prospect of alginate lyase from bacteria associated with brown algae *Hydroclathrus* sp. to be used as antibiofilm of infectious bacteria. It is expected to lead to a valuable alternative treatment of infection related with pathogenic bacteria in developing countries.

Key Words: antibiofilm agent, biofilm, bioprospection, symbiont bacteria.

Introduction. Infectious disease is among the leading causes of death, yet the disease still could not be eradicated by the millennium era. Statistical data from the World Health Organization in 2016 showed that infectious diseases were the 1st cause of death in low-income countries or the 4th in the world (Jamal et al 2018). In the last 2 years, infectious disease showed the highest fatality rate worldwide due to the worldwide outbreak of Coronavirus Disease 2019 or Covid-19 (Grewelle & De Leo 2020).

Several reasons for the difficulty of eradicating infectious diseases include the following: a. new infectious diseases continue to emerge; b. old infectious diseases increase the incidence or geographical distribution; c. old, previously controlled infections began to reappear; d. increased pathogen resistance to current antimicrobial drugs; e. damage in the public health system and communication between countries (Taylor 2019; Tuite et al 2020). The National Institutes of Health (NIH) revealed that up to 80% of cases of chronic infection of pathogenic microorganisms are related to biofilm formation (Topka-Bielecka et al 2021). The threat is increased in developing countries due to poor sanitation conditions, a climate that is suitable for the proliferation of microorganisms, and low economic resources (Founou et al 2017).

It is acknowledged that around 40-80% of bacterial cells on earth could form biofilms (Flemming & Wuertz 2019). However, biofilms are more frequently associated with pathogenic bacteria causing chronic health issues in human (Jamal et al 2018). The main problem related to infection of microorganisms involving biofilm formation is due to

resistance to the human immune system, antibiotics and other treatment (Vestby et al 2020).

The main component of the biofilm is alginate. An enzyme capable of depolymerizing alginate, the main component of biofilms, is alginate lyase (enzyme code: EC 4.2.99.4). Therefore, alginate lyase is often called antibiofilm material. Biofilm degradation ability by alginate lyase is crucial in eradicating biofilm-producing pathogenic bacteria for the following reasons:

a. alginate lyase is a degrader of biofilms that can reduce the virulence of pathogenic bacteria. Biofilm itself is a form of the mechanism of self-defense of infectious bacteria (Roy et al 2018);

b. biofilm degradation by alginate lyase can increase the phagocytosis of pathogenic bacteria in the human body;

c. alginate lyase increases the efficacy of antibiotics so that it can suppress the occurrence of antibiotic resistance in pathogenic bacteria (Blanco-Cabra et al 2020).

Screening of alginate lyase-producing bacteria, both in aquatic and terrestrial environment, has produced a number of new bacterial species such as *Vibrio furnisii* H-1 (Zhu et al 2018); *Paenibacillus algicola* sp. nov. (Zhu et al 2020), *Alteromonas portus* HB161718T (Huang et al 2021), *Formosa algae* KMM 3553T (Belik et al 2020), *Pseudofluviitalea phaphyphila* sp. nov. (Ji et al 2019), *Bacillus* sp. nov. (Zilda et al 2019), and *Vibrio cincinnemicentroti* sp. nov. (Kim et al 2013), or the discovery of novel alginate lyases (Zhu et al 2018; Belik et al 2020; Zhu et al 2020; Huang et al 2021).

In the ocean, large quantity of alginate is produced by brown algae, and becomes the main source of organic carbon for bacteria. Alginate is found as the main polysaccharide constituent of brown algal cell wall matrix (Salmeán et al 2017). In the industrial sector, alginates are produced also from the group of marine brown algae (Peteiro 2018). Brown algae are included in the phylum Phaeophyta, which has more than 1500 species. However, industrial exploration of brown algae for their chemical and biological benefits mostly only come from the genera *Sargassum*, *Laminaria*, and *Turbinaria*. The rest, including the genus *Hydroclathrus* has not been adequately researched or utilized.

Hydroclathrus sp. is known as an edible, yellowish-brown, sponge-like macroalgae commonly found in relatively shallow sea water surrounding islands. Genus *Hydroclathrus* is represented by a number of species including *H. clathratus*, *H. minutus*, *H. rapanuii*, *H. tenuis*, *H. tilesii*, and *H. tumulis* (Santiañez et al 2018). The data related with the biochemical composition and bioactivity of genus *Hydroclathrus* have just been published in the last few decades (Wang et al 2010a; Wang et al 2010b; Vimala & Poonghuzhali 2017; Rashedul & Zafar 2018; Alzahrani et al 2020). Extract of *Hydroclathrus* sp. was reported to contain tannins, saponins, flavonoids, alkaloids, phenols, glycosides, terpenoids, coumarin, steroids, phyto steroids, anthraquinones, and phlobatanin (Vimala & Poonghuzhali 2017). A high alginate content (46.0%) of a species of *Hydroclathrus*, *H. clathratus* from Bangladesh was just reported in 2018 (Rashedul & Zafar 2018).

