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Microbial Biofactories for the Synthesis of Antimicrobial Peptides

Shilpa S. Ruikar[1], Sameer Rastogi[2], Pooja Sharma[3], Girish R. Pathade[4], Nitin Tandon[5], Shital Yadav[6]

[1]Assistant Professor, Krishna Institute of Science and Technology, Krishna Vishwa Vidyapeeth (Deemed to be University), Near Dhebewadi Road, Malkapur, Karad, Satara, Maharashtra, India.
shilpa_ruikar@yahoo.co.in
[2]Professor Department of School of Pharmacy Noida International University, India.
dean.sop@niu.edu.in
[3]Department of Pharmacy, Sri Sai College Of Pharmacy, Pathankot-145001, Punjab, India.
poojasharma21012@gmail.com
[4]Professor, Krishna Institute of Science and Technology, Krishna Vishwa Vidyapeeth (Deemed to be University), Near Dhebewadi Road, Malkapur, Taluka-Karad, Dist Satara, Maharashtra, India,
girishpathade@yahoo.co.in
[5]Department of Applied Sciences, CT University, Punjab, Ludhiana, 142024, India
tandonnitin12004@gmail.com
[6]School of Applied and Life Sciences, Uttaranchal University, Dehradun, Uttrakhand, India,
Email: yadavshital97@gmail.com

ABSTRACT

Making antimicrobial peptides (AMPs), which show great potential as novel approaches to treat illnesses resistant to medications, depends on microbial biofactories, which have become very helpful. Effective against a spectrum of microorganisms, AMPs are a class of naturally occurring peptides. This makes them feasible choices for combat of newly emerging viral infections. Making AMPs via conventional chemical synthesis techniques might therefore present challenges like cost, lack of numerous AMPs, and limited availability of them. This has spurred research on bacterial systems as biofactories producing AMP.Yeasts, bacteria, and fungi among other microorganisms are being altered to overexpress AMP genes and produce peptides into the growth media. This increases production's cost-effective scalability. Thanks to synthetic biology technologies, metabolic engineering, and fermentation optimisation, the manufacturing of AMP in microbial systems has become much more efficient and prolific. To demonstrate that they can support the high-level translation of bioactive peptides, Escherichia coli, Bacillus subtilis, and Saccharomyces cerevisiae have been employed effectively as host organisms to create various AMPs. The ability of microbial biofactories to generate a wide range of diverse AMPs with varying antibacterial characteristics is among their greatest advantages. By use of natural biochemical pathways or by generating novel peptide sequences via genetic engineering, microorganisms may produce peptides that target certain bacteria or halt resistance mechanisms in their tracks. Furthermore, the synthesis of AMPs by microorganisms makes it possible to add post-translational modifications such as glycosylation and cyclisation, which can increase their stability, bioactivity, and therapeutic value. Notwithstanding these developments, numerous issues still need to be resolved before we can completely maximise the AMP generation by microorganisms. These include host-cell toxicity, peptide solubility, and processing techniques. Future research aims primarily to raise the efficiency and volume of AMP manufacturing. Creating more stable microbial strains, improving fermentation techniques, and researching novel microbial species that especially produce AMP can help to accomplish this. Furthermore crucial for producing novel AMPs more effective in killing bacteria is the use of computer algorithms and systems biology approaches.

KEYWORDS: Microbial biofactories, Antimicrobial peptides, Synthetic biology, Metabolic engineering, Drug resistance, Peptide production.

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INTRODUCTION

One of the main dangers to world health in the twenty-first century is now the rise of antimicrobial resistance (AMR.). It increases the difficulty of treating diseases and results in higher global mortality. Many antibiotics have become useless against pathogens like viruses, bacteria, and fungi as they have evolved resistance to many of them. This implies that normal medication is useless. Experts have so sought for other approaches to treat the illness, and antimicrobial peptides (AMPs) seem to be a suitable option. AMPs are naturally occurring small peptides that are very good at killing microbes. This makes them useful for treating illnesses caused by microbes that are immune to many drugs. They have many benefits over standard antibiotics, such as the ability to kill a wide range of bacteria quickly and with little chance of resistance developing. In addition, AMPs can attack pathogens that are bacteria or fungi, as well as viruses, parasites, and even cancer cells.Even though AMPs have a huge amount of promise as medicines, they are hard to make for a number of reasons. In the past, AMPs were either made chemically or taken from natural sources like plants, animals, or bacteria. Although chemical synthesis allows you to create whatever peptide sequence you choose, it cannot be mass-produced as it is costly and usually yields low amounts. Furthermore restricted by the scarcity of sources and the waste of gathering significant quantities of them are natural ways of obtaining compounds from plants or animals. These issues are driving increased interest among individuals in employing microbial systems as biofactories to produce AMPs.

Microbiome biofactories synthesis many therapeutic compounds using microorganisms like yeasts, fungus, and bacteria. They have attracted a lot of interest as a reasonably cheap and useful method of producing AMPs.

Among the many therapeutic compounds produced by microorganisms are peptides that kill bacteria. They are also quite simple to cultivate and highly versatile. They are thus a great instrument for producing novel AMPs as genetic engineering may also help to enhance the production of AMPs in microbial systems. Since they can create and release a lot of AMPs, microbial biofactories show great potential. This qualifies them as a suitable choice for conventional methods of synthesis. Two often utilised species of bacteria for AMPs are Escherichia coli (E. coli) and Bacillus subtilis as they may be genetically altered to generate peptide sequences with great efficiency. Since they can release proteins into the culture media without requiring complex extraction techniques, fungus and yeasts including Saccharomyces cerevisiae have also been found to be suitable hosts for producing AMP. Making a lot of AMP is ideal for microbiological fermenting techniques as they are simple to operate and scalable. Using microbial systems for AMP synthesis has the advantage of producing a lot of peptides and allowing one to alter and enhance the produced peptides. Synthetic biology and metabolic engineering let microorganisms be altered to produce peptides with specific traits such improved stability, activity, and selectivity. For instance, introducing post-translational modifications like glycosylation or cyclisation can help AMPs be more stable and bioactive, hence raising their therapeutic value. By altering their DNA, microorganisms may also be created to produce peptides effective against a broad spectrum of bacterial species. This aids in the solution of the growingly problematic antibiotic resistance issue. Although a lot of research has been done on producing AMPs utilising microbial biofactories, several issues still have to be addressed. Because they are difficult to dissolve and may be detrimental to host cells, AMPs in microbial systems can be challenging. Correct folding of peptides may sometimes be challenging. Certain AMPs may damage host cells, which would prevent their plentiful production [1]. Furthermore less valuable and bioactive generally are peptides produced in microbial systems as they may have difficulties dissolving and binding together. Researchers are focussing on enhancing the host strains via genetic editing, discovering fresh approaches to purify the AMPs, and optimising the fermenting conditions in order to handle these challenges. Furthermore, the need for AMPs capable of combat of a variety of drug-resistant bacteria has resulted in the synthesis of fresh synthetic peptides generated by computer modelling and peptide engineering. This approach allows researchers to create stable, easy-to-make peptides that not only combat resistant bacteria but also have other uses. Two computational approaches that have been used to identify possible peptide sequences more efficient at killing bacteria and less detrimental to cells are molecular docking models and bioinformatics [2]. These fresh findings enable the synthesis of AMPs with improved features for use in medicine.

