

# Chapter 5

## Biotechnology and Its Position in the Mitigation of Microbial Problems in the Food Industry



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### Introduction

In the Modern world, biotechnology has been devoted to every aspect of life. In foodstuffs production enterprises, biotechnology recreates a paramount part in processing food products (Dey & Nagababu, 2022). Biotechnology has various applications and aspects in the food processing industry represented in Figs. 5.1 and 5.2. The utilization of biotechnology can permit non-consumable and short-lived food items to be changed over into acceptable. Likewise, biotechnology can enhance the sensorial attributes, texture, and time span of food by stemming the maturation of microbes that provoke toxins in the food and by producing antimicrobial agents to eliminate putrefactive microbes (Miura & Okuda, 2023). The fermentation process is used to change the taste and texture of food in a desirable manner. As a consequence of fermentation, Polysaccharides are transformed into alcohols and CO<sub>2</sub>. ELISA is also employed to determine pathogens and traces of pesticides in raw and processed food products (Sannigrahi et al., 2023). Animal-based food products are highly perishable due to their high moisture, neutral pH, and nutritional content. The processing of these foods using appropriate methods is critical to preserve their quality and microbial safety. The preservation method includes chemical, physical, and biological. In contemporary years, Biopreservation has evolved for

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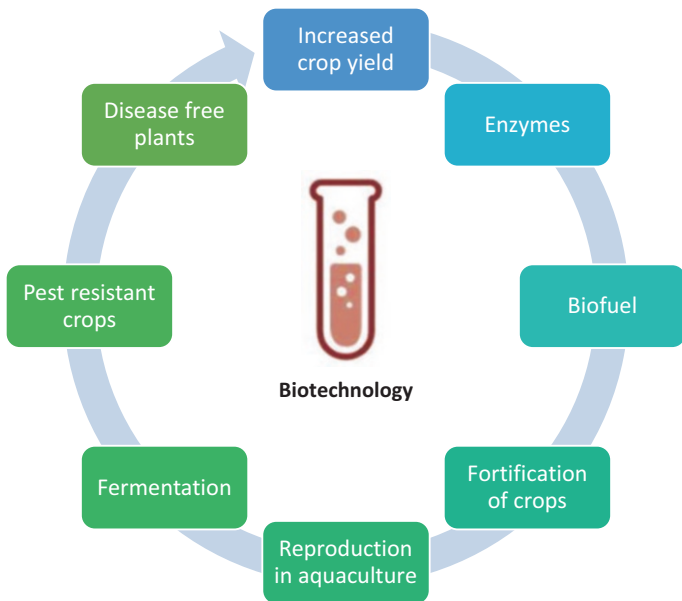
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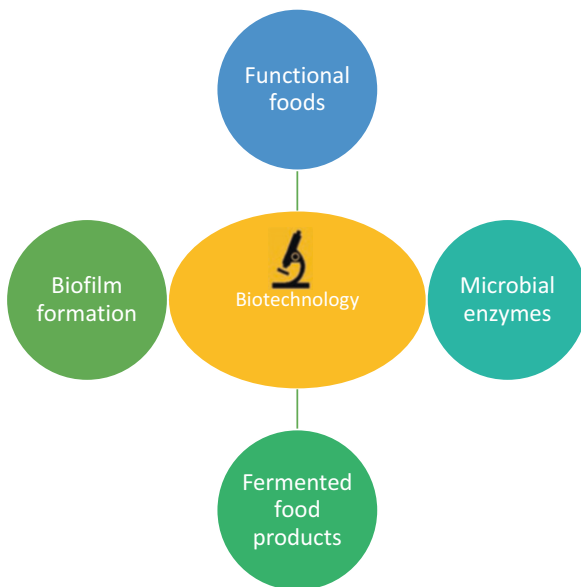
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**Fig. 5.1** Graphical representation for application of biotechnology in the food sector

**Fig. 5.2** Graphical representation of the role of biotechnology in the reduction of the microbial issue in food industries



improvement in food security and quality attributes. Biopreservation by Lactic acid bacteria (LAB) has been used instead of chemical preservatives. Bio-preservatives are safe, enhance nutritional value, and are considered to be clean labels (Rathod et al., 2021). Biopreservation has gained special attention among alternative food preservation techniques. The utilization of biosensors for the premature discovery of mycotoxins in food developments has evolved fundamentally to stay away from monetary misfortunes and disagreeable impacts on human well-being. The three main mycotoxins producing fungi in foodstuff are *Aspergillus*, *Fusarium*, and *Penicillium* (El-Sayed et al., 2022). Disclosure of the above-mentioned mycotoxins might be responsible for DNA deterioration & cell cessation. An additional quantification approach has been designed for the spotting of mycotoxins in foodstuffs. Among them, biosensors have concluded up being a viable instrument for the early recognition of mycotoxins (Oliveira et al., 2019). They are profoundly delicate and easy to utilize, consequently advancing quick and reproducible breakdown. Various types of transducers and biological materials are used for mycotoxin spoilage and its early detection. Other microbial mitigation and early detection techniques in food stuff include Biofilms, Biostimulants, Bioaugmentation, Bioremediation, etc. (Nwankwegu et al., 2022). Therefore, the aim of this chapter is to highlight the role of biotechnology in microbial mitigation and its early detection in the food industry.