Considering high demand for alginate lyase to combat biofilm-involving infection and the limited study exploring *Hydroclathrus* sp., the search for alginate lyase producing bacteria associated with the brown algae is interesting to do. Such investigation offers high potential to produce new bacterial and enzyme species. The present review was carried to foresee bio-prospect alginate lyase of bacteria associated with brown algae *Hydroclathrus* sp.

Material and Method

General strategy. To reach the research goal, a descriptive qualitative method adopted from Ethica et al (2018) was initially used to obtain detailed and accurate data reckoning on relevant papers for literature review. Next, elaboration between literature study and review analysis on available data were conducted from the most relevant reports and articles related with the use of alginate lyase produced by bacteria associated with brown algae as antibiofilm agent. The following step was extracting information about the use of

genetic engineering to overexpress alginate lyase coding gene *algL* from potential brown algae to scale-up the production of antibiofilm agent.

Eligibility criteria for the studies. Literature selection was based on the following inclusion criteria: (i) studies and reports with subjects limited to bacterial isolation from brown algal samples aiming to obtain alginate lyase for use as antibiofilm agent; (ii) studies and reports with subjects limited to overexpression of alginate lyase coding gene (*algL*) or genetically modified species other than as antibiofilm agent; (iii) mention of use of alginate lyase from the mentioned bacteria to scale up production of the enzyme; (iv) published in English languages. Publication date limited to the last 10 years was determined in the search strategy (Jaroń et al 2020). Next, the extracted data were displayed in charts or tables and the bio-prospect of alginate lyase from bacteria associated with brown algae and its genetic engineering was summarized to signify its use as agent to combat infection involving biofilm formation as one of major global health issues.

Identification of relevant studies. The current literature review was carried out both electrically and manually. "Electrically" means that literature search process was conducted primarily using a set of keywords applied to search engines of literature database. "Manually" is defined as manual literature search or hand literature search with the objective to identify studies that were missed by the primary search (Sigaard 2017). It was conducted based on the protocol and guidelines previously reported by Wright & McDaid (2011). The search of relevant literature was performed for two categories: (i) alginate lyase producing bacteria associated with brown algae worldwide; (ii) global application of alginate lyase of bacteria isolated from brown algae as antibiofilm agent. For both categories, electronic as well as manual search irrespective of publication date were done using MESH (Medical Subject Heading) terms through PubMed using keywords:

I. (((((((((alginate lyase[Title]) OR Alginate digesting enzyme[Title/Abstract]) OR Alginate breaking enzyme[Title/Abstract]) OR alginate degrading enzym[Title/Abstract]) OR "Polysaccharide-Lyases"[Mesh]) OR alginate lyase[Title/Abstract]) OR alginate lyases[Title/Abstract]) OR alginate lyase[Title/Abstract]) AND ((((((Antibiofilm[Title/Abstract]) OR anti-biofilm[Title/Abstract]) OR antibiofilm agent[Title/Abstract]) OR biofilm breaker[Title/Abstract]) OR biofilm degrader[Title/Abstract]) OR "Biofilms"[Mesh]) AND ((((((pathogen[Title/Abstract]) OR infectious disease[Title/Abstract]) OR pathogenic bacteria[Title/Abstract]) OR infection[Title/Abstract]) OR infectious bacteria[Title/Abstract]) OR "Infections"[Mesh]);
II. (((((((((alginate lyase[Title]) OR Alginate digesting enzyme[Title/Abstract]) OR Alginate breaking enzyme[Title/Abstract]) OR alginate degrading enzym[Title/Abstract]) OR "Polysaccharide-Lyases"[Mesh]) OR alginate lyase[Title/Abstract]) OR alginate lyases[Title/Abstract]) OR alginate lyase[Title/Abstract])) AND ((((((Brown macroalgae[Title/Abstract]) OR brown sea grass[Title/Abstract]) OR brown seagrass[Title/Abstract]) OR brown seaweed[Title/Abstract]) OR brown algae[Title/Abstract]) OR "Phaeophyta"[Mesh])

Both keyword groups were set on January 1, 2021. In addition, search from other sources such as Google Scholar and Science Direct was also performed. Studies excluded from the present review were: [i] studies conducted before 2016 [ii]; studies of alginate lyase producing bacteria isolated from algae other than brown algae, or the use of bacterial alginate lyase from wild type or genetically modified species other than as antibiofilm agent; [iii] mention of use of alginate lyase as antibiofilm agent in non-experimental studies (reviews); [iv] enzymes used as antibiofilm agents other than alginate lyases; [v] personal opinions or reports in news, magazine, personal web (blogs).

