FUNGAL BIOTECHNOLOGY: HISTORY TO CURRENT PERCEPTION

Abstract

In our biosphere, fungi are a common eukaryotic organism that is inextricably linked to other forms of life. Even though fungi are primitive eukaryotic creatures, they conduct biological functions that are closely linked to those of higher eukaryotic organisms. Both traditional and contemporary biotechnological procedures have utilised fungi. Since the beginning of time, they have been used in various ways to gain commercial value. The metabolic engineering of numerous significant yeasts and the production of a wide variety of novel chemical entities with various applications have both been made possible by technological breakthroughs. Synthetic biology and the use of microbes will aid in the construction of effective systems that unravel the complexities of industrial and medicinal biotechnology. In today's global economy, fungal biology plays a significant role. Microbial enzyme markets have seen a significant expansion because to recombinant DNA technology, which uses yeast and other fungi as hosts. The synthesis of enzymes, vitamins, polysaccharides, polyhydric alcohols, pigments, lipids, and glycolipids are only a few of the industrial processes that involve fungi. While some of the items are created for sale, others have potential biotechnological value. In the course of biotransformation, fungi are incredibly helpful. They can be utilised in novel, less-polluting production techniques compared to conventional chemical ones. A significant technological advancement that may soon have a significant effect on numerous industrial sectors is fungus biotechnology.

Keywords: Fungi, Biotransformation, RDT, Enzymes.

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I. HISTORY OF FUNGI

The history of fungi is intertwined with the evolution and development of life on Earth. Fungi are a diverse group of organisms that occupy a unique kingdom in the classification of living organisms. They play crucial roles in ecosystems, as decomposers, mutualists, and pathogens. Fungi are believed to have originated around 1.5 billion years ago during the Proterozoic Eon. The early fungi were likely simple, unicellular organisms resembling modern-day yeasts. They coexisted with early bacteria and algae, forming the foundations of life on Earth. During the Precambrian Period, fungi continued to evolve and diversify. Early fungi began forming symbiotic relationships with photosynthetic organisms, such as algae and cyanobacteria, giving rise to lichens. These mutualistic partnerships allowed fungi to access nutrients and provide shelter to their partners. By the late Proterozoic Eon, some fungal species started to develop multicellular structures. This marked a significant evolutionary milestone as it enabled fungi to form more complex and diverse structures, such as mycelium and fruiting bodies. Fungi were among the earliest organisms to colonize land during the Cambrian Period. Their ability to decompose organic matter was crucial in facilitating the breakdown of plant material and the formation of soil, making terrestrial ecosystems more habitable for other organisms. Like many other organisms, fungi were affected by mass extinction events, such as the Permian-Triassic Extinction around 252 million years ago. However, they survived and continued to play crucial roles in ecosystems. Mycorrhizal fungi, which form mutualistic associations with the roots of most land plants, underwent significant diversification during the Mesozoic Era. This symbiotic relationship was essential for the successful colonization of land by plants. Over time, fungi have continued to evolve and adapt to various environmental conditions. They have diversified into an estimated 2.2 to 3.8 million species, although only a fraction of these have been formally described.

The year 1859 served as a catalyst for biology. It was undoubtedly the year that Charles Robert Darwin's The Origin of Species was published, and it signalled the start of the acceptance of Darwin's theory that evolution had indeed taken place: that animals alive today evolved by progressive amendment from previous forms. Throughout the course of evolution, this sparked a tremendous wave of interest. Studying the evolution of animals is rather straightforward because a tonne of information about the morphology of the organisms has already been gathered, and most importantly, animal-specific features like bone and teeth have already begun to ossify. Studies on ossified fungi are rather uncommon for a number of reasons, one of which is the true absence of conclusive fossil evidence. Fungi, however, do not just have an easy morphology that makes it a poor predictor of any historical relationships. This hasn't stopped some mycologists from making phyletic assumptions about the beginnings and subsequent evolution of fungus, though. However, things have significantly improved since the recent introduction of molecular methods of connection assessment, including comparative analysis of molecular sequences (nucleic acid and proteins).

A significant and varied subgroup of the plant world are the fungi. They are categorised as part of the vast phylum Thallophyta because they resemble algae in many ways. In the world, there are 50,000–100,000 different kinds of fungi. There are 4,300 genera and 50,000 species of fungi, according to one estimate (Ainsworth, 1961), although this

number is continually rising due to the worldwide, never-ending hunt for these organisms. The fungi are regarded as their own kingdom in the five-reign method of classification.

