(7) Zygospores are provided with two wall-layers e.g., outer thick warty wall called exospore and the inner smooth wall called endospore.

(8) Meiosis of the diploid nuclei takes place during the germination of zygospore.

(9) In case of heterothallic species, germ-sporangium produces only spores of the same mating type i.e. either (+) or (–).

Common Indian species : *Mucor indicus; M. hiemalis; M. mucedo; M.javanicus*, etc.

G. *RHIZOPUS* :

Rhizopus belongs to the family Mucoraceae, order Mucorales, class Zygomycetes and the sub-division Zygomycotina and the division Eumycota.

The genus *Rhizopus* has about 14 species *(Invi et al,* 1965). But according to Hessltine and Ellis (1973) there are 120 species and varieties of *Mucor* have been reported. Most of the species are saprophytes and they form fleecy-white cottony mycelium on bread, cheese, jam and on other moist fresh organic matter in contact with soil. Of these species, *Rhizopus stolonifer* is very common and occurs frequently on bread and

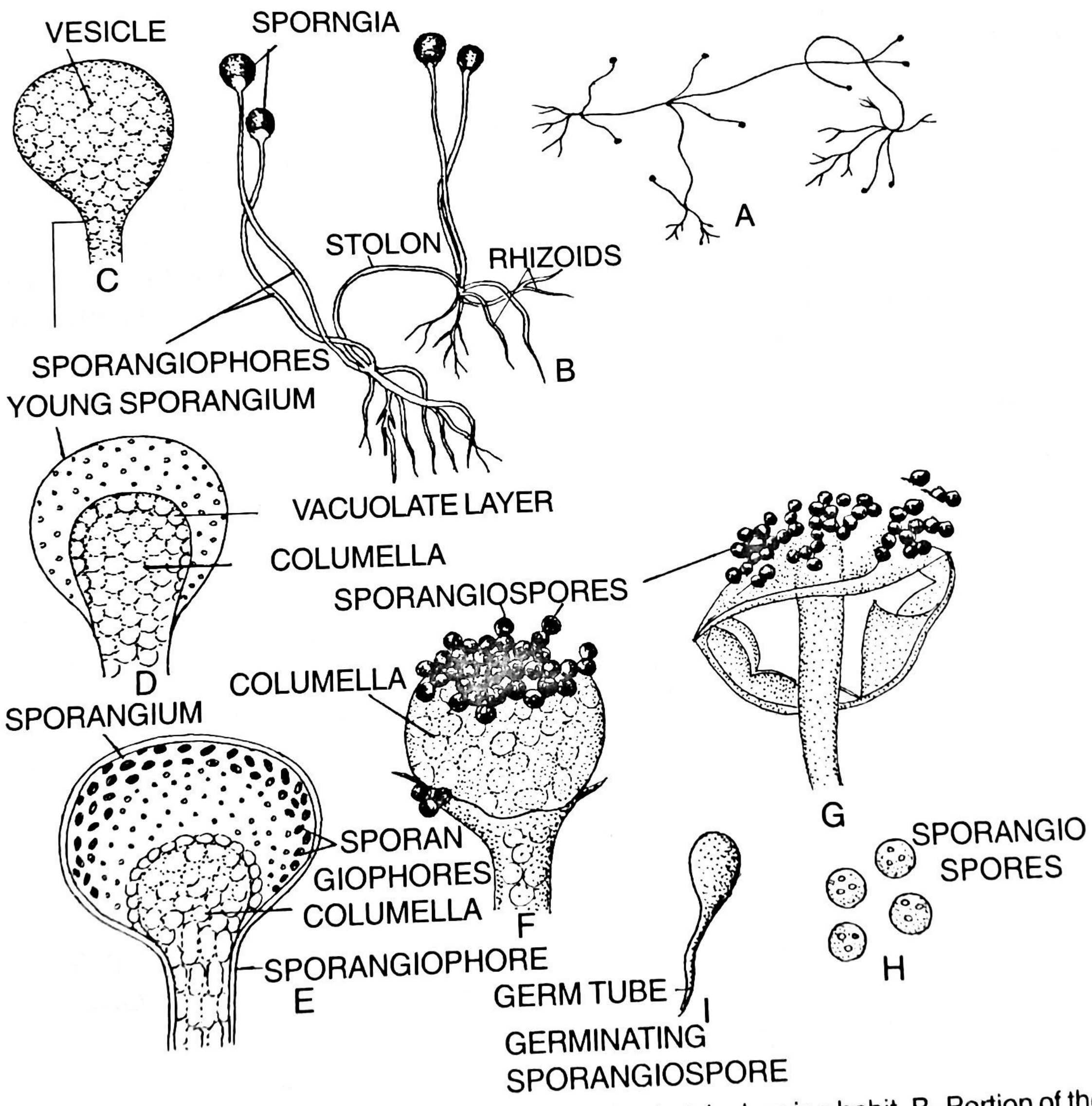

Fig. 3.30 *Rhizopus stolonifer* (*R. nigricans*) A– Diagrammatic sketch showing habit. B–Portion of the thallus showing different structures. C-E Sporangium devlopment and spore differentiation. F–Columella and attached spores. G–Invaginated columella. H–Sporangiospores. I– A germinating sporangiospore.

is therefore called as 'bread mould'— this species often behaves as parasites on sweet potatoes causing "soft rot" disease.

