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Symbiodinium IN CORAL REEFS AND ITS ADAPTATION RESPONSES TOWARD CORAL BLEACHING EVENTS: A REVIEW

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ABSTRACT

Symbiodinium is a category of symbiotic dinoflagellates commonly associated with various reef-building corals. Detrimental impacts of global climate change worsen the mutualistic association of coral-*Symbiodinium*, endangering the reefs to the bleaching and mass mortality phenomenon. Destruction of coral reef ecosystems has adverse effects not only on marine life but also on the human population. It has been proposed that to protect the coral reefs, an exclusive selection of thermal-tolerance traits in *Symbiodinium* will increase the survivability of coral reefs. However, there are still limited findings on the coral-endosymbiont resistance under adverse environments. Thus, this review aims to introduce shortly the coral reefs, *Symbiodinium*, and coral bleaching events, as well as to provide brief reviews of cellular and molecular responses in *Symbiodinium* to tackle thermal stress. Considering the potential applications of this knowledge to confront the threat of coral bleaching prevalence, more study especially in terms of cellular and molecular responses by omics approaches is needed to enhance the understanding of coral-*Symbiodinium* tolerance toward climate change, particularly heat stress.

Key words: Adaptation mechanisms, climate change, coral bleaching, *Symbiodinium*

INTRODUCTION

The coral reef is one of the most exclusive ecosystems in the world with 75% of global coral species located in the Coral Triangle with approximately 6 million km^2 area comprising multiple countries which include Indonesia, Philippines, Papua New Guinea, Timor Leste, and Malaysia (Gan *et al*., 2021). Coral reefs in Malaysia are located at the edge of the Coral Triangle, which covers approximately 4000 km^2 areas and shelters almost 550 hermatypic coral species in its water body (Loh *et al*., 2018). For the sake of conservation, protection, management, and restoration of the local coral reef ecosystems, Malaysia has gazetted a few coral reef areas along the shore, including 42 islands as Marine Park areas such as Pulau Perhentian, Pulau Redang, and Pulau Tioman (Abdullah *et al*., 2016; Loh *et al*., 2018; Ismail & Goeden, 2020).

It is estimated that around 1% of the ocean floor is covered by coral reefs, which are beneficial as a habitat for approximately 25% of aquatic organisms (Loh *et al*., 2018). As one of the most valuable ecosystems on earth, the staggering diversity of coral reef ecosystems is not only vital for marine species as food, shelter, and breeding ground but also important for humans (Abdullah *et al*., 2016). About half a billion people hinge on coral reef areas as shore protection from the harsh waves and tide, while in the meantime, coral reef areas contribute to the social economy of the community (Wong & Yong, 2020). Coral reefs are profitable for economic value generated from ecotourism, research, and fisheries sectors worth over US$ 375 billion each year globally, and remarkably US$ 563 million annually for Singapore and Malaysia (Abdullah *et al*., 2016; Tan *et al*., 2020).

Coral reefs form a symbiotic relationship with endosymbiotic dinoflagellates from the family Symbiodiniaceae, particularly the genus *Symbiodinium*. This beneficial relationship is known as mutualism where the coral acts as a host and provides protection to *Symbiodinium* and in exchange, *Symbiodinium* supplies organic compounds for the growth of the coral (Hoegh-Guldberg *et al*., 2017). Currently, minimal attention has been given to the inconspicuous dinoflagellates, especially *Symbiodinium*, in coral survival (Gardner *et al*., 2017; Nakajima *et al*., 2017).

However, global climate change is detrimentally affecting the mutualistic association of coral-endosymbionts, exposing the reefs to bleach and mass mortality, causing negative consequences to marine

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life and human populations (Ismail & Goeben, 2020; Dutra *et al*., 2021). The bleaching event that occurred in 1998 not only adversely impacted global coral reefs but also our Malaysia coral reefs with up to 40% of coral mortality due to climate change (Waheed & Hoeksema, 2014; Qin *et al*., 2019). The coral bleaching event occurs when oxidative stress is generated by reactive oxygen species in *Symbiodinium* cells (Chan *et al*., 2021). Comprehensive understanding of the underlying cellular mechanisms is still limited (Nielsen *et al*., 2018; Liu *et al*., 2018). Thus, an extensive understanding on the interaction of coral-*Symbiodinium* in response to thermal stress is crucial as a baseline to understand the coral reef responses to cope with the climate change.

CORAL REEFS AND THEIR ENDOSYMBIONTS

Coral is an animal that lives in clear water and relies on mutual relationships with endosymbiotic dinoflagellates to survive. Dinoflagellates, on the other hand, are plankton that can be found throughout the world's oceans (Nand *et al*., 2021). As in Figure 1, the mutual relationship is based on gas exchange, where coral offers carbon dioxide and water from cellular respiration while the endosymbiont provides oxygen as photosynthesis byproducts. Other than that, the phototrophic endosymbiont will provide organic carbon (energy) in the form of sugar, lipid, amino acid, glycerol, and other compounds from photosynthesis which are important for the growth and reproduction of coral (Hoegh-Guldberg *et al*., 2017; Petrou *et al*., 2018; Chan *et al*., 2021). The coral not only serves as a host for protection to the endosymbiont but also supplies it with inorganic nitrogen and phosphorus from its waste products (Tan *et al*., 2020; Reich *et al*., 2021).

This symbiosis relation, however, may deviate depending on the environmental conditions. In optimum conditions, their relationship will give benefit both parties, however during adverse environmental conditions, the cellular metabolisms may compromise and decrease the capacity for the carbon exchange between the host and endosymbiont (Hoegh-Guldberg *et al*., 2017; Petrou *et al*., 2018). Maintaining a healthy relationship between both coral and endosymbiont is essential in preserving their homeostasis and resilience (Petrou *et al*., 2018). Photosynthetic endosymbiont species which belong to the highly diverse family Symbiodiniaceae influences the survivability and fitness of the corals, especially under stress conditions (Tan *et al*., 2020; Dutra *et al*., 2021).