Selection of studies. Three of the authors [DSZ, WO & OO] independently identified studies meeting inclusion criteria set in this present review. First, evaluation was conducted on both titles and abstracts of the records resulted by the search to determine unsuitable studies, which then should be excluded according to the set exclusion criteria (Ahn & Kang 2018). Although the reference lists of review articles were recovered by the electronic search, they were not included. Full text articles of the remaining studies that suit the inclusion criteria were retrieved.

Control of bias assessment. To assess the risk of bias or quality in this systematic review, the following issues were included in: (i) completeness of reporting information regarding isolation of alginate lyase producing bacteria from brown algae for consecutive years; (ii) selective data reporting; (iii) selection of studies; (iv) study design; and (v) conflict of interest in the conduct of the study. If all criteria were met, then the overall plausible risk of bias was considered as moderate to low (Gambhir et al 2016).

Data collection, extraction and analysis. This review collected the data by following the guidelines set forth by Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) first reported by Liberati et al (2009). Two authors [SNE & MM] were responsible for extracting data from the obtained reports or studies. The pre-specified data was extracted from each of them, including alginolytic bacterial species, brown algae species as bacterial source, bacterial infection types (which are treated by alginate lyase), and studied brown algal origin all over the world.

Results and Discussion. The purpose of this review was to foresee bio-prospect of alginate lyase from bacteria associated with *Hydroclathrus* sp. as antibiofilm agent based on the trends in composition studies related with alginate lyase application on cases of biofilm-involved bacterial infections worldwide within the past 10 years. Alginate lyase producing bacteria associated with brown algae worldwide and the application of the bacterial alginate lyase as antibiofilm agent had become a central issue in this study. In particular, our study was focused on bio-prospect of *Hydroclathrus* sp. as a member of the brown macroalgae group known with their high alginate content as antibiofilm agent. Therefore, an insight into microbial biofilm formation, structure and component and its impact to pathogenic bacterial infection and treatment is first presented.

Biofilm formation and function. The microorganism biofilm was first observed by Van Leeuwenhoek on the surface of the tooth (Høiby 2017). Biofilm is an association of microorganisms firmly attached to a surface protected within an extracellular polymeric substance (EPS) matrix allowing character alteration of the microorganisms in terms of gene expression, protein synthesis, metabolic activities, and growth rate (Oxaran et al 2018; Abebe 2020). About 99% of the microorganisms present on earth live in communities known as biofilms, regardless they can form biofilm or not (Koo et al 2017). Biofilm-forming organisms spread in nature consist of partially known fungi and pathogenic bacteria (Jamal et al 2018). Because of biofilm formation, pathogenic bacteria are not only capable of causing the infection but are also able to survive in diverse environmental conditions (Hall & Mah 2017; Høiby 2017).

Steps of biofilm formation and development by bacterial cells are illustrated in Figure 1. As described in Figure 1, biofilm formation was initiated by the migration and adhesion of planktonic bacterial cells to an abiotic or biotic surface, which is considered as a critical step in the development of biofilms. Once attached to the surface, organisms will produce EPS, which helps biofilm formation (Lee & Yoon 2017).

A biofilm may develop into three-dimensional, multi-layered mature structures with high density of bacterial cells if conditions for sufficient growth and differentiation are suitable. The structure could be such as mushroom or tower-like structures enclosed with fluid filled channels in which nutrients, oxygen, and essential substances can be diffused and then circulate in various microenvironment. Later, the mature biofilm may detach microcolonies of cells from the main community allowing them to migrate to new

surfaces spreading the infection to different locations (Ansari et al 2017; Abu Khweek & Amer 2018; Rojo-Molinero et al 2019; Verderosa et al 2019).

