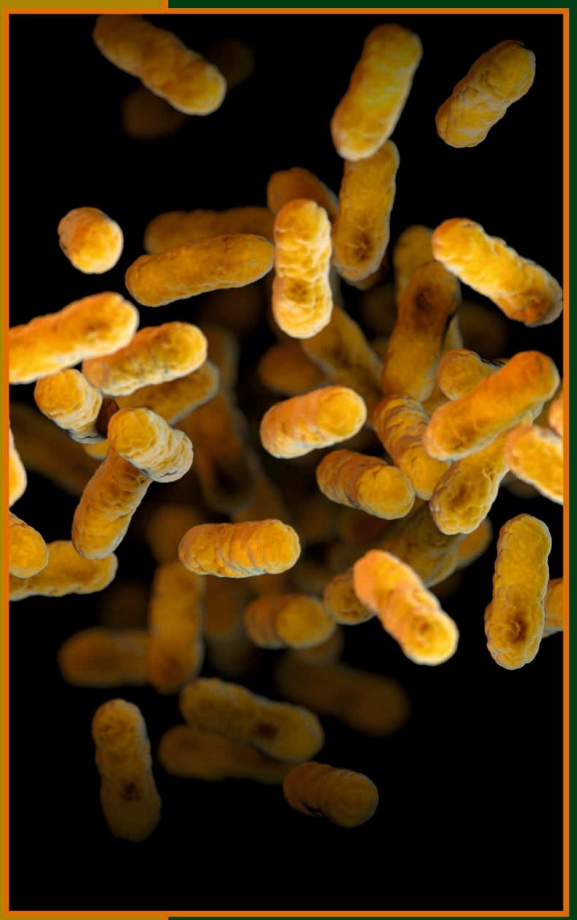
MICROBIOLOGY AND IMMUNOLOGY

Editor

Dr. Tanmay Ghosh



Kripa Drishti Publications, Pune.

MICROBIOLOGY
AND
IMMUNOLOGY

Editor
Dr. Tanmay Ghosh

Kripa-Drishti Publications, Pune.

Book Title: **Microbiology and Immunology**

Editor: **Dr. Tanmay Ghosh**

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1st Edition

ISBN: **978-93-90847-97-6**

Published: **Feb 2022**

Publisher:

KRIPA DRISHTI PUBLICATIONS

Kripa-Drishti Publications
A/ 503, Poorva Height, SNO 148/1A/1/1A,
Sus Road, Pashan- 411021, Pune, Maharashtra, India.
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Web: https://www.kdpublications.in

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ISBN: 978-93-90847-97-6

1. Aquaculture Microbiology

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1.1 Introduction:

Aquaculture is an age-old traditional practice of farming aquatic animals (e.g., fish, crustaceans, molluscs, algae etc.) by regulating the rearing process to increase their productivity. In the era of global food crisis, aquaculture stands as a sustainable approach to restore biodiversity and ensure nutritional security to a large populace. It is the most vibrant sector contributing to livelihood, food security, and rural employment.

Southern Asia, one of the biodiversity hotspots, is native to many indigenous freshwater fish species. Fish farmers use various conventional fishing gears and techniques to collect fishes from waterbodies.

They use *Ghuni* (Used to catch bottom dwelling fishes like *Trichogaster chuna, Anabas testudineus* etc.), *Palu'i* (Used to catch fishes in slime), *Dughārē* (Used to catch small indigenous fishes in shallow river), Baṛaśi (Used to catch small and medium sized fishes), *Cāp Jāl* (Used to catch small fishes), Drag net (Used to catch carps, small indigenous fishes of a pond), *Ghuṇ Jāl, Phāmḏi*, *Wheel chip, Jhim chip, Ṭōgi, Jaṭā* (Used to catch *Clarias orientalis, Monopterus cuchia, Clarias magur etc.*), *Laṛkā* (Used to catch *Channa. striata*), Kēmḏcā (Used to catch fishes like *Channa. striata*) and other fishing devices.

1.2 Limnological Parameters:

Aquaculture pond is a dynamic equilibrium which exhibits constant fluctuation due to prevailing physical and chemical processes. Water is a matrix of dissolved gases, inorganic substances, and organic matters.

The physico-chemical properties (e.g., pH, temperature, ammonia-, nitrate- and nitrite content, dissolved oxygen, biological oxygen demand, salinity, alkalinity, water depth) of water generally govern the life of aquatic organisms living in it.

Aquatic animals need to adapt to variable environment due to sudden fluctuation in water quality. The leftover feed, fecal matter of fishes and decomposition of organic material accumulate nitrogenous substances that are detrimental to aquaculture practices.

Industrial effluents, chemical fertilizers, pesticides and other anthropogenic activities in adjacent areas concomitantly pollute the aquatic environment.

In addition, pathogenic infections and algal bloom cause deterioration of water quality and depletion of aquatic diversity.

Aquaculture productions are thus depended upon maintenance of a steady state of those limnological parameters.

Proper exchange of gaseous substances, reduction of nitrogenous wastes, balancing of planktonic diversity and microbial population are crucial for fish growth and metabolism.

1.3 Aquaculture:

Aquaculture can be conducted through following approaches:

(1) Extensive: It is the farming of aquatic animals in their natural or semi-natural condition (e.g., large ponds, beels, rivers, lakes).

It is the most cost effective technique but the productivity generally remains relatively low compared to other approaches.

(2) Intensive: A fish farming procedure in which all the parameters are properly monitored to get the maximum yield in a specified time. It is generally conducted in small waterbodies or tanks maintaining high stocking density.

Fish are fed only with formulated feeds. However, the cost of investment remains very high.

(3) Semi-intensive: It is the most sustainable approach which maintains a delicate balance between the natural & artificial farming. It is also conducted in small waterbodies or tanks but with moderate level of stocking density.

Fish are generally fed on natural feeds with added feed supplements. It requires a modest level of monitoring system and hence becomes cost-effective. It generates a significant level of productivity.

However, intensification of aquaculture practices is often accompanied with many challenges and obstacles.

Massive urbanization, exploitation of natural habitats, uncontrolled introduction of allied exotic fishes, high- stocking density and microbial infections often poses a threat to the aquaculture sector. Abrupt use of pesticides in the adjacent agriculture fields also has made the situation more hostile.

1.4 Microbes and Aquaculture:

The production in aqua-sector is largely allied with its surrounding microbiota. Microbes possess both beneficial and harmful effects to its host organism (Figure 1.1). The harmful ones, known as pathogens, are involved in imparting infections to the susceptible species. While, the beneficial ones are regarded as probiotics. The utilization of probiotics in aqua-farming assists aquatic organisms in boosting the immune system against pathogenic diseases. The significance of probiotics in aquaculture is not just constrained to the gastrointestinal tract; but also it plays a key role in enhancing an organism's health quality, by stimulating growth, preventing diseases, enhancing immunity, and improving water quality by altering the microbiota of water and associated sediments.

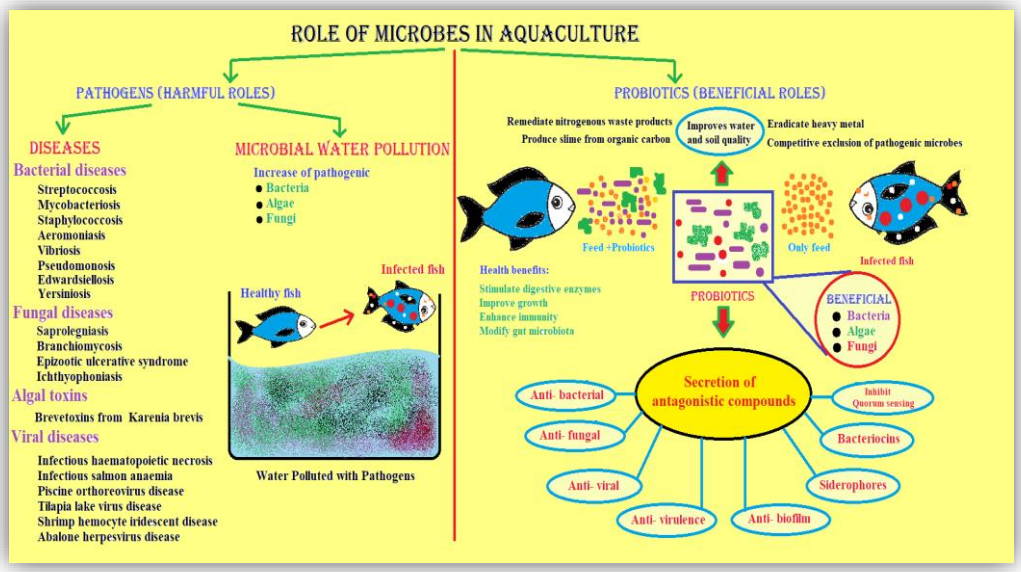

Figure 1.1: Role of microbes in aqua-sector

1.4.1 Microbial Diseases in Aquaculture:

Disease outbreaks are a major stumbling factor to the aquaculture sector and it acquire primacy owing to the ability to wipe out fish stocks, through spread of diseases.

Diseases in aquaculture are caused by complex interplay among the host, pathogen, and extrinsic stressors like environmental deterioration due to altering climate, eutrophication in native habitat, and inefficient fish monitoring systems.

Furthermore, increased stocking density exacerbates the prevalence and severity of infections.

Disease outbreaks have been considered as a major constraint in aquaculture sector. The epidemic diseases often result in massive financial damages.

Again, the convergence of persistent and emergent infirmities eventually constitutes a potential danger to the aquaculture sector, necessitating its quick management. Various microbial diseases allied with aquaculture are shown in Table 1.1.

Table 1.1: Microbial diseases in aquaculture sector

Pathogen		Host	Disease
Viral pathogens	*Herpesviruses*	*Cyprinus carpio*, *Anguilla* sp., *Acipenser* sp., *Gadus* sp.	Herpesvirus Diseases
	Cyprinid herpesvirus 3 (CyHV3)	*Cyprinus carpio*	Herpesvirus Diseases
	Channel Catfish Virus (CCV)	*Ictalurus punctatus, Clarias gariepinus, Hemibagrus gracilis, Pangasius hypophthalmus*.	Herpesvirus Diseases
	Iridovirus	*Epinephelus sp.*	Iridovirus Diseases
	Betanodavirus	*Epinephelus fuscoguttatus, Epinephelus lanceolatus, Trachinotus blochii, Lates calcarifer*	Viral Nervous Necrosis, Viral Encephalopathy and Retinopathy
	Tilapia Lake Virus	*Oreochromis* sp., *Barbonymus schwanenfeldii*	Syncytial hepatitis
	White Spot Syndrome Virus	*Penaeus monodon,* *Penaeus merguiensis*	White Spot Syndrome
	Macrobrachium rosenbergii nodavirus	*Macrobrachium rosenbergii*	White tail disease
	Infectious Hypodermal and Haematopoietic Necrosis Virus (Parvoviridae)	*Penaeus stylirostris, Penaeus vannamei, Penaeus monodon, Macrobrachium rosenbergii*	Infectious hypodermal and haematopoietic necrosis disease
	OMV	*Oncorhynchus masou, O. nerka, O. keta*	Oncogenic along with skin ulcerative condition with hepatitis

Pathogen		**Host**	**Disease**
	Spring viremia of carp virus	*Cyprinus carpio, Ctenopharyngodon idella, Carassius auratus, Leuciscus idus, Tinca tinca, Hypophthalamicthys, molitrix Danio rerio,* *Lebistes reticulates, Catostomus commersonii*	Spring viremia of carp
	Viral nervous necrosis virus	*Epinephelus akaara, Gadus morhua, Epinephelus coioides, Lates calcarifer, Oplegnathus fasciatus, Dicentrarchus labrax,* *Pseudocaranx dentex*	Nervous necrosis
	Walleye dermal sarcoma virus	*Stizostedion vitreum*	Sarcoma
Bacterial pathogens	*Vibrio harveyi, V. parahaemolyticus, V. alginolyticus, and V. anguillarum*	*Lates calcarifer, Epinephelus fuscoguttatus, Epinephelus coioides, Lutjanus* sp., *hybrid grouper (E. fuscoguttatus* x *E. lanceolatus), Oreochromis* sp., *Pangasius hypophthalmus, Scylla serrate, Portunus pelagius, Paphia textile, Meretrix meretrix, Cerithidea obtuse, Pseudosciaeana crocea*	Vibriosis
	Yersinia ruckeri	*Oncorhynchus mykiss*, Salmonids, *Anguilla reinhardtii*, Goldfish, Sole, Sturgeon and *Psetta maxima*	Yersiniosis, Enteric redmouth disease (ERM)
	Photobacterium damselae	*Chromis punctipinnis, Psetta maxima, Oncorhynchus mykiss, Trachinotus ovatus, Anguilla*	Photobacteriosis, skin ulcers