A. Background on antimicrobial resistance (AMR)

Antimicrobial resistance (AMR) is one of the biggest problems in world health in the 21st century. It makes treating infectious diseases a lot harder. AMR happens when germs like bacteria, fungus, viruses, and parasites change over time so they can't be killed or stopped from growing by drugs. This resistance can happen because of changes in genes, horizontal gene transfer, or the wrong or excessive use of antibiotics, all of which speed up the development of resistant types. A lot of antibiotics are used in agriculture, in human and animal health, and in other fields. This has helped resistance genes spread quickly, making it possible for bacteria to grow even when drugs are present. AMR has very bad effects, including longer hospital stays, the need for more specialised care, and higher death rates. Antibiotics used to make it easy to treat infections, but now they're harder to control [3]. This is making healthcare more expensive and putting more stress on healthcare systems around the world. Multidrug-resistant microbes like Clostridium difficile, Methicillin-resistant Staphylococcus aureus (MRSA), and Carbapenem-resistant Enterobacteriaceae (CRE) are some of the best known. These bacteria and others have become resistant to many types of medicines. This makes it harder to handle illnesses, do regular surgeries, and take care of long-term diseases that need effective antimicrobial treatments. Many cancer treatments, organ transfers, and other medical processes depend on being able to stop or treat illnesses, so AMR also poses a threat to their usefulness. The According to the World Health Organisation (WHO), AMR poses a serious danger to health that requires quick, global coordinated response. The sluggish pace at which new antibiotics are being developed and the proliferation of resistance to antibiotics microorganisms throughout the globe worries academics, legislators, and medical professionals. Not fast enough new forms of antibiotics have been developed to match the increase in resistance [4]. This has produced a therapeutic gap wherein infections that are developing resistance are either difficult, if not impossible, to cure. Given this, it is rather crucial to investigate various approaches of combat against AMR. The synthesis of antimicrobial peptides (AMPs), which provide a fresh approach of eradicating germs distinct from the action of conventional antibiotics, is another fascinating area. Finding new, potent antibiotics like AMPs has never been more crucial since AMR renders existing medications less effective.

B. Importance of antimicrobial peptides (AMPs) in combating AMR

It looks like antimicrobial peptides (AMPs) could be a good option to regular medicines in the fight against antimicrobial resistance (AMR). Natural occurring short peptides known as AMPs have broad-spectrum antibiotic effect against many kinds of pathogens, including bacteria, fungi, viruses, and parasites. From bacteria to plants, animals, and humans, many live entities rely on them as a component of their innate immune response. These peptides rapidly destroy bacteria and may compromise microbe cell walls, therefore preventing the survival and multiplication of pathogens [5]. In the battle against AMR, AMPs are very crucial because of their special mechanism, unlike that of ordinary antibiotics. Most antibiotics work by stopping specific microbial processes, like making cell walls or proteins. AMPs, on the other hand, work by directly connecting with the microbial cell membrane, breaking it down and killing the cell. Pathogens are less likely to become resistant to this method than to standard medicines. antibiotic peptides (AMPs) are very important in the fight against antibiotic resistance (AMR), as shown in Figure 1. Because AMPs don't work on the same targets that antibiotics do, they are much better at getting past the resistant mechanisms that many bacteria have built up.

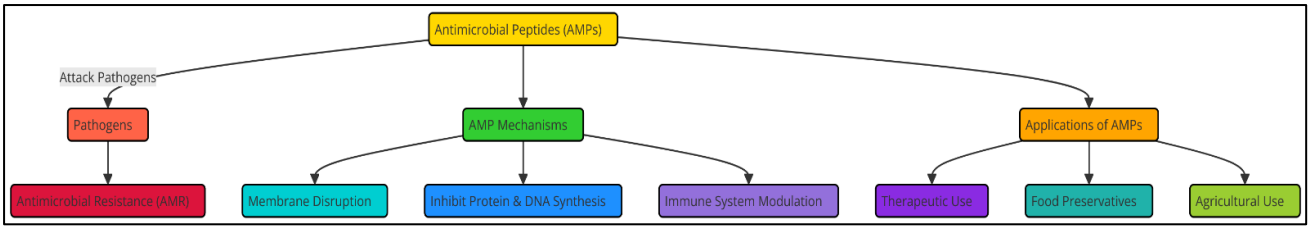

Figure 1: Importance of antimicrobial peptides (AMPs) in combating antimicrobial resistance (AMR)

Furthermore serving other purposes, AMPs may help wounds heal, lower inflammation, and control the immune system. They are very tempting for battling infections, aiding in tissue healing, and altering immune system response because they can do so many tasks [6]. Even resistant varieties like MRSA and VRE (vancomycin-resistant enterococci), such AMPs as defensins, cathelicidins, and lactoferrin have demonstrated to be very successful in treating a range of diseases. Moreover, AMPs break down organically and do not usually accumulate in the body, therefore reducing the long-term danger. AMPs are especially more valuable as medications as they can be developed and altered. By means of peptide engineering and synthetic biology, scientists may modify AMPs to make them more potent, stable, and selective against bacteria resistant to them. They may be altered, for instance, to increase their solubility, resistance to enzymatic breakdown, or ability to break through bacterial biofilms—common in chronic diseases and therefore less effective therapy is rendered [7].

MICROBIAL BIOFACTORIES

A. Definition and types of microbial biofactories

Microfactories are microbes intended for or used in the manufacture of functional molecules like antibiotics, enzymes, antimicrobial peptides (AMPs), and other useful agents. Either natural or synthetic techniques obtained from these bacteria might help in large-scale pharmaceutical compound production. They provide a scalable, competitively cost, sustainable substitute for existing chemical synthesis methods. The idea of microbial biofactories has changed dramatically because metabolic engineering, synthetic biology, and genetic engineering allow microorganisms to produce a vast range of chemicals. Various kinds of microbial biofactories are used in AMP production. Comprising mostly bacteria, yeast, and fungus, these biofactories are bioactive. Every group has different advantages and disadvantages when it comes to creating AMPs.

Bacteria: Bacteria, especially E. coli, Bacillus subtilis, and Lactococcuslactis, are some of the most common microbes that are used to make AMP. People often choose these creatures because they grow quickly, are easy to change genetically, and can make a lot of proteins. For example, E. coli is often used for recombinant protein production, which includes AMPs, because it can easily produce alien genes. Bacteria can also release the peptides straight into the growth medium, which makes handling later easy [8].

Yeasts: Saccharomyces cerevisiae and Pichia pastoris are two common types of yeast that are used as biofactories to make AMP. Yeasts are living animals that can make complex changes after translation, which are necessary for some AMPs to work and stay stable. A lot of the time, yeast systems are used to make AMPs with changes like glycosylation, which can make peptides more stable and active. Yeasts can also be grown in big bioreactors, which means they can be used for large-scale production.

Fungi: filamentous fungi like Aspergillus niger and Trichoderma reesei are being studied more and more for their ability to make AMP, especially for peptides that need to be folded in a complicated way or changed by enzymes. Since they can produce proteins and release them into the extracellular environment, fungus naturally has an advantage. Furthermore genetically altered to increase their productivity, fungi may produce peptides requiring post-translational modifications which might be crucial for the bioactivity of certain AMPs [9].