## **Microbial Contamination and Its Premature Spotting in Food Industry**

Microbiological irregularity directs to the nonessential or involuntary existence of pathogenic microorganisms. Microbial contamination is driven by transmissible microorganisms (Mostafidi et al., 2020). The infectious microorganisms live & originate maturation in the processing equipment, outcomes in the preface of pathogens into the food processing phases, whereby deteriorating the food. Food contamination by microorganisms has become the greatest concern for the majority of food industries, despite the fact that food contamination also impacts food packaging. Even though reasonable hygiene procedures are sustained in industriousness, it evolves and is challenging to overwhelm contamination. By creating contaminated toxic metabolites, pathogenic contaminants can induce several disorders, including botulism, food poisoning, and other specific intestinal diseases (Sohrabi et al., 2022).

### ***Traditional Approaches to Spot Pathogens***

Expectedly, the existence of illness-causing microbes in foodstuffs can be placed through a progression of examinations, including pre-and particular improvement and specific plating, trailed by biochemical screening and lastly affirmed by

serological testing (Vidyadharani et al., 2022). Albeit the conventional technique yields precise outcomes, it requires a lot of investment to finish every one of the vital stages and arrive at a resolution. This requires a fast strategy for microbial identification that recognizes microbial investigation of food more productively and quicker than current techniques. Thus, new procedures for identifying food tainting because of uncontrolled microbial development are being created, with the conventional strategies filling in as a benchmark. The ordinary and quick identification of Salmonella in specimens of distinctive cheddar was looked at in a review. Following the previously mentioned advances, the ordinary strategy was finished, while continuous polymerase chain response (PCR) was utilized for a quick conclusion. The outcomes uncovered that the International Organization for Standardization (ISO) announced that traditional methods are slower than PCR. Since time is a significant component that should be fulfilled in the food business, a fast determination is the predominant strategy for deciding microbial presence in food varieties. In certain cases, traditional strategies have been seen to create bogus positive or adverse outcomes, adding to the method's limits (Mata & Vanetti, 2012).

## *Quick Analysis of Food Contamination*

### **E-nose**

An E-nose or electronic nose is a novel widget that comprises the miscellany of electronic compound detectors with halfway explicitness and an example pattern framework equipped for perceiving basic and complex smells (Dhanekar, 2020). E-nose is extremely capable of cracking down the physical and substance attributes of staples and distinguishing the bacterial impurities available in milk and milk items, which makes sense for its rising use in the food business (Yadav et al., 2023). Tin sensors in an electronic nose were used to distinguish microbial tainting in meats. Around 98% of microbes can supposedly be screened by this gadget. In some cases, electronic noses are figured out with metal edifices, which have been exhibited to stand adequate for contrasting the existence of maturation in produce. Satisfactory analysis was executed utilizing sullied cereal grain kernels to examine the nature of device outcomes & to determine the existence of Fusarium and Penicillium species in the crops. The aforementioned illustrates the significance of involving an electronic nose for a fast conclusion of contamination (Moura et al., 2023). It additionally recognizes pathogenic microbial development that is answerable for the deterioration of milk quality. Assessing parasitic spores in cereals and prepared goods is likewise utilized. Thus, the gadget can be considered for assessing food quality to recognize suspicious microbial development.

## **Molecular-Based Detection**

To assess the microbial existence in foodstuff, genetic code successions of conceivably infectious microbes are distinguished. For the objective arrangement, reciprocal tests are hybridized. Multiplex PCR and quantitative PCR are generally acknowledged sub-atomic cycles for identifying tainting. Sub-atomic-based examinations, for instance, microarrays, loop-mediated isothermal amplification (LAMP) & nucleic acid sequence-based amplification (NASBA) can likewise identify pathogenic contamination in food (Melinte et al., 2022). The PCR cycle is adequately effective to create quicker and improved results. Progressively PCR, the method involved with deciding the pathogenic strains, requires almost 2 h after DNA extraction is finished (Gwak et al., 2020). Multiplex PCR can identify different foodborne microbes. This technique distinguishes numerous microorganisms in a single examination, making it valuable, quick, and straightforward (Chen et al., 2021). Numerous microbes were detected in shrimp. As per a review, Multiplex PCR positively detected these microbes in shrimp (Fakruddin et al., 2013). LAMP is performed isothermally with BstDNA polymerase. Bst DNA polymerase is utilized in LAMP rather than Taq DNA polymerase in PCR. Contrasting to PCR, LAMP should be possible at a consistent temperature. A few examinations uncovered that this measure has been fruitful in the location of microorganisms (Barkway et al., 2015).

## **Enzyme Based Immunoassays**

Immunochemical assay, particularly ELISA, gauges food impurities. These use protein form arrangement. A few new immunoassay techniques have been grown as of late particularly Fluorescence polarized immunoassay (FPIA), and Sidelong stream immunoassay. FPIA considers an exact assessment of the antigen or immune response. The strategy is basic and valuable. It uses a fluorescent dye that is energized via plane-polarized light; the revolution paces of particles are not set in stone by noticing the planes. Lateral flow immunoassay utilizes immunochromatographic sticks, dipsticks, and immunofiltration. This strategy isn't just more affordable than the regular ELISA, yet it is additionally more productive and dependable. In this test, the specimen streams in a parallel heading along the solid stage. Slim power is utilized for the parallel stream. It can be handily notorious with parallel stream dipsticks, though *E. coli* is ordinarily identified with immunochromatographic sticks (Zhao et al., 2014). As well as identifying bacterial microorganisms, the parallel stream method can likewise recognize the existence of viruses in foodstuffs.

