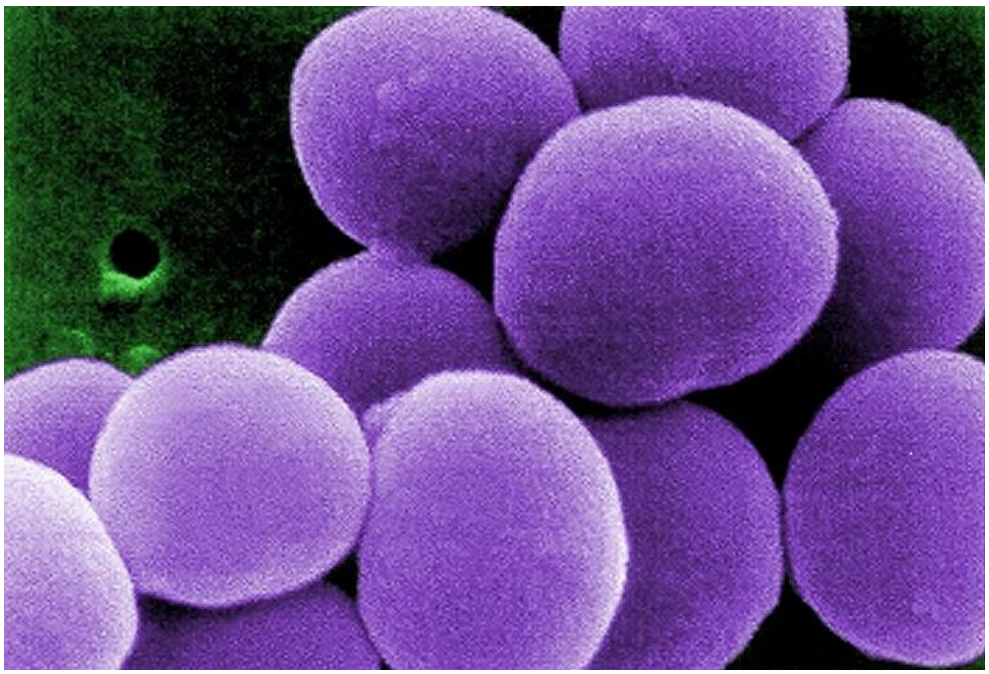
Chapter 1

Introduction



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1.1. General Introduction:

"Microorganism" is a general term which refers to a wide variety of a form of life requiring magnification to see, understand and resolve their structures. Ubiquitous in nature, they play a vital role in supporting and maintaining the balance of nature and life. Microorganism is mainly a unicellular organism that can be divided into its principal types—

1. Bacteria,
2. Yeasts,
3. Moulds,
4. Protozoa,
5. Algae,
6. Rickettsia

Viruses may also be referred as microorganisms, though they cannot live or reproduce themselves. They are considered as particles, not cells, because they consist of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA).

There is more than a thousand different species of bacteria are identified till date, but all of them are basically one of three different shapes. Some are rod or stick-shaped and called bacilli. Examples of rod-shaped bacteria include *E. coli*, *Salmonella*, and *Bacillus anthracis* which is the bacterium that causes anthrax in cattle. Rod-shaped bacteria include bent or curved rods; so *Vibrio* is a curved bacillus that causes cholera. Others are shaped like little balls and called cocci. Examples of bacteria with round cells include *Staphylococcus*, *Streptococcus*, and *Neisseria* (causes gonorrhea). Others still are twisted, helical or spiral in shape and called spirilla. These twisted cells can be flexible (*Treponema* which causes syphilis) or rigid corkscrew shape like Campylobacter (bacterium that causes food-borne illness). Some species of bacteria

vary in shape; this is called pleomorphism. Variations or lack of rigid cell walls causes microbes like *Mycoplasma, Corynebacterium,* and *Rhizobium* to appear swollen, curved, or club-shaped.

Yeast is widely found in nature with a wide variety of habitats, commonly found on plant leaves, flowers, fruits, soil, as well as on the surface of the skin and in the intestinal tracts of warm-blooded animals, where they may live symbiotically or as parasites.