Every microbial system has beneficial and negative aspects. Since bacteria have quicker growth rates and can be readily altered at the genetic level, they are ideal for producing many AMPs rapidly. Yeasts, on the other hand, provide a more consistent means of producing peptides requiring sophisticated modifications to function. For certain kinds of AMPs, particularly those difficult to produce in bacterial systems, fungi which can naturally produce and release proteins offer solutions.

B. Advantages of using microbes for AMP synthesis

Since they offer several advantages over conventional chemical synthesis or extraction from natural sources, microbial systems are a promising approach to generate antimicrobial peptides (AMPs). Among these advantages are cost-effective, scalable, easily changed genetically, and ability to create peptides with specified properties. The following are some key justifications for employing microorganisms to generate AMP:

Efficiency: Using microorganisms to produce AMP is one of the least costly methods available. Old-fashioned chemical peptide synthesis often requires expensive chemicals, sophisticated apparatus, and numerous processes of cleaning-up, which raises the manufacturing cost. Conversely, microbiological systems may be cultivated on inexpensive medium and bioreactors for fermentation let bacteria proliferate in large numbers, therefore producing a lot of AMPs for a much lower cost. Making AMP from bacteria also reduces the need for costly chemical procedures and additives, therefore streamlining the process even further.

Scalability: Microbial systems can be used to make a lot of since AMPs are very scalable. Once they are tuned for production of AMP, bioreactors may be used to cultivate microorganisms producing a lot of peptides. Making the fermenting process larger is

simple and allows one to produce a lot more AMPs required in industry [10]. Given antimicrobial resistance (AMR) is becoming a more significant issue, this capacity for growth is rather crucial for satisfying the growing need for many antibiotics.

Customizing and genetic manipulation: Particularly bacteria, yeasts, and fungus, microorganisms are rather simple for genetic creation. Scientists may therefore alter bacteria to improve their AMP production. Strains created by genetic engineering may have specific AMP characteristics like greater stability, bioactivity, and resistance to breakdown. Modifying the DNA of bacteria also allows one to include synthetic biochemical pathways that may not be present in nature and produce customised or novel AMPs. Being able to build and improve AMP production types makes it possible to create new antibiotics that are specifically made to fight bacteria that are resistant to them [11].

Complex Changes after Translation: To be fully bioactive, many AMPs need changes after translation, such as glycosylation, cyclisation, or the formation of disulphide bonds. In contrast to bacterial systems, microbial systems, especially yeasts and fungi, can make these changes no matter what. Adding carbohydrate groups to peptides is especially good at yeast systems, which can make them more stable and easier to dissolve. Fungi can make peptides that need to be folded and changed by enzymes in a complicated way. These changes are necessary for the cellular activity of some AMPs to work properly. Because they can do this, bacteria are perfect for making more complicated AMPs that need these changes to work.

C. Key microorganisms used in AMP production

1. Bacteria

Microorganisms like bacteria are often used to make antimicrobial peptides (AMPs) because they grow quickly, are easy to change genetically, and can make a lot of useful chemicals. Many times, bacteria like Escherichia coli, Bacillus subtilis, and Lactococcuslactis are used as hosts for the production of recombinant AMP. This is because their genes are well understood and they can easily produce and release foreign proteins. Escherichia coli (E. coli) is one of the most popular bugs that are used to make AMP [12]. People like this bacteria because it grows quickly, is easy to grow, and has well-known tools for changing its genes. You can change E. coli by adding plasmids that carry AMP genes. Then, using forced expression systems, you can make a lot of AMPs in a short amount of time. Furthermore, E. coli can release proteins into the growth medium, which makes the next steps of processing and purifying AMPs easier. Nevertheless, it can be hard to make some AMPs in E. coli because the peptides might be harmful to the bacteria, which can lower the yield [13]. You can lessen this problem by improving the conditions for fermentation, using inducible promoters, or using stronger bacterial types. Another popular choice for making AMP is Bacillus subtilis, which is a Gram-positive bacteria. Instead of staying in the cytoplasm like E. coli does, Bacillus subtilis naturally releases proteins into the external medium, which makes the cleaning process much easier. Also, different types of Bacillus are known to make a number of antibiotic chemicals, which makes them naturally good for making AMP. The strain can be genetically modified, and altered forms can be made to make more AMP. Bacillus systems are often used to make bigger, more complicated peptides that don't need many changes after they are made [14]. Key bacteria used to make antibiotic peptides (AMPs) are shown in Figure 2. But, like any bacterial system, making some AMPs can still be hard because they can be harmful to host cells and the peptides can become unstable.

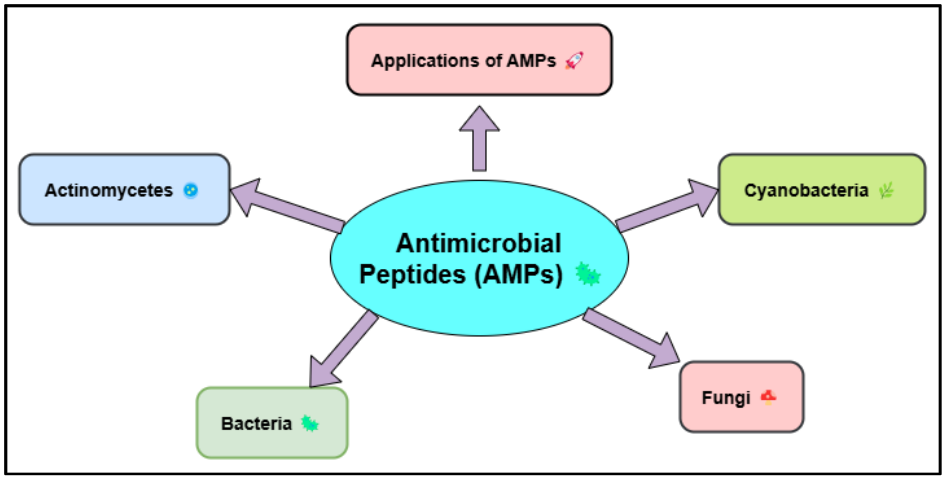

Figure 2: Illustrating key microorganisms used in antimicrobial peptide (AMP) production

A Gram-positive lactic acid bacteria called Lactococcuslactis is often used in food-grade uses, especially in the dairy business. It is also useful for making AMPs. Lactococcuslactis is known for being safe in food production and not making people sick. This makes it a great choice for making AMPs, especially medicinal peptides that people will eat. It can be modified to produce antibiotic peptides from both microbes and humans, and the fermentation process is easy to understand and can be scaled up [15]. Its natural production system also makes it easy for peptides to be released, which makes processing further down the line simpler.