Their mechanism of sustenance is either saprophytic, parasitic, or symbiotic because they lack chlorophyll and other photosynthetic pigments and are unable to synthesise their food from carbon dioxide and water in the presence of sunshine. When fungus behave as parasites, they attack living protoplasm and spread disease to plants, animals, and people. When fungi live as saprobes, they cause the degradation of organic things.

The majority of fungus have a relatively basic body made up of a network of branched filaments called hyphae. The mycelium is the tangled mass of hyphae. Mycelium is occasionally completely absent, as in the instance of synchytrium, while other times the plant body may be unicellular.

Regarding the history of fungus, there are two key points of view. According to a first glance, fungi evolved from different types of algae by achieving a saprophytic or parasitic style of existence. Numerous parasitic, colourless algae are what give fungi their appearance. According to the initial impression, the entire surface of the world was covered in water in the beginning, and when algae first emerged, they moved from the water to the land. Many of them died and lost their green coloration during this transmigration. There was a lot of dead organic matter accessible, and many algae evolved into the saprophytic fungi we see today. It is also hypothesised that the saprophytic fungi that eventually spread to other live plants gave rise to the parasitic fungi.

Fungi are eukaryotic, typically branch filamentous, branching, tiny, typically microscopic, creatures that produce spores but lack chlorophyll. Chitin and glucans, but not cellulose, make up the skeletal structure of fungi's cell walls. These are contained within a polysaccharide and glycoprotein matrix. Up until roughly 1990, the Oomycota, also known as oomycetes, a group of fungal-like creatures, were thought to be real fungi. The great majority of oomycetes lack chitin and instead have cell walls made of glucans and trace quantities of cellulose, with a few exceptions that do contain chitin. Despite the fact that the Oomycota now belong to the kingdom Chromista instead of the fungi, they are nevertheless referred to as fungi due to their many other similarities, at least in terms of how they infect plants and cause disease. The majority of the more than 100,000 species of known fungi are exclusively saprophytic, which means they survive on decaying organic matter and aid in its decomposition. roughly 50 species of fungi can cause disease in plants, most of which are superficial fungal infections, and roughly as many can cause disease in animals. Some types of fungi affect all plants, and each parasitic fungus can target a single plant type or a variety of them. Some fungi, referred to as obligatory parasites or biotrophs, can only develop and proliferate if they live their entire lives in close proximity to their host plants. Others, referred to as nonobligate parasites, can complete their life cycles on dead organic matter but still require a heated plant for a portion of their life cycles. They can also grow and reproduce on dead organic matter in addition to living plants. Depending on whether they are primarily parasitic or saprophytic, facultative saprophytes or facultative parasitic fungi might be nonobligate parasites.

Mycelium, a filamentous vegetative body, is present in the majority of fungus. The mycelium spreads in every direction. The individual mycelium branches, known as hyphae,

are normally homogeneous in thickness and range in diameter from 2 to 10 micrometres, though they can be up to 100 micrometres thick in some fungus. Some fungus have mycelium that is only a few micrometres long, while others have mycelium that is several metres long.

The mycelium of some fungus is made up of many cells with one or two nuclei each. In some, the mycelium is made up of several nuclei that may or may not be divided by septa (cross walls). The tips of the hyphae are where the mycelium grows.

Some lower fungi lack genuine mycelium and instead generate a network of strands with wildly different diameters, known as a rhizomycelium. Instead of mycelium, some microorganisms (myxomycota, plasmodiophoromycetes), which were once assumed to be early fungus but are now thought to be members of the Kingdome protozoa, create a naked, amoeboid, multinucleate body known as plasmodium.

The majority of plant pathogenic fungi dwell both in the soil or in plant detritus on the soil and on their host plants. Some fungi are only biotrophs, which means they live their whole lives on the host. Only the spores can fall to the ground, where they either perish or remain dormant until they are once more taken to a host where they can grow and reproduce. Others, like the apple scab fungus Venturia, are hemibiotrophs, which means that in order to complete their life cycle in nature, they must spend some of their lives as parasites on their hosts and some of their lives as saprophytes on the dead tissues of the same hosts on the ground. However, the latter category of fungi remains persistently connected to host tissues, whether they are alive or dead, and does not naturally grow on any other type of organic matter. A third category of fungi are facultative saprophytes, which are parasitic on their hosts but continue to live, grow, and reproduce on their dead tissues after they have died. They can also move from the host debris into the soil or other decaying plant matter, where they can only live, grow, and reproduce as saprophytes. They can colonise dead plant matter that has no connection to the host they can parasitize. These fungi can persist in the soil for long years without their hosts since they are typically soil pathogens with a wide host range. However, since prolonged and continuous growth of these fungi as saprophytes in the soil results in more or less rapid loss in their numbers, they may occasionally need to infect a host in order to increase their populations. Finally, some fungi are facultative parasites, meaning they may survive just fine as saprophytes in the soil or elsewhere but have the ability to parasitize and harm plants if they come into touch with a plant organ under the correct circumstances.