Vegetative body is the well developed mycelium composed of long, slender and much-branched hyphae, which ramify over the surface of the substratum. Hyphae are coenocytic (aseptate), but septa formation often takes place at the base of reproductive organs and occasionally when mycelium ages. The mycelium of *Rhizopus* very soon enters the reproductive phase and becomes differentiated into rhizoids, stolon and sporangiophores.

Stolons are the sub-aerial hyphae that grow horizontally for a distance above the substratum. The portion bending down functions as a node and forms a tuft of rhizoids.

Rhizoids arise in tufts from each node of the stolon towards the lower side. They penetrate the substratum for the absorption of water and other nutrients. Rhizoids also anchor the fungus securely.

Sporangiophores are erect, aerial and unbranched hyphae which grow upwardly (i.e. negatively geotrophic) in *clusters* at the point where the stolon forms *rhizoids. They* are asexual reproductive structures. Each sporangiophore bears terminally a sporangium.

Hyphal cell wall is composed of microfibrils of chitin, chitosan may be present abundantly; other polysaccharides *e.g.* glucosamine and galactose, proteins, lipids, etc. are also present. Hyphal cell within possesses granular streaming cytoplasm, vacuoles and mitochondria of varying sizes, numerous nuclei, endoplasmic reticulum, ribosomes, droplets of oils and glycogen as reserve food.

Reproduction takes place ***asexually*** and ***sexually***.

1. Asexual Reproduction takes place by non-motile unicellular sporangiospores formed within the black coloured globose sporangia (Figs. 3.29, B & 3.31). At the time of reproduction, one or more aerial hyphae are produced in cluster directly above the "rhizoids"— these are called *sporangiophores*. The tip of each sporangiophore swells up to form a globose structure called *sporangium*. During the development of sporangium, the cytoplasm carrying many nuclei flow into the young sporangium and accumulates in its periphery. The central portion of the sporangium becomes highly vacuolated and i.e. ultimately surrounded by a wall which separates it (vacuolated zone) from the peripheral zone. This central portion is the *columella*. The protoplasm of the peripheral zone becomes divided into large number of multinucleate segments. These segments secrete wall around each of them and metamorphoses into unicellular multinucleate globose or oval non-motile sporangiospores.

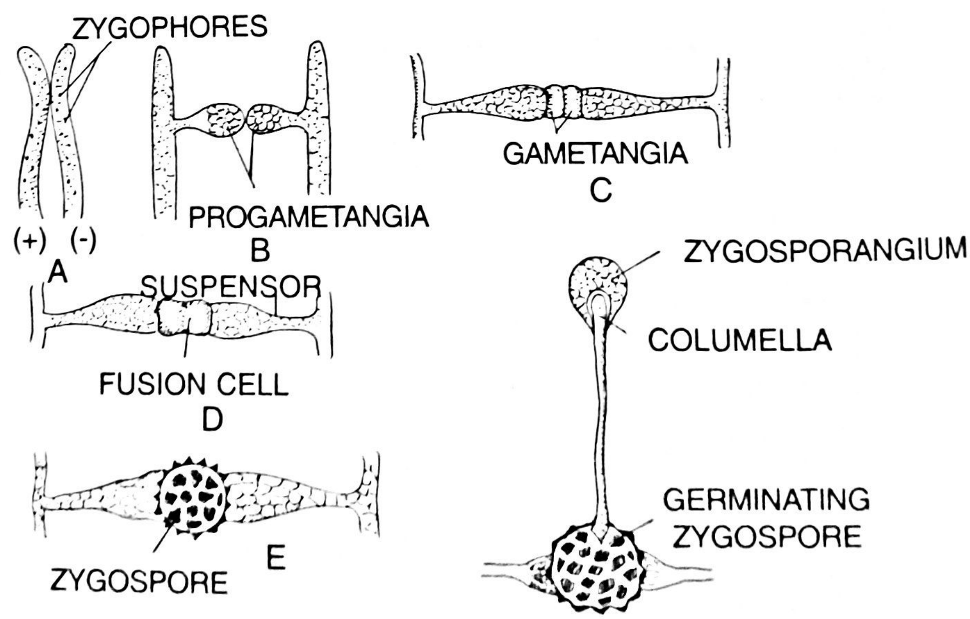

Fig. 3.31 *Rhizopus stolonifer*– Stages of sexual reproduction in the heterothallic species.

In *R. stolonifer* the columella is large (Fig. 3.30, F); as the sporangium dries, the columella collapses so that it looks like an inverted pudding bowl balanced on the end of a stiff sporangiophore (Fig.3.30, G). Associated with these changes in the shape of the columella, the sporangium wall breaks up into many fragments liberating the dry spores which eventually escape in wind currents. Under favourable conditions, each spore germinates by germ tube which develops into a fluffy, much-branched, white aerial mycelium.

Chlamydospore formation takes place rarely in old mycelia of *Rhizopus stolonifer* during unfavourable conditions. During chlamydospore formation the mature hypha become transversely septate. Some of this septate hyphae i.e. intercalary cells become thick walled and contains sufficient reserve food and represent chlamydospores. On return to favourable conditions, each chlamydospore germinates by producing new hypha.