Symbiodinium **(Symbiodiniaceae) diversity**

Symbiodiniaceae, the common coral endosymbionts with a diverse family of dinoflagellates, was previously grouped together according to the arbitrary letter such as A, B, C and usually referred to as "clade". However, these alphabetic clades from the family of Symbiodiniaceae have been systematically revised due to the diversity of species, comprising *Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, *Effrenium*, *Fugacium*, and *Gerakladium*, formerly known as clades A, B, C, D, E, F, and G, respectively (LaJeunesse *et al*., 2018). Abiotic and biotic environmental factors such

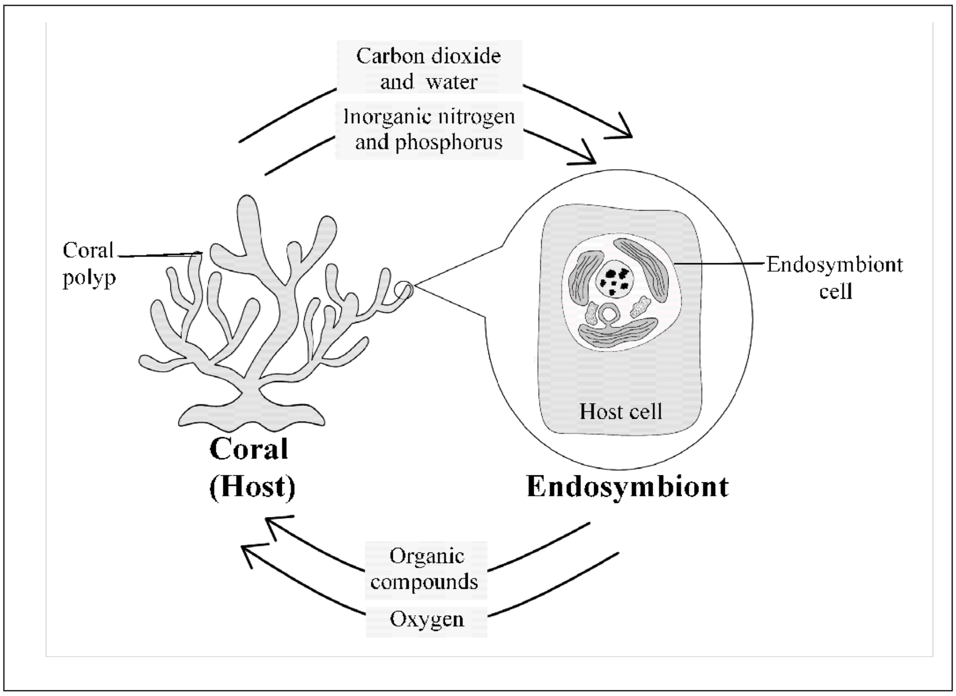


Fig. 1. The mutualistic relation between coral and its endosymbiont.

as light exposure, sedimentation, heat stress, salinity, competition between symbionts, host physiology, as well as symbionts' spatial and geographic distribution affect the diversity and abundance of Symbiodiniaceae in a coral (Tan *et al*., 2020). For instance, *Durusdinium* species mostly have higher heat resistance compared to other Symbiodiniaceae clades (Qin *et al*., 2019). Meanwhile, dinoflagellates from the genera *Breviolum* and *Fugacium* are usually observed in higher latitude, whereas *Cladocopium* can be found in tropical water (Tan *et al*., 2020). This showed that the Symbiodiniaceae response to the environmental conditions may vary depending on the characteristic of the clade.

Among the Symbiodiniaceae family, an astonishing diversity of *Symbiodinium* is the most recognized endosymbionts (Davies *et al*., 2018) which are commonly found in the shallow water of tropical and subtropical coral species (Baker, 2003). This genus can be classified into nine phylogenetic clades alphabetically from A to I, which clades A, B, C, and D are the most clades associated with scleractinian (stony) corals (Gierz *et al*., 2017; Goyen *et al*., 2017; Liu *et al*., 2018). These new clades of F to H are specified from former sub-clades F, which are F (formerly from Fr2 to Fr4), G (previously from Fr6), and H (specifically from Fr1), whereas clade I is a new clade that is a sibling group to clades B, C, F, and H (Pochon *et al*., 2004; Pochon & Gates, 2010).

Each clade has a distinct genetic lineage which makes each species unique. According to LaJeunesse *et al*. (2018), a total of 22 species have been identified which are separated into clade A (*S. linucheae, S. microadriaticum, S. natans, S. necroappetens, S. pilosum, S. tridacnidorum*), clade B (*S. aenigmaticum, S. antillogorgium, S. endomadracis, S. minutum, S. pseudominutum, S. psygmophilum*), clade C (*S. goreaui, S. thermophilum*), clade D (*S. boreum, S. eurythalpos, S. glynnii, S. trenchii)*, clade E (*S. voratum*), clade F (*S. kawagutti*), and clade G (*S. endoclionum, S. spongiolum*). Eight binomials related to *Symbiodinium* that have been mentioned by LaJeunesse *et al.* (2012) are clade A (*S.* '*cariborum*', *S.* '*corculorum*', *S.* '*fitti*', *S.* '*meandrinae*'), clade B (*S.* '*bermudense*', *S.* '*muscatinei*', *S.* '*pulchrorum*'), and clade E (*S.* '*californium*'), which are provisional taxa names that have not been fully identified yet mentioned in other research. Clades A, B, C, D, and F of *Symbiodinium* are commonly found as coral endosymbionts, while clade E is particularly present as free-living (Lin *et al*., 2018). The genetic diversity of *Symbiodinium* clades creates unique physiological traits such as the ability in light energy harvesting and utilization which impacts the coral-symbiont mutualism relation and coral fitness (Goyen *et al.*, 2017).

As the global temperature is rising perpetually, this change in climate has taken a toll on endosymbionts and their hosts, consequently impacting coral fitness and marine ecosystems. Therefore, more studies are required in order to understand the mechanisms and process of coral bleaching before a suitable approach can be contemplated to protect the coral reef.