Yeast is a single-celled microorganisms that are classified as members of the fungus kingdom alongwith 1,500 species identified till date (Kurtzman and Fell, 2006) and are estimated to constitute 1% of all described fungal species (Kurtzman and Piskur, 2006). Although Yeasts are unicellular, they possess a cellular organization similar to that of higher organisms by forming strings of connected budding cells known as pseudohyphae or false hyphae (Kurtzman and Fell, 2005). Depending on species and environment, Yeast sizes vary greatly measuring from 3–4 µm in diameter, although earlier it was reported that some yeasts can grow up to 40 µm in size (Walker *et al*., 2002). Most of the yeasts reproduce asexually by the asymmetric division process known as budding. The term "yeast" is often taken as a synonym for *Saccharomyces cerevisiae* (Kurtzman, 1994), because Yeasts do not form a single taxonomic or phylogenetic grouping. Yeasts are evolutionally diverse and are therefore classified into two separate phyla, Ascomycota or sac fungi and Basidiomycota or higher fungi. The budding yeasts ("true yeasts") are classified in the order Saccharomycetales ("What are yeasts?". Yeast Virtual Library. 13 September 2009).

Yeast has long been considered to produce a large variety of industrial products such as alcoholic beverages, bread etc (Legras *et al*., 2007). It is also a centrally important model organism in modern cell biology research, and is one of the most thoroughly researched eukaryotic microorganisms. Researchers have used it to gather information about the biology of the eukaryotic cell and ultimately human biology. (Ostergaard *et al*., 2000). The common "yeast infection" is typically caused by Candida albicans. In addition to being the causative agent in vaginal yeast

infections, Candida is also the cause of diaper rash and thrush of the mouth and throat. Yeasts have recently been used to generate electricity in microbial fuel cells, ("Bioprocess automation". Helsinki University of Technology, 2007) and produce ethanol for the bio-fuel industry.

Mould is a filamentous fungus, generally appearing as a circular colony that may be cottony, woolly, or glabrous. The filaments were known as hyphae, (Moore *et al*., 2011; Madigan *et al*., 2005) network of these tubular branching hyphae, called a mycelium, which is considered a single organism. Generally hyphae are appears to be transparent, that is why the mycelium appears like very fine and fluffy white threads over the surface.

Moulds are ubiquitous in nature; generally mould spores are a common component of household and workplace dust and can usually be found in damp, dark or steamy areas e.g. bathroom or kitchen, cluttered storage areas, areas with poor ventilation, basement areas, plumbing spaces and outdoors in humid environments, can cause allergic reactions and respiratory problems when mould spores are present in large quantities.

Though Moulds are considered to be microbes and do not form a specific taxonomic or phylogenetic grouping but there are thousands of known species of moulds found naturally, which have diverse life-styles. In the past, most moulds were classified within the Deuteromycota (Hibbett *et al*., 2007).

Moulds play an important role in biodegradation of natural materials. Now a day's moulds is widely used in different areas like biotechnology and food science in the production of different types of food, beverages for example *Aspergillus sojae* that have been cultured in many centuries of eastern Asia, used to ferment a soybean and wheat mixture to make soybean paste and soy sauce, antibiotics and pharmaceuticals andenzymes. Alexander Fleming's accidental discovery of the antibiotic penicillin involved a Penicillium mould called *Penicillium notatum.*

Protozoa generally have three different groups of shape. One group is the ciliates, which have hair-like projections called cilia on their flat tapered bodies. The

second group is the amoebae, which can be subdivided into the testate amoebae, which have a shell-like covering on their blob-like body, and the naked amoebae, which don't have this covering. The third group is the flagellates, which have one or several long, whip-like projections called flagella poking out of their cells.

Algae are simple plants that can range from the microscopic to large seaweeds, such as giant kelp that can be as big as more than one hundred feet in length. Algae are very diverse and found almost everywhere on the planet. They plays an important role in many ecosystems, including providing the foundation for the aquatic food chains supporting all fisheries in the oceans and inland, as well as producing around 70 percent of all the air we breathe.

Microalgae include both *Cyanobacteria*, (similar to bacteria, and widely known as "blue-green algae") as well as green, brown and red algae. Though there are many varieties of microalgae are exist in nature, but those are consider as the primary ones.

Algae can be grown using water resources such as brackish, sea, and wastewater unsuitable for cultivating agricultural crops. When using wastewater, such as municipal, animal and even some industrial runoff, they can help in its treatment and purification, while benefiting from using the nutrients present.

Most microalgae grow through photosynthesis by converting sunlight, CO_2 and a few nutrients, including nitrogen and phosphorous, into material known as biomass, this is known as "autotrophic" growth. Other algae can grow in the dark using sugar or starch (called "heterotrophic" growth), or even combine both growth modes (called "mixotrophic" growth).