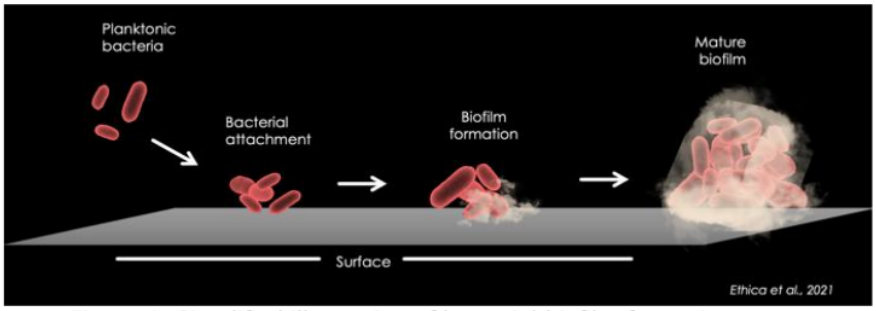

Figure 1. Simplified illustration of bacterial biofilm formation steps.

Biofilms are an example of a defense mechanism by microorganisms. Biofilms are produced by various species in their unique ways (Tasneem et al 2018). Biofilms are resistant to the mechanism of phagocytosis possessed by the human body's defense system (Jamal et al 2018). Biofilms can be found on various surfaces, such as biological tissues, medical devices, and water system pipes (Khatoon et al 2018). Biofilm formation depends on the type of microorganism and the surface it is attached to (Abu Khweek & Amer 2018). Biofilms tend to trap particles, including various minerals and host system components such as red blood cells, fibrin, and platelets. Biofilms are a cause of chronic, nosocomial, and medical-related infections (Khatoon et al 2018; Tasneem et al 2018).

Alginate as biofilm component and substrate of alginate lyase enzyme. Alginate, which is also known as algal polysaccharide, is reported as the major extracellular polymeric substances (EPS) in biofilms (Boutin et al 2019; Heriot et al 2019). Degradation of biofilm structural component allows the increased penetration of antibiotics which enhances the antibiotics efficiency (Sharma et al 2019). Thus, targeting alginate as major biofilm component has become an intriguing therapeutic strategy for handling biofilm-involved bacterial infections and involved antibiotic resistance. Alginate is a linear polysaccharide between β-D-manuronate (M) and its epimer, α-L-guluronate (G) which binds covalently (Jamal et al 2018; Taylor 2019; Grewelle & De Leo 2020; Tuite et al 2020), and is connected in a different order. Alginate is mainly used as a food additive to modify food texture due to its high viscosity and gelling properties (Xu et al 2018; Kothale et al 2020).

Alginate lyase, on the other hand, is a group of enzymes that catalyze the depolymerization of alginate into oligosaccharides. The enzyme degrades alginate as substrate by breaking the glycosidic bonds through an β elimination reaction, producing oligomers with 4-deoxy-L-erythro-hex-4-enepyranosyluronate at the non-reduction chain end (Vuoristo et al 2019).

Alginate lyase enzyme is a product of the expression of the alginate lyase gene (*algL*). The *algL* is a component of the alginate-producing operon which with glycosidase activity produces a primer to synthesize other enzymes in this cluster. Alginate lyase can lyse biofilms by reducing their adhesion to the surface, and increasing phagocytosis and antibiotic susceptibility (Ji et al 2019; Huang et al 2021). Alginate lyase is a pharmaceutical ingredient that is important because it increases the performance of antibiotics in killing biofilm forming bacteria (Jamal et al 2018; Del Pozo 2021).

Alginate lyase has been isolated from a variety of organisms with different substrate specificities, including algae, marine mollusks, marine and terrestrial bacteria, and some viruses and fungi (Zhu et al 2016; Ji et al 2019; Zilda et al 2019; Belik et al 2020;Zhu et al 2020; Huang et al 2021). With the advancement of structural biology, many types of alginate lyases and other families of lyase polysaccharides have been identified as crystalline structures, and with known catalytic mechanisms (Xu et al 2018). The product of the enzymatic reaction between alginate lyase and the alginate substrate

is alginate oligosaccharide (AOS) which because of its various biological activities can act as prebiotic, immune modulation, anticoagulant, antioxidant, and anticancer. In addition, AOS is also able to stimulate endothelial cell growth and stimulate the secretion of cytotoxic cytokines from human macrophages (Liu et al 2019; Xing et al 2020).

Trend of data in Table 1 shows that worldwide, the most studied infection cases caused by biofilm-producing bacteria treated using bacterial alginate lyases in the last 5 years are cystic fibrosis caused by *Pseudomonas aeruginosa*. Though rare, gastric and urinary tract bacterial infections caused by *Helicobacter* sp. and *Enterococcus* sp. have also been reported to be treated using alginate lyase isolated from various beneficial bacteria.