CORAL BLEACHING INFLUENCED BY CLIMATE CHANGE

Coral bleaching is classified by the extreme loss of coral coloration, which has commonly been attributed to the remarkable loss of endosymbiont cells due to the expulsion of endosymbionts from their host corals (Figure 2) (Hoegh-Guldberg *et al*., 2017; Matthews *et al*., 2020; Szabó *et al*., 2020). This phenomenon is triggered by stress events caused by climate change, particularly ocean warming comprising, but not limited to, the elevation of sea surface temperature in conjunction with upraised sunlight irradiance (Nand *et al*., 2021). Rising sea surface temperature is adversely influencing the growth of corals, as well as reef building-corals structures by reducing their skeletal density, augmentation, and calcification rate (Dutra *et al*., 2021). Therefore, if initiatives to reduce climate change causes are not implemented, coral bleaching events are projected to cause coral reef loss in the future (Buerger *et al*., 2020; Dutra *et al*., 2021).

In 2016 and 2017, it has been reported that half of the corals were decimated, whereas 89% of coral larvae declined when summer heatwaves hit the largest coral reef in the world, the Great Barrier Reef (Buerger *et al*., 2020). In addition, it is projected that 10% of entire global coral reefs are decimated beyond recovery and 30% are in danger of extinction, which prompts mortality within one to two decades. Meanwhile, there is a probability of coral reefs being exterminated within 3 more decades (2050) with the presence of multiple threats like bleaching, illness, climate change, anthropogenic activities, and overfishing if no conservation attempts are taken seriously (Abdullah *et al*., 2016). This is an alarming sign as the natural rate of coral adaptation is unable to cope with the great loss of corals for an extended period (Buerger *et al*., 2020). About 40% of coral reefs in Malaysia are prone to high and very high threat stages arise from the local threats comprising coastal development and pollution, as well as global threats such as thermal stress and ocean acidification based on the threat analysis method conducted by World Resources Institute (Burke *et al.,* 2011). These threats have significant impacts on coral reefs, for instance, hard corals were negatively affected by coral bleaching during the El Niño phenomena in 1998 and 2010 (Khodzori *et al*., 2021). However, the survival of corals may rely on the short-term thermal acclimatization related to phenotypic plasticity, coral adaptation involving the genetic composition of the heat-stress coral population, as well as physiological thermal tolerance of their endosymbionts, *Symbiodinium* within the host corals (Morikawa & Palumbi, 2019; Buerger *et al*., 2020; Carballo-Bolaños *et al*., 2020).

Mechanisms of coral bleaching

Coral bleaching, the whitening of corals can diverge into several definitions based on its mechanisms which are the loss of symbionts with or without disappearance of photosynthetic activity, loss of symbionts' pigmentation, as well as degradation of obligate symbionts or their host cells (Nielsen *et al*., 2018; Szabó *et al*., 2020). It was reported that the bleaching can be observed by naked eyes when corals start to lose 50 to 70% of their endosymbionts (Douglas, 2009). As presented in Table 1, previous findings reported several possible coral bleaching mechanisms like exocytosis, host cell detachment, symbiophagy, apoptosis or necrosis, and autophagy (Szabó *et al*., 2020). These bleaching mechanisms may happen in parallel depending on the severity and duration of the various factors like thermal stress, but further research is necessary to evaluate their significant impacts on *Symbiodinium* cells (Oakley & Davy, 2018; Kenkel *et al*., 2020).

Reactive oxygen species in *Symbiodinium* triggers coral bleaching

The primary source of coral bleaching events is initiated by photoinhibition when oxidative damage induced by excess reactive oxygen species (ROS) is generated by heat stress in water, mainly by anomalous temperature and light exposure (Liu *et al*., 2018; Chan *et al*., 2021). Although the study of ROS related to coral bleaching is still sparse, studies on coral symbiotic oxidative stress associated with disparities in endosymbiont photosystems are growing in number (Solayan, 2016). Intracellular ROS of *Symbiodinium* emerges via several mechanisms in the chloroplast, mitochondria, and peroxisome. There are many forms of ROS production in *Symbiodinium* such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), and singlet oxygen ($^1O_2$) (Wietheger *et al*., 2018).

In chloroplasts, ROS are correlated to an electron transfer in Photosystem I (PSI) and Photosystem II (PSII) (Solayan, 2016; Szabó *et al*., 2020). The first possible way of ROS generation is by the most common source $^1O_2$ production from photosynthesis in P680 (pair of chlorophyll molecules located at the reaction center) of PSII, thus damaging the D1 and D2 proteins and in chlorophyll-containing light-harvesting antenna complexes (Grégoire *et al*., 2017; Szabó *et al*., 2020). PSII has often been associated as the primary site of ROS generation and the most susceptible to thermal stress (Diaz & Plummer, 2018). The second way is O_2^- production generated by the overproduction in P700 (a pair of chlorophyll molecules located at the reaction center) of PSI and the action of ferredoxin (Wietheger *et al*., 2018; Szabó *et al*., 2020). Other than that, the Mehler reaction, which is associated with PSI by ferredoxin soluble electron carriers also triggers H_2O_2 production when oxygen photoreduction in chloroplast takes place, which can diffuse easily across membranes, consequently leaking from *Symbiodinium* cells into host cytoplasm (Solayan, 2016; Diaz & Plummer, 2018). O_2^- and H_2O_2 also can be produced in the matrix and intermembrane space of mitochondria which is initiated by complex I and complex III, as well as within the peroxisome membrane and matrix tru cytochrome b, peroxisome membrane polypeptide, and several oxidoreductases such as glycolate oxidase (Diaz & Plummer, 2018). At low concentration, these ROS are important in a coral and its endosymbiont as a cascade signalling for the second messenger system (Szabó *et al*., 2020), however, high levels will cause major damage to both coral and *Symbiodinium* such as protein oxidation, lipid peroxidation (membrane damage), and DNA degeneration (Solayan, 2016; Tolleter *et al*., 2018). Although researchers have identified the causes of coral bleaching, the studies related these compounds in coral-endosymbiont are still limited due to their complex structures and roles (Szabó *et al*., 2020).

The removal of endosymbionts from the host may impact the coral's health and survival, which lead to coral mortality, especially if the process continues for a prolonged period of time. The loss of vast coral species at one time without recovery usually indicates a mass bleaching event (Chan *et al*., 2021). In 1998, the first remarkable mass coral bleaching event was reported in the inshore reefs of Malaysia with around 40% of coral mortality when the water temperature reached 32 °C due to El Niño phenomena (Waheed & Hoeksema, 2014). The photooxidation process of bleaching occurs by the physiological failure of thermal susceptible *Symbiodinium* due to extreme photosystem damage and the generation of detrimental ROS, whereas thermal tolerant *Symbiodinium* possesses superior defense mechanisms (Matthews *et al*., 2020).