## ***Biosensors and Their Role in Microbial Mitigation***

Biosensors are logical gadgets that incorporate a natural detecting component with a physicochemical transducer to assemble a sign proximate to the specimen focus. As a reason for biosensor development, Ivnitski et al. (1999) investigated numerous

qualitative attributes for prompt & ambiguous confirmation of microbes, as well as miscellaneous spectroscopy and chromatography approaches (Ivnitski et al., 1999). As natural detecting components, biomolecules like compounds, immunoglobulin, genetic code, enzymes, and nucleic acids. Identified specimens incorporate explicit microorganisms, toxins, saccharides, and insect sprays. Ordinarily, biosensors are classified in the form of their primary transduction strategy. The acknowledgement signals comprise electrochemical, optical, and various transducers. Contingent upon the biochemical responses on a transducer surface and the estimating boundaries, biosensors can likewise be sorted as immediate or circuitous location frameworks. Direct-recognition biosensors are intended to identify bio-specific responses progressively by estimating the actual changes coming about because of the reagent's association. In biosensors utilizing circuitous identification, a biochemical response goes before the discovery of the response's items. Biochips and CPUs, which can recognize many particles related to foodborne and waterborne microorganisms, have gotten a lot of concentration over the course of the last years. Biochips can be intended to identify a wide range of waterborne microbes by engraving various antibodies or DNA particles against explicit microorganisms for concurrent recognition of microbes on a similar mark (Jaywant & Arif, 2019).

### **Surface Plasmon Resonance Based Biosensors**

Biosensors established on Surface Plasmon Resonance theory are used for spotting and the predominance of microorganisms has expanded significantly lately. Miscellaneous examinations have shown that immune response immobilization by authentic adsorption is inadequate for antigen restriction. Oh et al. (2004) led a progression of investigations utilizing SPR biosensors to recognize salmonella species. Detection of Salmonella was carried out by biosensor, and the surface was wrapped with 11-mercaptoundecanoic acid SAM, followed by the maturation of a coating of protein G & immobilization of monoclonal antibodies. The gadget's responsiveness went within the limits of  $10^3$  and  $10^9$  Colony forming units/ml. Oh et al. (2004) conducted an analysis of Salmonella by employing this biosensor. The sandwich technique was utilized to recognize Salmonella serotypes. Thusly, the gadget's recognition level was between  $10^3$  and  $10^8$  CFU/ml.

### **Tire-Based Biosensors**

TIRE is an optical strategy for concentrating on surfaces and conditions that depend upon the exploration of the amplitude and stage modifications of light. As far as total internal reflection, the chance of using stage changes of mirrored light has recently been explored. The TIRE technique was first portrayed in 1976 (Abelès, 1976). Contrasted with traditional ellipsometry and SPR innovation, deciding ellipsometry boundaries as far as complete inner reflection has altogether expanded awareness and identification levels. Consequently, the TIRE technique gives a

degree of assurance inside  $5 \times 10^7$  RIU, though this record is  $10^5$  RIU for ellipsometric estimations (Iwata & Maeda, 2010). Since the mirrored wave is shaped on a limit between optically differentiating conditions, ellipsometric estimations give data regarding the visual design of region and the cycles impacting its optical attributes (Arwin et al., 2004; Baleviciute et al., 2013). In an analysis, the TIRE technique was employed in *S. Typhimurium* recognition in model specimens Starodub et al. (2011). The outcomes signified that TIRE-based biosensors exhibited higher responsiveness than the SPR. The high responsiveness of ellipsometry-based biosensors can fluctuate for various substances; however, it ordinarily arrives at a couple of nanograms. The examination doesn't need huge amounts of reagents and can be achieved with microliters of specimens. Likewise, there is a compelling reason need to name reagents, there are no harmful impacts on the object of study, the constant investigation is conceivable, the estimating range is huge (from nM to M), and the examination time is short (Qi et al., 2023).

### PhL Based Biosensors

PhL is a strong innovation for the improvement of optical biosensors in light of the fact that it doesn't need bioreceptor readiness, convoluted electrical circuits, or costly gear. The working rule of PhL-based biosensors remains toward analyzing deviations in cutting-edge PhL spectra of nanoparticles brought about by the communication of organic parts. Utilizing photoluminescent nanoparticles, various substances, including particles, DNA atoms, dopamine, and *S. Typhimurium*, have been effectively distinguished (Liang et al., 2014; Qian et al., 2014; Viter et al., 2014). Viter et al. (2014) portrayed an innovative technique for recognizing *Salmonella* by utilizing biosensors with PhL of ZnO nanorods at room temperature. The outcomes showed that the communication of adsorbed proteins with the ZnO surface expanded the photoluminescent power of ZnO nanorods. A lessening in photoluminescent power was noticed following the expansion of *S. Typhimurium* cells and the Antigen-Antibody connection. In the scope of  $10^1$ – $10^6$  cells/ml, the biosensor showed signal adjustments. As indicated by Viter et al. (2014), the biosensor's location limit was around  $10^2$  cells/ml. Giving surface functionalization to the covalent restricting of antibodies can possibly increment sensor responsiveness. Graphene has a grandly unambiguous surface region, electronic conductivity, thermal stability, mechanical stability, covalent holding, and polymer mixing abilities and additionally has wide applications in Biosensing and fluorescence imaging (Abdelhamid & Wu, 2013).

### ISFETs-Based Biosensors

Semiconducting potentiometric gadgets such as Ion-selective field effect transistors (ISFETs) are generally utilized in biosensors. ISFET biosensors are adjusted for diverse applications, such as glucose and urea identification and clinical

investigations. The miscellaneous benefits of ISFTs are their portable size, capacity to put numerous cathodes on a solitary semiconductor chip, and modest large-scale manufacturing. Contingent upon the undertaking, various organic substances can be utilized to change the outer layer of FETs, yet most of the adjustments include catalysts, immunoglobulins, and DNA molecules. Starodub and Starodub (2000) used resistant ISFET-based biosensors effectively identify the herbicide simazine. According to the outcomes, in the wake of being treated with a corrosive arrangement and water, ISFET can be reutilized on different occasions without deterioration of signal. Taking into account every preliminary step, the examination required about 1 h.