Rickettsia is a type of nonmotile, Gram-negative, nonspore-forming, highly pleomorphic bacteria, which are obligate intracellular parasites causing many disease in humans such as typhus, rickettsialpox, boutonneuse fever, African tick bite fever, Rocky Mountain spotted fever, Flinders Island spotted fever etc. its route of transmission is by bloodsucking parasitic arthropods such as fleas , lice and ticks. The survival of *Rickettsia* is depends on entry, growth, and replication within the host cells

(Walker, 1996). *Rickettsia* can be present as cocci (0.1 μm in diameter), rods (1–4 μm long), or thread-like (10 μm long). *Rickettsia* is gram-negative and multiplies via binary fission only inside the host cells. They may occur singly, in pairs, or in strands.

Rickettsia is named after its discoverer, an American pathologist Harold Taylor Ricketts, who died of typhus in Mexico after confirming the infectious agent of that rickettsial disease.

Although some bacteria are harmful and can cause diseases in animal but the vast majority of them are not harmful instead are beneficial to humans and the environment. Microbes play an important role in Earth's ecosystems as they keep the nature clean by removing various toxins from water and soil, and degrade organic matters of dead plants and animals keeping the soil fertile. Without microbes, the earth would become a permanent huge waste dump. For example, *Cyanobacteria* or their DNA in the chloroplasts in plant cells were the main source of most of the free oxygen during the time of formation of earth atmosphere. They are also useful to human body as an aid in digestion and prevent the invasion of harmful bacteria. Now a day, microorganisms have been the sources of the production of different kind of antibiotics that have enabled to cure numerous human and other animal diseases.

Microorganisms are also able to produce different types of enzymes necessary for building up and breaking down many of the organic compounds. In recent times bacteria are widely 'employed' by humans in different fields like Biotechnology, Pharmaceuticals, and Food industry, for the interest of bioremediation, making medicine, making foods by fermentation and in many other activities.

"Bioactive molecules" are extra-nutritional constituents that typically occur in small quantities in the body of different form of life. They are being intensively studied to evaluate their effects on health. Bioactive molecules are substances that have an effect on the cellular function of an organism or living tissue. The bioactivity of such molecules may include both beneficial and adverse effects on the living cell.

Bacteria synthesize and secrete extracellular proteins and this is of interest to scientists from variety of disciplines. The bioactive molecules act as a useful criterion in bacterial identification and classification.

An extracellular protein is a peptide that resides in body fluids outside the cells. The extracellular proteins exist in the medium around the cell having originated from the cell without any alteration to the cell structure. For example, Some *Staphylococcal* strain synthesizes a large number of extracellular proteins that have been postulated to play a role in bacterial virulence. Extracellular protein can function in enzymatic action and cell recognition or signalling. Together with other organic compounds and ions, extracellular protein is important in maintaining water balance in the intracellular and extracellular spaces by acting as solutes.

Staphylococcus (from the Greek: staphylē, means "grape" and kókkos, "granule") is referred to a genus of Gram-positive bacteria. These are spherical cells, and form in grape-like clusters under the microscope (Ryan and Ray, 2004). This strain characteristically group together in grape-like clusters. *Staphylococci* are gram-positive, facultative anaerobes, lack spores and flagella, and grow by aerobic respiration, or fermentation that produces lactic acid. Though most of the species have a relatively complex nutritional requirement, but in general they require an organic source of nitrogen, supplied by at least 5 to 12 essential amino acids, e.g. arginine, valine, and B vitamins, including thiamine and nicotinamide (Kloos and Schleifer, 1986; Wilkinson, 1997).

Staphylococcus aureus is a gram positive bacterium, so the cell wall consists of a very thick peptidoglycan layer. They are spherical, form clusters in 2 planes and have no flagella.

At least 40 species of the *Staphylococcus* genus has been identified so far, out of which, nine have two subspecies and one has three subspecies (Harris *et al.,* 2002). Though the best-known species of the *Staphylococcus* strain reside normally on the skin and mucous membranes of humans and other warm-blooded animals but reported to be harmless. Some species like *S. aureus* can spread from person-to-person or from other sources and cause infection when it enters a normally sterile site as a result of

trauma or abrasion of the skin surface such as scratches, burns, puncture wounds or surgery. It is also considered as one of the most common causes of food poisoning. This species can be found worldwide (Madigan *et al.,* 2005). Though many staphylococcal strains preferentially colonize the human body (Kloos and Bannerman, 1994), but *Staphylococcus aureus* and *Staphylococcus epidermidis* are the two most characterized and studied strains.