Table 1

Studies reporting application of bacterial alginate lyase as antibiofilm agent in bacterial infection in the last 5 years worldwide

Infectious bacteria	*Infection case*	*Alginate lyase producing bacteria*	*Country*	*Authors*
Helicobacter pylori	Gastric	*Flavobacterium* sp.	Italy	Bugli et al (2016)
Pseudomonas aeruginosa	Cystic fibrosis	*Azotobacter vinelandii*	Korea	Jang et al (2016)
Pseudomonas aeruginosa	Cystic fibrosis	unspecified	Portugal	Alves et al (2016)
Pseudomonas aeruginosa	Cystic fibrosis	unspecified	Korea	Cho et al (2016)
Pseudomonas aeruginosa	Cystic fibrosis	unspecified	NZ	Germoni et al (2016)
Enterococcus faecalis & *Enterococcus faecium*	Urinary tract	unspecified	Italy	Torelli et al (2017)
Pseudomonas aeruginosa	Cystic fibrosis	*Pseudomonas aeruginosa*	Iran	Tavafi et al (2018)
Pseudomonas aeruginosa	Gastric	*Flavobacterium* sp.	Italy	Bugli et al (2016)
Pseudomonas aeruginosa	Cystic fibrosis	*Azotobacter vinelandii*	Korea	Jang et al (2016)
Pseudomonas aeruginosa	Cystic fibrosis	unspecified	India	Patel et al (2019)
Pseudomonas aeruginosa	Cystic fibrosis	unspecified	China	Li et al (2019)
Pseudomonas aeruginosa	Cystic fibrosis	*Pseudoalteromonas sp.*	Canada	Daboor et al (2019)
Pseudomonas aeruginosa	Cystic fibrosis	*Flavobacterium multivorum, Zobellia galactanivorans, Sphingomonas* sp.	Spain	Blanco-Cabra et al (2020)
Pseudomonas aeruginosa	Cystic fibrosis	unspecified	China	Wan et al (2020)
Pseudomonas aeruginosa	Cystic fibrosis	*Cellulophaga algicola*	India	Mahajan et al (2021)

Application of alginate lyases as antibiofilm agents to treat bacterial infection cases have been continuously conducted worldwide. Table 1 shows last reports investigating the use of bacterial alginate lyase as antibiofilm agents against biofilms produced by pathogenic bacteria causing infections (Bugli et al 2016; Daboor et al 2019; Mahajan et al 2021).

Aside of *P. aeruginosa*, *Helicobacter* sp. and *Enterococcus* sp., there are still many other infectious bacteria associated with the formation of biofilms including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterococcus faecalis*, and *Escherichia coli* (Macià et al 2018). Considering that recent studies (see Table 1) were successful in demonstrating the role of

alginate lyase as antibiofilm agent of *P. aeruginosa*, studies about the same application against other bacteria in different cases should be enhanced

Brown algae as main sources of alginolytic bacteria. Alginate lyase-producing organisms are very diverse. They have reported to be found in decomposed seaweed, seawater, etc. However, the group of bacteria associated with brown algae is the most widely reported to produce this enzyme. Table 2 shows studies worldwide reporting brown algae associated bacteria as source of alginate lyase in the last 5 years.

Table 2

Studies reporting brown algae (Phaeophyta) associated bacteria as source of alginate lyase in the last 5 years

Association of brown algae and alginate lyase producing bacteria		*Country*	*Authors*
Bacterium	*Brown algae*		
Helicobacter pylori	Gastric	Italy	Bugli et al (2016)
Bacillus litoralis	*Sargassum horneri*	Australia	Wang et al (2016)
Pseudomonas stutzeri MSEA04	unspecified	Egypt	Beltagy et al (2016)
Cobetia sp. Nap1	unspecified	Taiwan	Yagi et al (2016)
Algibacter alginolytica sp. nov.	*Laminaria japonica*	China	Sun et al (2016)
Microbulbifer sp. ALW1	unspecified	China	Zhu et al (2016a)
Bacillus weihaiensis	unspecified	China	Zhu et al (2016b)
Exiguobacterium sp. Alg-S5	*Sargassum* sp.	Barbados	Mohapatra (2017)
Bacillus halosaccharovorans	*unspecified*	China	Wang et al (2017)
Vibrio furnissii H1	unspecified	China	Zhu et al (2018)
Formosa algae	*Fucus evanescens*	Russia	Belik et al (2019)
Defluviitalea phaphyphila	unspecified	China	Ji et al (2019)
Marinimicrobium sp. H1	unspecified	China	Yan et al (2019)
Martelella sp. MAK4	unspecified	Egypt	Ali et al (2020)
Staphylococcus arlettae, S. pasteuri, Bacillus megaterium, Alteromonas macleodii	*Sargassum mcclurei*	Vietnam	Dieu et al (2020)
Psychromonas sp. C-3	*Laminaria* sp.	China	Xu et al (2020)
Paenibacillus algicola sp. nov.	unspecified	China	Zhu et al (2020)
Cobetia sp. nov.	*Sargassum fusiforme*	China	Cheng et al (2020)
Pseudoalteromonas sp. Alg6B	*Laminaria japonica*	China	Sun et al (2020)
Thalassomonas sp. LD5	unspecified	China	Zhang et al (2020)
Tamlana sp. s12	unspecified	China	Yin et al (2021)
Alteromonas portus HB161718^T	unspecified	China	Huang et al (2021)