2. Yeasts

More and more, yeasts like Saccharomyces cerevisiae and Pichia pastoris are being used as microbial biofactories to make antimicrobial peptides (AMPs). This is because they can change proteins after they are translated and can be used in large-scale fermentation processes. Yeasts are better at making AMP than prokaryotic bacteria because they are eukaryotic and can handle

complex molecules, like peptides, in ways that prokaryotic bacteria can't. Saccharomyces cerevisiae is one of the most studied yeast species. It has been used to make AMP because it can digest well, is easy to change genetically, and can release proteins into the growth medium. This yeast can naturally make changes after translation, like glycosylation, that are important for keeping many peptides stable, soluble, and bioactive [16]. By adding carbohydrate groups to peptides, S. cerevisiae can greatly improve their stability and activity. This makes it a great choice for making AMPs, which need these changes to work properly. It is also known that S. cerevisiae is a safe organism that can be used in food and medicine, which makes it an even better

choice as a host for producing therapeutic AMPs. A methylotrophic yeast called Pichia pastoris is also often used to make AMP, especially for peptides that need to be folded or changed in complicated ways. Pichia has been improved for high-density fermentation and can make a lot of modified proteins, such as AMPs. Pichia pastoris, unlike S. cerevisiae, can use methanol as its carbon source. This lets it have very dense cell populations, which leads to higher production. This yeast is great for making bioactive AMPs because it can handle complex post-translational changes like glycosylation, disulphide bond formation, or peptide cyclisation. Pichia's ability to release peptides into the growth medium makes the purification process even easier because cells don't have to be broken up or complicated extraction steps have to be done as much.

3. Fungi

Researchers are looking more and more into fungi, especially filamentous species like Aspergillus niger and Trichoderma reesei, to see if they could be used to make antimicrobial peptides (AMPs). AMPs can be made in large amounts by genetically engineering fungi. Fungi naturally make a lot of different secondary metabolites, some of which are antibacterial chemicals. They are better than bacteria and yeasts in many ways, especially when it comes to making more complicated peptides or ones that need a lot of changes after they are made. A well-known cylindrical fungus called Aspergillus niger has been used in industry to make different enzymes and secondary compounds [17]. This organism, A. niger, is a good choice for making AMP because it can release a lot of proteins into the growth medium. Naturally, this fungus can make a number of antimicrobial chemicals, and its genes can be changed to make it make more of certain AMPs. Because it can make peptides that need complex modifications like glycosylation, acetylation, and phosphorylation, it is very useful for making AMPs that need these modifications to work biologically. A. niger has also been studied a lot in industrial settings, which makes the fermentation and production processes pretty easy to understand and easy to scale up [18]. Trichoderma reesei is another filamentous fungus that is known for making cellulases and other enzymes. It is also being looked into for making AMP because it can secrete strongly, which makes it perfect for making a lot of peptides at once. T. reesei is great for making AMPs that need complicated tertiary structures or changes made by enzymes. Table 1 summarizes microbial biofactories, highlighting their roles in producing valuable bioactive compounds. The fact that mushrooms can naturally release proteins into the culture medium makes the purification process simpler, which is a big plus when looking to make a lot of useful peptides.

Table 1: Summary of Microbial Biofactories

Aspect	Related Work	Future Trend	Benefits
Microbial Biofactories	Microbial systems such as bacteria, yeast, and fungi used for AMP production.	Continued development of more efficient microbial systems and alternative strains.	Sustainable and scalable production of AMPs for diverse applications.
Applications	Production of AMPs for treating infections, plant protection, and food preservation.	Expansion of AMP applications beyond infection treatment, including cancer and wound healing.	Non-toxic, biodegradable, and eco-friendly alternative to chemical treatments.
AMP Production	Microbial strains engineered for enhanced production of AMPs using synthetic biology.	Improved yield, stability, and specificity of AMPs with advanced microbial strain engineering.	Microbial systems enable high-yield production at lower costs compared to chemical synthesis.
Pharmaceutical Uses	Clinical trials and preclinical studies using AMPs as therapeutic agents for infections.	Potential for AMPs to address antibiotic-resistant bacteria and fungal infections.	AMPs provide a novel approach to treating infections, including those resistant to antibiotics.
Agricultural Use	Development of AMPs for controlling plant diseases and as biopesticides.	Incorporation of AMPs in crop genetic engineering for disease resistance.	Reduces pesticide dependency and supports organic farming with bio-based solutions.
Industrial Use (Food) [19]	AMPs as natural preservatives in food, extending shelf life and reducing spoilage.	Growing demand for clean-label, preservative-free food products with AMP-based preservation.	AMPs help reduce food spoilage, increase shelf life, and minimize reliance on synthetic preservatives.
Genetic Engineering	Use of CRISPR-Cas and gene editing techniques to enhance AMP production.	Enhanced control over microbial genetic pathways and expression systems for optimized production.	Enhanced microbial strains lead to improved production efficiency and AMP quality.
Synthetic Biology	Creating custom microbial strains using synthetic	Synthetic biology driving innovation in novel, non-natural	Customized AMPs can be designed for specific target pathogens and applications.

	biology to produce novel AMPs.	AMP design and production pathways.	
Metabolic Pathway Optimization	Optimization of microbial metabolism to increase precursor availability for AMP production.	Greater precision in controlling metabolic networks for optimal precursor production.	Optimized metabolic pathways increase the efficiency of AMP production.
Environmental and Culture Conditions	Optimizing temperature, pH, and nutrient levels for improved AMP yield in fermentation.	Integration of AI and data analytics for real-time optimization of fermentation and culture conditions.	Environmental control leads to more consistent yields and higher AMP quality.
Scalability	Development of larger-scale bioreactors and continuous fermentation systems.	Adoption of large-scale bioreactors for continuous AMP production in biotechnological settings.	Larger-scale systems make it possible to meet industrial demands for AMPs.
Cost-effectiveness	Lower production costs compared to chemical synthesis and traditional antibiotics.	Reduction of production costs through process optimization, automation, and bioprocess integration.	Cost-effective and more sustainable than traditional antibiotic production.
Safety and Regulatory Concerns	Regulatory testing, safety evaluations, and GMP standards for AMP-based products.	Clearer regulatory guidelines and streamlined approval processes for AMP-based therapeutics.	Safer, with regulatory processes ensuring high standards for AMP-based products.

MECHANISMS OF AMP SYNTHESIS IN MICROBIAL BIOFACTORIES

A. Genetic pathways involved in AMP synthesis

Made in microbial biofactories via intricate genetic pathways, antimicrobial peptides (AMPs) are These channels regulate the synthesis, processing, and release of genes creating peptides. Although every kind of microorganism follows a series of transcriptional and translational processes that produce bioactive peptides, these channels vary for each one of them. Synthetic biology and metabolic engineering are two examples of genetic engineering techniques that have made it feasible to enhance these mechanisms so that microorganisms can produce more AMP. Usually in bacteria, AMPs are produced via mechanisms under control of ribosomes. One well-known example is the synthesis of lantibiotics, a kind of AMP produced by bacteria like Lactococcus lactis and Streptococcus pneumoniae. Usually, gene groups with a precursor peptide gene code for these peptides. One creates an inactive precursor from this gene. Enzymes convert the precursor peptide to become the adult AMP. Some promoter areas regulate the activation of the genes in these groups in response to outside stimuli include the amount of nutrients, stress, or the existence of germs that are rival with them. It is known that the non-ribosomal peptide synthesis (NRPS) route is another way that genes make AMP.