## **Bio Preservatives and Their Role**

Bio-preservation is characterized as the utilization of antimicrobial substances obtained from foodstuff or formed by microbial maturation towards handling and quality of food. A bio-preservative is a compound that forestalls or impedes waste brought about by chemical or natural degradation, in this way drawing out the time span of usability of an item. These mixtures are frequently obtained from normal sources, while some are shaped in food. As of late, bio-preservatives have become progressively significant because of the developing interest in nutritious and top-notch food varieties. Bio preservatives are extracted from floras, faunas, or microbes normally utilized in the aging of food varieties. Unlike manufactured or customary additives, most regular options are bio-additives; however, this term is seldom utilized. The most well-known and contemplated bio-preservatives obtained from plants, chiefly from spices, are Essential oils (EOs). Enzymes are acquired from animal items, like eggs or milk. Be that as it may, as of late there have been reports with respect to bacteriocins delivered by different microscopic organisms, including lactic acid microorganisms. Other regular additives utilized are reuterin (aldehyde) created by Lactic acid reuteri, diacetyl, and Hydrogen peroxide extracted from LAB, natural acids (lactic, malic, citrus, fumaric) extracted from various scope of microorganisms, and natamycin delivered by the few types of streptomycetes. Aside from these, most food sources are safeguarded with LAB, yet ongoing investigations have shown that food microbiota can safeguard against decay microorganisms. Bio preservatives ought to be protected, modest, compelling in lower concentrations, and permissible by regulatory bodies.

### ***Essential Oils***

Essential oils, or EOs, are basically extracted by actual strategies like squeezing and refining and are perplexing combinations of aromatic and unstable mixtures delivered by plant materials like Roots, Peels, leaves, Pulp, wood, seeds, Buds, and barks. With regard to the commercialization creation of EOs, steam refining is the



**Table 5.1** Major components in EOs

Plant	Major component
Oregano	Carvacrol
Clove	Eugenol
Peppermint	Menthol
Thyme	Thymol
Cinnamon	Cinnamaldehyde
Lemongrass	Citral
Coriander	Linalool
Rosemary	1,8-Cineole
Spearmint	Carvone
Fennel	Trans-Anethole

most usually utilized technique. Recent examinations in the food use of EOs it has a vast amount of antibacterial, antifungal, and antioxidant attributes. As a general rule, EOs are portrayed by their chemical structure; notwithstanding, a couple of significant constituents are liable for their antimicrobial properties. The significant parts of chosen EOs are displayed in Table 5.1.

EOs are chosen for their antimicrobial properties as bio preservatives; nonetheless, they ought to likewise be considered for their sensorial attributes. Thyme and oregano natural oils have shown elevated degrees of antibacterial action because of their significant phenolic parts carvacrol, thymol, and eugenol. In contrast to different substances, for example, ethers, phenolic substance's structure phenoxy radicals that connect with alkyl substituents. Also, minor parts of EOs can have synergistic antimicrobial impacts with significant parts. Oxygen accessibility likewise impacts EOs' antibacterial activity. EOs have wide antimicrobial spectra, which can restrain or hinder Gram-positive as well as Gram-negative microorganisms. Subsequently, various food applications have been accounted for in the earlier years. In this manner, they are of distinct fascination as bio preservatives.

### ***Mode of Action***

EOs' hydrophobic properties make them especially appropriate for connecting with the lipid bilayer that makes up the membranes of microbes. As layer porosity increments, particles, and other cell parts spill out (Lambert et al., 2001), At the end prompting cell death. In addition, cytoplasmic aggravations, for example, proton thought motive force, electron flow, active transportation, coagulation of cell contents, and changed lipid-protein collaborations in the membrane (Vergis et al., 2015), are incorporated into cell rupture; the blend of these variables brings about cell death. Varieties in the compositions of natural oils should be possible hence, their wide assortment of EOs can be utilized as bio-preservatives. A few significant parts are likewise liable for cell rupture, and in this way, EOs additionally shows antimicrobial properties.

Carvacrol expands the ease of fluidity, which permits protons and potassium particles to spill out, causes membrane harm, and forestalls ATP synthesis (Ultee et al., 2002). Then again, terpenes slowed down and infiltrated the lipid structure of the cell wall of microorganisms, subsequently denaturing protein and disturbing the cell wall, which prompted cell lysis and cell death (Barry-Ryan & Bourke, 2012). The antimicrobial property of EOs, boosting the speed of the process, is not entirely settled by the cell structure contrasts between Gram-positive and Gram-negative microbes. When contrasted with Gram-negative microbes, which are more delicate to EOs since they contain lipopolysaccharide, a hydrophilic part that forestalls EO collection on the layer, Gram-positive microscopic organisms act all the more rapidly because of EOs. The grouping of EOs expected to inactivate or restrain Gram-positive microbes is normally lower than that expected for Gram-negative microorganisms. There is an absence of information on fungi species. However, the latest reports give proof with respect to the method of activity of EOs. Rosemary natural balm (0.3–1.5  $\mu\text{l/ml}$ ) restrained the development of *Aspergillus flavus* by diminishing how much ergosterol was in the cell film; ergosterol is exceptionally fundamental for mass layer capability, smoothness, and penetrability.