2. **Sexual Reproduction** takes place by gametangial copulation method. *Rhizopus stolonifer is* heterothallic, hence the presence of two physiologically distinct and compatible mycelia (+) and (–) is required for sexual reproduction. When the two hyphae of opposite strains come in contact with one another, copulating branches, called *progametangia,* are formed (Fig.3.31). Many nuclei and much cytoplasm flow to the contacting tip of *progametangia,* next progametangia begin to enlarge. A septum then forms near the tip of each progametangium, separtating it into two cells— the terminal cell forms the *gametangium* and the basal cell forms the *suspensor cell.* Each gametangium contains dense and multinucleate protoplast, such protoplast constitute an isogamete which is also known as *coenogamete.* In the meantime the walls of the two contacting gametangia dissolve at the point of contact and the protoplast of the two coenogametes unite to form a new cell. This new cell enlarges considerably, its wall thickens, and its surface becomes black and warty— this thick-walled warty structure is the *zygospore.*

Life Cycle of Rhizopus sp.

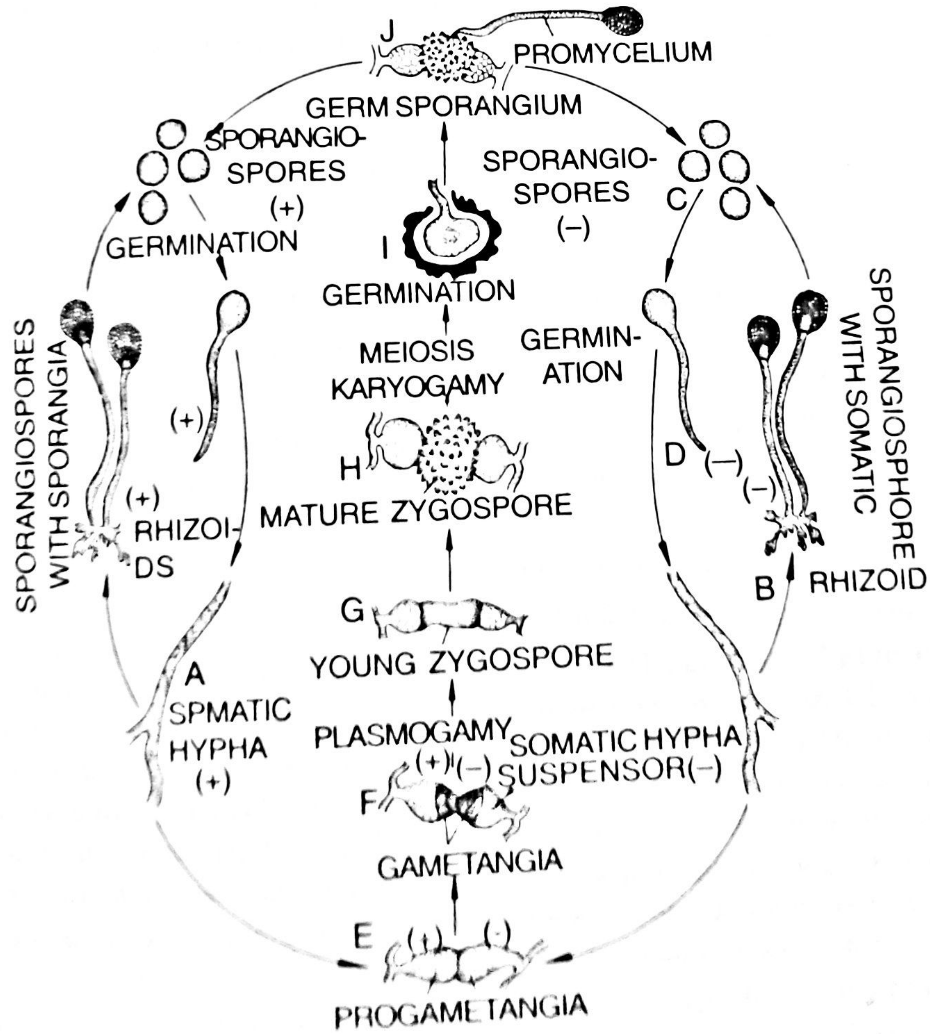

Fig. 3.32 Life cycle of *Rhizopus stolonifer.*

Germination of zygospore— After a considerable period of rest, the zygospore germinates. In zygospore many nuclei fuse in pairs, but some remain unfused. The unfused nuclei degenerate and diploid nuclei undergo meiosis after the rest period, just before germination (Cutter, 1942). Half the haploid nuclei resulting from meiosis are of the (+) and half of the (–) mating type. At the time of zygospore germination, the outer wall (*exosporium*) cracks open and the inner delicate wall (*endosporium*) protrudes in the form of a tube called *promycelium:* the promycelium bears at its tip a simple spherical *germ sporangium*. Within germ sporangium two types of nuclei (+ and –) along with much cytoplasm flow, which later on constitute sporangiospores in the usual manner. According to Cutter (1942), germ sporangium which contains both spore-types receive both (+) and (–) nuclei. But Gauger (1961) from his experiments think that the germ sporangium contains either all (+) or all (–) spores, or a mixture of both. Each of the spores after liberation germinates into a fresh germ tube which ramifies and produces new mycelium.

Parthenogenesis—Gametangia often fail to copulate and as such a single gametangium may develop directly into a thick-walled structure called *parthenospore* or *azygospore*. Parthenospore resembles the typical zygospore in external structure and germinates into new mycelium in due course.

