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Algicidal Bacteria to Prevent Eutrophication in Natural Water Body

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Abstract: Eutrophication is found to be one of the major issues worldwide. Freshwater as well as marine water bodies are affected due to rise in dominant harmful Algal Blooms (HABs) & depletion of oxygen in water. Cultural eutrophication enhances harmful effects of natural eutrophication causing various diseases due to bioaccumulation of toxins released by HABs in marine animals & seafood consuming human beings. Use of algicidal bacteria is one approach to restrict growth or lyse HABs in waterbodies. Commensal, mutualistic, parasitic relationships between species of algae & algicidal bacteria studied throughout the world. Variation in killing, resistance, regulation mechanisms found between algae & algicidal bacteria. Different isolation techniques of algicidal bacteria help to study different relationships among them. Algicidal effect of bacterial strain varies from species to species of different alga. Some of the HABs producing algal species are found to be of *Alexandrium*, *Aphanizomenon*, *Chattonella*, *Cylindrospermopsis*, *Gymnodinium*, *Oscillatoria*, *Pseudo-nitzschia* genus. The main objective of the review article is to study isolated algicidal bacteria found commonly in different countries.

Keywords: **Eutrophication, HABs, Algicidal bacteria, *Alexandrium*, *Chattonella*, resistance, regulation.**

I. INTRODUCTION:

Eutrophication process is defined as the increase in the rate of dissolved organic & inorganic matter in water bodies which lead to increase in algal blooms. Word Eutrophication comes from the Greek word *eutrophos* having meaning as "well-nourished", has become a major environmental problem [15] (Figure 1).

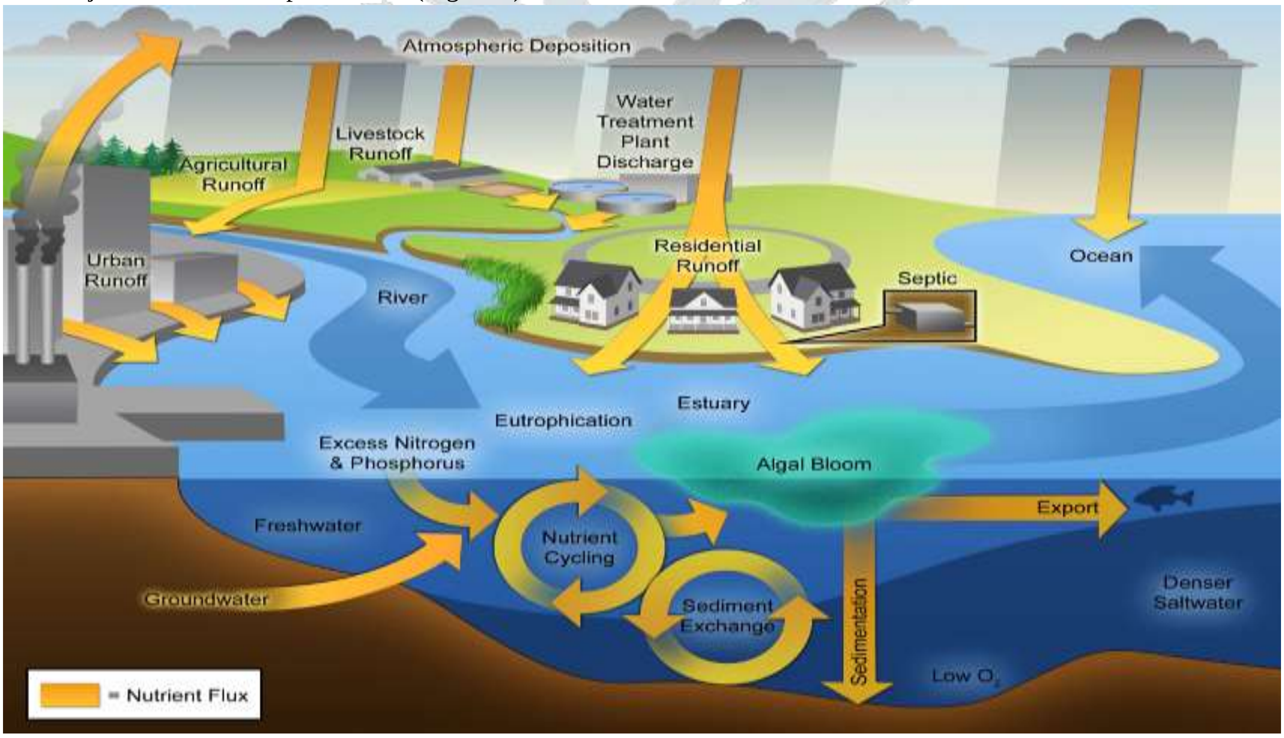


Figure 1: Process of Eutrophication [10].