Trend of data in Table 2 shows that most of the studies reported the alginate producing bacteria from unspecified species of brown algae. In terms of the specified species of brown algae as source of alginate lyase producing bacteria studied are limited from genera *Laminaria* and *Sargassum*. It also appears that in the last 5 years, most publications related to the discovery of bacterial alginate lyases associated with brown algae were from China.

Based on our literature review, we underline that brown algae are definitely a rich source of alginate lyase-producing bacteria, regardless of the limited information about the algal species. This is actually not surprising because brown algae usually have a high alginate content. However, the data suggests that brown algae species other than *Sargassum* and *Laminaria* were limitedly reported.

Brown algae as main sources of alginolytic bacteria. *Hydroclathrus* sp. (Figure 2) belongs to a family of photosynthetic multicellular brown algae having yellowish-brown in color, with an average length of 6-10 cm, forming a sponge-like lump, which is very porous and chain-like with irregularly rounded thallus. The outer zone consists of palisade cells that extend radially, cylindrical, and are less compact (Vimala & Poonghuzhali 2017).

Figure 2. Morphology of brown algae *Hydroclathrus* sp. found in South Eastern Sulawesi sea of Indonesian (Documented by Ethica et al in 2021).

Brown macroalgae are included in the Stramenopiles Kingdom, which is not closely related to land plants and green algae. The cell wall consists mainly of polyanionic alginate polysaccharides and fructose-containing sulfate polysaccharides (Salmeán et al 2017) Brown algae or seaweed is included in Phaeophyta Phylum which has the largest number of algae species and is complex compared to other algae groups. Phaeophyta has a fucoxanthin pigment that is not owned by red and green algae, so it is included in the kingdom Chromista (Al Ashwal & Abdelbary 2021). Brown algae are a large phylum consisting of a variety of marine and photoautotrophic organisms, consisting of more than 250 genera and more than 2000 species (Mekinić et al 2019; El Rashed et al 2020). Interestingly, since it has been discovered in 1920, the taxonomic status *Hydroclathrus* sp. is currently in the verification process and categorized as distinct (Santiañez et al 2018).

The distribution of *Hydroclathrus* sp. includes the tropics to subtropics; from the Atlantic Ocean to the Indian and Pacific Oceans; and across various islands including Cape Verde, Maldives, Hawaii, Samoa, and Polynesia. These macroalgae are found in various countries including the United States of America, South Africa, Mexico, Japan, Bangladesh, China, Australia, New Zealand, the Philippines, and also Indonesia (Jamal et al 2018; Rashedul & Zafar 2018; Santiañez et al 2018; Kepel et al 2019; Santiañez et al 2020).

Hydroclathrus sp. has been used for centuries in traditional cooking and medicine in the Hawaiian Islands. But it is only in the last few decades that the macroalgae has been known to have anticancer and antitumor, antiviral, antiherpes, anti-inflammatory, anticoagulant, and antimicrobial abilities (Vimala & Poonghuzhali 2017; Alharbi 2020; Santiañez et al 2020). Compared with other groups of brown algae species such as *Sargassum* spp., *Laminaria* spp., and *Turbinaria*, *Hydroclaratus* spp. is still not much researched and utilized. For example, the content of mixed-linked MLG glucan related to alginate in many species of brown algae has been reported, but in *H. clathrathus* it is unknown. In the world, the alginate content of this algae group was just recently reported in 2018 by 45.7%, from samples obtained at rocky shore of the St. Martin's Island, Bangladesh (Rashedul & Zafar 2018). In fact, bacteria associated with *Hydroclathrus* sp. are also limitedly studied. In Indonesia *Hydroclathrus* spp. are quite abundant however they are considered to be of less economic value because they don't belong to the main group of agar and carrageenan producers. *Sargassum* should be explored for more opportunities to obtain novel alginolytic bacteria.