Economic importance of *Rhizopus*— *R. stolonifer* is used commercially for the manufacture of fumaric acid and for some steps in the manufacture of cortisone. Considerable quantities of alcohol is obtained from *R. oryzae*. Various species of *Rhizopus* (*R. sinensis, R. nodosus,* etc.) are capable of forming large quantities of lactic acid. In Indonesia a delicious food known as *'tempeh'* is prepared from soyabeans with the help of some strains of *Rhizopus.*

R. stolonifer can cause a rot of sweet potato or fruit of apple, strawberry and tomato (Webster 1980). This fungus is also responsible for 'leak' disease of strawberries in storage. *Rhizopus* species cause fungal diseases 'mucormycosis' of domestic animals and man.

Sailent features of *Rhizopus* :

1. Saprophytic in nature—occurs in soil, on fruit and other foods, all types of decaying materials and as a laboratory contaminant.

2. Vegetative body is the much branched coenocytic mycelia.

3. The characteristic features are the presence of rhizoids at the base of sporangiophores and the stoloniferous habit.

4. Sporangiophores grow in clusters; they are stout and stiff.

5. Most species are heterothallic.

6. The term sporangium contains either one type of meiospores (+ or –) or two types of meiospores (+ and –).

7. Meiosis occurs at the time of zygospore germination.

Common Indian species :— *Rhizopus stolonifer, R. oryzae, R. sinensis,* etc.

● **3.3 Heterothallism in Mucorales :** The species or individuals in which the sexes are seggregated in separate thalli and in which two different thalli are required for sexual reproduction are called *heterothallic,* and the condition exemplified by heterothallic species is known as *heterothallism.* Sometimes self sterile *i.e.,* self incompatible species are also referred to as heterothallic by some workers; for sexual reproduction, these self-sterile species therefore requires the union of two compatible thalli regardless of the possible presence of both male and female sex organs on the same species. But species in which sexual reproduction takes place in a single thallus i.e., self compatible species are called *homothallic* and the condition exemplified by homothallic species is known as *homothallism.*

In some members of Mucorales, the formation of zygospores as a result of conjugation occurs frequently in nature while in other members zygospore formation does not take place frequently. However, Ehrenberg (1820) and De Bary (1864) first observed zygospore formation in *Sporodinia grandis,* a member of the order Mucorales. These earlier workers were unable to explain the actual cause of the formation of zygospores and hence the problem remained unsolved for long time. However the phenomenon of sexual incompatibility i.e.

4.2 Comparison between Phycomycetes and Ascomycetes

PHYCOMYCETES	ASCOMYCETES
1. Members of Phycomycetes are mostly aquatic, semi-aquatic in habitat some are terrestrial.	1. Aquatic species are few; majority of Ascomycetes are saprophytes or parasites.
2. Normally the mycelium is aseptate and coenocytic.	2. The thallus structure may be unicellular (yeasts), otherwise the mycelium is always septate.
3. Somatic hyphae are not organised into fungal tissue.	3. The hyphae show a tendency to aggregate into fungal tissues.
4. Asexual reproduction takes place by the formation of motile zoospores formed in zoosporangium.	4. Asexual reproduction takes place by exogenously produced conidia. Zoospores are not formed as all.
5. Sexual reproductive structures become progressively complicated from lower to higher Phycomycetes.	5. Progressive simplification and disappearance of sexual reproductive structures from lower to higher forms.
6. Plasmogamy is immediately followed by karyogamy.	6. Plasmogamy is not immediately followed by karyogamy in most members.
7. Diplophase is represented by zygospore which is usually long lived, i.e. require long resting period before germination.	7. Diplophase is represented by ascus, this phase is not long lived— the diploid nucleus divides very soon to form haploid nuclei.
8. Well developed fruit bodies are not formed.	8. Well organised fruit bodies i.e ascocarps are formed in almost all members.

4.3 Structure and life history of some typical Ascomycetes :

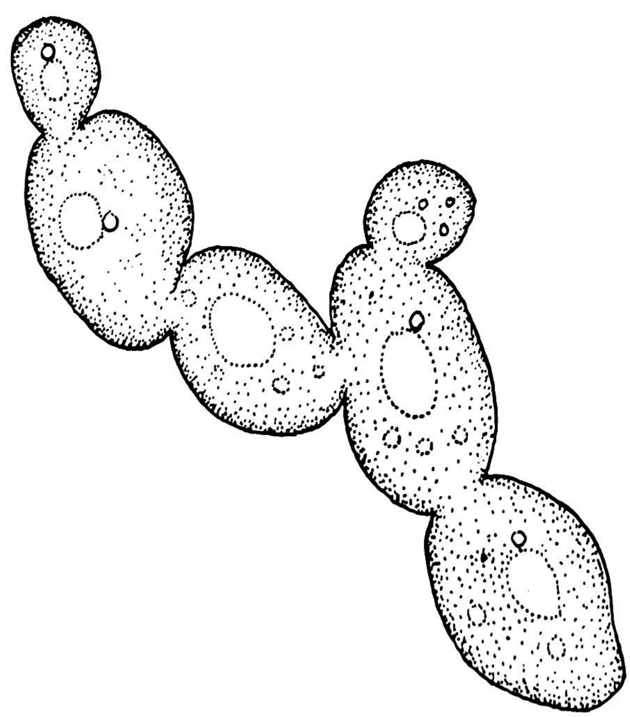

Fig.4.17 Chain of Yeast cells (pseudomycelium) formed by budding (after Alexopoulos, 1962)

A. *SACCHAROMYCES* :

Species of *Saccharomyces* are commonly known as the yeasts in English. The genus *Saccharomyces* belongs to the sub-family Saccharomycetoideae, family Saccharomycetaceae, order Endomycetales, class Hemiascomycetes, sub-division Ascomycotina and the division Mycota.