In this case, NRPSs are big groups of enzymes that work together to make peptides. These peptides are put together step by step through enzymatic processes. Some filamentous fungi, like Aspergillus and Penicillium species, use these routes all the time. They make secondary substances that kill microbes. The NRPSs are modular enzymes made up of different regions that are in charge of adding certain amino acids to the peptide chain. By changing the enzymes' substrate selectivity, genetically manipulating these NRPS pathways makes it possible to make new AMPs. This is useful for making peptides with specific antibacterial properties. Similar processes involving precursor peptides are used by yeast and fungi systems. However, these systems also include the host's own post-translational changes to improve AMP activity. AMP production is often controlled by modified plasmids that carry genes for the AMP of interest in yeasts such as Saccharomyces cerevisiae and Pichia pastoris. The genes are then copied and turned into peptides. The yeast's own enzymes may change these peptides even more to make sure they fold correctly and stay stable. Changing these systems genetically can improve the structure of the precursor peptide to help it secrete better or make the end product more stable and effective.

B. Enzymatic processes for AMP production

Enzymatic processes are very important for making antimicrobial peptides (AMPs) in microbial biofactories because they turn raw peptides into useful chemicals. Some of these processes are peptide maturity, post-translational changes, and breaking of peptide precursors. They are all necessary to make functional AMPs that can kill microbes. Depending on the type of bacteria and the AMP that is being made, these steps involve different enzymes. Proteolytic cleavage is one of the most important enzyme processes in the production of AMP. A lot of AMPs are made as precursor peptides that are not active. To become active, they need to be cut up into signal sequences or propeptides. There are proteases, like procaspases and lipases that are often involved in this step in bacterial systems. Bacteriocins are a type of AMPs that are made by Lactococcuslactis. They are made by cutting the precursor peptide by certain proteases, which frees the active antimicrobial fragment. In the same way, Escherichia coli, a Gram-negative bacteria, can make AMPs with signal sequences that can be cut. These are then handled by special bacterial proteases during or after release. AMP that is physiologically active needs to be made through this breakdown. Along with protease breakdown, changes to the structure of the peptide are also necessary for AMPs to work properly. One of these changes is cyclisation, which turns the peptide into a circle shape that makes it more stable and less likely to be broken down by enzymes. At this point, enzymes like cyclases speed up the process by helping the N- and C-termini of the peptide form a chemical link. Some AMPs, like lantibiotics and defensins, are often made through cyclisation, which makes them more resistant to proteolysis

and better at killing microbes. Glycosylation is another important enzymatic change that happens when carbohydrate molecules are added to the backbone of a peptide.

Step 1. Synthesis of Peptide Precursors (Gene Expression & Translation)
mRNA → Ribosomes → Precursor Peptide
Step 2. Cleavage of Signal Peptide (Signal Peptidase Activity)
Precursor Peptide → Signal Peptidase → Mature Peptide + Signal Peptide
Step 3. Cyclization (Cyclase Enzyme Activity)
Linear Peptide → Cyclase → Cyclic Peptide
Step 4. Glycosylation (Glycosyltransferase Activity)
Peptide + Sugar → Glycosyltransferase → Glycosylated Peptide
Step 5. Disulfide Bond Formation (Disulfide Isomerase Activity)
Peptide with Cysteine Residues → Disulfide Isomerase → Peptide with Disulfide Bonds
Step 6. Protease Cleavage for Activation (Protease Activity)
Inactive Precursor Peptide → Protease → Active AMP + Cleaved Fragments
Step 7. Secretion (ATP-binding Cassette (ABC) Transporters)

$$Mature\ AMP\ +\ ATP\ \rightarrow\ ABC\ Transporters\ \rightarrow\ AMP\ in\ Extracellular\ Medium$$

C. Regulation of AMP biosynthesis in microorganisms

Microbial biofactories synthesis antimicrobial peptides (AMPs). Many mechanisms carefully regulate them to ensure proper synthesis in response to environmental cues. This guarantees accurate formation of these molecules. When AMPs are needed, microorganisms may create them particularly in response to stress—that which results from infection or proximity to rival species. Understanding and modifying how AMP production is regulated would help one to maximise the benefits from industrial microbial systems producing these peptides. In bacterial systems, two-part signal transmissions methods are often utilised to regulate AMP synthesis. Usually operating in these systems is a sensor kinase. It gathers cues from the surroundings, such as those related to the availability of nutrition or the presence of infections. These signals subsequently switch on a response regulator, which initiates the synthesis of AMP-producing genes. In Streptomyces species, for example, a two-part system monitors cell count and initiates the expression of AMP genes in response to quorum sensing that is, when a certain number of bacteria are present controlling the synthesis of lantibiotics. Another often used method by bacterial systems to regulate events is the interaction of activator and repressor proteins. Attaching to promoter areas in the AMP gene clusters; these proteins control the activation of genes generating AMP. Sometimes activators are required to increase AMP synthesis when the microorganism is under stress or is competing with another microbe; repressors halt the transcription of AMP genes when things are going well. For instance, Bacillus subtilis produces certain AMPs in response to environmental stimuli such pH fluctuations or the presence of other bacteria. An activator protein controls this. In yeast and fungi, the management of AMP generation is often more difficult as epigenetic modifications and transcription factors are involved.

METHODS OF ENHANCING AMP PRODUCTION IN MICROBIAL BIOFACTORIES

A. Genetic engineering approaches

1. CRISPR-Cas technology

By making it simpler, quicker, more flexible to alter the DNA of germs used to create antimicrobial peptides (AMPs), Crispen-Cas technology has revolutionized genetic engineering. First discovered as a means of bacterial self-defence against infection were CRISpen-Cas systems. These days, they are extensively used to modify certain genes. By altering genes involved in peptide biosynthesis, CRISpen-Cas systems may be employed in microbial biofactories to increase AMP generation, therefore strengthening strains' resistance to harm, and modifying metabolic pathways to generate more. Among the primary applications of CRISpen-Cas technology in AMP manufacturing is gene editing. Scientists may utilise CRISpen-Cas9, for example, to eliminate genes that interfere with AMP synthesis—that is, those related to stress or metabolic processes that rob resources away from producing peptides. Turning down these genes helps more of the resources of the microorganisms to be directed towards AMP synthesis. Additionally utilised to introduce AMP generating genes to the genome of bacteria or enable these genes to function better is Crispen-Cas9. Furthermore, this increases the synthesis of AMPs by ensuring sufficient of the required enzymes or precursor peptides.

The ability of CRISpen-Cas systems to simultaneously create accurate alterations to the genome is among their finest features. Unlike more conventional techniques like as random mutagenesis or normal plasmid-based cloning, CRISpen-Cas9 enables you modify only certain genomic regions, therefore reducing the possibility of unintended consequences. This precise editing in microbial biofactories reduces the possibility of introducing harmful characteristics or undesired modifications, therefore stabilising production strains. Furthermore, synthetic biology techniques—which modify whole AMP production pathways in bacteria—can be used using CRISpen-Cas9. Synthetic operons containing multiple genes involved in producing sophisticated AMPs, for instance, may be inserted into the genome of a host microorganist to facilitate the expression and release of those genes. Beyond just adding or deleting genes, Crispen-Cas systems may do. They can also boost the activity of genes that make AMP. CRISPR interference (CRISPRi) can stop the translation of negative regulators by hitting the promoter regions of these genes. This makes it easier for genes that make AMP to be translated. This method works especially well for fine-tuning AMP creation in response to things in the surroundings, like stress or the supply of nutrients.