ADAPTATION RESPONSES TO CONFRONT CORAL BLEACHING EVENTS

To endure bleaching events, corals are adapting to the changing circumstances by adjusting cellular and biochemical processes (Petrou *et al*., 2018). However, the rapid changes in the climate are beyond the adaptation of coral, leading to the persistent degradation of coral reefs. To overcome this issue, more research has been carried out to boost the resilience and tolerance of corals, especially to heat constraints, by focusing on their endosymbionts, *Symbiodinium*. Previous studies have revealed that *Symbiodinium* possesses various adaptations to survive in adverse conditions via their cellular and molecular responses, which will be inherited by the next generation (Morikawa & Palumbi, 2019). Cellular responses include behavior, morphology, and photophysiology, whereas molecular responses include genetics and biochemistry (LaJeunesse *et al.*, 2018). The main challenge nevertheless would be to understand the complex mechanisms of *Symbiodinium* responses to adapt to thermal stress (Qin *et al.*, 2019).

Cellular responses toward thermal stress

In an effort to understand *Symbiodinium*'s mechanisms under heat stress, the main aspect that needs to be focused on is the cellular level responses. Previous findings demonstrated that most *Symbiodinium* cultures can survive up to 30 °C temperature by possessing various cellular adaptations compromising behavior (host-symbiont specificity), morphology (cell size), and photophysiology (photosynthetic efficiency) (Chakravarti & van Oppen, 2018; LaJeunesse *et al.*, 2018; Petrou *et al.*, 2018). Notwithstanding, a complete understanding of the cellular mechanisms is still sparse (Nielsen *et al.*, 2018). Therefore, a comprehensive understanding of the cellular metabolisms of the endosymbionts is crucial to help identify the process of bleaching and improve the survival rate (Petrou *et al.*, 2018).

Behavioral protection

A protective behavior is any voluntary or involuntary reaction of an individual in regards to protecting itself from threatened environments (Rohrscheib & Brownlie, 2013). Behavioral protection of *Symbiodinium* may involve host-symbiont specificity, where the host is selectively chosen by the endosymbionts (Tonk *et al.*, 2013; LaJeunesse *et al.*, 2018). Other than host shape, thickness, and pigment, local environmental conditions like light and temperature also play an important role in host-symbiont specificity which controls the ability of coral's tolerance to stress (Tonk *et al.*, 2013; Reich *et al.*, 2021).

Selection pressure on the occurrence of *Symbiodinium* populations in corals is referred to as the adaptive bleaching hypothesis or the *Symbiodinium* shift hypothesis (Louis *et al.*, 2016). The light associated with depth gradients from shallower water (5 to 25 m) that extend down to mesophotic water (more than 100 m) is the main factor for *Symbiodinium* distinct selection pressure, which leads to specificity between *Symbiodinium* and corals (Bongaerts *et al.*, 2015; Grégoire *et al.*, 2017; Reich *et al.*, 2021). Previous findings revealed that *Symbiodinium* '*fitti*' is associated with Caribbean acroporids, *Acropora cervicornis* and *Acropora palmata* as well *A. cervicornis* in Florida. Although located in the same region (Florida), depth and light intensity play a major role in the exclusive selection pressure of the host-endosymbiont (Lohr *et al.*, 2019; Reich *et al.*, 2021). In addition, the symbiont acquisition strategy whether through the environment or parent colony as well as morphological variation will also influence the selection pressure of *Symbiodinium* (Tonk *et al.*, 2013).

Morphological protection

Symbiodinium possesses different morphologies such as cell size, chromosome number, as well as chloroplast size, number, and arrangement which influence thermal adaptations (Biquand *et al.*,

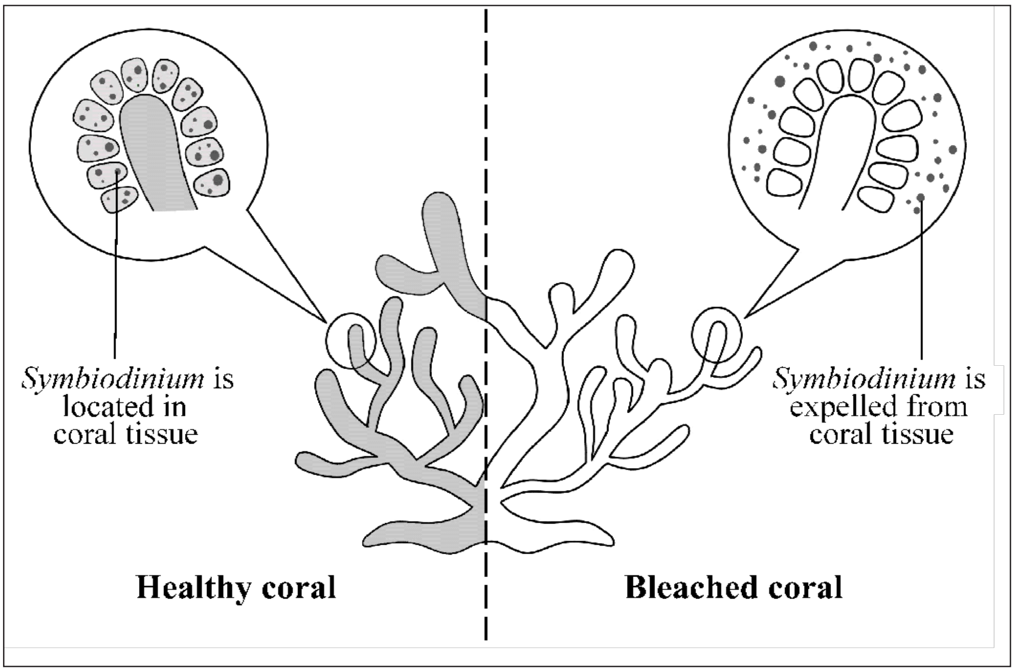
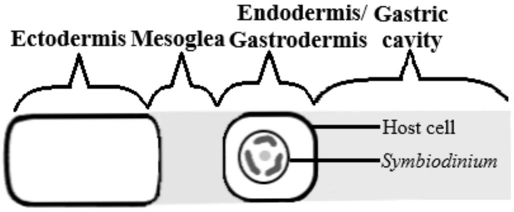
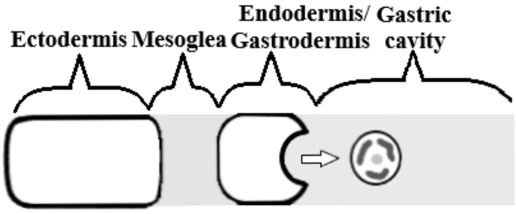
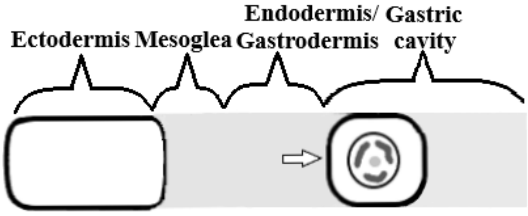
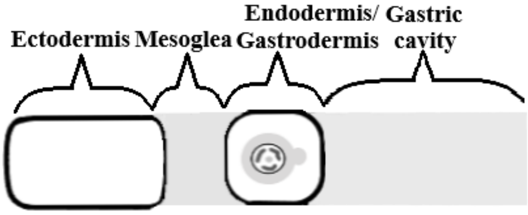
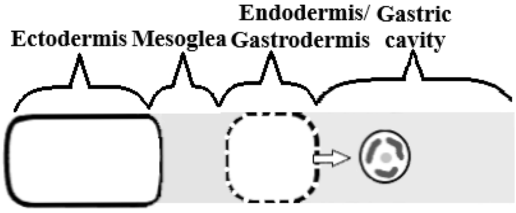
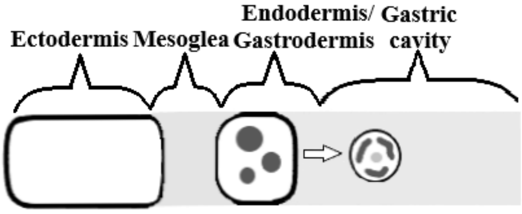