The term 'yeasts' actually refers to Ascomycetes which are predominantly unicellular, which reproduce vegetatively by budding, fission or both, which produce ascospores in a naked ascus developing from a zygote or parthenogenetically from a single vegetative cell and which when placed in sugar solution carry out alcoholic fermentation producing alcohol and carbon dioxide.

Species of *Saccharomyces* are cosmopolitan i.e. found every where over the surface of the earth. They are abundantly found to occur saprophytically in substrata which contain sugars such as sugar solution, on the surface of ripe fruits, in the nectar of flowers, etc. They are also found in soil, in milk, in animal excreta and on decaying vegetables. Some are found to occur as parasites in plants and animals including man (Gwynne-Vaughan and Barnes, 1927).

The genus *Saccharomyces* contains about 40 species, of which the best known is *Saccharomyces cerevisiae*.

Vegetative structure— Vegetative body of *Saccharomyces* is very simple, and consists of single cell. Vegetative cells sometimes adhere in chains forming pseudomycelium (Fig. 4.17).

Cells are round, spherical, oval or elliptical; the size of cells usually ranges between 6-8μ x 5-6μ (Webster 1980). The cell wall surrounds the protoplast, cell wall is thin and delicate, firm and chitinous. The cell wall along with chitin, contains glycogen and mannan (polysaccharides), lipids, phosphates, and proteins. According to Agar and Douglas (1957) the cell wall consists of two layers, of which outer electron dense layer is about 0.5μ thick and inner microfibril containing layer is less electron dense and is about 0.2 μ thick. According to Matile *et al* (1969) the cell wall of *Saccharomyces cerevisiae* is thick and composed of three layers— the outer layer consists of mannan protein and some chitin, middle layer of glucan and the inner composed of protein glucan.

Individual cells are hyaline but they appear coloured (creamish or brownish) in colonies on artificial media.

The cell wall possess circular raised bud-scars at some points where buds have arisen. Beneath the cell wall, there lies the cytoplasmic membrane, i.e. plasmalemma, it is the limiting membrane of the cytoplasm. At certain points the cytoplasmic membrane is invaginated. Inner to the cytoplasmic membrane i.e. in the cytoplasm, all cell inclusions i.e. Golgi apparatus, ribosomes, endoplasmic reticulum, mitochondria,

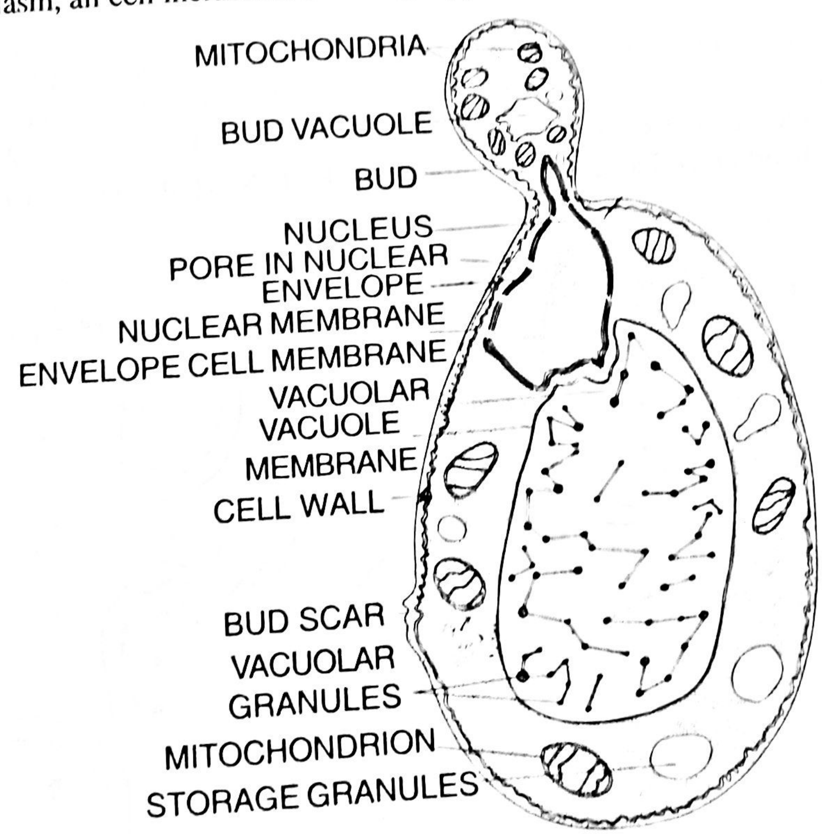

Fig.4.18 Diagram of a section of a budding yeast (*Saccharomyces cerevisiae*) as seen under electron microscope (*after Webster,* 1980).

lipid granules in the form of sphaerosomes and nucleus are present. A large, well developed, centrally located vacuole is present in mature cells of *Saccharomyces cerevisiae*. The vacuole is surrounded by single vacuolar membrane called *tonoplast*. Vacuole is filled up with water, lipid granules and granules of polymetaphosphate (probably volutin). Electron microscopic studies (Agar and Douglas 1957, Moens and Rapport, 1971; Hartwell, 1974; Webster, 1980) suggest that nucleus is distinct, it is surrounded by a nuclear envelop composed of double unit membrane perforated by pores. The young nucleus is provided with a cup-shaped nucleolus and dome-shaped nucleoplasm at the time of budding.