Fungi take on different positions in respect to the cells and tissues of the plant during the parasitic phase. Some fungus develop on the surface of plants but insert their feeding organs inside the plant's epidermal cells. Some, like the fungus responsible for apple scab Venturia, only grow between the cuticle and the epidermal cells. Others spread haustoria inside the cells as they grow in the intercellular gaps between the cells. Still others indiscriminately spread out among and through the cells. Fusarium and other fungi that cause vascular wilts develop inside the xylem vessels of diseased plants, while so-called endophytic fungi, which are mostly found in symptomless plants and develop intercellularly in the various plant organs, grow inside the cells of symptomless plants. Because they are unable to feed on dead cells, obligatory parasites can only develop in conjunction with living cells. However, the macerating enzymes or poisons of some nonobiligate parasites kill the plant cells before the mycelium even grows, preventing their mycelium from ever coming into

touch with living plant cells. To ensure their quick and effective spread, the reproductive bodies of the fungus are often generated at or very near the surface of the host tissues, regardless of the position of the mycelium within the host.

II. HISTORY OF FUNGAL BIOTECHNOLOGY

The history of fungal biotechnology dates back thousands of years when humans unknowingly began using fungi for various purposes. However, the intentional and systematic application of fungi in biotechnological processes started to gain momentum in the 20th century. Biotechnology is defined as "any technique that uses living organisms, or substances from these organisms, to form or modify a product, to reinforce plants or animals, or to develop microorganisms for specific uses" by the geographical point of Technology Assessment of the general assembly (see Balasabramaniam, 1996; Subramanian, 1992). If the term "biotechnology" is used in a broader sense, it is obvious that "flora biotechnology" is not Associate in Nursingexclusively equipment practise, but rather that its roots go back thousands of years, "ever since initial|the primary toast was projected over a shell filled with wine and additionally the initial loaf ofleavened bread was baked" (Alexopoulos, 1962). In reality, humans have used a variety of fungi for a wide range of purposes since ancient times, including as food, to make sour drinks, and to make sourdough bread. They have also been used in ceremonies and for medical purposes. Although several ethnic groups used various fungus in their own ways, such practises were not exclusive to any particular area of the planet. Even today, indigenous tribes and ethnic groups employ plants and fungi in their own unique ways. The science of "ethno mycology" is concerned with the use of fungi in rituals and cognitive content from ancient times to the present. The late Dr. R.G. Wasson of latest royalty, his wife V.P. Wasson and R. Heim have created important contributions to the science of ethnomycology (Wasson and Wasson, 1957; Heim et aI., 1967).

Fungi continue to be of significant interest in various biotechnological fields. They are used in the production of enzymes, pharmaceuticals, biofuels, and bioactive compounds. Mycotechnology, a branch of biotechnology focused on fungi, is gaining prominence. Fungal biotechnology is a rapidly evolving field with numerous applications in various industries. Ongoing research and technological advancements are likely to unveil new opportunities and novel uses of fungi in the future.

1. **Fungi as a food:** Fungi have been utilized as food by humans for thousands of years. They offer a variety of culinary delights, and many edible fungi have become important ingredients in traditional cuisines worldwide.

   Mushrooms are perhaps the most well-known and widely consumed edible fungi. There are numerous mushroom varieties with unique flavors and textures, such as button mushrooms, portobello mushrooms, shiitake mushrooms, oyster mushrooms, and more. Mushrooms are used in soups, salads, stir-fries, pasta dishes, and as toppings on pizzas.

   Truffles are a highly prized delicacy known for their distinctive and intense flavor. They grow underground in association with the roots of specific trees, and truffle hunting has become a specialized activity in certain regions. Truffles are used sparingly to add depth and aroma to various dishes.