Step 1. Identification of Target DNA Sequence
- A specific DNA sequence (target sequence) is identified in the genome to be modified.

- Target DNA = Sequence to be edited
Equation:
Target DNA = Specific sequence in genome
Step 2. CRISPR RNA (crRNA) Synthesis
- The CRISPR array is transcribed into a precursor RNA (pre-crRNA), which is processed into smaller crRNA molecules that guide the Cas9 enzyme.
Equation:
CRISPR Array → Pre-crRNA → crRNA (guide RNA)
Step 3. Formation of Cas9-crRNA Complex
- The Cas9 protein binds with the crRNA to form the Cas9-crRNA complex, which guides Cas9 to the target sequence.
Equation:
Cas9 + crRNA → Cas9-crRNA Complex
Step 4. Target DNA Recognition and Binding
- The Cas9-crRNA complex recognizes and binds to the target DNA sequence complementary to the crRNA.
Equation:
Cas9-crRNA Complex + Target DNA → Cas9 Binding to Target DNA
Step 5. DNA Strand Cleavage
- Cas9 introduces a double-strand break (DSB) in the DNA at the target site.
Equation:
Cas9 + Target DNA → Cas9 Cuts DNA → Double-Strand Break (DSB)
Step 6. DNA Repair Mechanism Activation
- The cell activates DNA repair mechanisms (e.g., Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR)) to fix the break.
Equation:
Double-Strand Break (DSB)→ Repair Mechanism (NHEJ or HDR)
Step 7. Insertion/Deletion/Modification
- If HDR is used, a template DNA sequence can be provided for precise editing (insertion of new genes or modifications).
- If NHEJ is used, insertions or deletions may occur, leading to a loss of function or gene knockout.
Equation:
HDR (with Template)→Insertion/Modification of DNA
OR
NHEJ → Indels (Insertions/Deletions)
Step 8. Resulting Modified DNA
- The DNA is repaired, and the desired genetic modification (edit, knockout, insertion) is now integrated into the genome.
Equation:
Modified DNA = Desired Genetic Edit

2. Overexpression of biosynthetic genes

Another very effective genetic engineering method to boost the production of antimicrobial peptides (AMPs) in microbial biofactories is to overexpress metabolic genes. It is possible for researchers to make a lot more of these beneficial molecules by boosting the output of genes that help make AMPs. This method can be used with different microorganisms, like bacteria, yeasts, and mushrooms, to improve the production process and make the microbial creation of AMPs more efficient. Strong promoters are often used to make target genes produce themselves at a high level, which leads to overexpression of metabolic genes. To make AMP genes work better in bacteria like Escherichia coli and Bacillus subtilis, strong promoters like T7 or P43 are often used. It is the job of these promoters to make sure that a lot of transcription happens so that enough of the AMP precursor is made. For instance, in E. coli, recombinant plasmids carrying the AMP gene of interest are put into the host cells. Strong regulators then help the peptide be highly expressed. Overexpression of this peptide can cause it to build up in the microbe cells or leak out into the surrounding medium, based on the systems that are in place for release. Figure 3 shows how metabolic genes can be overexpressed to make more antibiotic peptides.

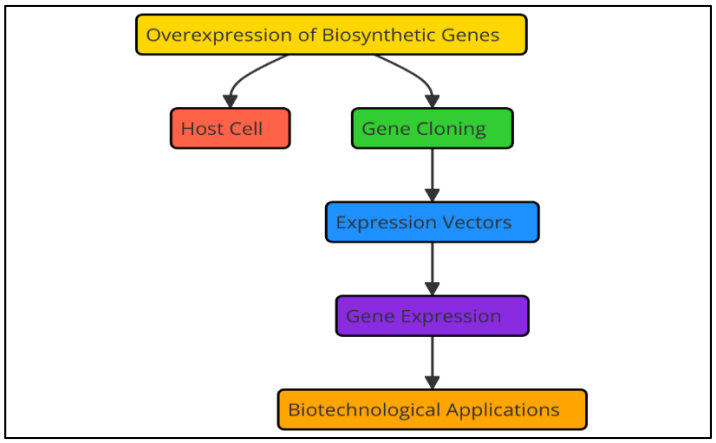

Figure 3: Illustrating the overexpression of biosynthetic genes

For more complicated peptides that need changes or folding after translation, like those made in yeasts or mushrooms, promoter optimisation is very important. Yeasts like Saccharomyces cerevisiae and Pichia pastoris are often modified to produce biosynthetic genes from other living things. To increase AMP production, strong, inducible promoters like AOX1 (methanol-inducible) or GAL1 (galactose-inducible) are used. These promoters allow limited translation, which means that the genes are only turned on when they are needed. This keeps the microbial system from getting too busy. This managed increase makes sure that the machinery inside the cells doesn't get too busy and that resources are used well for making peptides. Overexpressing AMP synthesising genes in fungi like Aspergillus niger or Trichoderma reesei can be done by putting the genes into high-expressing sites or making the promoters that make them work better. It is known that these fungi can release proteins into the culture medium. This makes them perfect for making AMPs that need to be folded or changed in complex ways. If you add more than one copy of the AMP gene or use strong promoters, you can speed up the biosynthesis machinery in these fungi, which will make more of the chemical you want.

B. Metabolic pathway optimization

One important way to improve the production of antibacterial peptides (AMPs) in microbial biofactories is to optimise metabolic pathways. Microorganisms make AMPs and other useful chemicals by using complicated metabolic networks. Researchers can guide the microorganism's metabolic flow towards making AMPs by studying and changing these metabolic pathways. This increases both the yield and efficiency of production. To make metabolic pathways work better, the core metabolic network needs to be fine-tuned, biosynthetic pathways need to be engineered, and intermediates need to be sent to the right pathways less often. In the process of making AMP, one of the main goals of metabolic pathway optimisation is to make sure that there are plenty of the precursor molecules that are needed for AMP formation. This is done by improving the processes that make amino acids, cofactors, and energy sources that are needed for the production of peptides in bacteria like Escherichia coli or Bacillus subtilis. Amino acids, which are the building blocks of peptides, are often needed in large amounts for the production of AMPs. By improving the amino acid biosynthesis pathway, scientists can speed up the flow of molecules that are used to make important amino acids like leucine, tryptophan, and methionine. Either essential enzymes that aid in the synthesis of amino acids are overexpressed or mechanisms using these amino acids for other purposes are disabled to accomplish this. Apart from producing amino acids, the carbon cycle of the microorganism is also rather crucial for generating AMP.