Fig. 2. *Symbiodinium*'s location in healthy and bleached coral, modified from Petrou *et al.* (2018).

Table 1. Several possible bleaching mechanisms in corals, modified from Szabó *et al*. (2020)

Bleaching mechanism	Explanations
Normal 	□ Healthy *Symbiodinium* is an endosymbiont to coral cell, which is located in inner endodermis.
Exocytosis 	□ *Symbiodinium* is expelled out from the inner endodermis or gastrodermis of the host cell to the gastric cavity (Szabó *et al*., 2020). □ *Symbiodinium* expulsion normally happens to retain *Symbiodinium* population and density (Fujise *et al*., 2014; Oakley & Davy, 2018).
Host cell detachment 	□ A host cell containing *Symbiodinium* is released from the endodermis or gastrodermis to the gastric cavity (Szabó *et al*., 2020).
Symbiophagy 	□ *Symbiodinium* is digested within the host-derived symbiosome (Oakley & Davy, 2018; Szabó *et al*., 2020).
Host cell apoptosis or necrosis 	□ A host cell is degraded and consequently releases *Symbiodinium* from the host cell into the gastric cavity (Szabó *et al*., 2020).
Autophagy 	□ A host cell is disintegrated due to cellular breakdown with or without *Symbiodinium* (Szabó *et al*., 2020).

2017). Amongst morphological studies, cell size has a significant impact on cellular function, DNA content, growth, and photosynthesis of *Symbiodinium* (LaJeunesse *et al*., 2012). In addition, *Symbiodinium* cell size also acts as an additional factor that may influence host specificity in coral communities. The variation size of coral endodermis, ranging from 10 to 25 µm, forces the coral to selectively choose the suitable size of *Symbiodinium* cell to be engulfed by phagocytosis (Biquand *et al*., 2017).

The *Symbiodinium* cells size range from 5.5 to 12.4 µm and vary amongt clades and species (Hansen & Daugbjerg, 2009; LaJeunesse *et al*., 2001; Lee *et al*., 2014; LaJeunesse *et al*., 2018) as shown in Table 2. The *Symbiodinium* cell size can be divided into three groups based on cell diameter: small (less than 8.0 µm), medium (8.0 to 10.0 µm), and large (more than 10.0 µm) (Biquand *et al*., 2017). Cell size is considered as an important *Symbiodinium* morphological protection towards coral bleaching due to its relation to photosynthetic efficiency via the variability of PSII light harvesting and utilization (Suggett *et al*., 2015).

It was reported that the increment of *Symbiodinium* cell size will significantly increase volume-specific chlorophyll a and b, elevate the photosynthetic efficiency, and initiate the whitening of corals and bleaching events (Suggett *et al*., 2015; Malerba *et al*., 2018; Hoadley *et al*., 2019). Previously, *Pocillopora* coral colonies associated with *S. glynnii* have been reported can sustain their symbiont populations from bleaching and mortality even during extreme warm water events in the Eastern Tropical Pacific compared to *S. trenchii* from the same coral species. The contradiction between bleaching tolerance in both *Symbiodinium* species is associated with the cell size variation (Wham *et al*., 2017). Therefore, the size of *Symbiodinium* cells could provide an insight into photophysiological adaptation which is closely related to coral's health. More research is needed to unravel the interconnection between *Symbiodinium*'s cell size and the coral bleaching events.