Eutrophication has 2 types- i) Natural Eutrophication caused by natural processes due to ageing of living beings present in water bodies. ii) Cultural Eutrophication caused by anthropogenic activities which enhance effects of natural eutrophication. Nitrogen-N & phosphorous- P content in water bodies mainly controls eutrophication [26]. But other than N and P, several algae use complex organic molecules as nutrients & energy such as mixotrophs- species that mix their strategies like extracellular oxidation, hydrolysis for taking up nutrients [26]. Some metal concentrations also rise eutrophication caused by specific algae like Selenium- Strains of the dinoflagellate *Gymnodinium catenatum* [4], Silica [27]. Eutrophication rises due to following reasons such as- 1] Coastal pollution because of increase in population growth leads to sewage discharge & runoff from populated & agricultural areas [5]. 2] Increase in the use of N and P chemical fertilizers [5]. 3] Transfer of nutrients from benthic to pelagic environment [27]. 4] Overexploitation of Piscivorous fishes [27]. This eutrophication gives rise to Harmful Algal Blooms (HABs) in various regions like increased abundance of toxic diatom *Pseudo-nitzschia*, in the Mississippi River of the United States [21], blooms of *Phaeocystis globosa* in Northern European waters [12], outbreak of toxic dianoflagellates *Pfiesteria piscicida* and *P. shumwayae* in estuaries and coastal waters of the mid-Atlantic and south-eastern United States [12]. Algal blooms in water bodies further cause depletion of oxygen content.

There are evidences of practices performed to control issue of Eutrophication-

1. Controlling P and N inputs:
   Scientist Vollenweider's simple model based on lake where he changed the formulations of laundry detergents which contain 50% P & he also removed P from human sewage for reduction in algal blooms [34] but this technique has many limitations like its time consuming and tedious process.
2. Release of Piscivorous predators:
   In Wisconsin, small lakes were selected for experiment where study of phytoplankton content was carried out in presence & absence of Piscivorous predators. Result evident that Piscivorous predators containing lakes showed low levels of algae [19].
3. Use of Algicidal organisms:
   In this biological system used for killing algae found to be more efficient as these techniques are more cost effective & easy to imply. One approach is to make use of algicidal bacteria [37].
4. Physical method:
   In which various machines are used for removal of algae from water bodies [37].

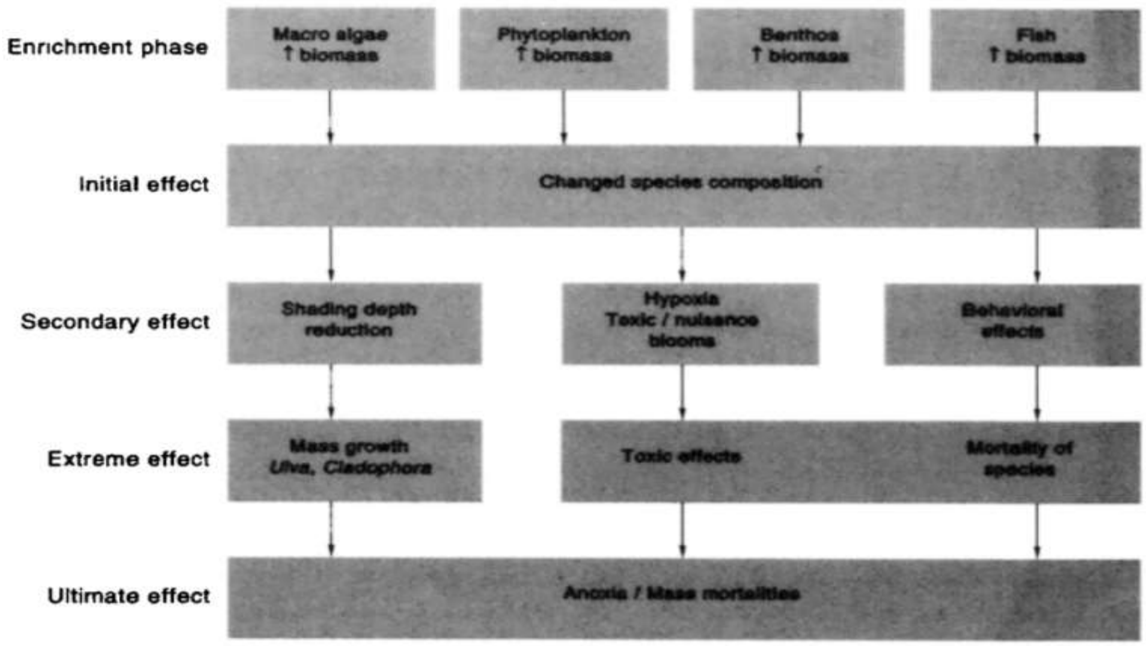


Figure 2: Phases of Eutrophication [26].