Microorganisms initiate the process of biosynthesis and generate energy and biomass from carbon sources like glucose, glycerol, and methanol. By means of bettering the carbon metabolism path, researchers may ensure appropriate usage of carbon to produce AMP. Changing the carbon supply and using the metabolic pathways that convert this carbon into the building blocks for AMP synthesis helps the carbon flow in yeasts like Pichia pastoris to be altered from growth to peptide synthesis. One approach to maybe enhance metabolic pathways is flux balance analysis (FBA). It forecasts and enhances chemical movement across microbial networks by use of mathematical models. FBA helps researchers determine how variations in one component of the metabolic network could influence AMP generation generally. By modelling various genetic variations and environmental variables using computer tools, scientists may determine the optimal strategies to increase AMP generation. This allows one to employ customised metabolic engineering to increase outputs and reduce the manufacturing of waste products, therefore addressing a common issue in large-scale microbial fermentation. Also, lowering the production of byproducts is an important part of optimising metabolic pathways. While fermentation is going on, many microorganisms, like bacteria and yeasts, make waste products like organic acids (like acetic acid). Table 2 summarizes metabolic pathway optimization strategies to enhance antimicrobial peptide production in microorganisms.

Table 2: Summary of Metabolic pathway optimization

Aspect	Related Work	Key Finding	Challenges	Scope
Metabolic Pathway Optimization	Optimizing microbial metabolism to enhance production of AMPs.	Efficient metabolic pathway design can significantly increase AMP yields.	Balancing the redirection of metabolism while maintaining microbial viability and growth.	Metabolic pathway optimization holds immense potential for increasing AMP yields and expanding the range of peptides produced.
Gene Overexpression	Overexpression of key biosynthetic genes to boost production efficiency.	Gene overexpression boosts the availability of AMP precursors, improving production.	Achieving high expression levels without causing metabolic imbalance or toxicity.	Gene overexpression techniques can be applied to optimize the production of a wide variety of AMPs.
Amino Acid Biosynthesis	Increasing amino acid production pathways for higher peptide yields.	Targeted amino acid biosynthesis pathway optimization leads to higher peptide synthesis.	Ensuring that amino acid biosynthesis pathways do not interfere with other essential metabolic processes.	Amino acid pathway optimization can provide a foundation for the development of cost-effective peptide production platforms.
Carbon Metabolism Engineering	Redirection of carbon flux to enhance AMP precursor availability.	Redirection of carbon flux reduces metabolic bottlenecks and increases AMP production.	Controlling carbon flux precisely in order to optimize precursor production for AMPs.	Carbon flux redirection can be adapted to improve a variety of microbial systems for AMP production.

Pathway Reconstruction	Reconstructing metabolic networks in microorganisms to produce novel AMPs.	Metabolic network reconstruction enables the design of microorganisms for novel AMP production.	Complexity of metabolic network reconstruction and ensuring that engineered pathways function as expected.	Pathway reconstruction enables the design of microbes with novel capabilities for AMP production, opening new opportunities.
Flux Balance Analysis (FBA)	Use of computational models to optimize metabolic flux and improve AMP yields.	FBA models can predict and optimize metabolic flux to enhance the efficiency of AMP production.	FBA optimization requires accurate modeling and may not always predict real-world outcomes.	FBA can be used to identify the most efficient metabolic routes for AMP synthesis, offering clear directions for optimization.
Optimizing Nutrient Utilization	Optimizing nutrient media to balance microbial growth and AMP production.	Optimizing nutrient utilization leads to more efficient use of resources, boosting AMP yields.	Nutrient optimization can be difficult, as variations in media can lead to inconsistent results.	Nutrient optimization strategies can be broadly applied to various microbial systems to boost AMP yields.
Reducing Byproducts	Modifying microbial pathways to reduce unwanted byproducts during fermentation.	Reducing byproducts in fermentation processes improves the overall yield of AMPs.	Minimizing byproducts requires careful metabolic tuning, which may be resource-intensive.	Minimizing byproducts is crucial for reducing costs and improving the sustainability of AMP production.
Metabolic Engineering in Yeast	Optimizing yeast metabolism to improve AMP synthesis efficiency.	Yeast systems can be engineered to balance growth and AMP production, increasing efficiency.	Yeast systems may require complex manipulation of their native pathways, leading to unpredictable results.	Yeast engineering for AMP production is a promising area with potential for large-scale, cost-effective synthesis.
Metabolic Engineering in Bacteria	Engineering bacterial systems for optimized AMP biosynthesis.	Bacterial strains engineered with optimized metabolic pathways improve AMP yields and stability.	Bacterial systems can face challenges in optimizing metabolic pathways without causing instability or toxicity.	Bacterial systems provide a versatile platform for AMP production, with ongoing improvements in metabolic engineering.
Microbial Strain Development	Developing new microbial strains with enhanced metabolic pathways for AMPs.	Custom-engineered microbial strains can produce higher yields of specific AMPs.	Development of microbial strains with enhanced metabolic pathways may require extensive screening and iteration.	Microbial strain development is key to meeting the growing demand for AMPs across industries.
Synthetic Biology Tools	Leveraging synthetic biology tools to design and optimize AMP-producing pathways.	Synthetic biology allows for the design of efficient, customizable biosynthetic pathways for AMPs.	Synthetic biology approaches require high precision in designing biosynthetic pathways and may encounter unexpected challenges.	Synthetic biology offers a powerful framework for designing customized AMP biosynthetic pathways tailored to specific needs.
Scalability and Process Integration	Scaling up optimized processes for industrial-level AMP production.	Optimized metabolic systems can be scaled up for large-scale AMP production, reducing costs.	Scaling up optimized processes while maintaining efficiency, stability, and low costs is a significant challenge.	The future of AMP production lies in integrating optimized metabolic systems into large-scale production processes.

C. Environmental and culture conditions influencing AMP yield

The climate and culture conditions in which bacteria are growing have a big impact on how antimicrobial peptides (AMPs) are made in microbial biofactories. These things are very important for controlling the activity of metabolic genes that make AMP, as well as the general output of the desired peptides. Making the best choices about things like temperature, pH, food supply, air levels, and the type of growth medium is important for getting the most AMP out of microbial systems. One of the most important things that affects the growth of microbes and the production of AMP is temperature. There is a best temperature range for most microbes where they can grow the fastest and make the most chemicals, including AMPs. For example, Bacillus subtilis and Escherichia coli flourish at rather 37°C. Conversely, yeasts such as Saccharomyces cerevisiae and Pichia pastoris thrive at somewhat lower temperatures, perhaps 25 to 30°C. But temperature also affects the stability of the created AMPs. While certain AMPs are more stable at lower temps, some may need higher temperatures to fold correctly and remain functional. The fermentation temperature has to be adjusted to balance peptide lifetime with bacterial development for optimum AMP outputs. Furthermore heavily influencing the synthesis of AMP is the pH of the growing media.

Many bacteria thrive in somewhat acidic or neutral pH levels. The pH, however, may also affect the stability and dissolution ability of the AMP. Like certain AMPs, which are ubiquitous in environments where some bacteria thrive naturally, others might be more suited in acidic surroundings. Conversely, certain forms of peptides could need a somewhat neutral or slightly alkaline environment. Fermentation produces organic acids, hence the pH could change. This can halt the generation of AMPs and stop the growth of bacteria. Usually, one adds buffers or modifies the composition of the culture medium to maintain the pH level at the ideal range for both bacterial growth and AMP generation and monitor on it.