Reproduction— *Saccharomyces* reproduces *vegetatively* by budding only and therefore this genus is commonly called "budding yeast".

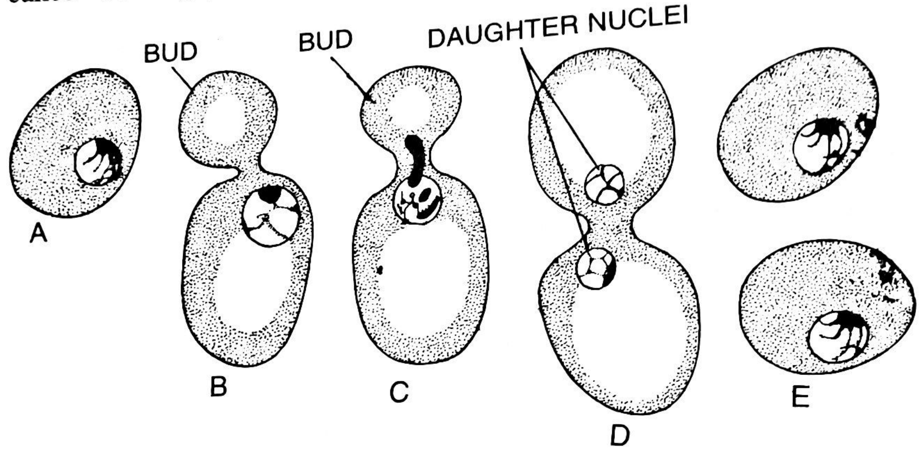

Fig. 4.19 *Saccharomyces cerevisiae*. A-E—Different stages in budding and the formation of daughter cells.

1. VEGETATIVE REPRODUCTION TAKES PLACE BY : ***Budding***— At the time of budding a small outgrowth or protuberance is formed at one end of the mother cell. According to Webster (1980), the nucleus appears to divide by constriction and the nuclear envelop *i.e.* membrane does not break down. A portion of the constricted nucleus along with other cell organelles enters the bud. Next the cytoplasmic connection

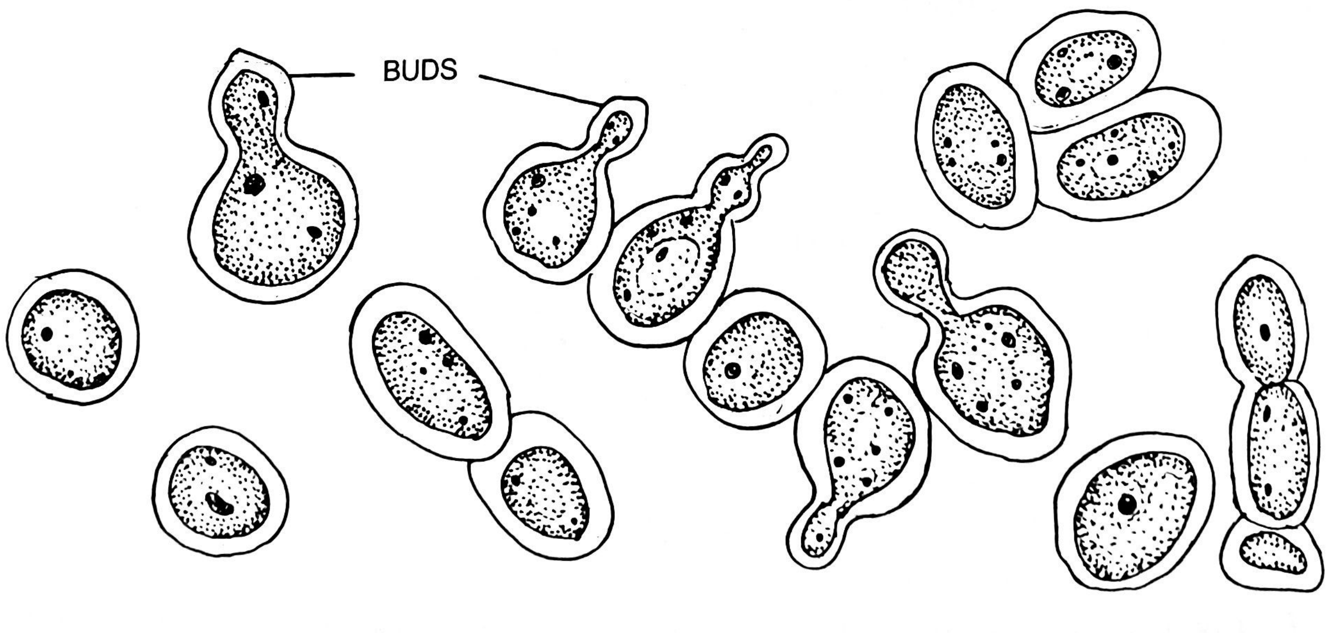

Fig. 4.20 *Saccharomyces cerevisiae* with buds as seen under high power of a light microscope.

between the parent cell and the bud is closed by laying down of wall material. The bud then assumes normal size which also undergoes budding in the same manner— as a result chain of yeast cells i.e. buds are produced within a short time and the cells of which remain attached with each other for some time forming *pseudomycelium* (Fig. 4.17), or the bud may separate from the parent cells (Fig. 4.19) leaving its point of attachment as a birth or bud scar. Sometimes chain of cells may be branched due to budding from several points on the parent cell (multipolar).