Pathogen		**Host**	**Disease**
		reinhardtii, Sparus aurata, Dicentrarchus labrax, Seriola quinqueradiata, Pagrus Auriga, Diplodus sargus, Argyrosomus regius	
	Streptococcus iniae, S. agalactiae	Asian seabass, Hybrid tilapia	Streptococcosis
	Aeromonas hydrophila, A. veronii	Giant freshwater prawns, tilapia, catfishes	Motile Aeromonas Septicemia
	Mycobacterium marinum, M. fortuitum, M. chelonae	Wide range of Marine, brackish and freshwater fishes.	Piscine Tuberculosis
	Edwardsiella tarda	African catfish, tilapia, *Monopterus albus*, *Trichogaster pectoralis*, Asian seabass	Edwardsiellosis
Fungal pathogens	*Saprolegnia sp. (Saprolegnia parasitica, Saprolegnia diclina, Saprolegnia ferax)*	Carp, Catfish, Eels, Salmonids	Saprolegniasis
	Branchiomyces sanguinis, Branchiomyces demigrans	Carps, Tench, Pike, Largemouth bass, Striped bass	Branchiomycosis (gill rot)
	Aphanomyces invadans	*Cyprinus capio, Oreochromis niloticus, Chanos chanos*	Epizootic Ulcerative Syndrome (EUS), Red Spot Disease, Mycotic Granulomatosis, Ulcerative Mycosis
	Ichthyophonus hoferi	*Clupea harengus,* freshwater salmonids	Ichthyophoniasis, granulomatosis

Pathogen		Host	Disease
	Achlya sp.	*Puntius sophore, P. conchonius, P. ticto, Colisa fasciata, Chanda ranga, Labeo rohita (fingerlings), L. bata (fingerlings), Notopterus notopterus, Anabas testudineus, and Channa punctatus.*	Saprolegniasis

1.4.2 Prevention of Aquatic Diseases:

A. Antibiotics and chemical substances:

Prevention and control of aquatic diseases often focuses on the use of chemotherapeutic substances, immunostimulants and veterinary medicines that often make the situation more hostile. The emergence and vertical transfer of antibiotic-resistant genes has become a serious threat to public health. It happened most dramatically in the shrimp industry where massive increases in production, high- stocking density and overuse of antibiotics had led to the emergence of antibiotic resistant bacteria and production crashes in many Asian countries. There are many reports regarding sharp decrease in productivity due to abrupt use of anti-microbial drugs.

B. Probiotics:

Probiotics are live beneficial microorganisms which when consumed in adequate amount confer health benefit to the host. They are eco-friendly biocompatible substances that are alternatively used to prevent and control aquatic diseases in recent time. Aquaculture probiotics colonizes the gastrointestinal tract of aquatic species and confer protection against pathogens by limiting nutritional resources through the process of competitive exclusion. Probiotic isolates often secrete extracellular enzymes (e.g., amylases, proteases, lipases) or growth factors (e.g., vitamins, siderophores, fatty acids, amino acids) which can digest indigestible food components more efficiently and promote fish nutrition. The consortium of probiotics may also be effective and consistent than a single strain due to their synergistic nature.

Probiotic also enhances the quality of culture water through a cost-effective technology to monitor waste removal and reduce water pollution. Probiotics often exert signaling molecules to stimulate humoral or cellular immune response against pathogenic invasion. Application of probiotic *Lysinibacillus sphaericus* provided protection to *Clarias batrachus* against pathogenic *Vibrio harveyi*. Probiotics are used as functional feed additives to inhibit pathogenic infections, reduce biofilm formation, and to enhance fecundity.

C. Algal extracts:

Algae and algal extracts are enriched with proteins, vitamins, minerals and are used as feed ingredient in aquaculture sector. They often function as prebiotic supplement to provide nutrition to gut micro-flora. Potential algal extracts are also capable of producing secondary metabolites, expression of the pro-inflammatory genes (e.g., IL-1β and TNF-α) that might be used as immunotherapeutics against infection. For e.g., addition of a mixture of algal extracts (*Fucus vesiculosus, Ulva rigida* and *Nannochloropsis gaditana*) to the basal diet of Zebra fish increases C3b transcription that provides immunity against viral diseases in Zebra fish larvae.

D. Vaccination:

Vaccination can be an effective strategy to induce immunity in fish against viral infections. Several oral delivery techniques have been employed, including cholera toxin B molecular carrier, encapsulation of recombinant G protein with polyethylene glycol (PEG), and active *Aeromonas salmonicida* delivering the antigen. The benefit of live attenuated vaccination was first obtained against Channel Catfish Virus (CCV) of *Ictalurus punctatus*. An innovative live recombinant IHNV vaccine has been designed by French researchers by means of reverse genetics approaches.

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ISBN: 978-93-90847-97-6

2. Complement System

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2.1 Introduction:

The complement system is an integral part of the innate immune response and acts as a bridge between innate and acquired immunity. This system is composed of a series of proteins that are mostly (although not exclusively) synthesised in the liver, and exist in the plasma and on cell surfaces as inactive precursors (zymogens). Complement mediates responses to inflammatory triggers through a co-ordinated sequential enzyme cascade which leads to clearance of foreign cells through pathogen recognition, opsonisation and lysis. Complement also possesses anti-inflammatory functions: it binds to immune complexes and apoptotic cells, and assists in their removal from the circulation and damaged tissues. The complement proteins are activated by, and work with IgG and IgM antibodies, hence the name 'complement'. Many complement proteins exist in a 'precursor' form and are activated at the site of inflammation. The complement system is more complex than many enzymatic cascades as it requires the formation of sequential non-covalently associated activated protein fragments. These in turn become convertases and cleave components for the next enzymatic complex in the cascade, and the rapid dissociation of these complexes (and loss of enzymatic activity) forms an integral part of the elegant regulation of complement activity.

2.2 History:

In the late 19th century, the focus of scientific research was on the human body's defence against microbial infection. The 'Theory of Metchnikoff' proposed that phagocytes in the blood were capable of ingesting and destroying the invading bacteria, thus providing the basis of innate cellular immunity.

This phagocytic theory was challenged by many pathologists initially on the basis that the phagocytic leucocytes were 'truly causal in the successful response to infection'. Buchner and colleagues (1891) found a heat labile factor in blood that was capable of killing bacteria, and named it 'alexin' (in Greek, means 'to ward off').

Jules Bordet supported this 'humoral theory' (immunity conferred due to antitoxic and bactericidal substances in body fluids) by demonstrating that immune lysis required the presence of two factors: a heat labile lytic factor (similar to alexin) and a heat-stable factor, which he termed sensitiser (which we now know was antibody).

Paul Ehrlich described the side-chain theory of antibody formation, especially the mechanisms of antibody neutralisation by toxins that induced bacterial lysis with the help of complement (which has replaced the historical term alexin).

According to his theory, the immune cells contained receptors that could recognise antigens, and following immunisation, these receptors multiplied and were shed into the circulation as 'amboceptors' (now called antibodies).

These antibodies attached not only to specific antigens but also to a heat-labile antimicrobial component called 'complement'. Ehrlich's theory proposed that the antibody and complement combined to form a complex enzyme capable of attacking and killing cells and micro-organisms.

2.3 The Complement Components:

The proteins and glycoproteins that compose the complement system are synthesized mainly by liver hepatocytes, although significant amounts are also produced by blood monocytes, tissue macrophages, and epithelial cells of the gastrointestinal and genitourinary tracts.

These components constitute 5% (by weight) of the serum globulin fraction. Most circulate in the serum in functionally inactive forms as proenzymes, or zymogens, which are inactive until proteolytic cleavage, which removes an inhibitory fragment and exposes the active site. The complement-reaction sequence starts with an enzyme cascade.

Complement components are designated by numerals (C1–C9), by letter symbols (e.g., factor D), or by trivial names (e.g., homologous restriction factor). Peptide fragments formed by activation of a component are denoted by small letters. In most cases, the smaller fragment resulting from cleavage of a component is designated "a" and the larger fragment designated "b" (e.g., C3a, C3b; note that C2 is an exception: C2a is the larger cleavage fragment).

The larger fragments bind to the target near the site of activation, and the smaller fragments diffuse from the site and can initiate localized inflammatory responses by binding to specific receptors. The complement fragments interact with one another to form functional complexes. Those complexes that have enzymatic activity are designated by a bar over the number or symbol (e.g., C4b2a, C3bBb).

2.4 Pathways of Activation:

There are three known pathways for complement activation: Classical, Alternative and Lectin pathway (Fig. 1). The final steps that lead to a membrane attack are the same in all pathways.

2.4.1 The Classical Pathway Begins With Antigen-Antibody Binding:

The classical pathway is initiated by IgM or IgG antigen/ antibody complexes binding to C1q (first protein of the cascade) leading to activation of C1r, which in turn cleaves C1s. This in turn activates the serine proteases that lead to cleaving of C4 and C2, leading to formation of C4b2a (C3 convertase), which in turn cleaves C3 into C3a and C3b. While C3a acts as a recruiter of inflammatory cells (anaphylatoxin), C3b binds to the C4b2a complex to form C5 convertase (C4b2a3b).

The C5 convertase initiates the formation of the Membrane Attack Complex (MAC) that inserts into membrane creating functional pores in bacterial membranes leading to its lysis. The classical pathway can also be activated by other danger signals like C-reactive protein, viral proteins, poly-anions, apoptotic cells and amyloid, thus providing evidence that classical pathway could be activated independent of antibodies

2.4.2 The Alternative Pathway Is Antibody-Independent:

Fifty years after the discovery of the classical activation pathway, Pillemer et al. proposed a highly controversial alternative activation pathway. Initially, this was rejected by the scientific community and only substantiated and accepted more than a decade later. Pillemer's hypothesis was based on observations that the complement system could be activated by direct binding of bacteria and yeast independent of antibody interaction.

It was originally named the 'properdin pathway' and is now known as the alternative pathway. The alternative pathway is not so much an activation pathway, as it is a failure to regulate the low level continuous formation of a soluble C3 convertase.

The internal thioester bond of C3 is highly reactive and undergoes spontaneous hydrolysis resulting in a molecule known as C3 (H_2O) which resembles C3b. This can then bind to factor B, and be processed into a short lived soluble C3 convertase that can generate more C3b.

If this C3b binds to a nearby surface that is incapable of inactivating it (such as bacteria/yeast cells or damaged host tissues), this then leads to amplification of the alternative pathway.

The presence of complement regulators in healthy cells ensures the spontaneous hydrolysis of C3 is kept in check. C3 activation takes place when C3b binds to factor B and is then cleaved by factor D (a process which is stabilised by magnesium ions and properdin).

The enzymatic action of factor D acts as the rate limiting step of the alternative pathway and cleaves factor B, the larger fragment of which remains bound to C3b to form the alternative pathway C3 convertase–C3bBb. C3b is able to create new C3 convertase in the presence of Factors B and D, thus acting as an 'amplification loop' for other pathways, as well as the alternative pathway.