As seen in the mediaeval painting of the church of Plaincorault in France (1291 a.C.) where Adam and Eve are seen eating from a "fungus-tree" as part of the well-known temptation scenario, fungi have long been utilised as food.

One of the oldest instances would be the production of milk products like cheese. When man transitioned from being a gatherer and hunter to being a wanderer and then a farmer, herds of domesticated oxen provided meat, milk, and other goods. One of the most nutrient-dense but also perishable natural products is milk. Through the action of microbes, milk can either be destroyed or transformed into a stable, nutrient-dense diet like cheese. One of the earliest records of milk use is a nearly 4,000-year-old relief found in the Sumerian city of Ur, which details the entire process from getting milk to making butter and/or cream (Kurtzman, 1983).

Another historical example is the making of bread from ground cereals, in which yeasts are responsible for leavening the dough by producing CO2 when the appropriate conditions (water, temperature) are met (Legras et al., 2007). Early evidence of bread-making can be found in an Egyptian geological relief from about 4500 years ago. Beer and wine, both alcoholic beverages that are produced in large quantities now, have historically played a significant role as both necessary foods and social beverages. Here, yeasts (fungi) transform the sugar in grapes and cereals into alcohol and dioxide (Legras et al., 2007).

The earliest examples would be the fact that edible fungus for human consumption are currently grown in highly automated, computerised, and even mechanised businesses all over the world (Hall et al., 2003). Due to expanding demand, numerous efficient methods, and falling costs, this output is always increasing. Low cost substrates, including waste products like straw, no requirement to use tillable land, excellent flavour, high nutrient and mineral content, and in some cases even beneficial health effects, such as in the case of golden oak mushrooms, are all advantages of growing edible mushrooms (Hall et al., 2003). Currently, more than four million tonnes of cultivated edible mushrooms are produced annually throughout the world, with the cultivar Agaricus bisporus taking the lead, followed by the agaric, agaric, Shii-take, and golden oak mushrooms (Morin et al., 2012).

2. **Fungi Use as a Metabolic Products:** Fungi are valuable sources of various metabolic products with significant applications in different industries. These metabolic products are produced by fungi as part of their biochemical processes and can be isolated and utilized for various purposes. Some of the key metabolic products obtained from fungi i.e., enzyme, pharmaceuticals, secondary metabolite, bioremediation agent and bioactive compound for agriculture.

Fungi are prolific producers of enzymes with diverse catalytic activities. Fungal enzymes, such as amylases, cellulases, proteases, lipases, and ligninases, find applications in various industries, including food processing, brewing, detergent manufacturing, textile processing, and biofuel production. These enzymes help break down complex substrates, facilitating industrial processes and reducing waste.

Fungi are rich sources of bioactive compounds with pharmaceutical potential. Penicillin, one of the most well-known fungal products, is an antibiotic used to treat bacterial infections. Other fungal-derived compounds, such as statins (used to lower cholesterol) and immunosuppressants (e.g., cyclosporin), have significant medical applications.

Fungi produce a wide array of secondary metabolites, such as alkaloids, terpenoids, polyketides, and peptides. These compounds often exhibit interesting biological activities, including antimicrobial, anticancer, antiviral, and immunomodulatory properties. Fungal secondary metabolites have the potential for use in drug discovery and development.

Some fungi are capable of degrading and detoxifying various pollutants, including organic contaminants, heavy metals, and petroleum products. Fungal metabolic products, such as enzymes and organic acids, are involved in the bioremediation of contaminated environments, contributing to environmental cleanup efforts.

Fungi can produce compounds with biocontrol and plant growth-promoting properties. For example, certain fungal metabolites and secondary metabolites can act as natural biopesticides to control plant pathogens or pests, reducing the need for chemical pesticides.

Overall, the metabolic products derived from fungi have diverse applications in medicine, agriculture, industry, and environmental management. The study of fungal metabolism and the discovery of novel metabolic products continue to be areas of active research with significant potential for biotechnological advancements.

One of the most frequent instances is when fungi are employed to produce particular metabolites that serve a variety of purposes. Alcohol is still a crucial agricultural crop. It has changed, however, in that it is increasingly used as a component of pharmaceuticals and for artificial and prepared business processes in addition to being used in beverages. However, alcohol produced from prokaryotes is becoming more common recently (Homann et al., 2005).