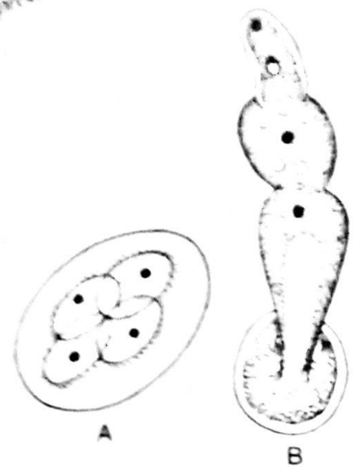

Fig. 4.21 *Saccharomyces* sp. A—Formation of ascospores. B—An ascospore germinating into new daughter cells by budding.

2. **Asexual Reproduction** — Asexual reproduction in *Saccharomyces cerevisiae* can be induced to form asci and ascospores by growing cells on a nutrient rich medium. The protoplast of the cell then rounds itself to form usually 4 (or fewer) *endospores*, known as ascospores. *Saccharomyces cerevisiae* is a heterothallic species. Out of 4 haploid ascospores two are of one mating (A) and the rest two are other mating type (a). The yeast cell then itself turns into an ascus. These spores are finally liberated by the breaking up of ascus wall. These haploid cells of different mating types usually multiply by budding. The haploid buds thus formed has independent life and keep on producing new haploid cells for several generations (Fig.4.20).

3. **Sexual reproduction**— It occurs under unfavourable conditions. Two somatic cells of opposite mating type behave as gametangia and undergo sexual fusion (Fig. 4.22) — as a result plasmogamy and karyogamy takes place and a diploid *zygote* (2n) is formed (Fig.4.22). Zygotic cells are ellipsoidal and larger then the haploid cells.The zygote also undergoes budding for several generation. These diploid cells also had independent life like haploid vegetative cell.

During unfavourable condition (at low temperature, shortage of food and water, etc.) the diploid cells function as asci. The diploid nucleus undergoes meiosis and four haploid daughter nuclei are formed. It

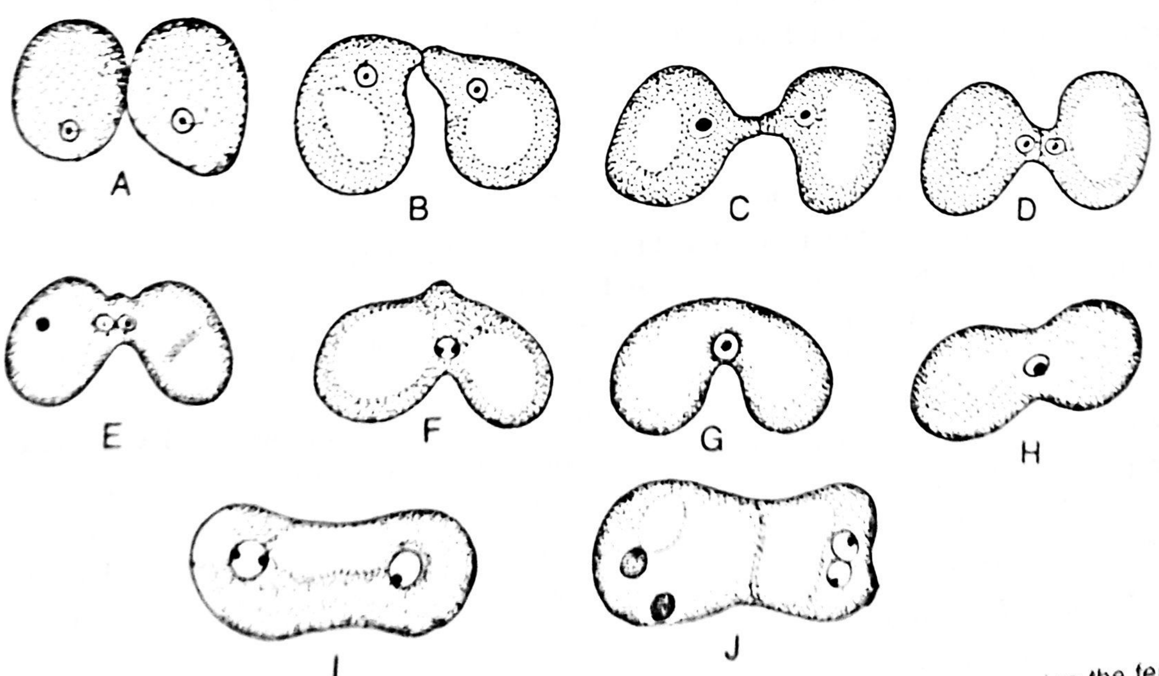

Fig 4.22 Different stages (A-J) of sexual reproduction in *Saccharomyces* sp. showing the fermation of ascus with 4 ascospores (J).

has been observed that the nuclear membrane remain intact during meiosis and the four haploid nuclei remain within the original membrane for sometime. This type of nuclear division is named *uninuclear* (Moens and Rapport (1971). Of these four haploid nuclei two are of one mating type (a) and rest two are of other mating type (A). All these haploid nuclei after accumulating cytoplasm around them change into ascospores and the cell containing ascospores is called ascus. These ascospores (n) then rupture the ascus wall, come out and behave as fresh haploid cells of *S. cerevisiae*.