II. SOME OF THE ALGAL BLOOMS PRESENT IN DIFFERENT PARTS OF WORLD:

There is difference between Marine & Freshwater as P, N content found to be limited in freshwater & marine water respectively leads to difference in phytoplankton [26]. The information of some algal blooms found in the United States, Spain, India & their effects seen on environment as well as living beings are listed in Table 1.

Table 1: Algal blooms found in different parts of the World and their effects on living beings.

Sr. No.	Name of algae	Tax group	Places of Occurrence	Effect	Ref.
1.	*Aphanizomenon spp*	CYNO	1. Washington State, United States.	Production of freshwater toxins: Microcystin, Anatoxin-a, Cylindrospermopsin.	[32]
2.	*Cylindrospermopsis spp*	DIAT	1. Washington State, United States. 2. Spain.	1. Production of freshwater toxins: Anatoxin-a, Cylindrospermopsin, Saxitoxin. 2. Fish killing & Bloom forming.	[32] [33]
3.	*Alexandrium spp*	DINO	1. Washington State, United States. 2. Spain. 3. India.	1.Production of marine toxin: Saxitoxin. 2.Paralytic Shellfish poisoning	[32] [33] [20]
4.	*Pseudo-nitzschia spp*	DIAT	1. Washington State, United States. 2. Spain. 3. India.	1. Production of marine neurotoxin: Domoic acid. 2. Fish killing & Bloom forming.	[32] [33] [20]
5.	*Gymnodinim spp*	DINO	1. Spain. 2. Karnataka, India.	1. Fish killing & Bloom forming. 2. Paralytic Shellfish poisoning. 3. Red colouration of water.	[33] [3]
6.	*Heterocapsa spp*	DINO	Spain.	Fish killing & Bloom forming.	[33]
7.	*Prorocentrum spp*	DINO	1. Spain. 2. India.	Fish killing & Bloom forming.	[33] [20]
8.	*Scrippsiella spp*	DINO	Spain.	Fish killing & Bloom forming.	[33]
9.	*Chaetoceros spp*	DIAT	1. Spain. 2. India.	Fish killing & Bloom forming.	[33] [20]
10.	*Chattonella spp*	ALG	India.	Fish & Animal mortalities.	[20]
11.	*Cochlodinium spp*	DINO	Off Goa,India.	1. Fish & Animal mortalities, Algal Bloom	[3]
12.	*Dinophysis spp*	DINO	1. Washington State, United States. 2. India.	Production of marine toxin: Diarrhetic Shellfish toxin.	[32] [20]
13.	*Coscinodiscus spp*	DIAT	Kerala, India.	Brownish-red discolouration of water.	[3]
14	*Noctiluca spp*	DINO	1. Kerala, India. 2. Goa, India. 3. Off Kerala coast, India.	1. Discolouration of water. 2. Mortality of fish. 3. Oxygen depletion. 4. Bleaching of Corals. 5. Death of fishes & Sea animals.	[3]
Sr. No.	**Name of algae**	**Tax group**	**Places of Occurrence**	**Effect**	**Ref.**