The C3bBb generated in the alternative pathway can activate unhydrolyzed C3 to generate more C3b autocatalytically. As a result, the initial steps are repeated and amplified, so that more than $2 \text{ X } 10^6$ molecules of C3b can be deposited on an

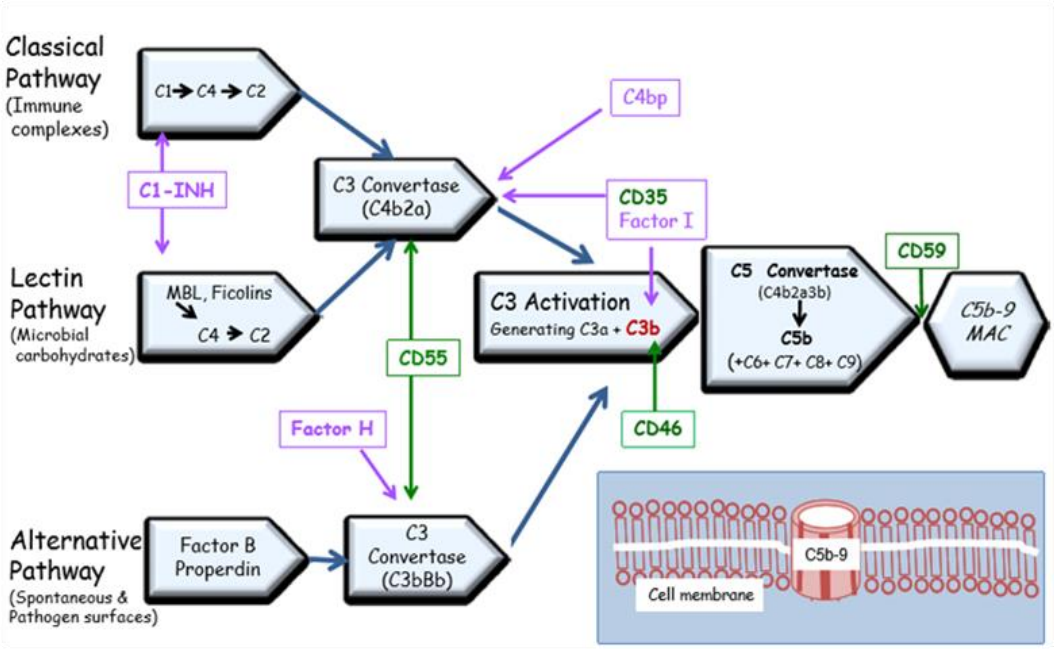


Figure 2.1: Pathways of complement activation: classical, alternative and lectin pathway: IgM or IgG antigen/antibody complexes binding to C1q, the first protein of the cascade, initiates the classical pathway. The alternative pathway is not so much an activation pathway, as it is a failure to regulate the low level continuous formation of a soluble C3 convertase.

The third pathway is known as MBL (Mannosebinding lectin)/MASP (MBL associated Serine Protease) pathway. The initiating molecules for the MBL pathway are multimeric lectin complexes that bind to specific carbohydrate patterns uncommon in the host, leading to activation of the pathway through enzymatic activity of MASP.

The sites of action of the membrane bound complement regulators–CD35, CD46, CD55 & CD59 (green boxes) and the fluid phase regulators – C1-INH, Factor H, Factor I and C4bp (violet boxes) are represented with arrows.

Insert: Membrane Attack Complex (MAC). The interaction of C5b with C6, C7, C8 and C9 leads to formation of C5b–9 or Membrane Attack Complex (MAC), a multimolecular structure that inserts into the membrane creating a functional pore leading to cell lysis.

Antigenic surface in less than 5 minutes. The C3 convertase activity of C3bBb generates the C3bBb3b complex, which exhibits C5 convertase activity, analogous to the C4b2a3b complex in the classical pathway. The nonenzymatic C3b component binds C5, and the Bb component subsequently hydrolyzes the bound C5 to generate C5a and C5b; the latter binds to the antigenic surface. The alternative pathway omits the components C1, C2 and C4.

2.4.3 The Lectin Pathway Originates with Host Proteins Binding Microbial Surfaces:

Forty years after the proposal of the alternative pathway, the MBL (mannose-binding lectin)/MASP (MBL-associated serine protease) pathway was discovered. This pathway was characterised by using proteins isolated from rabbit liver and serum, but its function remained unclear initially. The initiating molecules for this pathway are collectins (MBL and ficolin), which are multimeric lectin complexes. These bind to specific carbohydrate patterns uncommon in the host, leading to activation of the pathway through enzymatic activity of MASP. There are structural similarities shared between MBL and C1 complexes (MBL- with C1q-associated serine proteases, MASP-1 and MASP-2 with C1r and C1s, respectively), leading to the belief that complement activation by MBL and C1 complexes are similar. MASP-2 cleaves C4 and C2 to form C3 convertase, while MASP-1 may cleave C3 directly bypassing the C4b2a complex, albeit at a very slow rate. Another serine protease, MASP-3 was shown to down-regulate the C4 and C2 cleaving activity of MASP-2. Following the initial characterisation of MBL, 3 other lectins (known as ficolins) have been shown to interact with MASP: ficolin-1 (or M-ficolin), ficolin-2 (or L-ficolin) and ficolin-3 (or H-ficolin or Hakata antigen). The ficolins activate the lectin pathway by forming active complexes with MASP [51, 52]. More recently, a new C-type lectin (CL-11) was shown to interact with MASP-1 and/or MASP-3 and could activate the lectin pathway.

2.4.4 Other Activators of The Complement System:

Various serine proteases belonging to the coagulation system have also been shown to activate the complement cascade independent of the established pathways. In vitro findings suggested that the coagulation factors FXa, FXIa and plasmin can cleave both C5 and C3, leading to generation of anaphylatoxins C5a and C3a [54].

Studies have documented FVIII and von Willebrand factor to possess lectin activity. Vice versa, complement factors are also known to interact with the coagulation system. C1 inhibitor was shown to block the endogenous coagulation pathway, while C5a was shown to induce tissue factor (membrane glycoprotein that serves as a cofactor for blood coagulation factor VIIa) activity on endothelial cells.

Individual cells have also been implicated in activating certain elements of complement pathway. It has been showed that phagocytic cells, especially lung macrophages could generate C5a from C5 independent of the plasma complement system using cell bound serine proteases.

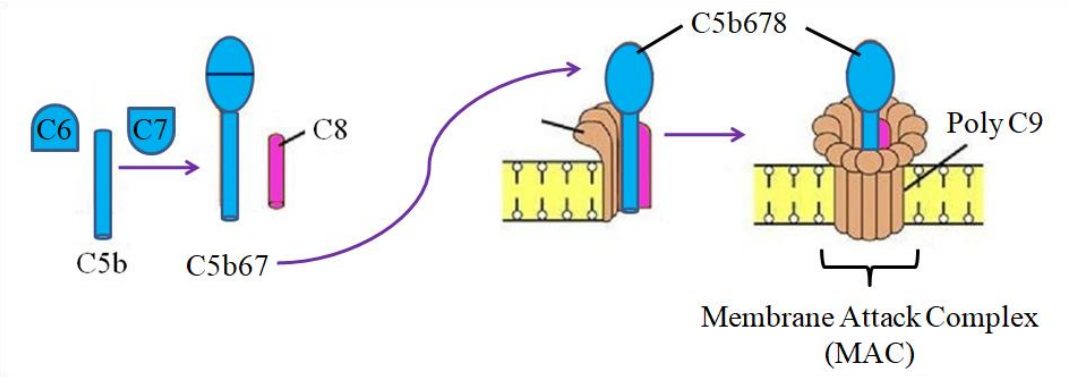
C-reactive protein is an acute phase reactant that can activate the classical pathway of the complement system, and its role in the complement led ischemia–reperfusion injury (IRI) has been shown in intestinal and myocardial animal IRI models. Similarly, cross-talk between complement and toll-like receptors has shown to be possible due to mitogen activated protein kinases in renal IRI setting.

Cross-talk between complement system and other systems will exist, and future research will be aimed at evaluating these ‘communicators’ between systems.

2.5 Complement Cascade - The Three Complement Pathways Converge At the Membrane-Attack Complex:

The principal function of the complement system is protection of the host from infection/inflammation by recruiting (chemotaxis) and enhancing phagocytosis by innate immune cells (opsonisation), leading to lysis of the target cells. All three pathways lead to the generation of C3 convertase that cleaves the C3 protein into C3a and C3b. While C3a acts as an anaphylatoxin, C3b covalently binds to the activating surface and participates in the self-activation loop of complement activation via the alternate pathway. C3b also associates with C3 convertases (C4b2a or C3bBb) to form the C5 convertase, which cleaves C5 complement into C5a and C5b. Interaction of C5b with C6, C7, C8 and C9 leads to formation of C5b–9/MAC (Membrane Attack Complex) (Fig. 2), a multimolecular structure that inserts into the membrane creating a functional pore leading to cell lysis.

MAC can cause lysis of some cells (e.g. erythrocytes) with a single hit, but some nucleated cells required multiple hits, or rather, multiple channel formation to cause cell lysis.



However, studies have shown that when the number of channels assembled on the cells is limited, sublytic C5b–9 can activate transcription factors and signal transduction, leading to inhibition of apoptosis and cell homeostasis.

Figure 2.2.: A Model for MAC Formation. The complement terminal pathway is initiated by the cleavage of C5 to C5b. C6 traps a labile conformation of the C5b TED (Thioester-containing domain) domain to form C5b6, a platform for the stepwise assembly of components C7, C8, and C9. Regulatory proteins in the plasma block MAC assembly in solution by binding exposed hydrophobic regions and sterically inhibit C9 oligomerization. Binding of C5b8 to membranes recruits multiple C9 molecules.

The anaphylatoxins (C3a and C5a) are key players in the recruitment of inflammatory cells and release of mediators that amplify the inflammatory response. C5a is probably the principal anaphylatoxin mediating inflammation. C5a binds to C5a receptor (C5aR or CD88) that is widely present on inflammatory and noninflammatory cells.

Apart from recruiting the neutrophils, C5a also increases neutrophil adhesiveness and aggregation. C5a causes secretion of pro-inflammatory cytokines and lysosomal enzymes from the macrophages and monocytes, thus leading to chemotaxis. C5a also up-regulates adhesion molecules such as α-integrin and β 2-integrin; in particular, Mac-1, in polymorphonuclear leukocytes. C5a was shown to be an important inflammatory mediator for the early adhesive interactions between neutrophils and endothelial cells in the acute inflammatory response.

It is responsible for up-regulation of vascular adhesion molecules such as P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [29, 72]. C3a does not act as a chemoattractant for neutrophils, but aids migration of eosinophils and mast cells. C3a and C5a also act on their receptors expressed on innate immune cells such as dendritic cells, thus playing a role in initiating and regulating T cell responses. In the IRI setting, MAC has been shown to mediate IR injury, and its inhibition was shown to attenuate the IRI effect.

2.6 Biological Consequences of Complement Activation:

Complement serves as an important mediator of the humoral response by amplifying the response and converting it into an effective defense mechanism to destroy invading microorganisms. The MAC mediates cell lysis, while other complement components or split products participate in the inflammatory response, opsonization of antigen, viral neutralization, and clearance of immune complexes (Table 1).

2.6.1 The Membrane-Attack Complex Can Lyse A Broad Spectrum Of Cells:

The membrane-attack complex formed by complement activation can lyse gram-negative bacteria, parasites, viruses, erythrocytes, and nucleated cells. Because the alternative and lectin pathways of activation generally occur without an initial antigen-antibody interaction, these pathways serve as important innate immune defenses against infectious microorganisms. The requirement for an initial antigen-antibody reaction in the classical pathway supplements these nonspecific innate defenses with a more specific defense mechanism. In some instances, the requirement for antibody in the activating event may be supplied by so-called natural antibodies, which are raised against common components of ubiquitous microbes. Nevertheless, antibody and complement do play a role in host defense against viruses and are often crucial in containing viral spread during acute infection and in protecting against reinfection. Most, perhaps all, enveloped viruses are susceptible to complement mediated lysis. The viral envelope is largely derived from the plasma membrane of infected host cells and is therefore susceptible to pore formation by the membrane attack complex. The complement system is generally quite effective in lysing gram-negative bacteria.