Based on data from literature review, high levels of alginate in *Hydroclathrus* sp. offer high chance of finding alginolytic enzyme-producing bacteria associated with the brown algae. The reason is because alginate is a specific substrate of alginate lyase. Hence, the abundance of *Hydroclathrus* sp. in nature gives the added value of bacteria as an easily grown enzyme producer, and potential for novelty and invention related to the isolation of alginolytic bacteria from brown algae are among strength and opportunity to bring good prospects of *Hydroclathrus* sp.

Strategy to develop alginate lyase from bacteria associated with Hydroclathrus sp. *Hydroclathrus* sp. is an attractive substrate for alginate degrading bacteria due to its high alginate content. Bacteria are among beneficial enzyme producers because they could be grown in large quantities only in short span of time, could be targeted for genetic manipulations to enhance the enzyme production source (Subu et al 2017). On the other hand, to date, many feasible facilities are available to isolate, purify and characterize extracellular alginolytic enzymes produced by bacteria. They include techniques to immobilize the enzymes (Alves et al 2016; Li et al 2019; Patel et al 2019) or to test their ability to degrade biofilms of pathogenic bacteria (Bugli et al 2016; Germoni et al 2016; Tavafi et al 2018; Vuoristo et al 2019). Alginate lyase could even be purified only in the one-step stage using the affinity chromatography method, while its characterization could be done by the SDS PAGE Method (Ghadam et al 2017; Zilda et al 2019). The specificity of the alginate lyase enzyme could then be determined later using molecular methods including genomic and proteomic analysis (Pilgaard et al 2019). Thus, it is much possible to obtain biofilm agent from groups of alginolytic bacteria isolated from brown algae *Hydroclathrus* sp. by following those previously reported techniques.

A detailed scheme as a strategy to obtain alginate lyase from bacteria associated with *Hydroclathrus* sp. according to those reported successful methods is displayed in Figure 3.

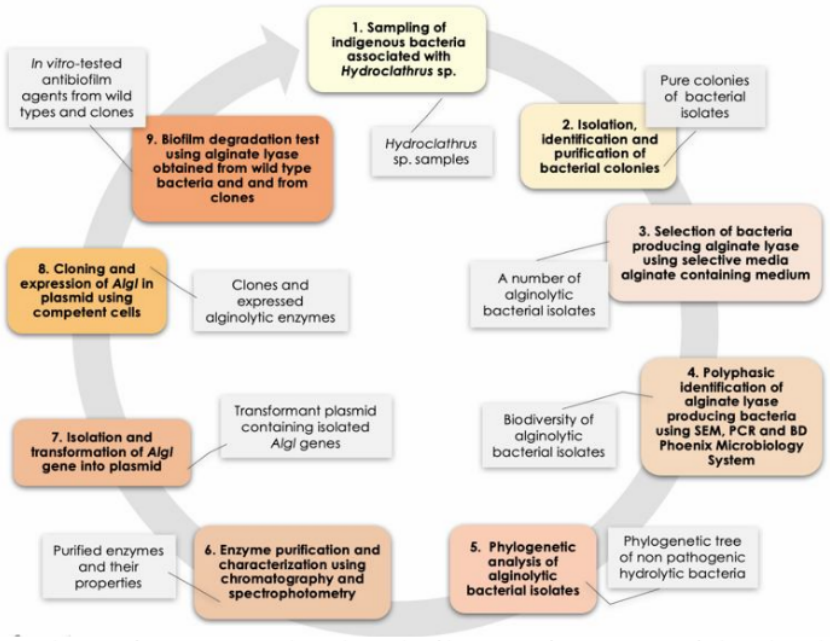

Figure 3. Scheme of steps required to obtain biofilm agent from groups of alginolytic bacteria isolated from brown algae *Hydroclathrus* sp. and the targeted outcomes of each step.

Bioprospection of alginate lyase as antibiofilm agent in infection treatment. Treatment of infections all over the world in the last decade using antibiotics has faced ineffectiveness problems due to the resistance mechanism of bacteria by biofilm formation (Jamal et al 2018; Vestby et al 2020; Topka-Bielecka et al 2021). Hence, the development of an antibiofilm agent targeting most infectious species of bacteria is a necessity. Based on all data obtained from our literature study, a diagram describing factors supporting the importance of bioprospection of antibiofilm agent development from alginate lyase producing bacteria associated with *Hydroclathrus* sp. could be generated (Figure 4).