Since the introduction of the anti-baby pill and in conjunction with other medicinal uses, steroids have become essential (Cresnar et al., 2009). Due to their capacity to undergo extremely precise one- or two-step changes in steroids, fungi such as several Aspergillus strains were crucial in the manufacturing of such drugs (Calam et al., 1939).

Everyone is aware of the significance of such metabolites in medicines because Fleming found the assembly of the primary antibiotic, Penicillin, from the imperfect flora fungus genus notatum in 1929 (Fleming, 1929). Only about four hundred of the over 6000 antibiotics that are currently known, at least by their formula, are employed in human medications. A small fraction of them are produced by fungi. Since infectious microbes continue to develop resistance to the widely used antibiotics, it is imperative that novel antibiotics are continually sought after and discovered (Brian et al., 1951).

Numerous flora enzymes have been produced commercially and have become essential instruments for research and business. They have a wide range of uses, from food processing to medical diagnosis to organic chemical analysis.

Amino acids, organic acids, and nucleic acids With an annual production of approximately 600.000 tonnes, acid is the most productive organic acid of flora origin. It is mostly used in the food and beverage industry. However, commercially produced amino acids and nucleic acids are also derived from fungus and are used widely (Taylor et al., 1979).

3. **Current status of fungi:** In the contemporary age, groups of fungi have been identified based on their shared molecular ancestry, with additional traits serving as supporting evidence. Classifications in the past were solely based on morphological and physiological traits, which failed to accurately reflect the history of biological process (Naumova et al., 2005). The simplicity with which DNA from eukaryotes can be cloned in prokaryotes has made it possible to study and analyse eukaryotic DNA sequences. Large quantities of these kinds of cloned sequences are simple to get, and they can be altered in vivo using bacterial genetic techniques, in vitro using bacterial genetic techniques, and in vitro by changing certain enzymes (Ropars et al., 2014). To determine the effects of these experimentally produced alterations on the function and expression of eukaryotic genes, the rearranged sequences must be removed from the bacteria in which they were cloned and reintroduced into a eukaryotic cell (Cheeseman et al., 2014). Despite the fact that prokaryotes lack many eukaryotic cell activities, including the localisation of ATP-generating systems to mitochondria, interaction of DNA with histones, mitosis and meiosis, and mandatory cell differentiation, many of these functions are shared by eukaryotic cells. It is necessary to examine the genetic regulation of these processes in a eukaryotic setting.

   In a perfect world, these eukaryotic genes would be returned to the original creature. The possibility of cloning these genes in Saccharomyces cerevisiae and other fungi will be discussed in this section (Liti et al., 2006). It should go without saying that yeast cells are more easier to grow and alter than cells from plants and animals. Additionally, their regulation and cellular biochemistry closely resemble those of higher eukaryotes.

III. INTRODUCTION OF DNA IN TO FUNGI

Similar to Escherichia coli, it takes artificial ways to introduce foreign DNA into fungi because they are not naturally capable of transformation. Spheroplasts, or cells without a wall, are one technique that was first created for the yeast S. cerevisiae. This technique involves the enzymatic removal of the cell wall, followed by the fusion of the resultant spheroplasts with ethylene glycol in the presence of DNA and CaCl2 (Dandan et al., 2017). Then, in a stabilising solution containing 3% agar, the spheroplasts are allowed to produce new cell walls. The subsequent retrieval of cells is cumbersome as a result of the latter phase. Spheroplasts can be used instead of electroporation because it is simpler and more practical (Dandan et al., 2017). On the surface of solid medium, electroporated cells can be chosen, making further manipulation easier. Numerous yeasts and filamentous fungi have been electroporated and subjected to the spheroplast method.DNA can also be inserted into

filamentous fungus by conjugation. Enterobacterial plasmids like R751 (INcPb) and F (IncF), according to Sikorski et al. (1989), can help with plasmid transfer from E. coli to S. cerevisiae and Schizo saccharomyces pombe.

IV. PLASMID VECTORS FOR FUNGI

Plasmid vectors that can replicate in the fungal host are needed if the heterologous DNA delivered into fungus is to be maintained in an extrachromosomal condition. Yeast episomal plasmids, yeast replicating plasmids, yeast cntromere plasmids, and yeast artificial chromosomes are the four different types of plasmid vectors that have been created (Stephen et al., 1985; Adrio, 2003). They all share traits with one another. They all have distinct target sites for a variety of restriction enconucleases, to start with. Second, they are all capable of replicating in E. coli, frequently in large numbers. This is crucial because, in many investigations, the vector DNA must be amplified in E. coli before being transformed into the final recipient yeast (Broach, 1982). Last but not least, they all use markers that can be easily chosen in yeast and that frequently complement the corresponding mutations in E. coli. These four markers are the most frequently used: His3, Leu2, Trp1, and Ura3.