Life cycle : In *Saccharomyces cerevisiae* two haploid vegetative cells of two opposite strains (+ and –) unite with each other, as a result a diploid zygote cell (2*n*) is produced which again by budding produces many diploid somatic cells; each diploid cell in turn behaves like an ascus and produces four haploid (*n*) ascospores (2+, and 2–) through meiosis, which are finally liberated and they start budding producing thereby many haploid vegetative (*n*) or somatic cells of two different strains (+ and –).

In this type there is distinct life cycle where two phases viz. haplophase and diplophase are equally well represented and alternate in regular succession (Fig. 4.23). This type of life cycle is called *haplodiplobiontic* life cycle.

In this life cycle of heterothallic strain of *S.cerevisiae* indicates the existance of independent haploid and diploid phases of equal importance.

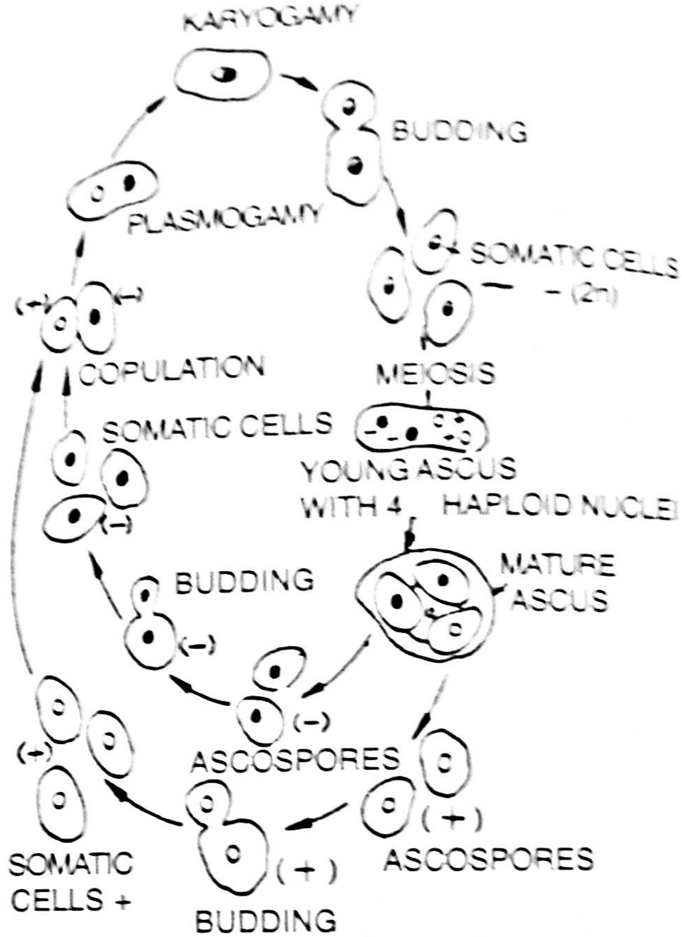

Fig. 4.23 Diagrammatic life cycle of *Saccharomyces cerevisiae*. [Redrawn from Alexopoulos (1962) [after Guillermond, 1905].

Economic importance— Different strains of *Saccharomyces* (yeasts) are among the economically most important fungi. For the fermentation products like alcohol and CO_2. *Saccharomyces cerevisiae, S.cerevisiae* var. *ellipsoideus*, etc. are used by brewers and bakers in making wines and bread respectively. In the brewery, the alcohol is the industrial product. In the bakery, the CO_2 is the important product and the alcohol is the waste. The carbon dioxide from yeasts makes the dough to rise in the baking ovens and gives the bread its spongy nature. Yeasts used in breweries are of two types viz. '*top yeasts*' and '*bottom yeasts*'. Former produces alcohol at the top of the sugar solution to be fermented, while latter remain in the lower portion where oxygen supply is poor. Top yeasts have greater fermentive power. Yeasts are sold in the market as dried yeast cakes or cakes of commerce, generally used in the laboratory and alcohol industry. One species has been found to synthesize proteins from molasses and ammonia— this activity of yeasts is very important in the production of protein foods. As yeasts are great sources of vitamins (vitamins B and C) and proteins, they are as good as valuable food. Yeasts are also employed to impart flavour to cocao beans.

Salient features of *Saccharomyces* :

(1) Thallus i.e. vegetative structures is very simple, being represented by a single cell. Cells may be haploid (*n*) or diploid (2*n*). The chromosome number of the diploid cell is 8.

(2) The cell wall is distinct and composed mainly of pectin, lipids and polysaccharides; chitin may be present.

(3) True nucleus with definite membrane is present; the nucleus is distinct from the vacuole.

(4) Various other cell inclusions such as fats, glycogen (as reserve food), endoplasmic reticulum, mitochondria, etc. are present within cytoplasm.