Gram-positive bacteria are generally resistant to complement-mediated lysis because the thick peptidoglycan layer in their cell wall prevents insertion of the MAC into the inner membrane. Although complement activation can occur on the cell membrane of encapsulated bacteria such as *Streptococcus pneumoniae*, the capsule prevents interaction between C3b deposited on the membrane and the CR1 on phagocytic cells. Lysis of nucleated cells requires formation of multiple membrane attack complexes, whereas a single MAC can lyse a red blood cell. Many nucleated cells, including the majority of cancer cells, can endocytose the MAC.

2.6.2 Cleavage Products of Complement Components Mediate Inflammation:

The complement cascade is often viewed in terms of the final outcome of cell lysis, but various peptides generated during formation of the MAC play a decisive role in the development of an effective inflammatory response. The smaller fragments resulting from complement cleavage, C3a, C4a, and C5a, called anaphylatoxins, bind to receptors on mast cells and blood basophils and induce degranulation, with release of histamine and other pharmacologically active mediators. The anaphylatoxins also induce smooth-muscle contraction and increased vascular permeability. Activation of the complement system thus results in influxes of fluid that carries antibody and phagocytic cells to the site of antigen entry. C3a, C5a, and C5b67 can each induce monocytes and neutrophils to adhere to vascular endothelial cells, extravasate through the endothelial lining of the capillary, and migrate toward the site of complement activation in the tissues.

2.6.3 C3b and C4b Binding Facilitates Opsonization:

C3b is the major opsonin of the complement system, although C4b also has opsonizing activity. The amplification that occurs with C3 activation results in a coating of C3b on immune complexes and particulate antigens. Phagocytic cells, as well as some other cells, express complement receptors (CR1, CR3, and CR4) that bind C3b or C4b. Antigen coated with C3b binds to cells bearing CR1. If the cell is a phagocyte (e.g., a neutrophil, monocyte, or macrophage), phagocytosis will be enhanced. Activation of phagocytic cells by various agents, including C5a anaphylatoxin, has been shown to increase the number of CR1s on activated cells, greatly facilitating their phagocytosis of C3b-coated antigen.

Recent studies indicate that complement fragment C3b acts as an adjuvant when coupled with protein antigens.

C3b targets the antigen directly to the phagocyte, enhancing the initiation of antigen processing and accelerating specific antibody production.

Table 2.1: Summary of biological effects mediated by complement products.

Effect	**Complement product mediating***
Cell lysis	C5b–9, the membrane-attack complex (MAC)
Inflammatory response	
Degranulation of mast cells and basophils†	C3a,C4a, and C5a (anaphylatoxins)
Degranulation of eosinophils	C3a, C5a
Extravasation and chemotaxis of leukocytes at inflammatory site	C3a, C5a, C5b67
Aggregation of platelets	C3a, C5a
Inhibition of monocyte/macrophage migration and induction of their spreading	Bb
Release of neutrophils from bone marrow	C3c
Release of hydrolytic enzymes from neutrophils	C5a
Increased expression of complement receptors type 1 and 3 (CR1 and CR3) on neutrophils	C5a
Opsonization of particulate antigens, increasing their phagocytosis	C3b, C4b
Viral neutralization	C3b, C5b–9 (MAC)
Solubilization and clearance of immune complexe	C3b
*Boldfaced component is most important in mediating indicated effect.	
†Degranulation leads to release of histamine and other mediators that induce contraction of smooth muscle and increased permeability of vessels.	

2.6.4 The Complement System Also Neutralizes Viral Infectivity:

For most viruses, the binding of serum antibody to the repeating subunits of the viral structural proteins creates particulate immune complexes ideally suited for complement activation by the classical pathway. Some viruses (e.g., retroviruses, Epstein-Barr virus, Newcastle disease virus, and rubella virus) can activate the alternative, lectin, or even the classical pathway in the absence of antibody. The complement system mediates viral neutralization by a number of mechanisms. Some degree of neutralization is achieved through the formation of larger viral aggregates, simply because these aggregates reduce the net number of infectious viral particles. Although antibody plays a role in the formation of viral aggregates, in vitro studies show that the C3b component facilitates aggregate formation in the presence of as little as two molecules of antibody per virion. The binding of antibody and/or complement to the surface of a viral particle creates a thick protein coating. This coating neutralizes viral infectivity by blocking attachment to susceptible host cells. The deposits of antibody and complement on viral particles also facilitate binding of the viral particle to cells possessing Fc or type 1 complement receptors (CR1). In the case of phagocytic cells, such binding can be followed by phagocytosis and intracellular destruction of the ingested viral particle. Finally, complement is effective in lysing most, if not all, enveloped viruses, resulting in fragmentation of the envelope and disintegration of the nucleocapsid.

2.6.5 The Complement System Clears Immune Complexes From Circulation:

The importance of the complement system in clearing immune complexes is seen in patients with the autoimmune disease systemic lupus erythematosus (SLE). These individuals produce large quantities of immune complexes and suffer tissue damage as a result of complement-mediated lysis and the induction of type II or type III hypersensitivity. Although complement plays a significant role in the development of tissue damage in SLE, the paradoxical finding is that deficiencies in C1, C2, C4, and CR1 predispose an individual to SLE; indeed, 90% of individuals who completely lack C4 develop SLE. The complement deficiencies are thought to interfere with effective solubilization and clearance of immune complexes; as a result, these complexes persist, leading to tissue damage by the very system whose deficiency was to blame.

The coating of soluble immune complexes with C3b is thought to facilitate their binding to CR1 on erythrocytes. Although red blood cells express lower levels of CR1 (~5 X 10^2 per cell) than granulocytes do (~5 X 10^4 per cell), there are about 10^3 red blood cells for every white blood cell; therefore, erythrocytes account for about 90% of the CR1 in the blood. For this reason, erythrocytes play an important role in binding C3b-coated immune complexes and carrying these complexes to the liver and spleen. In these organs, immune complexes are stripped from the red blood cells and are phagocytosed, thereby preventing their deposition in tissues. In SLE patients, deficiencies in C1, C2, and C4 each contribute to reduced levels of C3b on immune complexes and hence inhibit their clearance. The lower levels of CR1 expressed on the erythrocytes of SLE patients also may interfere with the proper binding and clearance of immune complexes.

2.7 Complement Deficiency:

Genetic deficiencies have been described for each of the complement components. Homozygous deficiencies in any of the early components of the classical pathway (C1q, C1r, C1s, C4, and C2) exhibit similar symptoms, notably a marked increase in immune-complex diseases such as systemic lupus erythematosus, glomerulonephritis, and vasculitis. These deficiencies highlight the importance of the early complement reactions in generating C3b, and the critical role of C3b in solubilization and clearance of immune complexes. In addition to immune complex diseases, individuals with such complement deficiencies may suffer from recurrent infections by pyogenic (pusforming) bacteria such as streptococci and staphylococci. These organisms are Gram-positive and therefore resistant to the lytic effects of the MAC. Nevertheless, the early complement components ordinarily prevent recurrent infection by mediating a localized inflammatory response and opsonizing the bacteria. Deficiencies in factor D and properdin, early components of the alternative pathway, appear to be associated with Neisseria infections but not with immune-complex disease. Patients with C3 deficiencies have the most severe clinical manifestations, reflecting the central role of C3 in activation of C5 and formation of the MAC. Individuals with homozygous deficiencies in the components involved in the MAC develop recurrent meningococcal and gonococcal infections caused by *Neisseria* species. In normal individuals, these gram-negative bacteria are generally susceptible to complement-mediated lysis or are cleared by the opsonizing activity of C3b.

MAC-deficient individuals rarely have immune-complex disease, which suggests that they produce enough C3b to clear immune complexes.

Interestingly, a deficiency in C9 results in no clinical symptoms, suggesting that the entire MAC is not always necessary for complement-mediated lysis. Congenital deficiencies of complement regulatory proteins have also been reported.

The C1 inhibitor (C1 Inh) regulates activation of the classical pathway by preventing excessive C4 and C2 activation by C1.

The deficiency gives rise to a condition called hereditary angioedema, which manifests clinically as localized edema of the tissue, often following trauma, but sometimes with no known cause.

Investigations of in vivo complement activity in these animals has allowed dissection of the complex system of complement proteins and the assignment of precise biologic roles to each.

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ISBN: 978-93-90847-97-6

3. The Salubrious Role of Actinomycetes Towards Plant Growth: A Step Towards Sustainable Agriculture

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Abstract:

Sustainable agriculture implies the use of environmentally benign processes to increase crop productivity. Microbes nourish the soil and increase nutrient availability for crops without distorting the environment. Actinomycetes are a group of prokaryotic gram-positive bacteria that have been linked to increased agricultural productivity by producing a wide range of growth promoting compounds that aid in the growth of agricultural crops while also acting as a biocontrol agent. Besides actinomycetes have the ability to follow both direct as well as indirect ways to promote plants growth. They are capable to produce growth inducing compounds that assist in N_2 fixation, indole 3-acetic acid production (IAA), phosphate solubilizations, siderophore production, etc. The actinomycetes are also competent to reduce the biotic as well as abiotic stresses and protect the crops from harmful pathogens. Furthermore, actinomycetes are known to elute certain growth promoting compounds that not only assist in growth and development of the crops but also enable them to withstand harsh environmental conditions like drought, salinity, waterlogging etc. In the present chapter, we intend to discuss on the significant role of actinomycetes in enhancing soil health, suppressing pathogens, and increasing crop production by their direct and indirect mechanisms. Furthermore, we will briefly discuss the current applications and benefits of using actinomycetes for sustainable agriculture.

Keywords: *Sustainable agriculture, crop productivity, actinomycetes, biocontrol agent.*

3.1 Introduction:

The copy warned the Little Blind Text, that where it came from it would have been rewritten with declining soil nutrition, conventional agriculture is struggling to meet the food needs of growing population around the world. However to restore depleting soil nutrition, reliance on the use of harmful and toxic chemical fertilizers have also increased. (Djebaili *et al.,* 2020).

The excessive use of toxic chemical fertilizers thus enter into the food cycle which eventually led to development of many human diseases and ecological disparity. The natural way to replenish the soil nutrition is a slow and time consuming process which thus impact the overall food production. (Abbamondi *et al.,* 2016). The burgeoning of the human population has led to the rise in the food demand, in contrary to inadequate supply of food (Djebaili *et al.,* 2020; Hamedi *et al.,* 2015). To bridge the gap between the supply and demand of the food a sustainable approach towards agriculture is the most pressing need. Though to some extent the need has been fulfilled by the genetically modified crops (GMO) but required to supplement with chemical fertilizers for their growth (Dalal *at al.,* 2014; Rojas e*t al.,* 2014). Therefore, an eco-friendly and sustainable approach is required to scale up the crop production using microbes that are capable of supplementing the plants with a plethora of growth regulators and defence towards pathogen (Gopalakrishnan *et al.,* 2015).

In the late 1970s, Kloepper and co-workers coined the term plant growth-promoting rhizobacteria (PGPR) to describe isolated bacteria from the rhizosphere (Kumar and Jacob, 2019). Furthermore, De Bary (1866) have coined the term "Endophyte" for the microorganisms that are present inside the plant tissue. (Compant *et al.,* 2012).

Actinomycetes, also called Actinobacteria, are consider to be an intermediate group between fungi and bacteria. The name Actinomycetes was derived from Greek word *aktis* (a ray) and *mykes* (fungus) due to its close morphological resemblance to fungus. They are free living bacteria characterized by tough powdery and pigment forming colonies (Srinivasan *et al.,* 1991).

The rhizospheric and endophytic actinomycetes have a wide range of natural bioactive chemicals that provide plants with different nutrients, hormones required for their growth. The bioactive compounds also protects the plant from any phytopathogens.

The rhizospheric and endophytic actinomycetes possesses an arsenal of various natural bioactive compounds. The bioactive compounds from actinomycetes provides the plants with required nutrition for their growth, various hormones necessary for developments in plants and the antibiotics required to fight against phytopathogens. (Qin *et al.* 2015; Shan *et al.*, 2018).