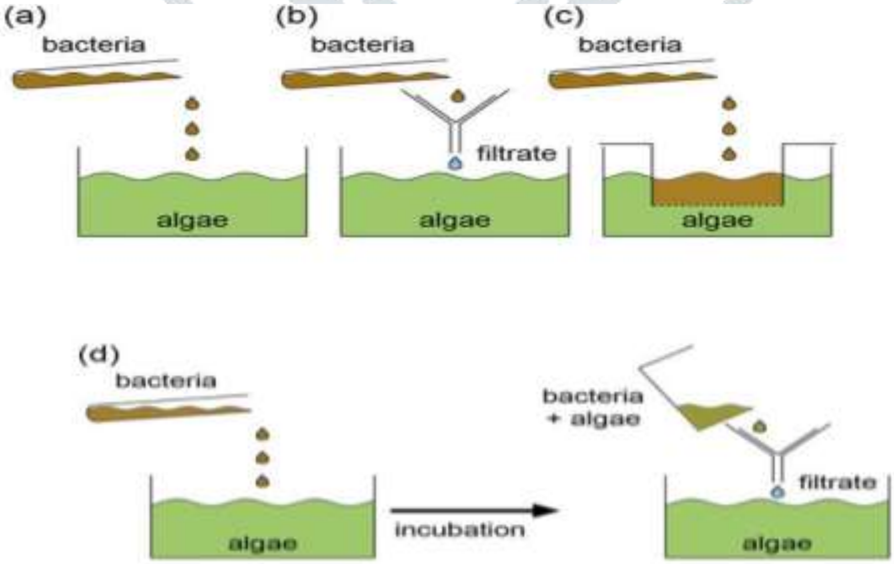
15	*Kerenia spp*	DINO	Kerala, India.	1. Intense brownish colouration of water, Mass fish mortality.	[3]
16	*Trichodesmium spp*	CYNO	1. Lakshadweep, India. 2. Manglore-Quilon, India. 3. Gulf of Mannar, India	1. Adverse effects on Tuna fisheries. 2. Discolouration of water. 3. Mortality of fish, crabs.	[3]
17	*Microcystis spp*	CYNO	Kerala, India.	1. Discolouration of water, Irritation of skin among local people.	[3]
18	*Hornellia spp*	RAPH	North Kerala, India.	1. Green discolouration, Fish & Faunal mortality.	[3]
19	*Oscillatoria spp*	CYNO	Washington State, United States.	Production of freshwater toxin: Anatoxin-a.	[32]
20	*Skeletonema spp*	DIAT	Spain.	Fish killing & Bloom forming.	[33]

CYNO- Cyanobacteria, DIAT- Diatom, DINO- Dinoflagellate, ALG- Algae, RAPH-Raphidophyte

III. ALGAL BLOOMS AND ALGICIDAL BACTERIA:

Algae & Heterotrophic bacteria present in freshwater as well as marine water both need Dissolved Organic Matter (DOM) for their growth [14]. Relationship between algae & bacteria is a dynamic relationship. There organic matter produced by algae utilized by bacteria present in water establishing Commensalism & mutualist relationship by exchanging organic & inorganic matter between alga-bacteria [24]. But there are some bacteria which under nutrient stress lyse the algae present in the water for their growth. The scientists researched various bacteria & algal relationships. Algicidal bacteria can be defined as bacteria that have biochemical potential to kill algae. It should have specialized properties to kill algae in any conditions & satisfy Koch's postulates in an environmental context [14].

To determining relationship between algae & algicidal bacteria following methods applied by Scientists:



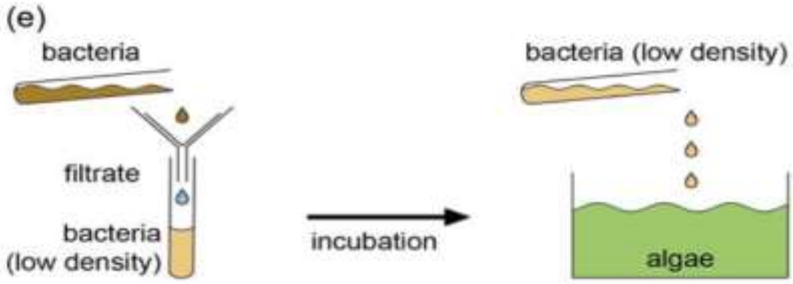


Figure 3: Methods for determining relationship between algicidal bacteria & algae [17].

(a) Direct contact Cultivation: Identification of algicidal bacteria independent of their mechanism.

(b) Cell-free medium (filtrate): Identification of indirect killing mechanism of algicidal bacteria.

(c) Non-contact co-culturing with semipermeable membranes facilitating passage for only algicide: Identification of signalling compound present for algicide production.

(d) Cultivation with direct contact followed by testing the spent medium (filtrate) of bacteria-killed algae in new strain of algae for analysis of cross-signalling algicidal activity.

(e) To provide initial evidence for a Quorum Sensing (QS)-type mechanism in which low density QS algicide bacterial culture is added into high density culture of algae.

There is evidence in papers that bacterial media containing some components show algicidal activities. Before the discovery of ribosomal DNA sequencing for identification of bacteria, analysis of bacterial taxa & its physiological conditions used to be carried out by biochemical & morphological methods [14].