### *Applications in the Food Industry*

Food compositions, for example, macronutrients, water activity, pH balance, and enzyme activity can lessen the antimicrobial impact of EOs. Low pH upgrades EO solvency and antimicrobial action. Particularly, the antimicrobial movement of EOs relies on their concentration values. However, off-flavor major areas of strength, or sometimes their odour limits their application in foodstuff. The application strategy for EOs in food is fundamental for microbial control, sensorial attributes, quality, and the time span of usability. The most appropriate application strategy relies upon the kind of food, introductory microbial burden, target microorganism, sensory effect, EO flavour similarity with food, and capacity temperature. Direct expansion (as an ingredient of food plan or on food surfaces by spraying or dipping/coating), vapor stage lemongrass, and rosemary EOs have been utilized in meat products also on some fruits and vegetables like tomato lettuce via an active packaging approach. Food-borne pathogenic microorganisms are EO targets. Every nutritional category has agent studies. For every food type, a synopsis of a few delegate studies is displayed in this section.

#### **Fruit and Fruit Products**

Natural oils or certain methods in blending with EOs have additionally been utilized to bio safeguard fruit and fruit juices endlessly. The most well-known and broadly acknowledged method for deactivating microorganisms in natural product juices includes hot temperature treatment, which can modify the juice's colour and flavour.

Hot temperature treatment at 55 °C with 190 parts for each million-orange fruit. EO in squeezed orange pulp abbreviated the time expected to inactivate the microbe *E. coli* O157:H7 by 2.5 times. The sensorial harm of squeezed orange was relieved by the incorporation of orange EO, which cut the hot temperature treatment time down by 50% (Espina et al., 2014). 2log reduction of *Saccharomyces cerevisiae* was achieved in apple-orange (1:1) juice, which was thermally treated (for 2 min at 90 °C) and shelf stored at 25 °C for 8 days. As an outcome, it helped to retain its quality attributes, colour, and flavour (Tyagi et al., 2013).

### **Vegetable Products**

EOs can change the flavours of vegetables, which have a short time span of usability because of microbial deterioration and other variables. It is important to utilize a suitable application mode to ensure sensorial worthiness. Ponce et al. (2011) analyzed EOs to Romaine lettuce leaves in polyethylene terephthalate plate to expand their time span of usability at 6 °C. EOs incorporated samples in cases of repressed local microorganisms for 1 week. However, they were successful in maintaining sensorial attributes of microbial decay.

### **Dairy Products**

It has been proposed that EOs could be utilized to save dairy items; in any case, mindful methodologies have been accounted for on the grounds that numerous milk items are aged or matured food sources. Thusly, EOs could hinder LAB or molds that are liable for the improvement of the flavour, smell, surface, and sensorial attributes of cheeses and yogurts. Oregano and thyme EOs at 0.1 mL/100 g (splashed on a superficial level) were viable to inactivate 5 log reduction of a *Listeria monocytogenes*. Likewise addition of EOs in Feta cheddar was shelf stored at 4 °C for 20 days. Following 20 days of refrigeration, both EOs helped to 4log reduction of *E. coli* O157 (Govaris et al., 2011). When labneh (concentrated yogurt) was shelf stored at 6 °C and 0.3% cinnamon oil was added, the time span of usability was improved by up to 3 weeks (Thabet et al., 2014).

### **Meat Products**

Meat items deteriorate basically because of fats oxidation and bacterial development, in spite of the fact that molds might create a few issues in the development of meat products. Meats might become tainted with microorganisms during butchering or handling. LAB, a huge bacterial gathering related to meat deterioration, advances undesirable quality changes. Also, food-borne microorganisms can develop on newly slaughtered meats. *Penicillium* is a predominant family tracked down in developing meat products. EOs decreased and restrained microbial burden as well

as upgraded quality credits like hindered fat oxidation; EO of *Salvia officinalis* at low fixations (0.1%) repressed the development of *Salmonella Anatum* and *S. Enteritidis* in minced hamburger shelf stored at 5 °C for 2 weeks. Centralizations of 2% of Essential oil inactivated *Salmonella* up to manifolds following 2 weeks of shelf storage investigation at 5 °C for 2 weeks (Hayouni et al., 2008).

### ***Mycotoxins Detection and Its Control***

A mycotoxin is acquired from the Greek word “mykes,” which implies fungus, and the Latin word “toxicum,” which implies poison. Particularly, mycotoxins are low-sub-molecular weight intensifies conjoined by filamentous fungi during secondary metabolism; their chemical configuration can vary from simple 4-carbon mixtures to complex particles. The utilization of mycotoxins by humans or animals prompts obsessive or unwanted physiological reactions. At the point when mycotoxins are gulped unexpectedly, they cause mycotoxicosis (Soares Mateus et al., 2021).

30<sup>5</sup> fungal secondary metabolites are known to exist, among them six mycotoxins that are significant according to a farming perspective. Mycotoxin-creating parasites can grow in cereal grains under normal circumstances during handling and storage (Awuchi et al., 2022). A great many substrates and ecological circumstances are helpful for mycotoxins growth naturally (Nji et al., 2022). Particularly, the utilization of food technology essentially decreases mycotoxins yet doesn't dispose of them. Mycotoxins affect DNA, RNA, and protein union and might cause changes in physiological capabilities, including propagation, development, and advancement (Yang et al., 2020). Notwithstanding these various activities, mycotoxins might influence the digestive system, cause skin problems, make hematological impacts and diminish development.