Plant Growth promoting Actinobacteria (PGPA) have the ability to induce various mechanisms to supress the harmful pathogens by production of lytic enzymes, siderophores, antibiotics and various biometabolities. Also PGPA assist the plant to overcome various abiotic stress such as water scarcity, heavy metal toxicity, depletion of soil nutrition, salinity etc. by several mechanisms. The various PGPA mechanisms comprises of regulating the ethylene level in plants by production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, Nitrogen fixation, siderophore production etc. (Anwar *et al.*, 2016). Thus, in this chapter we are going to discuss about the PGP activity of the actinomycetes, its antagonist activity against wide variety of plant pathogens and the biometabolities that support the growth and development in plants.

3.2 Actinomycetes and Its Role as Plant Growth Promoters (Pgps):

Plant growth is governed by various biotic and abiotic factors (Figure 1) that impact the total crop production. To overcome these obstacles use of synthetic fertilizers has increased over the past few years which had led to different environmental pollutions. Actinomycetes are ubiquitous groups of microorganisms that are widespread in diverse ecosystems around the world. (Djebaili *et al.*, 2020).

They are the active producer of various economically important bioactive compounds. They covers 70 to 80 % of the commercially available antibiotics. (Srinivasan *et al.*, 1991; Lacey 2008). Other antibiotic producing genus includes *Micromonospora*, *Actinomadura*, *Streptoverticillium* and *Thermoactinomycetes* (Colquhoun *et al.*, 1998, Waksman, 1954).

Actinomycetes occur both as rhizospheric bacteria and as endophytic bacteria. They are an excellent source of biometabolities which promote plant growth and ensure its good health using its direct and indirect PGP traits (as shown in Figure 2). Furthermore, actinomycetes assist the plant growth by production of phytohormones such as indole-3-acetic acid (IAA) (Franco-Correaa *et al.,* 2010; Rungin *et al.,* 2012). Moreover, actinomycetes exhibits antagonist activity against a wide range of plant pathogens by production of beneficial enzyme such as chitinase, ACC deaminase, wide range antimycotics and antibiotics. Moreover, they also have the ability for phosphate solubilization, HCN production and nitrogen fixation (Sreevidyaa *et al.,* 2016; Chukwuneme *et al.,* 2020).

It has also been reported that strains of various genera belonging to Actinobacteria have the ability to produce IAA. Report suggest various actinobacterial strains such as VAI-7; VAI 40; SAI 13; SAI 29, *S. pseudovenezuelae* (MG547870), *A. arilaitensis* (MG547869) and *Streptosporangium becharense* SG1 are excellent producers of indole-3-acetic acid (IAA). (Sreevidyaa *et al.,* 2016; Chukwuneme *et al.,* 2020; Boukaya *et al.,* 2018).

As an endophytes, actinomycetes grow a symbiotic association with the host plant and internalize the tissue without harming the host. Furthermore, endophytic actinomycetes have played a significance role in plant growth by supressing various phytopathogens and secreting various beneficial biometabolities. Endophytic actinomycetes such as *Streptomyces mutabilis* NBRC 12800^{T}, *Streptomyces cyaneofuscatus* JCM 4364^{T}, *Streptomyces asterosporus* NRRL B-24328^{T} have been investigated to eliminate plant disease such as damping of *R. solani, Fusarium* root rot along with additional biological activities and PGP traits (Goudjal *et al.,* 2013; Goudjal *et al.,* 2016). Actinomycetes are able to produce Polyamines (PAs) which are a low molecular weight bioactive compounds and reported to produce by both animal as well as plants. Two variant of the actinomycetes strains of *Streptomyces griseoluteus* had been studied which includes PNPM (Polyamine non-producing mutant strain) and wild type isolate (WT). The PNPM was incapable to promote plant growth whereas when WT variant was used it showed significant plant growth due to the activation of various plant growth regulators that includes IAA, GA_3. (Nassar *et al.,* 2003). So the study provides an important link between the Polyamines that supports the PGRs traits. Actinomycetes are therefore an indispensable part of future sustainable agriculture.

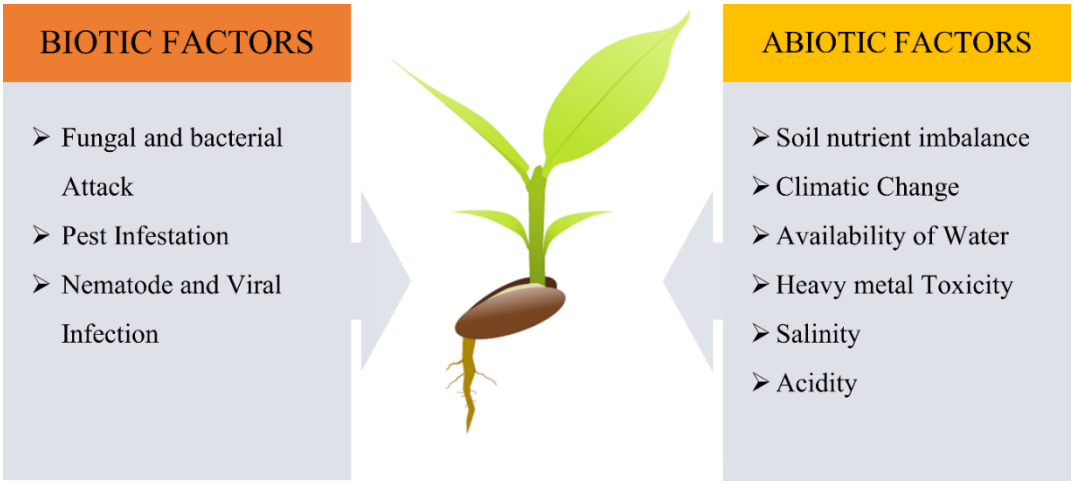

Figure 3.1: Schematic illustration for biotic and abiotic stress in plants.

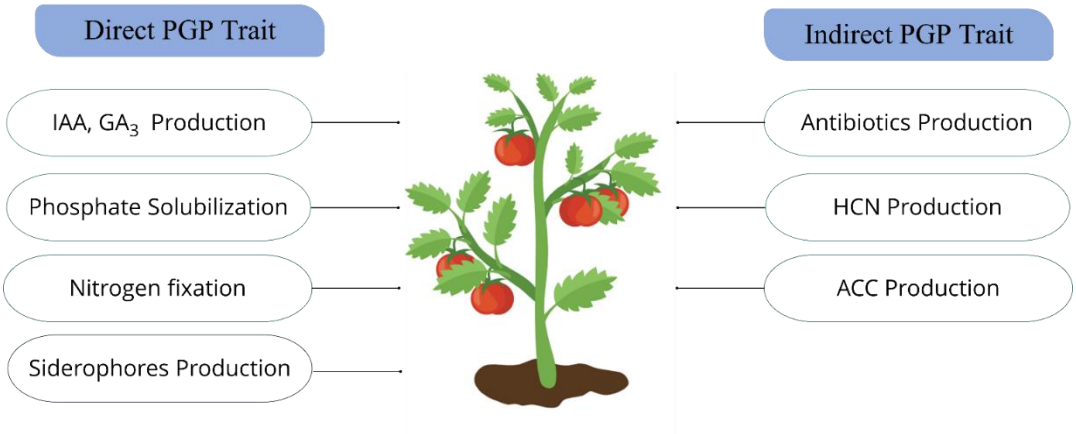

Figure 3.2: Schematic diagram for the direct and indirect PGP traits.

3.3 Actinomycetes Traits as Plant Growth Promoter:

3.3.1 Indole-3-Acetic Acid (Iaa) Production:

Auxin is one of the most important plant hormones essential its growth and development. Auxin is required to regulate physiological process such as ontogeny, organogenesis, vascular differentiation, and root and shoot formation, fruit ripening etc. Among all phytohormones of the Auxin class, indole-3-acetic acid (IAA) is the most common naturally occurring hormone involved in plant growth and development. IAA producing strains have been successfully isolated from both the rhizospheric soil as well as from different parts of

plants (Kudoyarova *et al.,* 2019; Canellas and Olivares, 2014). Lasudee *et al.* (2018), reported to have isolated IAA producing actinomycetes strain *S. thermocarboxydus*, from the spores of *Funneliformis mosseae* an Arbuscular mycorrhizal fungi (AMF).

The AM fungi and the actinomycetes formed a symbiotic relationship that had also benefited the host plant. *S. thermocarboxydus* showed excellent PGP traits when inoculated with Thai jasmine rice under low soil nutrition and induced drought stress. (Lasudee *et al.,* 2018).

Jog *et al.* 2014, isolated five strains of *Streptomycetes sp.* from both the rhizospheric soil and roots tissues of *Triticum aestivum* which were capable to produce PGPs traits including IAA, phytase etc. (Jog *et al.,* 2014). Therefore, along with other PGP traits, IAA helps the plant resist stress and improve plant growth.

3.3.2 Gibberellins Production:

Gibberellins are naturally occurring phytohormones belonging to the class of diterpenoid which play a pivotal role in healthy modification of plants. Gibberellins improves the growth of plant tissue along with cell elongation. (Vandenbussche *et al.,* 2007). Solans *et al.,* (2011) reported to isolate saprophytic actinomycetes from the actinorhizal plant (*Ochetophila trinervis)*.

The isolated actinomycetes belongs to the genus *Streptomyces*, *Actinoplanes* and *Micromonospora*. The roots of *O. trinervis* were inoculated with different genera of actinobacteria separately and in combination to establish the co-inoculation effect on the plant growth. The result revealed the development of intense root hairs with different shapes. The presence of Gibberellic Acid (GA_3) was confirmed in the study using GCMS analysis. (Solans *et al.,* 2011). According to Nassar *et al.* (2003) the variant WT of actinomycetes strain *Streptomyces griseoluteus* have been reported to produce number of plant growth regulator including gibberellic acid (GA_3). GA_3 was found significantly in the root and the shoot of *Phaseolus vulgaris* L. (Nassar *et al.,* 2003). Therefore GA_3 produced by the actinomycetes strains have significant effect in plant growth promotion and overall health.

3.3.3 Phosphate Solubilization by Actinomycetes:

Phosphorus is an essential nutrient for the plant growth and development and are naturally present in the soil. Microbes have the ability to solubilize the mineral form of phosphorus making it freely available for plants. (Romero-Perdomo *et al.,* 2021). Actinomycetal strains belonging to the genus *Streptomyces* have been reported to possess the ability to solubilize the phosphate from the soil.

The strains isolated from the rhizospheric region of *T. repens* were reported to solubilize inorganic phosphate from the soil. Actinomycetes able to solubilize phosphate in the soil with the help of phosphatase enzyme. The strains MCR26, MCR9 and MCR24 showed a correlation and synergistic effect with the Arbuscular mycorrhizal (AM) *Glomus mosseae* in terms of plant growth and Phosphate acquisition. (Franco-Correaa *et al.,* 2010). Minima *et al.,* (2018) have also reported to isolate a total of 191 number of actinomycetes strains from the roots and stem of Rice plant (*Oryza sativa* L.) majority of which were capable of solubilizing phosphate belongs to the genera of *Streptomyces*. Other actinomycetes genera involve in solubilizing phosphate are *Actinomadura*, *Actinomycetospora*, *Kribbella*, *Microbispora*, *Nocardia* and *Pseudonocardia* (Mingma *et al.,* 2018).

3.3.4 Nitrogen Fixation:

Nitrogen is considered to be a vital element required by plants for their normal growth and development. The Nitrogen is the main constituent found in chlorophyll, thus is an imperative element in the development of photosynthetic components in the plant cell. (Bassi *et al.,* 2018). Franco-Correa *et al.,* (2010) reported that actinomycetes with the nitrogen fixing ability can grow on N-Free media indicating the ability to fix Nitrogen (Franco-Correaa *et al.,* 2010). The various strains of actinomycetes capable of fixing N includes *Frankia sp., Streptomyces thermoautotrophicus* UBT1 (Valdés *et al.,* 2005).

Furthermore, studies suggests that bacteria belong to the group *Frankia* when inoculated with the actinorhizal plants in a nutrient deficient environment improves the plant growth and development. (Diagne *et al.,* 2013). Therefore, actinomycetes are an invaluable source for nitrogen fixation, thus supporting plant growth promotion (PGP).