Bacteria secrete compounds which reach towards algae after dissolving in water bodies to lyse them by indirect attack while bacteria lyse the algae using physical contact by direct attack. Interaction between algicidal bacteria & algae found to be of 3 types (figure 3):

1. Phycosphere interaction [17]:

In this type of interaction microbe-alga interaction environment is present called ‘Phycosphere’ in which indirect killing of algae is carried out by releasing algicidal compounds by interacting algicidal bacteria. There are chances of release protecting compounds such as toxins in the phycosphere by alga to protect itself from killing.

2. Contact interaction [17]:

In this type of interaction, killing of algae is carried out by direct attaching to the surface of bacteria.

3. Free-living interaction [17]:

In this type of interaction, killing of algae carried out by random freely present bacteria by secreting algicidal compound.

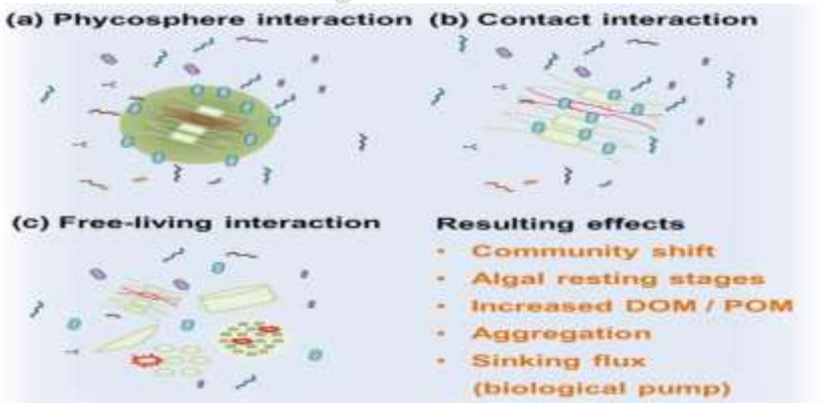


Figure 4: Types of association of algicidal bacteria with phytoplankton [17].

(a) Algicidal bacteria-blue, phycosphere-in green. (b) Algicidal bacteria-blue, affecting cell-red.

(c) Algicidal bacteria-blue, Algal lysis by bacteria-red, affecting cell-red.

Bacteria secrete compounds which reach towards algae after dissolving in water bodies to lyse them by indirect attack while bacteria lyse the algae using physical contact by direct attack. Some of the algicidal bacteria use proteases to kill algae. A hypothesis proposed that some algal mutants show cell surface polysaccharides which disable bacterial compounds to kill mutant algae.

Regulation of interaction between alga-algicidal bacteria found to be of 2 main types:

1. Allowance to algicidal activity:

   Following processes allow algicidal bacteria to lyse algal cells.

- Chemotaxis: Some chemical gradients gather algicidal bacteria to lyse particular algae. *C. prasina* betaproteobacteria shows algicidal activity against *T. pseudonana* because of chitinase [13] [17] (Figure 5).

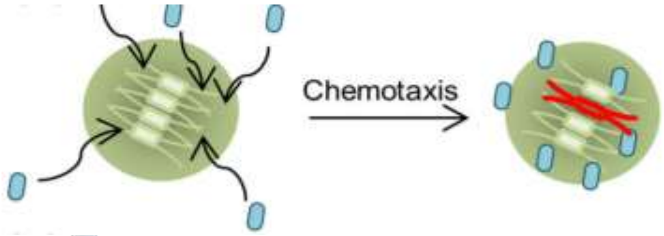


Figure 5: Chemotaxis (Normal algal cells- Grey, Affecting algal cell-red) [17].

- Nutrient availability: Presence of nutrients initiates algicide production for lysis of algae. Bacteria against Cyanobacterium *O. laetevirens* produce an algicidal compound which is regulated by nutrient availability [25] [17].
- Quorum Sensing (QS): Intra or inter-species cell to cell communication carried out using signal molecules leads to formation of algicidal compounds. *K. Algicida* shows more protease production against diatom *Skeletonema costatum* by QS mechanism [22] [17] (Figure 6).

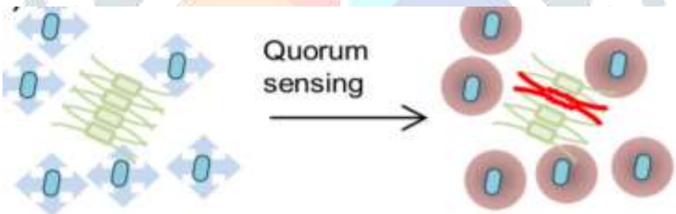


Figure 6: Quorum Sensing (Signal molecules- blue arrows, Normal algal cells- Grey, Affecting algal cell-red) [17].

2. Restriction to algicidal activity:

   Following processes restrict algicidal bacteria to lyse algal cells.