### **Mycotoxin-Producing Fungi**

Fungi are unicellular, polynuclear, eukaryotic organic entities that are heterotrophic and whose cells are comprised of chitin. Mycotoxins are either grown during pre-harvest level or throughout postharvest (storage, transport, and handling). Mycotoxins are generally created by filamentous fungi that are adapted to the earthly environment. Mycotoxins are delivered by specific types of filamentous fungi having a place with types of the genera *Aspergillus*, *Penicillium*, and *Fusarium* that attack crops at the field level and may develop on food varieties during capacity when they got ideal conditions for growth (Syamilah et al., 2022).

## ***Chemical Structures, Toxicity, and Mode of Action of Mycotoxins***

### **Aflatoxins**

Aflatoxins are composed of heterocyclic mixtures that are exceptionally oxygenated. *Aspergillus flavus*, *A. parasiticus*, and *A. nomius* form aflatoxins B1, G1, B2, and G2. Aflatoxin B1 is the most cancer-causing type, causing liver malignant growth in humans (Baird et al., 2006). Global Office for Exploration on cancer growth characterized AFB1 as a group A cancer-causing agent (Soares Mateus et al., 2021). AFM1 and AFM2, monohydroxylated subordinates of AFB1 and AFB2, are usually found in milk (Popescu et al., 2022). AFB1 has been connected to essential liver disease in people, where it cooperates with HBV contamination to cause malignant growth, and it has been marked a group 1 cancer-causing agent in people. Insect harm might make grain crops more powerless to mycotoxin-delivering growths (Winter & Pereg, 2019). *Aspergillus* strains that cause aflatoxin to flourish in high dampness/humidity, hot temperatures, or potentially poor drying. This implies that grains put away in these circumstances could become contaminated (Awuchi et al., 2021).

### **Zearalenone**

Zearalanol can cause mature adolescence in kids, and Estrogenization can be caused by the F-2 toxin (Yli-Mattila et al., 2022). Mature advancement of breasts and, furthermore estrogenic impacts in females, as well as preputial development in males, is the most notable impact of ZEA. Fundamentally rats, cows, and chickens, pigs are the most delicate and seriously impacted species.

### **Fumonisin**

The fumonisins are a type of nonfluorescent mycotoxins which are fundamentally formed by *F. verticillioides* and *F. proliferatum* (Santos et al., 2022). Fumonisin B1 (FB1), the most predominant of the various fumonisin analogs, was designated a group 2B cancer-causing agent (Wild & Gong, 2010). The cancer-causing effects of fumonisins have all the irrelevant of being inconsequential to DNA interaction (Coulombe, 1993). Its closeness to sphingosine proposes a potential job in the biosynthesis of sphingolipids (Shier, 1992). Sphingolipids are subsequently restraining sphingolipid biosynthesis causes serious cell activity issues. In specific human populations, fumonisins are associated with causing esophageal growth. No matter what their impacts on humans, fumonisins particularly cause liver harmfulness by slowing down sphingolipid metabolism, their essential method of activity.

## Patulin

*P. expansum*, typically comprehended as the blue mould pathogen of apples and some climacteric fruits, is nature's direct patulin producer. Patulin is excessively harmful to plant and animal cells and should react with the terminal sulfhydryl groupings of proteins in edibles (da Silva et al., 2022). Patulin deters DNA synthesis and hampers immunosuppression. Patulin exposure is coupled to immunity, digestive, and neurological disorders. (Esheli et al., 2022).

## *Methods for the Detection of Mycotoxins in Foods*

### Chromatographic Techniques

- Thin-layer chromatography

The standard TLC approach is viewed as an adequate evaluating device for the existence of aflatoxins and a solid analytical measurement procedure. TLC is a broadly involved strategy for quantitative estimations of mycotoxins utilizing fluoro-densitometry and visual systems with a detection limit of up to **0.01** ppm. Silica gel is the base of the TLC approach. Silica gel is usually infused with organic acid and has allegedly been employed to determine normal mycotoxins like aflatoxins, citrinin, and fumonisin (Pradhan & Ananthanarayan, 2020).

- HPLC

HPLC is the considerably well-known chromatographic process for quantitative investigation of mycotoxins, particularly aflatoxins. Various kinds of solid stage or reverse stage columns, elution combinations and gradients, identification techniques, and test preparation and purification methodologies determined these HPLC strategies. Unique mixtures are isolated on the basis of their association with the column matrix and the mobile phase solvent by infiltrating dragged samples into a standard or reverse-phase HPLC chromatography (column (Vaudreuil et al., 2020). A few mycotoxins, such as ochratoxin and citrinin, have biological fluorescence and can, in this manner, be identified straight by HPLC-fluorescence identification (Singh & Mehta, 2020).

- LCMS

Liquid chromatography combined with mass spectrometry (LC-MS) or couple mass spectrometry (LC-MS/MS) is an adequate technique for mycotoxin recognition and recognizable proof, particularly for those toxins with low UV/Vis absorbance or local fluorescence (Tsagkaris et al., 2019). Flores and González-Peas (2017) detailed the concurrent measurement of **16** mycotoxins in cattle milk, including aflatoxins M1, B1, B2, G1, and G2 (Leite et al., 2021).

- Gas chromatography-mass spectrometry (GCMS) technique

Gas chromatography can be utilized to see whether mycotoxins are sufficiently volatile at the column temperature or can be modified into volatile subordinates. It is, in many cases, utilized in additional specialized labs to analyze certain mycotoxins, particularly type-A mycotoxins, which are not handily examined with HPLC (Luo et al., 2022). It tends to be combined with various discovery frameworks; however, most continually electron-capture detectors (ECD) and mass spectrometry (MS) are utilized (Lobato et al., 2021).