3.3.5 Siderophore Production:

Siderophore are low molecular weight organic compounds with metal chelating activity. The name Siderophore was derived from Greek word meaning '*iron carrier'* and have a high specific affinity towards iron. Many microorganisms are able to produce siderophores which in return help them to acquire iron as a source of nutrients from the environment. (Ahmed *et al.,* 2014). According to Rungin *et al.* (2012), actinomycetes strain Streptomyces *sp.* GMKU 3100 which is able to produce siderophore has induced the root and shoot growth of rice and mungbean plants. Literature studies suggest that siderophore producing actinobacteria develop antagonist activity against phytopathogens (Rungin *et al.,* 2012). The two *Streptomyces* strains UPMRS4 and UPMRS28 isolated from the rhizospheric soil of both healthy and blast infected rice plants has also been reported to produce siderophores (Awla *et al.*, 2017). Dimkpa *et al.,* have successfully isolated a Nickle resistant strain *Streptomyces acidiscabies* E13 which have the ability to produce siderophore under nickel stress. The *S. acidiscabies* E13 produce hydroxamate siderophore such as DFOB, DFOE and Cch in the presence of Ni and absent of Fe in the media. (Dimpka *et al.* 2008). Verma at *al.*, (2010) studied the three *Streptomyces* strains isolated from the root of *Azadirachta indica* that were capable to produce siderophore and have exhibited antagonistic activity against *Alternaria alternate* (Verma *et al.,* 2011) Therefore, actinomycetes are an excellent source for the production of siderophores and plays a vital role as plant growth promoters.

3.3.6 Antibiotics Production:

Actinomycetes are the biofactories for the production of numerous bioactive metabolites. Control of phytopathogens by production of antibiotics is one of the PGP trait of actinomycetes. Both the endophytic as well as rhizospheric actinobacteria are reported to have showed antagonistic activity against bacterial and fungal strains. Samac *et al.,* (2003) reported the antibiotic producing strain of actinomycetes that was able to colonise *Medicago sativa* L. plants and inhibits the growth of *Phoma medicaginis* var. *medicaginis.* (Samac *et al., 2003*). Similarly, *Streptomyces philanthi* RM-1-138 which has been reported to inhibit the growth of fungal pathogen *R. solani*. *Streptomyces violaceusniger* strain YCED-9 have also showed antagonist activity against a broad range of fungal pathogens. The strain produce polyene-like compounds similar to guanidylfungin A, nigericin, and geldanamycin

(Trejo-Estrada *et al.,* 1998). Thus, antibiotics produced by actinomycetes can be incorporate in near future as biologically control agent (BCA) in agricultural practice thus eliminating the use of harmful pesticides.

3.3.7 Hcn Production:

Hydrogen cyanide (HCN) is a volatile secondary metabolites. As reported the HCN is a toxic compounds use to counter the phytopathogens of plants. Goudjal Y *et al.,* 2016, isolated HCN producing *Streptomyces sp.* strain from roots of *S. nigrum*. The strain has further been reported to show antagonistic activity against pathogenic fungi such as F. *oxysporum* f. sp. *radicis* lycopersici, *F. solani* and *F. oxysporum,* (Goudjal *et al.*, 2016). Other reported actinobacterial strains equipped with HCN production are *Streptomyces sp.* UPMRS4 and *Streptosporangium becharense* SG1 (Awla *et al.,* 2017; Boukaya *et al.,* 2018). Therefore, HCN is an essential candidate that promotes growth and protects the plant from unwanted microbial attacks.

3.3.8 1-Aminocyclopropane-1-Carboxylic Acid (ACC) Deaminase Activity by Actinomycetes:

ACC (1-aminocyclopropane-1-carboxylic acid) deaminase activity is one of the crucial activity involving the growth and development of the host plant. ACC which is an immediate precursor for the ethylene production are secreted by plants root exudates under abiotic and biotic stress condition (Zarei *et al.,* 2020). According to the Chukwuneme *et al.,* (2020) when ACC gets cleaved to ACC Deminase it release α-ketobutyrate and ammonia in the process which can be measured to determine the ACC deminase activity. In the study it was observed *Streptomyces pseudovenezuelae* was the highest producer of deminase activity (0.903 ± 0.024 μmol αKB mg−1 h−1). Other actinobacterial strains includes *A. arilaitensis , Streptomyces indiaensis, M. oxydans, Streptomyces spp., S. werraensis* and *S. luteogriseus* respectively (Chukwuneme *et al.,* 2020).

The Actinomycetes strain *S. thermocarboxydus* isolated from the spores of *F. mosseae* an Arbuscular mycorrhizal fungi (AFM) that are used widely in horticulture as a bioinoculant. The Actinomycetes strain is capable to produce ACC deminase when grown on DF salt

medium supplied with ACC as sole nitrogen source. Furthermore, the ACC deminase equipped bacteria provides the host plant to withstand in water deficient environment. The strain *S. thermocarboxydus* produce both ACC and IAA which synergistically induce growth promotion in Rice (*Oryza sativa*) KDML105 under induced drought stress. (Lasudee *et al.,* 2018)

3.4 Conclusion and Future Applications:

The book chapter has unequivocally exemplified the multifunctionality of actinomycetes in the field of sustainable agriculture. Actinomycetes possess diverse sets of plant growth promoting traits without exerting any negative impact into the environment. Actinomycetes have also exhibited the symbiotic relation between the bacteria and the Arbuscular Fungi which will revolutionized the agriculture sector by providing multifarious ways to resolve issues related to crop improvement. The literature study suggest that the plant growth promoting actinomycetes can be used as a bioinoculant to improve crop yields as well as induce disease resistant capabilities to the plant. Using bioinoculant as a substitute for chemical fertilizer will reduce the environmental pollution and its harmful impact in humans. Also actinomycetes plays a major role in strengthening the plant to withstand various abiotic and biotic stress. Thus, actinomycetes is the future for sustainable agriculture.

Actinomycetes Strains	**Isolation source**	**Plant Growth promoting Trait**	**PGPR Trait on inoculated Plant**	**References**
Streptomyces mutabilis NBRC 12800T and *Streptomyces cyaneofuscatus* JCM 4364T	Plants root of Cleome Arabica; *Astragallus armatus*	Antifungal*;* Biocontrol of *R. solani* damping-off	*R. solani*	Goudjal *et al.,* 2013
Streptomyces sp. strains VAI-7;VAI 40; SAI 13; SAI 29	Herbal vermicompost	IAA-Production	Chickpea.	Sreevidyaa *et al.,* 2016

Actinomycetes Strains	**Isolation source**	**Plant Growth promoting Trait**	**PGPR Trait on inoculated Plant**	**References**
Streptomyces lydicus DSM 40002T	Oryza sativa L.	Antifungal; siderophores and solubilize phosphate	Thai Jasmine Rice	Mingma *et al.,* 2018
Streptomyces sp. GMKU 3100	Roots of Oryza sativa L. cv. KDML105	Siderophore production; Phosphate solubilization and IAA production	*Vigna radiata* (L.) CN72) and *Oryza sativa* L. cv. KDML105	Rungin *et al.,* 2012
Streptomyces sp. UPMRS4	Rhizospheres soil of both healthy and blast disease infected rice	Biocontrol of rice blast disease (*Pyricularia oryzae*); siderophore production; Phosphate solubilizing; (HCN)	MR219 rice variety	Awla *et al.,* 2017
S. pseudovenezuelae (MG547870) and *A. arilaitensis* (MG547869)	rhizosphere soil of maize plantations	IAA; Siderophore; phosphate solubulization and ACC production	Maize plants	Chukwuneme *et al.,* 2020
Streptosporangium becharense SG1	Saharan soil from Béchar	Antagonist activity aganist Root rot disease caused *by Fusarium culmorum*; Promote	durum wheat	Boukaya *et al.,* 2018

Actinomycetes Strains	**Isolation source**	**Plant Growth promoting Trait**	**PGPR Trait on inoculated Plant**	**References**
		growth of durum wheat; IAA production; Phosphate solubilisation; HCN; Siderophore		
Streptomyces spp.	Herbal vermicompost	Antifungal Activity; Biocontrol of *Fusarium oxysporum* wilt of chick pea; IAA production; Siderophore Production	Sorghum and rice	Gopalakrishnan *et al.* 2013
Streptomyces acidiscabies E13	-	IAA; Siderophore production; Promote growth of Cowpea under the presence of nickel contamination by binding iron and nickel	Cowpea (*Vigna unguiculata* L.)	Dimpka *et al.* 2008
S. thermocarboxydus isolate S3	Spores of *Funneliformis mosseae* CMU-RYA08	PGP Properties; IAA production and ACC deaminase activity; Phosphate	Thai jasmine rice (O. sativa and Mung beans (Vigna radiata)	Lasudee *et al.*, 2018

Actinomycetes Strains	**Isolation source**	**Plant Growth promoting Trait**	**PGPR Trait on inoculated Plant**	**References**
		solubilizing; Siderophore		
Streptomyces spp.	Rhizosphere Soil samples *Trifolium repens* L.	Nitrogen fixation; Phosphate solubilization; N_2 Fixation	Clover plants	Franco-Correaa *et al.* 2010
Streptomyces asterosporus NRRL B-24328^T	*S. nigrum*	Antagonist activity against *Frl* root rot; HCN; Siderospore; IAA; Phosphate Solubulizatio n; β-1,3-glucanase activities	Tomato seedlings.	Goudjal *et al.*, 2016
Streptomyces spp.	Moroccan phosphate mines Soil	Phosphate solubilization; IAA production	Wheat plant (*Triticum durum* L.)	Hamdali *et al.* 2008
Streptomyces spp.	Rhizophere and roots of *Triticum aestivum*	IAA, siderophore production; Phosphate solubilization;	*Triticum aestivum* (wheat)	Jog *et al.* 2014
Streptomyces griseoluteus WT	rhizosphere soil of Phaseolus vulgaris	IAA production; GA_3	Bean (Phaseolus vulgaris L.)	Nassar *et al.* 2003

Actinomycetes Strains	**Isolation source**	**Plant Growth promoting Trait**	**PGPR Trait on inoculated Plant**	**References**
Streptomyces sp., Nocardia sp., Nocardiopsis sp., Spirillospora sp., Microbispora sp. and *Micromonospora sp.*	Leaves, branches, and roots of mandarin root stocks and trees	Phosphate-solubilizing activity, IAA production	Mandarin seedlings	Shutsrirung *et al.*,2013
Streptomyces sp.	Root of Azadirachta indica A. Juss. (Meliaceae), plants	IAA, siderophore production; Phosphate Solubulizing; Antagonistic activity aganist *Alternaria alternata*	Tomato (*Solanum lycopersicum*)	Verma *et al.* 2011
Frankia sp.; Streptomyces sp.; Actinoplanes sp. and *Micromonospora sp.*	Isolated from rhizosphere and rhizoplane of *Ochetophila trinervis*	IAA; GA_3; Zeatine	*O. trinervis* and Alfalfa plants	Solans *et al.*,2011

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ISBN: 978-93-90847-97-6

4. Antibodies

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Abstract:

In the battle of fighting against pathogens or antigens, the well-known leading key players that come into picture are the antibodies. Antibodies also called as immunoglobulins shield us by its unique Y-shaped structure serving as a "bullet of the battle". Antibodies which are a part of adaptive immune system hunt down the pathogens when they encounter antigens by neutralizing, capturing and eliminating it from the body. Not only do they attack but they mediate other biological functions as well. These antibodies are well known for its protective effect but sometimes they turn out to be evil by posing deleterious effects on the host tissue itself. Nevertheless, antibodies play the lead role in the cavalry to invade the invaders by activating cascade of events in the pool. Following section will enlighten more about the outlook of antibodies, its role and working.