It is possible to find non-reverting mutants for recessive mutations in the cognate chromosomal markers. Ura3 and Lys2, two yeast selectable markers, have the benefit of allowing for both positive and negative selection. Positive selection favours auxotrophy's complementarity. Negative selection looks for the capacity to flourish in an environment where cells expressing the wild tye function are prevented from growing. It is 5-fluoro-orotic acid in the case of Ura3, and -aminoadipate in the case of Lys2. These inhibitors make it possible to quickly identify those uncommon cells that have experienced a recombination or loss event and isolate the plasmid DNA sequences from those cells. Due to its size and the presence of several common restriction site locations within its coding sequence, the Lys2 gene is not widely used.

1. **Yeast Episomal Plasmids:** Yeast episomal plasmids are small, circular DNA molecules that can exist independently in the cytoplasm of yeast cells. These plasmids replicate autonomously within the yeast cell, separate from the chromosomal DNA, and can be passed on to daughter cells during cell division. The ability to maintain and replicate independently makes them useful tools for genetic engineering and molecular biology studies in yeast. Beggs (1978) originally created YEps by reincorporating the naturally existing yeast 2m plasmid with an E.Coli cloning vector. This plasmid is 6.3 kb in size, replicates 50–100 times per haploid cell, and is thought to have no purpose.

2. **Yeast Replicating Plsmids:** Yeast replicating plasmids, also known as yeast expression plasmids or yeast expression vectors, are small, circular DNA molecules that are capable of replicating autonomously in yeast cells. These plasmids are specifically designed for use in yeast genetic engineering and molecular biology studies, allowing the introduction, maintenance, and expression of genes of interest in yeast. Yeast replicating plasmids serve as essential tools in yeast molecular biology and are widely used in yeast genetics and functional genomics research. They are employed for a variety of applications, such as:

    - Expression of heterologous genes for protein production and functional studies.

- Gene knockout or knock-in experiments for studying gene function.
- Construction of yeast libraries for screening purposes.
- Genome editing using CRISPR-Cas9 or other site-specific nucleases.
- Yeast two-hybrid assays for protein-protein interaction studies.

Yeast replicating plasmids are versatile and valuable tools that have greatly contributed to the understanding of yeast biology and have practical applications in biotechnology and bioengineering.

YRps were developed for the first time by Struhl et al. in 1979. They found DNA fragments from chromosomes that have the necessary sequences for E. coli vectors to grow in yeast cells. Autonomously reproducing sequences, or ars, are these kinds of sequences. The difference between an ars and a centromere is that the former serves as the site of replication. The former is engaged in chromosome segregation, whereas the latter.

Although yeast is transformed by plasmids with an ars very effectively, the resulting transformants are extremely unstable. YRps tend to stick with the mother cell for unknown reasons and are not properly transferred to the daughter cell. There are a few stable transformants that are occasionally discovered, and these seem to be instances where the complete *YRp* has integrated into a homologous area on a chromosome in a way that is identical to that of *Yips*.

3. **Yeast Centromere Plasmids:** Using a YRp vector, Clarke and Carbon (1980) recovered a variety of hybrid plasmids containing DNA segments from the region surrounding the centromere-linked yeast leu2, cdc 10, and pgk loci on chromosome III. Most of the recombinants were unstable in yeast, as was to be expected from plasmids harbouring an ars. One of them, though, was kept alive permanently thanks to meiosis and mitosis. The stability segment was restricted to a 1.6 kb region between the leu2 and cdc 10 loci, and when it was present on plasmids carrying either of the two ars examined, those plasmids behaved like minichromosomes. A scentromere linked genes and was unlinked from genes on other chromosomes, serving as linked markers segregating on the minichromosomes in the first meiotic division.

   The peculiar chromatin structure found in the centromere region of yeast chromosomes is shared structurally by centromere sequences found on plasmids. In yeast cells, three chromosomal features are functionally displayed by YCps. First off, in the absence of selective pressure, they are mitotically stable. Second, they separate in a mendelian way during meiosis. Finally, they are discovered in the host cell at low copy numbers.