Photophysiological protection

Photophysiology, a short term for photosynthetic physiology, is the physiology responses of photosynthetic processes to change in environments, which is commonly assessed by measuring chlorophyll fluorescence (Harrison *et al*., 2015). The most common photophysiological parameter that has been widely used to measure photoprotection and stress detection is the photosynthetic efficiency of PSII. Photosynthetic efficiency is associated with the ability of *Symbiodinium* to convert inorganic carbon dioxide into organic compounds via a carbon fixation process, such as maximum quantum yield (F_v/F_m) measurement

Table 2. Cell sizes of *Symbiodinium* species

Symbiodinium clade	*Symbiodinium* species	Cell length (µm)	Cell width (µm)	References
Clade A	*S. linucheae*	8.5	8.0	(LaJeunesse *et al*., 2018)
	S. microadriaticum	7.6 to 10.0	5.8 to 7.7	(Lee *et al*., 2014)
	S. natans	9.5 to 11.5	7.4 to 9	(Hansen & Daugbjerg, 2009)
	S. necroappetens	9.2	8.5	(LaJeunesse *et al*., 2018)
	S. pilosum	10.5	9.8	(LaJeunesse *et al*., 2018)
	S. tridacnidorum	8.1 to 10.1	5.6 to 7.1	(Lee *et al*., 2014)
Clade B	*S. aenigmaticum*	7.5	6.8	(LaJeunesse *et al*., 2018)
	S. antillogorgium	7.7	7.1	(LaJeunesse *et al*., 2018)
	S. endomadracis	6.6	6.0	(LaJeunesse *et al*., 2018)
	S. minutum	6.8 to 9.4	5.5 to 7.8	(Lee *et al*., 2014)
	S. pseudominutum	8.1	7.3	(LaJeunesse *et al*., 2018)
	S. psygmophilum	7.9 to 12.3	6.4 to 9.7	(Lee *et al*., 2014)
Clade C	*S. goreaui*	9.7 to 11.1	8.7 to 10.0	(LaJeunesse *et al*., 2001)
	S. thermophilum	9.3	8.6	(LaJeunesse *et al*., 2018)
Clade D	*S. boreum*	9.5	8.7	(LaJeunesse *et al*., 2018)
	S. eurythalpos	9.0	8.2	(LaJeunesse *et al*., 2018)
	S. glynnii	9.0 to 10.0	7.6 to 9.0	(LaJeunesse *et al*., 2001)
	S. trenchii	9.5 to 10.5	8.5 to 10.0	(LaJeunesse *et al*., 2001)
Clade E	*S. voratum*	8.5 to 12.4	6.4 to 9.8	(Lee *et al*., 2014)
Clade F	*S. kawagutti*	8.7	8.4	(LaJeunesse *et al*., 2018)
Clade G	*S. endoclionum*	8.5	7.6	(LaJeunesse *et al*., 2018)
	S. spongiolum	8.6	7.8	(LaJeunesse *et al*., 2018)

(Louis *et al*., 2016; Hull *et al*., 2021). Reductions of photosynthetic efficiency in *Symbiodinium* have been considered as an indicator of heat and bleaching susceptibility (Davies *et al*., 2018). During the elevated levels of sea temperature, the photosynthetic protein complex of PSII is negatively impacted. High temperature influences the carbon metabolism and electron transport which inhibits the photosynthesis activity of *Symbiodinium* cells (Grégoire *et al.,* 2017). However, the performance of photosynthesis varies depending on the *Symbiodinium* species, which can be the photophysiological indicator of thermal stress (Grégoire *et al.,* 2017).

It has been proposed that *Symbiodinium* clade D has higher tolerance towards the increase of temperature and radiance compared to other clades. Studies conducted by Rowan (2004) showed that *Symbiodinium* clade D produced higher F_v/F_m with increasing temperature (32 °C) as a photoprotection strategy in the warm water corals such as *Pocillopora* spp. In another study, *Symbiodinium* in seven different coral species which are *Acropora aspera*, *Acropora formosa*, *Acropora millepora*, *Platygyra daedalea*, *Porites cylindrica*, *Porites lutea*, and *Montipora digitata* exhibited a descending pattern of F_v/F_m with the extended period of thermal treatment (32 °C), however, the reduction point is differ among the coral-host species depending on their heat sensitivity (Fisher *et al*., 2012).

Apart from that, Chakravarti and van Oppen (2018) demonstrated different results where only *Symbiodinium* clade F (*S. kawagutii*) was able to grow in higher temperatures up to 34 °C throughout approximately one year compared to clade A, D, and G, which clade F maintained its F_v/F_m values between control (27 °C) and higher temperatures (30 °C, 33 °C, and 34 °C). Contrarily, *Symbiodinium* clade F shows a reduction pattern of F_v/F_m throughout 28 days of thermal treatment at 31 °C, suggesting photoinactivation of PSII (Gardner *et al*., 2017; Gierz *et al*., 2017). Therefore, various strains and clades of *Symbiodinium* possess different photosynthetic efficiency depending on the environmental temperature. However, it should be noted that the threshold level of photosynthetic efficiency varies depending on the increment of the temperature and duration of the exposure, thus more study is needed, especially the mechanism behind the photoactivation of each clade.

Molecular responses toward thermal stress

Apart from the cellular responses, molecular responses of *Symbiodinium* such as genetics (gene expression) and biochemistry (proteins and metabolites) are also important to be comprehended to avoid complete perish of coral reef ecosystems (Szabó *et al.*, 2020). Over the past few decades, molecular levels have been extensively studied by researchers via "omics" approaches such as transcriptomics, proteomics, and metabolomics to unveil inclusive pathways and mechanisms although there are difficulties in collecting large amounts of tissue for analysis (Oakley & Davy, 2018; Petrou *et al*., 2018). This helps the researchers to in-depth understand the intricacies of the metabolic state of the coral-*Symbiodinium* and uncover the molecule derived from the metabolic process (Gierz *et al*., 2017; Petrou *et al*., 2018).

Transcriptomic approaches

Gene expression profiling by RNA sequencing (RNA-Seq) analysis has been broadly used to investigate transcriptomic responses of *Symbiodinium* to thermal stress in an effort to curb coral bleaching events (Gierz *et al*., 2017). However, there is no standard primer set for reference genes that can be used across different *Symbiodinium* clades and strains (Goyen *et al*., 2017). Due to its high sensitivity, throughput, and cheap cost, gene expression profiling has become a significant and popular way of assessing *Symbiodinium* response to experimental circumstances (Oakley *et al*., 2018). To investigate heat tolerance in *Symbiodinium*, identification of differentially expressed genes (DEGs) analysis will be emphasized more than non-significant differential expression genes or 'core' genes that mostly catalytic activity and binding (Gierz *et al*., 2017; Lin *et al*., 2018). DEG is usually used to express the difference in the expression level of a gene from various samples tested.