Keywords: *antibodies, classification, structure.*

4.1 Where Do They Come From?

Well, the journey of production begins from the progenitor lymphoid cell in the bone marrow where it matures to form either progenitor T cell or progenitor B cell. The progenitor T cell migrate to thymus for maturation while the progenitor B cell undergoes somatic recombination to form immature B cell. Each progenitor B cells that give rise to

millions of immature B cell through somatic recombination end up having unique antibodies projecting on their surface. Eventually both the immature B cell and naïve T cell end up in lymph nodes for maturation. Once the antigen presenting cells present the antigen to the naïve T cells in the lymph node, they get so very active that they immediately begin to proliferate and differentiate to form cytotoxic T cells and T helper cells.

With that joy, T helper cells come forward to help its inmate to get activated. Not only that but B cells also get activated on its own by just seeing its antigen. To combat the enemy even more efficiently, they strengthen themselves by expanding its squad and become large in number. And this is called clonal expansion where they differentiate either to memory B cells or antibody secreting plasma cells. And that's how our hero antibody gives its entry in the battle [1].

4.2 But How Do They Look Like?

Antibodies (Ab) aka immunoglobulin (Ig) aka "our heroes" that are released in to the blood stream by plasma cells after the recognition of antigen look alike with almost similar structural components. These antibodies look like Y-shaped arms and it is made of 2 pairs of polypeptide chains. We generally categorize them into heavy and light chains. It is these chains, i.e., 2 identical heavy chains (H) and 2 identical light chains (L) that orient together to form this Y shaped arm. Within these two chains lie two different regions, the variable (V) and constant region (C).

The variable portion in light chain and heavy chain are termed as VL and VH whereas the constant region in light and heavy chain are termed as CL and CH respectively. The main difference between these two regions is that the variable regions with an amino group while the constant region terminate with a carboxy group. The tip portion of the antibody structure to where the antigens generally bind are made of variable regions to enhance specificity against different antigens whereas the remaining portion is made of constant regions.

Now to make the entire structure stable by holding it together, there are di-sulfide bonds that bridge the heavy chains to each other and the heavy chains to light chains. So, in total an antibody has a variable region and a constant region in the light chain.

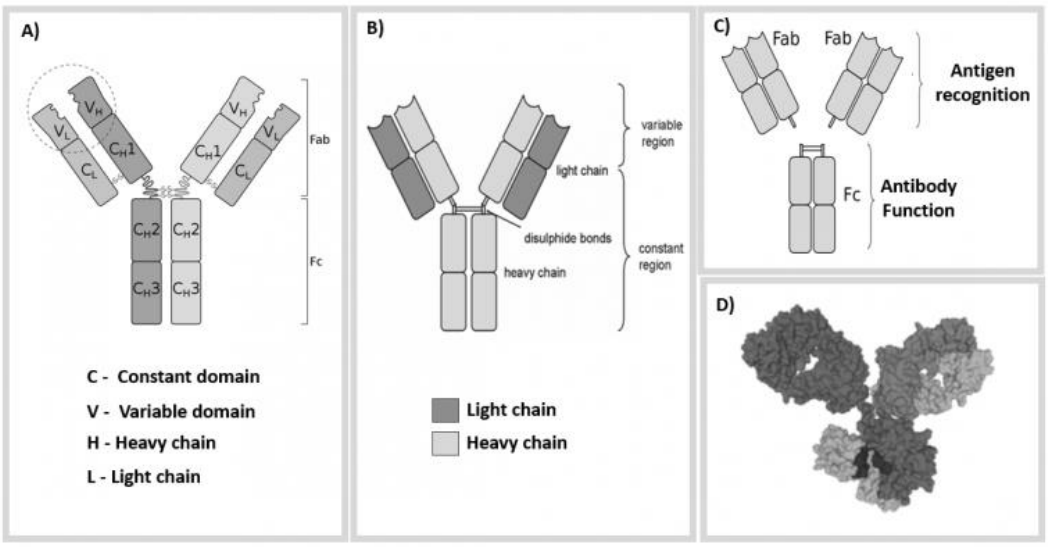


Figure 4.1: (A) constant and variable regions in heavy and light chains (B) light and heavy chains (C) Fab and Fc region (D) 3D structure of antibody (source: https://teachmephysiology.com/immune-system/adaptive-immune-system/antibodies/)

The variable portions of one pair of heavy and light chain, VH and VL come together to form an antigen binding domain (Fab) in the antibody to entrap the antigens. Thus, an antibody has two antigen binding domains. By making differences in the sequence of variable regions, it showcases its unique ability of binding to varied types of antigens.

Wherein the rest of the constant regions in the heavy chain is called the fragment crystallizable region (Fc) [2]. In addition to these, there is something notable in the structure of antibody like the hinge region and the hypervariable region.

The hypervariable regions or the complementarity determining regions (CDRs) are loops that are a part of Fab portion of antibody that are complementary to the sequences found in antigens. To bind to any antigen, an antibody not only requires complementary sequence but also it needs to be flexible so as to enhance its ability to bind to different array of antigens on different surfaces. This flexibility is bestowed by the hinge region, made of amino acids which can be stretched, that is present in the middle portion of heavy chains of IgG and IgA only [3].

There is one other thing called joining (J) chain, a polypeptide protein, part of antibodies dimeric IgA and pentameric IgM, that is released into mucosa and regulates polymeric formation of immunoglobulins [4].

4.3 Do They Work Alone?

Of course not, they do have siblings to get done other works and functions at different sites. They are almost similar in structure except for the constant domain parts and each type of antibody carry out its own notable functions at different locations.

Well, the main five different types of siblings that we come across in our body are immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin E (IgE), immunoglobulin D (IgD) where IgG, IgE and IgD exist in monomeric state and IgM exist in pentameric state while the IgA in dimer form.

Researchers found that the sequence found in the heavy chain of antibodies fall under five categories and hence given the Greek names like (α) alpha, (μ**)** mu, (*δ*) delta, (γ) gamma and (ε**)** epsilon for the antibodies IgA, IgM, IgD, IgG and IgE respectively.

There are other subclasses for the heavy chains of IgA and IgG [5]. When it comes to the light chain of antibodies, there are again classes like kappa (k) and lambda ***(λ)*** and subclasses like **λ1, λ2, λ3 and λ4. Each class of antibodies can either have kappa or lambda light chains** the basic difference lies in the molecular weight of each antibody.

The largest structure being the pentameric IgM holds the highest molecular weight of about 900,000 Daltons. This is followed by the secretory IgA dimer with about 385,000 Daltons.

The rest of the monomeric antibodies i.e., IgE, IgD and IgG holds the next three consecutive places with about 200, 180 and 150 kilo Daltons respectively.

The difference not only lies in its molecular weight but also in terms of function, the number of antigen binding sites, distribution and even the quantity present in the serum. The following section and the table 4.1 encompass more insights about each type of antibody. Figure 4.2 shows the structure of the five main classes of antibodies

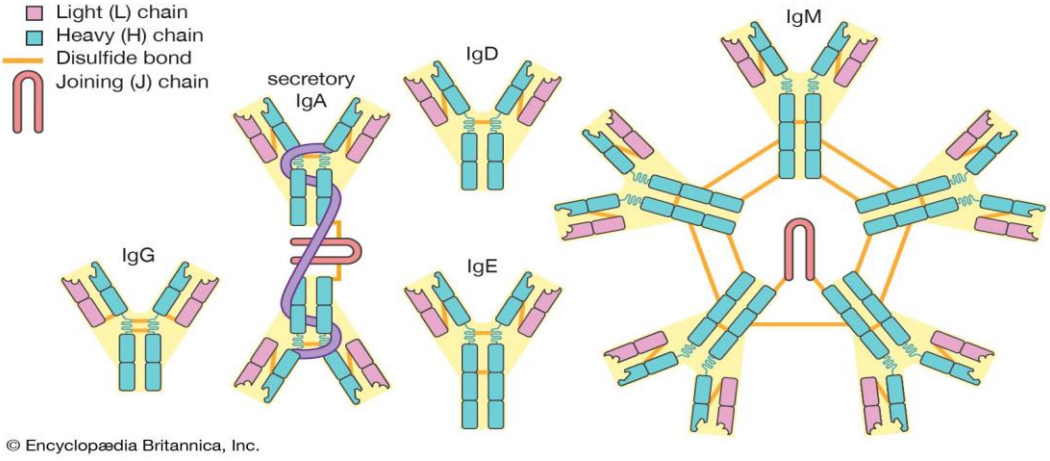


Figure 4.2: (source: https://www.britannica.com/science/immune-system/Classes-of-immunoglobulins)

4.3.1 In More Detail….

A. IgG: The only antibody that is present in ample quantity in the blood serum is this immunoglobulin G. We can also see them on the surface of matured B cells. These constitute about 80% in the serum. These are large globular protein molecules of about 150 KDa and plays a big part in fighting against harmful pathogens and infections.

They have the capacity to bind to pathogens like bacteria, virus, and fungi. IgG is so very important because it provides long term protection against pathogens as they can persist for years after the first exposure. In addition to this, they are also able neutralize the toxins.

The unique function of IgG is that they are the only class of immunoglobulins that have the capacity to pass through placenta to provide humoral immunity to the fetus unlike any other class of molecules because of its low molecular weight [6].

On the other hand, these are associated with type II and type III hypersensitivity reactions.

B. IgA: These are dimeric molecules with a J chain as a secretory molecule of 385 kDa and constitute about 13% in serum. We can find these antibodies specifically in mucous membranes like the respiratory and digestive tracts and also in saliva, breastmilk and tears

to act as first line of defense. Just like IgG, these antibodies also neutralize the bacterial toxins and provides resistance against infections. But these also do the elimination of antigens by using their secretory component around the J chain.

These secretory antibodies provide defensive mechanism against pathogens by enhancing adaptive humoral immunity in the mucosal membranes. So they eliminate the pathogen by this IgA mediated excretory pathway [7].

C. IgM: Five monomeric units attach together to form these largest pentameric IgM antibodies. It's mainly involved in the ABO blood group antigens on the surface of red blood cells.

In addition to this, they are also responsible for mediating phagocytic clearance of pathogens. The very first antibody that forms after exposure to antigens is IgM. Just like IgA, these antibodies also exist in secretory form.

Typically, this antibody displays low binding affinity to antigen but all the 10-arms in the protein come and work together so as to strongly bind to the antigen. Having a molecular weight of 900 kDa, they occupy just 6 % in the serum [2].

D. IgE: This monomeric antibody, IgE, is exclusively seen only in mammals. Only a very minute amount is present in serum of not more than 0.002 %. These are believed to be protective against certain parasites and venoms.

Nevertheless, it's mainly involved in allergic reactions by fighting against allergens and is therefore associated with type I hypersensitivity reactions. They also participate in immune response events by binding to basophils and mast cells [8].

E. IgD: One other monomeric antibody, IgD, constitute only about 1 % in the serum. Though it's in minute amount, antibodies present on the surface of B cells plays its role in induction of production of antibodies.

Just like IgE, they activate basophils and mast cells to mediate the production of anti-microbial factors thereby participating in respiratory human defense [9].

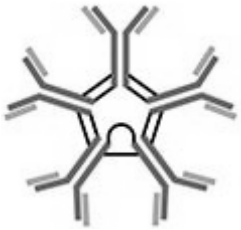
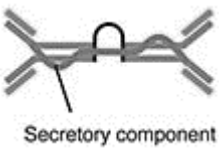
Properties	**IgM**	**IgG**	**IgA**	**IgE**	**IgD**
Number of units	pentamer	monomer	dimer	monomer	monomer
Structure					
Heavy chains	μ	γ	α	ε	δ
Number of Antigen binding sites	10	2	4	2	2
Molecular weight (Daltons)	900,000	150,000	385,000	200,000	180,000

Table 4.1: Details of five major classes of antibodies

4.4 Do They Take Part only in Antigen-Binding?

Well, one thing that has to be remembered is that antibody not only acts as opsonin and recognizes the pathogen by mediating phagocytosis via their Fc region but they also block different parts of pathogens to prevent its entry and to neutralize them. On the other hand, IgM or IgA antibodies activates the complement system as they bind to different microbial surfaces and mediate the production of anaphylatoxins and other components so as to form membrane attack complex.