- Quorum Quenching (QQ): Development of interference or resistance to signal molecules by algae to restrict lysis carried out by QS. Some bacteria & algae raise pH to disrupt QS mechanism [36] [17].
- Evasive Strategy:

  Lysis from bacteria prevented by induced resting stage in algal cells. *Roseobacter* spp bacteria induce cyst formation in *A. tamarense* dinoflagellate to restrict it from lysis [1] [17] (Figure 7).

Figure 7: Evasive strategy (Normal algal cells- Grey, algal cells at resting stage- orange) [17].

- Induced resistance:

  Active compounds created by algae for production of resistance against algicidal bacteria. Induced production of proteases by algae for counterattacking towards lytic enzymes produced by algicidal bacteria [22] [17] (Figure 8).

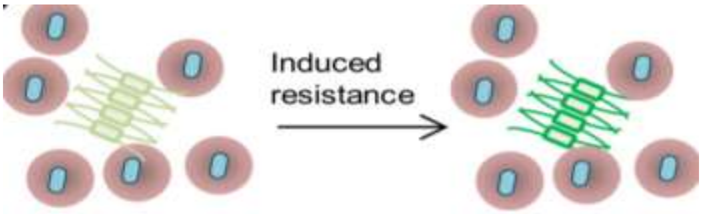


Figure 8: Induced resistance (Normal cells- Grey, Resistant cells- Green) [17].

Various studies have found prey specificity of different algicidal bacterial species as mentioned in the Table 2.

Table 2: Types of algicidal bacteria and their effects on algae [14].

Strain	Genus	Phylum	Target Algae
A5Y	*Cytophaga*	Bacter.	Bacil., Raph.
MC8	*Cytophaga*	Bacter.	Raph.
LR2	*Cytophaga*	Bacter.	Rhod., Chlor.
AA8-2	*Cytophaga*	Bacter.	Dino.
C49	*Flavobacterium*	Bacter.	Raph.
5N-3	*Flavobacterium*	Bacter.	Dino.
SS-K1	*Saprospira*	Bacter.	Bacil., Hapt.
SS98-5	*Saprospira*	Bacter.	Bacil.
ACEM 20	*Zobellia*	Bacter.	Raph., Dino.
K12	*Alteromonas*	y-Proteo.	Bacil.
SR-14	*Alteromonas*	y-Proteo.	Bacil.
ANSW2-2	*Alteromonas*	y-Proteo.	Dino.
E401	*Alteromonas*	y-Proteo.	Dino., Raph.
MC27	*Alteromonas*	y-Proteo.	Raph.
GY21	*Alteromonas*	y-Proteo.	Raph.
S	*Alteromonas*	y-Proteo.	Raph., Dino., Bacil.
Y	*Paseudoalteromonas*	y-Proteo.	Raph., Dino.
Strain	**Genus**	**Phylum**	**Target Algae**
A25	*Paseudoalteromonas*	y-Proteo.	Bacil.

ACEM 4	*Paseudoalteromonas*	y-Proteo.	Raph., Dino.
B42	*Vibrio*	y-Proteo.	Bacil.
G62	*Vibrio*	y-Proteo.	Raph., Bacil.
G42	*Pseudomonas*	y-Proteo.	Raph.
ACEM 32	*Bacillus*	Gram +	Raph., Dino.
LG-1	*Micrococcus*	Gram +	Raph., Dino.

Bacter.: Bacteroidetes, y-Proteo.: y-Proteobacteria, Raph.: Raphidophyceae, Dino.: Dinophyceae,

Bacil.: Bacillariophyceae, Chlor.: Chlorophyceae, Rhod.: Rhodophyceae, Hapt.: Haptophyceae,

IV. DIFFERENT ALGICIDAL BACTERIA TO LYSE DIFFERENT ALGAL BLOOMS:

There are different algicidal bacteria isolated for different types of algae as per research done by Scientists.

1. **Algicidal bacteria against blooms caused by *Alexandrium tamarense* [35]:**

This Dinoflagellate related Harmful Algal Bloom (HABs) are involved in toxin production which causes poisoning in Shellfish. It causes economic effects by causing alteration in estuarine phytoplankton communities which affects fisheries, biochemical cycling.

Non-axenic *A. tamarense* ATGD98-006 culture selected for study isolated from China. Algicidal activity shown by *Joostella sp*. DH77-1 bacteria isolated from the surface water samples of *P. donghaiense* accompanied with *A. tamarense*. This bacterial strain shows indirect attack on algae by releasing active compounds. These compounds alter enzymatic antioxidant systems, pigment content, protein content and membrane integrity in *A. tamarense* lead to cell death.