According to Lin *et al*. (2018), *Symbiodinium kawagutti* (clade F) exhibited 357 DEGs (4.42%) specifically for thermal stress during high temperature (30 °C) compared to control (25 °C). Among the DEGs, the expression of heat shock proteins like Hsp40, Hsp70, and Hsp90 are widely spotted as target genes for thermotolerance detection (Cziesielski *et al*., 2018). The increment of Hsp70 was noticed in *S. kawagutti* to confront heat stress (Lin *et al*., 2018). Similar to *S. kawagutti*, the upregulation of Hsp70 was also found in *Symbiodinium* sp. clade F at 31 °C temperature (Gierz *et al*., 2017). On the contrary, Hsp70 in *Symbiodinium* sp. clade C isolated from *Acropora aspera* declined by 59% and 69% when exposed at 32 °C with fast (18 hours) and gradual (120 hours) thermal stress, accordingly (Rosic *et al*., 2011). Hsp70 in *S. microadriaticum* was also downregulated under high temperatures (Chen *et al.*, 2017). Intriguingly, previous studies carried out by Putnam *et al*. (2013) and Barshis *et al*. (2014) recorded no significant changes in the Hsp70 expression of *Symbiodinium* sp. after 9 days and 72 hours of high temperature, correspondingly. The variation findings might be correlated with the ability of the *Symbiodinium* to control the size and composition of the protein pool as thermal protection (Barshis *et al*., 2014).

Apart from heat-shock proteins, the cytochrome P450 (Cyt P450) gene is also an important DEGs in *Symbiodinium* for cell defense and detoxification from detrimental ROS during thermal stress (Rosic *et al*., 2010). The upregulation of Cyt P450 was detected in *S. kawagutii* (Lin *et al*., 2018). Chen *et al.* (2017) found that *S. microadriaticum* exhibited a negative impact of Cyt P450 during heat stress. Rosic *et al*. (2010) also reported a consistent finding where expression of Cyt P450 was dropped in *Symbiodinium* sp. clade C when treated at very high temperature (32 °C) under rapid (18 hours) and gradual (120 hours) thermal stress. However, there were no significant effects of high temperature on Cyt P450 expression in *Symbiodinium* spp. clades C and D (Barshis *et al*., 2014). The studies on the Cyt P450 gene expression in *Symbiodinium* under thermal stress are still limited to understanding the full mechanisms. However, Barshis *et al*. (2014) suggested that variation in *Symbiodinium* physiologies like modification in thylakoid membrane might be correlated to the regulation of the gene expression.

It is also interesting to note the expression of oxidative stress-related genes such as superoxide dismutase (SOD), glutathione S-transferase (GST), and catalase peroxidase (KatG) which can scavenge the harmful ROS (Gierz *et al*., 2017; Cziesielski *et al*., 2018). SOD has a cytoprotective function against oxidative damage by major superoxide radicals with can be found in several isoforms such as copper/zinc SOD (Cu/ZnSOD), manganese SOD (MnSOD), and iron SOD (FeSOD) (Roberty *et al*., 2016; Moghaddam *et al*., 2021). Gierz *et al*. (2017) reported thermal stressed *Symbiodinium* sp. (clade F) in the study upregulated Cu/ZnSOD and MnSOD. In another study, *Symbiodinium* clade A and clade D exhibited an overexpression of selected gene MnSOD in heat treated culture as oxidative protection (Goyen *et al*., 2017). Other than that, *Symbiodinium* spp. isolated from the Great Barrier Reef's coral, *Acropora tenuis* at Magnetic Island showed the upregulations of Cu/ZnSOD and FeSOD after 9 days of heat stress exposure (32 °C) compared to *Symbiodinium* spp. from South Molle Island (Levin, 2017). Nevertheless, it is essential to note that *Symbiodinium* cells do not only depend on the SOD expression alone to confront oxidative stress. Therefore, further comprehensive studies related to oxidative stress-targeted genes can be performed.

Based on previous studies conducted, it can be concluded that there is a continuous argument related to the gene expression of *Symbiodinium* in response to stress. The DEGs analysis of *Symbiodinium* towards the rising temperature differed depending on the adaptation of *Symbiodinium* clade itself and most of the DEGs studies were not using specific *Symbiodinium* species. Thus, a huge gap in transcriptomic areas urges further studies, especially towards the gene expression of different clades and species of *Symbiodinium* to be done.

Proteomic approaches

Proteomic approaches are useful tools to uncover *Symbiodinium*'s response to cellular stress at the protein-level (Mayfield *et al*., 2018). While there has been growing research into thermal tolerance mechanisms at the transcriptomic level, there has been little research into proteomic (Cziesielski *et al*., 2018). One of the main dynamic range issues in proteomic studies is the amount of proteins present in the sample varies by several orders of magnitude, confusing the capacity to detect small proteins amid the very abundant ones (Oakley *et al*., 2018). During high-temperature conditions, the mRNA splicing process to translate mRNA into proteins will be suppressed, resulting in an alteration of the difference in the expression of protein levels, which are also known as differentially expressed proteins (DEPs) (Cziesielski *et al*., 2018; Mayfield *et al*., 2018).

After 8 weeks of treatment, a study by Mayfield *et al*. (2018) demonstrated a notably second rise of stress response protein known as DnaJ protein in heat-stressed *Symbiodinium* sp. Similarly, Baumgarten *et al*. (2013) also noted an up-regulation of DnaJ protein in *S. microadriaticum* under high temperatures. DnaJ protein serves as co-chaperones to prevent improper interactions between proteins in non-native conformations. The rise in *Symbiodinium*'s DnaJ protein is an alarming sign of protein denaturation and symbiont mortality, which can cause hosts to lose their symbionts (Ladner *et al*., 2012). The expression of the stress response proteins was involved in a diverse array of important cellular processes in *Symbiodinium* and high numbers of DEPs indicate adverse effects of temperature on *Symbiodinium*.