4. **Yeast Artificial Chromosomes:** Even the YCp vectors, which have yeast centromeres, are all three autonomous plasmid vectors described above are maintained in yeast as circular DNA molecules. As a result, none of these vectors resemble the typically linear-structured yeast chromosomes. All yeast chromosome ends feature distinctive structures known as telomeres, just like those of all other linear eukaryotic chromosomes and those of other yeast chromosomes. The telomere structure has evolved as a device that frequently cannot be completed by the ordinary mechanisms of DNA replication in order

to retain the integrity of the ends of DNA molecules. By cloning yeast telomeres into a YRP, Szostak and Blackburn (1982) created the first vector that could be maintained as a linear molecule, so simulating a chromosome. YACs, or yeast artificial chromosomes, are the name given to these vectors.One benefit of YACs over other plasmid vectors is that as insert size grows, so does their stability. YACs are crucial tools in any genome sequencing research since they have no practical size limit. Burke et al. (1987) created the technique for condensing lengthy DNA sequences in YACs.

V. EXPRESSION OF CLONE GENE IN FUNGI

When it became possible to manipulate genes in fungi, several fruitless attempts to express heterologous genes from higher eukaryotes or bacteria were made. According to this, fungus promoters have a unique structure, which was originally seen in S. cerevisiae. The typical yeast promoter contains four structural components. At the transcription initiation site, a number of consensus sequences are first discovered. More than half of the known yeast initiation sites are represented by two of these sequences, TC(G/A)A. Higher eukaryotes do not have these sequences at transcription-initiation sites, demonstrating a mechanistic divergence between those organisms' transcription machinery and that of yeast (Sun, 2019).

The TATA box is the second motif in the yast promoter. The classic sequence TATA/AAT/A is present in this AT-rich region, which is 60–120 nucleotides before the initiation site. It is comparable to the pribnow box of E. coli promoters in terms of functionality.

Upstream activating sequences (UASs) and upstream repressing sequences (URSs) are the third and fourth structural components, respectively. In genes whose transcription is controlled, they are present. Positive control protein binding to UASs speeds up transcription, and UAS deletion stops transcription completely. UASs must have at least one region with dyad symmetry in order to function. When negative control proteins bind to URSs, the transcription rate of the genes that require negative regulation decreases.

Sequences known as downstream activating sequences (DASs), which are found inside the gene itself, can influence the level of transcription. When used, the phosphoglycerate kinase itself accumulates to above 50%, according to Chen et al. (1984). These disappointing levels of heterologous protein represent mRNA levels that were caused by a lower level of initiation rather than a shorter half-life for m-RAN. The presence of a DAS was demonstrated by the restoration of mRNA transcription upon addition of downstream PGK sequences.

VI. EXPRESSION OF PROTEIN IN FUNGI

The earliest over expression systems created were for the yeast S. cerevisiae, and they utilised promoters from genes encoding numerous glycolytic enzymes, such as alcohol dehydrogenase (ADHI), PGK, or glyceraldehydes 3-phosphate dehydrogenase (GAP). These are potent promoters, and mRNA produced from them can account for up to 5% of the total. They were formerly believed to be constitutive, but it was later discovered that glucose can cause them. There are now a wide range of natural and modified promoters accessible, each

with a different level of potency, control, and induction ratio. (Romanos et al., 1992; Zubieta et al., 2018; Sakekar et al., 2021; Aerts et al., 2019).

A closely controlled promoter is ideal in order to separate the induction phase from the growth phase. As a result, fewer cells are chosen for non-expression, and it is possible for cells to produce proteins that are harmful to them. Additionally, the perfect promoter will have a high induction ratio. The GAL1 gene promoter is one that possesses these qualities and is now the most popular.

VII. CONCLUSION

This chapter concentrated on the function of fungi and microbial biotechnology, both historically and in the present. Microbial enzyme markets have seen a significant expansion because to recombinant DNA technology, which uses yeast and other fungi as hosts. The synthesis of enzymes, vitamins, polysaccharides, polyhydric alcohols, pigments, lipids, and glycolipids are only a few of the industrial processes that involve fungi. While some of the items are created for sale, others have potential biotechnological value. In the course of biotransformation, fungi are incredibly helpful. They can be utilised in novel, less-polluting production techniques compared to conventional chemical ones. Finally, it is clear that fungal biotechnology is a significant technology that will soon have a significant influence on a variety of industrial sectors.

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