2. **Algicidal bacteria against blooms caused by *Aphanizomenon flos-aquae* [28]:**

This Cyanobacterium is a causative agent of Paralytic Shellfish Poisoning (PSP). HABs of *A. flos-aquae* algae annually occur in Dianchi lake of China.

Pseudomonas mendocina DC10 bacteria isolated from Dianchi Lake, China showed algicidal activity against *A.Flos-aquae* strain. Production of Reactive Oxygen Species after inoculating bacteria & algae in the same media acts as a killing agent for algae. Analysis carried out by determining parameters such as Monoaldehyde, Catalase, Peroxidase, Superoxide dismutase production in *A. flos-aquae* algae.

3. **Algicidal bacteria against blooms caused by *Chaetoceros* genus [16]:**

Chaetoceros genus diatoms species commonly occur in marine waters can cause finfish mortalities, damages to gill tissues, haemorrhage of gill capillaries etc. [2]

C. socialis & *C. didymus* isolated from surface waters from Hegoland Roads & Mediterranean Sea respectively. Newly isolated strain of *Kordia algicida* showed algicidal activity against *C. socialis*, *C. elegans* while some strains of *C. pseudo-curvisetus*, *C. protuberans*, *C. didymus* found to be resistant against *K. algicida* bacterium.

Heterogeneity in susceptibility of *Chaetocerous* spp. to *K. algicida* is unknown but proteases & oxylipins involved in resistance mechanism of *C. didymus* against *K. algicida*.

4. **Algicidal bacteria against blooms caused by *Chattonella marina* [9]:**

C. marina is Raphidophycean flagellate & most noxious red-tide organism causes HABs in many parts of world. It produces fat-soluble neurotoxin.

Algicidal activity showing bacteria against *C. marina* isolated from coastal surface water of Uljin, Republic of Korea. Phylogenetic analysis of bacterial strain confirmed that the bacteria belong to *Bacillus spp. AB-4 strain*. Light microscopy technique applied to study mode of action for killing algae. Bacteria released heat stable algicide that disrupted cell integrity, broken cellular components of *C. marina* Algae.

5. Algicidal bacteria against blooms caused by *Cochlodinium polykrikoides* [8]:

HABs of dinoflagellate *C. polykrikoides* kill fish & Shellfish through massive mucus production & Oxygen depletion. It occurs annually in Korean coastal waters.

Isolation of algicidal bacteria carried out from Kamak Bay, Korea. Seven algicidal bacteria of genera *Bacillus*, *Dietzia*, *Janibacter* & *Micrococcus* isolated in which *M. luteus* SY-13 showed most algicidal activity against *C. polykrikoides*. *M. luteus* SY-13 releases algicide which restricts motility followed by swelling & lysis of algae *C. polykrikoides*.

6. Algicidal bacteria against blooms caused by *Cylindrospermopsis Raciborskii* [18]:

Harmful Cyanobacterial Blooms (HCBs) forming one of the common species of Cyanobacteria is *Cylindrospermopsis Raciborskii*. This species produces Hepatoxin named Cylindrospermopsin & Neurotoxin named saxitoxin involved in fish, domestic livestock & human mortalities.

Bacterial strain isolated from decayed cyanobacterial blooms present in Saudi eutrophic lake. Isolated bacterium *Bacillus flexus* SSZ01 shows ability to kill *C. Raciborskii* cynobacterial bloom with degradation Cylindrospermopsin toxin. Killing of algae carried out by secretion of extracellular cyanobacteria-lysing substances produced by bacteria which lyse the algae and Cylindrospermopsin toxin released into the medium which is further degraded by extracellular active compounds excreted by bacteria into the environment.

7. Algicidal bacteria against blooms caused by *Gymnodinium catenatum* [30]:

Dinoflagellate *Gymnodinium catenatum* produce Paralytic Shellfish Poisoning called Sulfamate Saxitoxin derivatives which accumulate in shellfish & cause Gastrointestinal & neurological problems in Shellfish consumers.

Pseudoalteromonas rubra, *P. ulva*, *P. tunicata* species showed algicidal activities against *G. catenatum*. *P. tunicata* produces 3 types of bioactive compounds such as antifungal, inhibition of algal spore germination & antifouling that act independently. These bioactive compounds are involved in lyse algae by extracellular exudation.