Apart from that, DEPs related to photosynthetic like ribulose-1,5-bisphosphate carboxylase-oxygenase (or known as Rubisco) also increased in *Symbiodinium* sp. under thermal stress. In addition, peridin-chlorophyll, a binding protein linked to photosynthesis, was escalated in heat-stressed *Symbiodinium* after four weeks of high-temperature exposure (Mayfield *et al*., 2018). Another study has been conducted by Pasaribu *et al*. (2015) on *Symbiodinium* sp. present in the sea anemone, *Exaiptasia pallida*, where DEPs detected in *Symbiodinium* sp. were related to photosynthesis consisting of photosystem and chlorophyll protein in response to increasing temperatures. The upregulation of DEPs correlated to photosynthesis in *Symbiodinium* sp. under heat stress suggests the photoprotective of *Symbiodinium* sp. to hinder the photosynthetic apparatus injury which may cause the bleaching events by the stress (Dang *et al*., 2019).

During the rising temperature, several DEPs in *Symbiodinium* sp. produced in high concentrations associated with transport protein like voltage-dependent T-type calcium channel subunit alpha-1H (Mayfield *et al*., 2018). This finding appears contrary to the previous research by Weston *et al*. (2015) that observed no detection of transport protein (voltage-dependent calcium channel alpha-2/delta invertebrate-type protein) in *Symbiodinium* sp. under elevated temperature. The transport proteins are involved in exocytosis as a functional process for the expulsion of endosymbionts from coral tissues during bleaching events (Weston *et al*., 2015). However, further study is required to enlighten the relation between transport proteins and exocytosis of *Symbiodinium*.

The protein regulation of *Symbiodinium* and the response of the host towards the protein regulation may indicate the relationship of host-endosymbiont. The rising temperature may level up the protein expression in *Symbiodinium*, which leads to the decrease of the osmotic pressure between host and symbiont. This eventually leads to bleaching events. Thus, more research is required especially in the integration of the proteomic with the transcriptomic and metabolic data to enhance the understanding of the proteomic function in the bleaching phenomena.

Metabolomic approaches

Metabolomics is the detection and quantification that focuses on metabolites, which is the intermediates and end products of cellular regulatory activities such as carbohydrates, lipids, amino acids, fatty acids, sugars, organic acids, and other secondary metabolites (Hillyer *et al*., 2017). *Symbiodinium* metabolomic profiling data are key elements that reflect the information on the endosymbiont's stress condition, as well as the physiological activities of their symbiotic partners (Oakley & Davy, 2018; Lohr *et al*., 2019). *Symbiodinium* provides the coral host with its metabolites like glucose, lipids, alanine, and glycolic acid as an exchange for the coral host's metabolites like ammonium, nitrate, and sulfate (DeSalvo *et al*., 2010).

Among all *Symbiodinium* clades, clade C can fix and translocate more organic carbon under normal conditions (Sogin *et al*., 2017). This is in agreement with Farag *et al*. (2018) as *Symbiodinium* clade C was composed mostly of sugars, which are primary products of photosynthesis. Sugars are essential for growth and adaptation to environmental restraints for metabolic processes. During severe thermal stress, *Symbiodinium* has been found to accumulate higher glucose as an alternative for energy supply (Hillyer *et al*., 2018). Apart from that, Sogin *et al*. (2017) reported that *Symbiodinium* clade C in the study produced more carbohydrates compared to lipid, suggesting that clade is actively in carbohydrates metabolism. However, many findings reported that *Symbiodinium* clade C exhibited a significant reduction in carbohydrates in terms of sugars, most remarkably the disaccharides melibiose under higher temperatures (Hillyer *et al*., 2017; Farag *et al*., 2018).

The heat-stressed *Symbiodinium* produced higher fatty acids in the form of saturated fatty acids (dodecanoic acid, C12:0), and polyunsaturated fatty acids (linoleic acid, C18:2, and linolenic acid, C18:3). High production of polyunsaturated fatty acids in *Symbiodinium* suggests the protection of chloroplast lipid membranes from any oxidative injuries due to excessive ROS, as a strategy to sustain hosts' endosymbiont during heat stress (Ladner *et al*., 2012). On the other hand, a previous study by Rosset *et al*., (2019) found that the increase of fatty acids was only observed among sulfoquinovosyldiacylglycerol (SQDGs) in *S. trenchii* (previously known as *Durusdinium trenchii*) as the main respond to a higher temperature (30 °C). SQDGs are important for maintaining the structure and function of the photosynthetic apparatus in the *Symbiodinium*. Meanwhile, further study conducted by Tchernov *et al.* (2004) recorded a lower proportion of polyunsaturated fatty acid in the thylakoids membrane of *Symbiodinium* isolated from clades A, B, and E which the clades were related as thermotolerant. These findings showed that *Symbiodinium* altered the production of fatty acid constituents to cope with the elevated temperature, however, the constituents differ depending on the symbiont strain present in the host.

Our capacity to analyze, estimate, and perhaps change the outcome of bleaching incidents is dependent on our understanding of the biological processes of *Symbiodinium*'s high-temperature acclimation (Oakley & Davy, 2018). Thus, it is crucial to investigate the emerging responses of *Symbiodinium* toward climate change, considering the significant role of *Symbiodinium* in the health and function of the corals.

CONCLUSION

This review article provides an insight into the relationship between coral and *Symbiodinium*, as well as the coral bleaching occurrence engendered by climate change. *Symbiodinium* has a significant impact on coral's health and survival. In addition, this review also offers an overview of cellular and molecular adaptation strategies possessed by *Symbiodinium* to cope with climate change, mainly thermal stress. Yet, understanding of the complete pathways responsible for the adaptation of the coral-endosymbiont relationship under unfavourable conditions is still lacking especially in the transcriptomic, proteomic, and metabolomic approaches of *Symbiodinium*. Although proving to be a powerful tool that might be the key to uncovering the bleaching event, limited research studies restrict the review in this paper. However, brief information gained from this review is believed to enlighten marine researchers to give consideration towards studies related to

Symbiodinium's responses toward stressors. Thus, this review suggests that more studies have to be done about coral and *Symbiodinium* mechanisms in an effort to reduce the mass coral bleaching occurrence to sustain the marine ecosystems. In addition, more serious efforts are required, including strong regulations, proper environmental strategies, effective administration, comprehensive community encouragement, and engagement to preserve our coral ecosystems for the next generation.

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