So far, the story of antibodies seemed very perfect and incredible by carry out multiple functions by recruiting multiple components to opsonize the pathogens. Most of the time our body keeps on fighting the real foreign bodies or enemies well but sometimes it goes wrong. That is where the entire event of immune response turns out to be a chaotic mess by attacking body's own cells. This problem arises when the antibodies cannot distinguish between one's own self and foreign cells resulting in the attack of host cells that is termed as auto-immune disorder.

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ISBN: 978-93-90847-97-6

5. Food Microbiology

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5.1 General Principles of Food Microbiology:

- Microorganisms are present everywhere on earth, which includes humans, animals, plants and other living creatures, soil,water and atmosphere.

Microbes are also an important and essential component of an ecosystem. Molds and bacteria play key roles in the cycling of important nutrients in plant nutrition particularly those of carbon, nitrogen and Sulphur.

- Food microbiology is the study of microorganisms, which have both beneficial and deleterious effects on the quality, and safety of raw and processed meat, poultry, and egg products.

Microbiology is important to food safety, production, processing, preservation, and storage. Microbes such as bacteria, molds, and yeasts are employed for the foods production and food ingredients such as production of wine, beer, bakery, and dairy products. Specifically, areas of interest which concern food microbiology are food poisoning, food spoilage, food preservation, and food legislation. Studies in animal models that determine the responses of probiotic microorganisms in the gastrointestinal tract.

- Fundamental physiology and gene expression studies of food/ beverage microorganisms, unless they directly relate to the food/ beverage ecosystem; The isolation and characterization of antimicrobial substances such as essential oils, bacteriocins etc., unless their efficacy is tested and validated in the food/beverage ecosystem; Development of new methods for the analysis of microorganisms, unless the method is tested and validated in the food/beverage ecosystem.

5.2 Overview of Beneficial Microorganisms and Their Role In Food Processing And Human Nutrition:

- Beneficial microorganisms are those used in food fermentation to produce products such as cheese, fermented meat (pepperoni), fermented vegetables (pickles), fermented dairy products (yoghurt), and ethnic fermented products such as sauerkraut, idli and kimchi. In fermented products (produced by natural or controlled fermentation), microorganisms metabolize complex substrates to produce enzymes, flavor compounds, acids, and antimicrobial agents to improve product shelf-life and to prevent growth of pathogens and to provide product attributes. Microorganisms employed by the food industry include bacteria, yeasts, and molds. Differ morphologically and physiologically according to which the media, fermentation methods and the downstream processes vary. Advantages of microbial fermented food products: Extended shelf-life, nutritional benefit, Enhancement of sensory characteristics.

- Microorganisms with their enzymes, also breakdown indigestible compounds to make the product more palatable and easy to digest. Examples of beneficial microorganisms are *Lactobacillus acidophilus, Lactobacillus arabinosus, Lactobacillus lactis, and Pediococcus cerevisiae.*

Food spoilage microorganisms are those which upon growth in a food, produce undesirable flavour (odour), texture and appearance, and make food unsuitable for human consumption. Sometimes uncontrolled growth of many of the beneficial microorganisms can also cause spoilage.

Table 5.1: Fermented Dairy Products

Name	**Micro flora**
Srikhand (chakka)	*L. delbrueckii subsp. bulgaricus.*
Lassi	*S. salivarius subsp. thermophilus,*
Yoghurt	*S, salivarius subsp.thermophilus, L. delbrueckii subsp.*
butter milk	*lactic subsp. diacetylactis,*

Table 5.2: Commercially Produced Secondary Metabolites

Secondary Metabolite	**Microorganism**
Penicillin	*Penicillium chrysogenum*
Streptomycin	*Streptomyces griseus*
Cyclosporin A	*Tolypocladium inflatum*

Table 5.3: Commercially Produced Enzymes

Microorganism	**Enzyme**
Saccharomyces cerevisiae	Invertase
Mucor pusillus	Microbial rennet
Trichoderma reesii	Cellulase
Saccharomycopsis lipolytica	Lipase
Aspergillus niger	Glucoamylase

5.3 Over View of Sources Of Microorganisms In Food Chain (Water And Air):

- Water can support the growth of many types of microorganisms. This can be advantageous. For example, the chemical activities of certain strains of yeasts provide us with beer and bread. As well, the growth of some bacteria in contaminated water can help digest the poisons from the water. Water microbiology is concerned with the microorganisms that live in water, or can be transported from one habitat to another by water. The presence of other disease causing microbes in water is unhealthy and even life threatening. For example, bacteria that live in the intestinal tracts of humans and other warm blooded animals, such as *Escherichia coli*, *Salmonella*, *Shigella*, and *Vibrio*, can contaminate water if feces enters the water.

- **Drinking Water as a Vehicle of Diseases:** Water is essential to life. An adequate, safe and accessible supply must be available to all. Improving access to safe drinking-water can result in significant benefits to health. Every effort should be made to achieve a drinking water quality as safe as possible. Many people struggle to obtain access to safe water. A clean and treated water supply to each house may be the norm. According to the WHO, the mortality of water associated diseases exceeds 5 million people per year. From these, more that 50% are microbial intestinal infections, with cholera standing out in the first place

Table 5.4: The most important bacterial diseases transmitted through water

Disease	**Causal bacterial agent**
Cholera	*Vibrio cholerae,* serovarieties O1 and O139
Gastroenteritis caused by vibrios	Mainly *Vibrio parahaemolyticus*
Bacillary dysentery or shigellosis	*Shigella dysenteriae*
Acute diarrheas and gastroenteritis	*Escherichia coli*

- **Air microbiology:** Is a scientific discipline that concerns the microorganisms, including bacteria, archaea, fungi and viruses, in the atmospheric air. It is a subdiscipline of environmental microbiology. Biological aerosols as a human hazard source. What types of dangers are connected to the presence of microorganisms in air? Infectious diseases (viral, bacterial, fungal and protozoan), Allergic diseases, Poisoning (exotoxins, endotoxins, mycotoxins). Bioaerosols may carry microorganisms other than those which evoke respiratory system diseases. The intestinal microorganisms contained in aerosols may, after settling down, get into the digestive system (e.g. by hands) causing various intestinal illnesses.
- **Infectious airborne diseases:** The mucous membrane of the respiratory system is a specific type of a 'gateway' for most airborne pathogenic microorganisms. Susceptibility to infections is increased by dust and gaseous air-pollution, e.g. SO2 reacts with water that is present in the respiratory system, creating H2SO4, which irritates the layer of mucous. Consequently, in areas of heavy air pollution, especially during smog, there is an increased rate of respiratory diseases. Bioaerosols may, among other things, carry

microbes that penetrate organs via the respiratory system. After settling, microbes from the air, may find their way onto the skin or, carried by hands, get into the digestive system (from there, carried by blood, to other systems, e.g. the nervous system).

5.4 Primary Sources of Microorganisms in Food:

- From the meat and poultry regulatory perspective, we will be addressing bacteria as a main source of food contamination. Keep in mind that there are other microorganisms like viruses, parasites, fungi, etc., that are able to contaminate food and cause food borne illnesses in animals and humans. Bacteria can be found virtually everywhere including humans and can enter food products through different routes. The following list outlines some of the most common ways in which microorganisms enter food products.
- Soil, water, and establishment environment: Many bacteria are carried in soil and water, which may contaminate food. In addition, the establishment environment is an important source of contamination because of the daily activities and pest infestation. *Listeria, Clostridium, Salmonella, and Escherichia* are good examples.
- **Animal feeds:** This is a source of salmonellae to poultry and other farm animals. It is a known source of Listeria monocytogenes to dairy and meat animals when fed silage. The organisms in dry animal feed are spread throughout the animal environment and may be expected to occur on animal hides, hair, feathers, etc.
- **Animal Hides:** The hide is a source of bacterial contamination of the general environment, hands of establishment employees, and skinned carcasses. Studies have shown that this may be a primary source for *E. coli O157:H7, Salmonella*, and *Listeria* in cattle.
- **Gastrointestinal Tract:** The intestinal biota consists of many organisms; notable among these are pathogens such as *Salmonella*, *Campylobacter*, *E. coli O157:H7*, and other microorganisms. Any or all of the *Enterobacteriaceae* may be expected in feces of livestock and poultry.
- **Food Handlers:** The microbiota on the hands and outer garments of handlers generally reflect the environment and habits of individuals (hygiene), and the organisms in question may be those from hides, gastrointestinal tracts, soil, water, dust, and other environmental sources.

5.5 Over View of Food Borne Pathogens:

Following is a list of three types of pathogens and infectious agents of public health concern

- **Gram Positive:**
  Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, B. anthracis, Clostridium botulinum, C. perfringens.
- **Gram Negative:**
  Salmonella spp, Campylobacter spp, Escherichia coli 0157:H7, Yersinia enterocolitica, Brucella spp
- **Viruses:**
  Hepatitis, Rotaviruses, Tapeworms, *Taenia spp*, Roundworms: *Trichinella spp*, Protozoa: -*Toxoplasma spp* -*Sarcocystis spp*.

5.6 Microbial Food Spoilage and Food Borne Diseases:

Food to become contaminated as it is produced and prepared. Many food borne microbes are present in healthy animals (usually in their intestines, hides, feathers, etc) raised for food. Meat and poultry carcasses can become contaminated during slaughter by contact with small amounts of intestinal contents or poor dressing procedures. Also, it has been shown scientifically that some Salmonella serotypes can infect a hen's ovary in such a manner that the internal contents of a normal looking egg can be contaminated with Salmonella even before the shell is formed.

In food processing, food borne microbes can be introduced from infected humans who handle the food, or by cross contamination from some other raw agricultural product and/or the establishment environment. For example, the unwashed hands of food handlers who are themselves infected can introduce bacteria and viruses. In the kitchen, microbes can be transferred from one food to another food by using the same knife, cutting board or other utensil to prepare both without washing the surface or utensil in between. The way that food is handled after it is contaminated can also make a difference in whether or not an outbreak occurs. Many microorganisms need to multiply to a larger number before enough are present in food to cause disease.

Given warm moist conditions and an ample supply of nutrients, one bacterium that reproduces by dividing itself every half hour can produce 17 million progeny in 12 hours. As a result, lightly contaminated food left out overnight can be highly infectious by the next day. If the food were refrigerated promptly, the bacteria would not multiply at all or at a very slow rate.

Microorganisms can cause a variety of effects in food products including spoilage, which primarily affects product quality, and food poisoning, which is generally caused by pathogens. As regulators, we are most concerned with the effects that microorganisms have on food that leads to food borne illness, because this affects public health.

A food borne illness (or disease) is exactly what the term indicates - a disease or illness caused by the consumption of contaminated foods or beverages. It would seem rather obvious that a food borne microbial pathogen, or a preformed microbial toxic product, or another poison such as a poisonous chemical that has somehow contaminated the food and/or beverage, leads to one of the many different food borne illnesses. There is no one "syndrome" that is representative of food borne illness/disease. Different diseases have many different symptoms. However, the microbe or toxin enters the body through the gastrointestinal tract, and often causes the first clinical signs such as nausea, vomiting, abdominal cramps and diarrhea, which are common symptoms in many food borne diseases.

5.7 General Principles and Techniques in Microbiological Examination of Foods:

Microbiological quality of foods: FOOD microbiology as applied to food processing uses microbial inoculants to enhance properties such as the taste, aroma, shelf-life, texture and nutritional value of foods. It may be necessary to carry out a microbiological examination of a food for one or more of a number of reasons. The determination of the microbiological quality of a food or food constituent may be required in order to estimate its shelf-life or its suitability for human consumption. Microbiology techniques are methods used for the study of microbes, including bacteria and microscopic fungi and protists.

They include methods to survey, culture, stain, identify, engineer and manipulate microbes.

5.8 Common Test Methods:

- Culture Media.
- Immunoassay.
- Polymerase chain reaction.

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Kripa-Drishti Publications
A-503 Poorva Heights, Pashan-Sus Road, Near Sai Chowk,
Pune – 411021, Maharashtra, India.
Mob: +91 8007068686
Email: editor@kdpublications.in
Web: https://www.kdpublications.in

ISBN: 978-93-90847-97-6

9 789390 847976