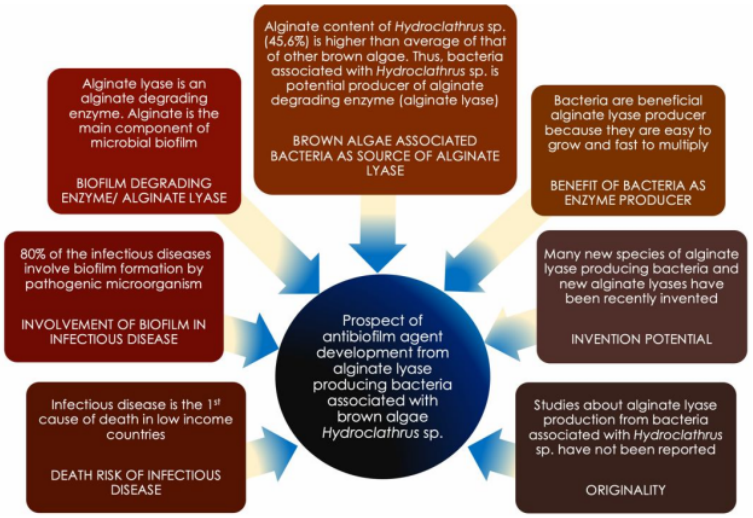

Figure 4. Factors contributing to the good prospect of antibiofilm agent development from alginate lyase producing bacteria associated with *Hydroclathrus* sp.

As mentioned in Figure 4, death risk of infectious disease and involvement of biofilm in infectious disease are among reasons why studies related with antibiofilm agent should be intensified. Alginate lyase potential as antibiofilm agent, brown algae as a rich source of alginate lyase producing bacteria, and bacteria as beneficial enzyme producer offer potentially new enzymatic treatment to biofilm involved infection.

As a summary, diagram in Figure 5 highlights our opinion and strategy about the bio-prospect of *Hydroclathrus* sp. as a potential source of important bacteria producing alginate enzyme lyase. Biofilms are known to be resistant to antibiotics, even to disinfectants such as chlorine. Because of such nature, the use of antibiotics alone is not effective in treating biofilm-related infections (Sun et al 2019; Yan et al 2020).

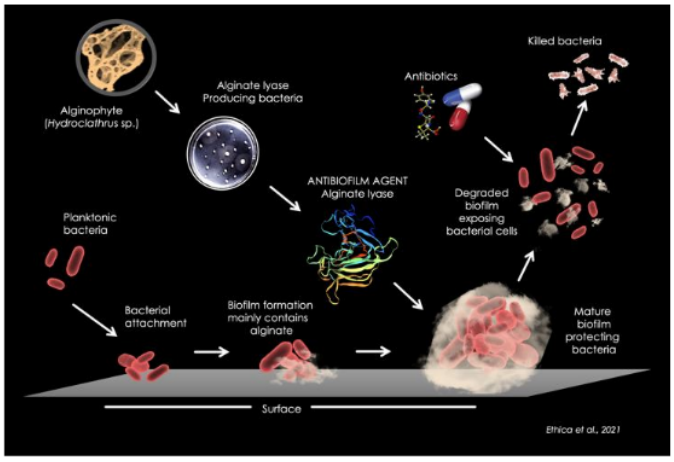

Figure 5. Bioprospection of alginate lyase isolated from bacteria associated with alginophyte *Hydroclathrus* sp. as antibiofilm agent of pathogenic bacterial biofilms.

The conceptual strategy mapped in Figure 5 is intended to help overcome the problem of biofilm-related infections that are troubling the world, particularly the developing countries today. Eradication of biofilms is not easy to do when counting only on antibiotics. Thus, we suggest the use of various species of brown algae, specifically those

with high content of alginate as breakthrough to obtain novel bacterial alginate lyases as new antibiofilm agent.

Conclusions. It could be concluded from our literature review that most studies on the application of alginate lyase as antibiofilm agent in the world were to cystic fibrosis case of infection caused by *Pseudomonas aeruginosa*. Along with this, it is also clear that unspecified brown algae, followed by *Sargassum* sp. and *Laminaria* sp. have been mostly targeted as source of bacterial alginate lyase without regards to their alginate contents. *Hydroclathrus* sp., a species of brown algae with high content of alginate, appears to be a prospective as a source of bacterial alginate lyase as antibiofilm agent in the treatments of bacterial infection cases. It is expected that in general, the use of alginate lyase as antibiofilm agent could be expanded to broader cases of troubling biofilm-related infections in developing countries, not only limited to cystic fibrosis cases.

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