### **Immunological Methods**

- ELISA

ELISA operates because an essential antibody that is well-defined for the toxin of interest or a marked toxin-enzyme conjugate form contends with the toxin in the specimen for a set number of restricted sites. The subsequent complex reacts with a chromogen, which can be estimated with a reading (Chu, 2019). ELISAs are ordinarily profoundly unambiguous, fast, and generally easy to use. Delicate microtiter plate immunoassays (in ELISA design) are financially accessible. Most of these units depend on a heterogeneous ELISA design in which the specimen toxin rivals a marked toxin (for example, a toxin enzyme conjugate) for a predetermined number of antibody-binding sites. More toxin is available in the specimen, the more vulnerable the limiting of the marked toxin and the more vulnerable the sign created by the assay (Geleta, 2022).

- Fluorescence polarization immunoassay

This strategy is predicated on the contest between free and fluorescein tracer-toxins for toxin-explicit monoclonal antibodies in the arrangement. In this procedure, no enzymatic response is expected for discovery. Besides, recognizing the bound and free labels is pointless. Such a measure has been produced for evaluating aflatoxin in the grains (Zhang et al., 2022).

## **Biofilms in Food Industry: Mitigation of a Microbial Issue by Introducing Biofilms in the Food Industry**

### ***Biofilms***

These are assorted biological systems created by single or multiple organisms submerged in a superficial environment of moving based on the type of nutrient-supplying environments and the varieties populating it. Fungi and Bacteria are instances of microorganisms that can develop these biofilms. The existence of numerous forms of bacteria holds critical conservation benefits since this stimulates

biofilm association with a surface. This could occur without any specific fimbriae in certain species. Antimicrobials like quaternary ammonium complexes and other biocides are more impervious to blended biofilms (Meyer, 2015). The extracellular network comprises polysaccharides, polypeptides, and nucleic acids. The aforementioned framework might be converged to tough surfaces (Tools, carriers, conveyance, biological stages and soil etc.) (Flemming et al., 2016). The superficial environment grid plays an underlying part, which represents the biofilms' wonderful steadiness in food enterprises. It makes convoluted inclinations for oxygen and supplement transport, contains extracellular enzymes for feed and food, permits cell correspondence particles to be moved, and safeguards the implanted cells from hurtful synthetics. Biofilm-related impacts (host specificity, rusting of metallic surface, change in organoleptic qualities because of lipase or protease digestion) are essential in particular enterprises, for example, dairy creation lines, where various designs and processes (Milk tanks, pipes, cheese capacity tank, pasteurizers, and packaging methods) capability as outer reactants arrangement at distinguishing temperatures. It might be comprised of *S. enterica*, *L. monocytogenes* and *Pseudomonas* species (Mizan et al., 2015).

Its existence in processing industries can emanate toxins. It can degrade compositions, generating personal or innumerable intoxications. This is why Biofilm networks in a food-production area jeopardize human well-being.

### ***Bacillus Cereus***

*Bacillus cereus* is a facultative anaerobic or spore-forming Gram-positive bacteria that can flourish in different environments and temperatures (4–50 °C) and be thermal safe. Compound treatments, and sunlight (Bottone, 2010). The staying power of vegetative strains of *B. cereus* on food equipment surfaces is risky to one's well-being. Moreover, because of the formation of endospores, this bacterium is equipped for enduring modern pasteurized activities. This hampers biofilm expulsion utilizing cleaning procedures (Auger et al., 2009) and can charm biofilm perseverance in milk plants, affecting dairy and its product storage of realistic usability (Gopal et al., 2015). Biofilms of *B. cereus* are habitually found close to various microorganisms in the industry (Majed et al., 2016). Their linkage is served by their perplexing association of polysaccharides, polypeptides, and nucleic acids (Vilain et al., 2009). The paramount relationship of these strains has a prerequisite influence this permits other bacterial species to connect rapidly, which would be cleared by various streams (Marchand et al., 2012). These strains are vastly discovered in dairy processing plants (Ehling-Schulz et al., 2015; Ruan et al., 2015).

An analysis of the processing plant creating sterilized milk in North America discovered around 6% of these had a  $10^6$  -colony forming unit of *B. cereus* per ml of tested specimens & that around 5% of these entities brought enterotoxins at decks that could lead to foodborne illness. *B. cereus* could deliver enterotoxin in this pasteurized milk after 1 week of storage. Essentially elevated *B. cereus* bacterium was found in items with a high butterfat content or those that were sterilized at increased



temperatures for a brief period (Saleh-Lakha et al., 2017). Customary markers, like oxygen-consuming settlement development and psychotropic counts. In an analysis utilizing sterilized milk, 18 *B. cereus* isolated were recognized.

### *E. coli*

These strains are usually found in the gastrointestinal tract and don't represent a well-being risk. In any case, a few sorts are hazardous foodborne microbes that can be transported through the water, milk & milk products, and meat & meat products. These items might have been tainted at the starting place or during the course of food processing. This contamination might happen in the food products during the pre-gather stage because of the utilization of spoiled water while creating the yields. This disease may likewise happen in storage and handling conditions, where it might emerge during the cleaning and handling of raw materials, yet additionally attributable to storage temperatures that permit the flow of bacterial contaminants to replicate rapidly (Carter et al., 2016). A few examinations have shown that kinds of *E. coli* may stick to many surfaces, together with steel material, Teflon, and polystyrene. (Van Houdt & Michiels, 2010).

### *Listeria Monocytogenes*

*L. monocytogenes*, a Gram-positive bacteria, is typical and causes serious foodborne illness. It isn't, notwithstanding, impervious to pasteurization techniques (Milillo et al., 2012). Fish, milk items, frozen yogurt, organic products, delicate cheeses, sugar-coated candies, and raw milk are a few instances of packed food sources known to convey this illness (Rothrock et al., 2017).