In some special condition, *Staphylococcus* act as a potent human pathogenic bacterium responsible for a wide range of acute and pyogenic infections, including abscesses, central nervous system infections, endocarditis, osteomyelitis, pneumonia, urinary tract infections and several syndromes caused by exotoxins and endotoxins, besides food poisoning, scalded skin and toxic shock syndromes (Lindsay *et al.,* 1998). A few *Staphylococcal* strains are also found as a main cause of hospital-acquired (nosocomial) infections of surgical wounds and infections related to indwelling medical devices (Archer, 1998). It is also reported that, a few *Staphylococcal* strain has the ability to produce protein having coagulase property. The ability of certain bacterial species to coagulate the plasmas of certain animals was first described in 1903 (Loeb, 1903). Later Gratia (1920) and Gross (1931) were also investigated the clotting of human plasma by a specific substance produced by *Staphylococci sp.* (Staphylocoagulase).

1.2. Background about Coagulase:

Staphylocoagulase or Coagulase is an extracellular protein produced by several *Staphylococcus* strains. The cell wall proteins are rich in hydroxyproline, proline, and lysine. Coagulase is an example of extracellular protein. Coagulase reacts with prothrombin in the blood and the interaction enables the conversion of fibrinogen to fibrin that results in clotting of blood (Miale, 1949). In the laboratory, it is used to distinguish between different types of Staphylococcus isolates. However, there is no overwhelming evidence that it is a virulence factor, although it is reasonable to speculate that the bacteria could protect themselves from phagocytic and immune defences by causing localized clotting. Coagulase reacts with prothrombin in the blood. The resulting complex is called staphylothrombin, which enables the enzyme

protease to convert fibrinogen to fibrin. Coagulase is tightly bound to the surface of the *Staphylococcus sp.* and can coat its surface with fibrin upon contact with blood. It has been proposed that fibrin-coated *Staphylococci* resist phagocytosis making the bacteria more virulent. Extracellular proteins are resides in body fluids outside the cell and primarily take part in enzymatic action and cell recognition or signalling. Coagulase also acts as solutes in maintaining water balance in the intracellular & extracellular spaces. Evidence of two forms of *Staphylococcal* Coagulase was reported so far. One is bound to the cell wall which is responsible for the slide test coagulation and the other is liberated as free coagulase into the culture medium and is responsible for the tube test (Duthie, 1954).

1.3. Importance of Coagulase:

As mentioned earlier coagulase is an extracellular protein produced by several microorganism with following importance-

1) It is used for distinguishing different types of *Staphylococcus* isolates.
2) Study about coagulase enzyme become important to have more understanding in pathogenicity of *Staphylococcus*. This will also help to know the role of the enzyme in development of *Staphylococcal* infection.
3) The study about the *Staphylococcus* strains as well as staphylocoagulase is also important due to the spread of antibiotic resistance (Mohamed, 2012) along with better understanding about the contribution of the enzyme in development of *Staphylococcal* infection (Said, 2010).
4) Researchers have discovered that coagulase can also act as a systemic hemostatic agent (Mojovic *et.al.*, 1969).

1.4. Statement of the problem:

In recent times the antibiotic resistant developed by many bacterial strain has became a problematic ecological phenomenon. Though several types of new antibiotics were prepared synthetically and semi-synthetically, resistant bacterial

strains are developing at a larger pace. Therefore, better understanding about these bacterial strains is required and there is an urgent need of better antibiotics.

Hence, a systematic study about coagulase of *Staphylococcal* origin will help to make more understanding about the nature of the strain which is responsible for several types of infection in human being. The coagulation properties of the extracellular protein have an important role in the homology of coagulation cascade. In depth study about staphylocoagulase may herald a new finding in human pharmacology.

Considering the above, the present study has been undertaken with the following objectives -

1) Isolation, identification and purification of Staphylococcus Sp. Cobs2Tis23.
2) Molecular characterization of Staphylococcus Sp. Cobs2Tis23 strain by standard method to get more purified extracellular protein.
3) Identification, Characterization & purification of extracellular protein (Coagulase) from the microbial extract. And the protein showing clotting of plasma will be purified for further homogenicity and the clinical assay with plasma coagulase.