8. Algicidal bacteria against blooms caused by *Oscillatoria* species [7]:

Harmful Cyanobacterial Bloom (HCBs) caused by different species of *Oscillatoria* such as *O. chlorina*, *O. tenuis, O. planctonica* secrete Lipopolysaccharide toxin that causes illness in humans varies from headache, fever, allergy to respiratory & gastro-intestinal illness [31].

Bacillus cereus CZBC1 strain shows algicidal effects against *Oscillatoria* species. It secretes alginolytic compounds which indirectly kill *Oscillatoria* species algae.

9. Algicidal bacteria against blooms caused by *Prorocentrum donghaiense* [6]:

P. donghaiense algae produces Shellfish toxins which further affects Shellfish consuming humans as that toxin proved as potent neurotoxin**.**

Bacterial strain FDHY-03 isolated from Xiapun Sea, China showed algicidal activity against dinoflagellate *P. donghaiense*. Phylogenetic study of bacterial strain identified that *Alteromonas macleodii* belongs to y-proteobacteria. It lyses algae by indirect mode where it secretes hydrolytic enzymes in the environment. *A. macleodii* showed algicidal activities against other HABs such as *A. pacificum*, *G. impudicum*, *S. costatum* etc.

10. Algicidal bacteria against blooms caused by *Pseudo-nitzschia* species [29]:

Some species of *Pseudo-nitzschia* diatom are toxic which produce Domoic acid toxin which primarily affects shellfishes & other marine organisms & their ingestion in human causes Seizures, Memory loss, Intestinal distress or sometimes leads to death [23].

The relationship between common algicidal bacteria group with *Pseudo-nitzschia* species algae carried out in which *Cellulophaga* spp & *Roseobacter* spp bacteria showed mutualistic & commensal interaction between P. pungens. Defence mechanisms of *Pseudo-nitzschia* species restrict activity of algicidal bacteria while two y-protobacteria and one Bacteroidetes enhance production of Domoic acid toxin.

Table 3: Algae Isolated from different countries & Effective algicidal bacterial strains show activity against them.

Sr. No.	Names of algal species	Effective algicidal bacterial strains	References
1	*Cochlodinium polykrikoides*	*Pseudomonas* species R Jin 1-1	[11]
		M. luteus SY-13	[8]
2	*Heterosigma akashiwo*	*Pseudomonas* species ZB Jin 2-3	[11]
3	*Heterocapsa triquetra*	*Marinomonas* species DZ Yeongu 1-4	[11]
4	*Prorocentrum minimum*	*Pseudoalteromonas* species M Dol 1-8	[11]
5	*Scrippsiella trochoidea*	*Pseudomonas* species MS Yeon 1-1	[11]
6	*Skeletonema costatum*	*Pseudoalteromonas* species M Dol 1-8	[11]
7	*Chattonella marina*	*Skatelama* species ZB Yeonmyeong 1-13	[11]
		Bacillus spp. AB-4	[9]
8	*Alexandrium tamarense*	*Joostella sp.* DH77-1	[35]
9	*Aphanizomenon flos-aquae*	*Pseudomonas mendocina* DC10	[28]
10	*C. socialis and C. elegans*	*K. algicida*	[16]
11	*Cylindrospermopsis Raciborskii*	*Bacillus flexus* SSZ01	[18]
12	*Gymnodinium catenatum*	*Pseudoalteromonas tunicate*	[30]
Sr. No.	**Names of algal species**	**Effective algicidal bacterial strains**	**References**
13	*Oscillatoria chlorina, O. tenuis, O. planctonica*	*Bacillus cereus* CZBC1	[7]
14	*Prorocentrum donghaiense*	*Alteromonas macleodii*	[6]

V. CONCLUSION:

Eutrophication can be caused due to dissolved organic & inorganic matter present in water bodies. Differences found in algae causing eutrophication based on the type of dissolved matter present in waterbodies. It is responsible for causing eutrophication by showing different effects phase-wise. The main demerit of eutrophication is rise in Harmful Algal Blooms (HABs). Different approaches implemented to reduce HABs & use of algicidal bacteria is one successful approach in it. Different strain algae show susceptibility towards different algicidal bacteria & the study of relationships between them found out too. Some bacteria change their mutual, commensal relationship to parasitic due to external factors & some bacteria show true parasitic relationship. Killing of algae carried out by direct & indirect ways by attaching to cells & releasing algicide compounds respectively. The study of killing & regulation mechanisms carried out by different isolation methodologies. Commonly found HABs & algicidal bacteria against them are studied with their mechanism of action which shows variation in action & resistance mechanism from species to species. This study helped to learn the evolution process between them. Future study should be carried out for the betterment of this approach to restrict eutrophication.

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