Biofilms of these strains are generally made of teichoic acid and shall foster on various surfaces across a food processing plant. These contaminations can further reproduce at lower temperatures (Silva et al., 2008). There is a record of five outbreaks that occurred due to *Listeria monocytogenes* from 2014 to 2016 (Burall et al., 2017). The presence of these strains demonstrates remarkable fidelity to numerous surfaces and restraint to chemical compounds, which are significant justification behind microbial migration reactions in the milk and meat products.

## **Advanced Biotechnological Tools for the Control of Microbial Mitigation**

Biotechnology is the use of technology and science to living creatures and their components, products, and models to change living or non-living elements and produce knowledge, products, and services. The diversity of plants and microbes makes

it more difficult to improve applied genetics (Alan, 2023). The successful gene modification of microorganisms has encouraged agricultural scientists to employ plant breeding technologies. The strategy of the plant breeder is determined by the specific biological elements of the crop being bred to facilitate the emergence of fresh varieties and sometimes even types of plants by evading current biological barriers to the interchange of genetic material. Emulsification offers food products a distinct look and sensory qualities that substantially influence customer perception. Because human perception is complex, food manufacturers typically require numerical criteria for quality control (Andreani et al., 2023).

Bioactive chemicals derived from natural sources are frequently incompatible with the food matrix, break down quickly during food preparation, and are vulnerable to digestive action in biological systems. As a result, these bioactive compounds are encapsulated for improved protection and simpler integration into the food matrix. Because of the acidic environment in the gastrointestinal tract, bioactive proteins disintegrate quickly and are digested by hydrolytic enzymes. Furthermore, hydrophobic chemicals, such as polyphenols, have a low water solubility, which makes integration into food and absorption in the digestive tract difficult (Maurya et al., 2023).

## ***Bioremediation***

Bioremediation is comprised of two words: “bios” (living animals) and “remediate” (to cure an issue). Bioremediation is a successful innovation/process that bridges the capacity of organic living beings to break down environmental contamination, subsequently helping the environment in supporting regular environmental factors. Bioremediation strategies are constituted of two approaches in-situ & ex-situ (Kirthi & Chaudhuri, 2023). The strategy for treating defilement in its local area under natural circumstances is known as in situ. However, the ex-situ method includes eliminating the pollutant and treating it far away from its local site, for instance, under research facility conditions. Bioremediation is an earth-useful, low-support, minimal expense, low-input, and long-haul way to deal with polluted site clean-up (Kumar et al., 2022).

The utilization of living creatures for eliminating pollutants depends on the possibility that organic entities feed on impurities for their development and digestion and thus can eliminate compounds from the environment. Many microbes, such as bacteria and fungi, degrade complex compounds relatively efficiently, and the resulting chemicals are usually safe for food. Fungi, on the other hand, can digest vast complex organic substances that are generally not digested by other creatures using their hyphae. Similarly, other biological creatures such as protozoa, algae, and plants have been discovered to be capable of absorbing nitrogen, phosphorus, sulfur, and a variety of minerals from contaminated foods (Raj & Das, 2023; Kirthi & Chaudhuri, 2023).

## ***Bioaugmentation***

Bioaugmentation is a novel approach to utilizing the ability of various microorganisms in their natural environments to clean up contaminants from polluted regions. The method employs the isolation of natural microorganisms (fungi, bacteria, etc.) and their eventual addition to contaminated locations after cultivating them under ex-situ conditions to increase their abundance and functional metabolism to remediate pollutants such as metal (Rafeeq et al., 2023). Bioaugmentation can be laid in the accompanying ways: (a) by dragging local pathogens from the objective tainted site and refining them under lab conditions before re-immunization; (b) dragged pathogens are not intrinsically inoculated to the wellspring of the local culture, and (c) extraction can be incorporated to the site with the assistance of crafted and transformed microorganisms (Raj & Das, 2023).

## ***Biostimulants***

To boost the metabolic step of a competent microbe for efficacious pollutant debase-ment, favorable changes in some parameters, such as oxygen, water, pH change, accessible phosphorous, and nitrogen supplies, must be utilized to boost the biore-remediation process. (Triozi et al., 2023). Biostimulation, which involves the addition of nutrients, has the promise of speeding up deterioration rates. Biostimulation is the arrangement of supplements, oxygen, pH and temperature changes, and different variables to the defiled site to build the mass action of local microorganisms reasonable for bioremediation. Thus, Biostimulation speeds up the pace of decay in dirtied areas by bringing restricting supplements into the arrangement of the microbial local area that lives there. Biostimulation assumes a significant part in making good circumstances, especially in enacting microbial populaces that have adjusted in their territories because of steady toxin openness at contaminated areas (Stinconone et al., 2023).

## **Conclusion**

The rise in popularity of genetically modified crops that are resistant to herbicides, insects, and pathogens has resulted in increased crop yields and decreased production costs. The primary focus of modern biotechnology is on the development of foods that exhibit better nutritional, taste quality, and safety characteristics. Biotechnology, as a holistic field, offers a wide of innovative devices which enable the detection of microbes and their toxins. These devices comprise several biosensors, Protein Misfolding Cyclic Amplification (PMCA), and ELISA analysis. The

PCR technique has been constructed for the purpose of identifying the presence of contagious pathogenic agents, including bacteria, viruses, fungi, and other relevant micro-organisms. Various researchers have claimed that the utilization of biotechnology in modern food production can result in the provision of a wider range of nutritionally enhanced, healthier, more palatable, secure, long-lasting, safe, and convenient food products to global populations, all at lower costs.

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