It is the content of nutrients in the growth media also influences AMP generation in great significance. Microbes mostly need carbon sources (like glucose and glycerol), nitrogen sources (like ammonium salts and peptone), and trace elements (like magnesium and potassium). Driving metabolic pathways towards the synthesis of AMP depends also on the concentration of carbon to nitrogen in the growth media. While low nitrogen levels may generate secondary compounds like AMPs, microorganisms thrive in conditions rich in carbon. Microbial fermentation makes extensive use of this phenomenon, in which the culture medium is altered specifically to reduce nitrogen availability, therefore initiating the synthesis of AMPs. Similarly, the amount of vital minerals and cofactorues such as magnesium, manganese, and zinc may affect the overall efficacy of AMP production as well as the activity of enzymes.

APPLICATIONS OF AMPS FROM MICROBIAL BIOFACTORIES

A. Pharmaceutical applications

Made by microbial biofactories, antibiotic peptides (AMPs) offer great potential in the medical industry particularly as alternative medications to combat the developing threat of antibiotic resistance (AMR). Research on AMPs' possible application in treating diseases brought on by resistant to many medications organisms is under progress. This is so because they fight a broad spectrum of pathogens, including viruses, bacteria, fungi, and parasites. In many respects, pharmaceutical-grade AMPs are superior than ordinary antibiotics. They kill germs in a larger variety of methods, for instance, quicker, and there is less likelihood that resistance would develop. AMPs find most frequent usage in medications to combat bacterial-caused diseases. Because they may break down bacterial cell walls, AMPs including defensins, cathelicidins, and polymyxins are under investigation as therapies for both Gram-positive and Gram-negative infections. For example, polymyxins are effective in killing immune to many medications Gram-negative bacteria such as Escherichia coli and Klebsiella pneumoniae. But additional issues like neptrotoxicity have forced these peptides to be altered to increase their specificity and reduce their toxicity. Perfect for this is microbial biofactories. AMPs are under testing for their capacity to combat illnesses brought on by more than only bacteria. Common antifungal medications often fail to treat fungal disorders, including species of Candida. This explains the need of discovering fresh therapies. Like histatins, AMPs are known to destroy fungus and are under investigation as potential component in creams used to cure fungal illnesses. Against many viruses, including HIV, influenza, and herpes simplex virus, AMPs including human defensins have demonstrated efficacy. They either directly neutralise viral particles or inhibit viruses from entering host cells. Another crucial field in which AMPs may be used is the treatment of skin infections and continuous wounds. This is so because they aid in healing of wounds and eliminate germs. One such a cathelicidin with both antibacterial and immunomodulation properties is AMP LL-37. This makes it an excellent alternative for healing wounds contaminated with antibiotic-resistant bacterial species.

B. Agricultural use

Because they may be used either in place of or in addition to conventional herbicides and medications to battle plant germs and animal illnesses, antimicrobial peptides (AMPs) are increasingly being used in agriculture. There is much potential in substitutes for conventional agricultural toxins known as AMPs. They less likely cause bacterial resistance and are beneficial for the surroundings. The antibacterial properties of AMPs make them quite helpful for animal care and plant protection. They may enhance animal welfare and aid to reduce food losses. Natural means of keeping plants safe from a variety of bacterial, fungal, and viral illnesses are attracting increased interest for AMPs. Chemical toxins contaminate the environment and leave behind potentially lethal remnants. Conversely, AMPs break down organically and have no effect on good bugs, animals, or humans. Big crop diseases are caused by plant pathogens include species of Fusarium and Xanthomonas. Promising in their management of these infections are AMPs including chitinases, defensins, and thaumatin. Genes that produce AMP may be incorporated into crops via genetic engineering. This renders plants disease-free, therefore eliminating the need for chemical herbicides. For instance, transgenic plants containing AMP genes are more suited to combat infections like Pseudomonas syringae, which causes tomato sickliness with bacterial speck disease.

Another approach AMPs may be used is with biocontrol products like biopesticides. One may apply them straight on genetically modified plants. These peptides may be blended into the ground or applied on crops to reduce the count of bacteria and halt diseases. Since synthetic chemicals are forbidden, many organic farmers find it beneficial to substitute AMPs as biopesticides instead. AMPs are a better option than chemical toxins for the environment as they originate naturally and do not linger there. AMPs are being investigation as potential alternatives to antibiotics in animal husbandry for preventing diseases and treating animals should they arise. Too many medications used in animal farms have let pathogens resistant to antibiotics flourish. This seriously compromises human and animal health. AMPs have potential as a means to prevent animals from acquiring diseases without aggravating medication resistance as they destroy a broad spectrum of bacteria. Salmonella, Escherichia coli, and Staphylococcus aureus-caused illnesses in chickens, pigs, and cattle have been treated using peptides including defensins and cathelicidins. It is possible to add AMPs to animal feed or make them a part of animal health goods to boost their immune systems and lower the need for medicines. Additionally, AMPs are being researched for their ability to help animals grow. Although some AMPs are antibiotic, they can also help animals stay healthy and grow faster by affecting their immune systems and reducing inflammation. AMPs can help make farming more sustainable and efficient by improving animal health and lowering the number of infections that happen.

C. Industrial applications (e.g., food preservation)

Antimicrobial peptides (AMPs) are becoming better known for the industrial uses they could have, especially in food preservation, where they can be used instead of chemical additives because they are safe and natural. People want barely prepared, chemical-free foods more and more, which has sparked interest in using AMPs to keep food safe, extend its shelf life, and stop it from going bad. Some of the best things about AMPs for food preservation are that they are biodegradable, have broad-spectrum antibacterial action, and are not harmful to people. When it comes to food preservation, AMPs can stop the growth of germs that make food go bad, like bacteria, yeasts, and moulds. Traditional chemical stabilisers, such as sulphur dioxide and sodium benzoate, work well, but they often hurt people or the environment. AMPs, on the other hand, break down naturally and can be used in very small amounts without harm. These chemicals can be used on meats, cheese, fruits, and veggies, among other foods, to kill microbes and keep people from getting sick from food. AMPs, such as nisin and nisin-like peptides, work especially well against Gram-positive bacteria like Listeria monocytogenes, which is a disease that is often found in dairy and deli foods. Clusters of bacteria stuck to surfaces are called biofilms. They are a big problem in places that process food because they are hard to clean and can make the food dirty. AMPs can be used to cover food-contact surfaces, like tools, packing, and food processing equipment, to stop biofilm formation because they can break down bacterial walls. Antimicrobial peptides (AMPs) are used in health, gardening, and food protection, as shown in Figure 4.

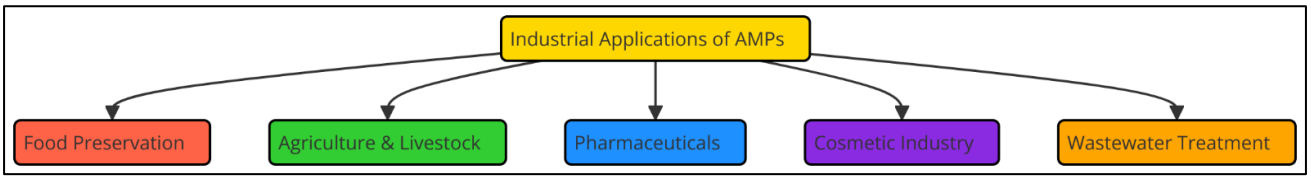

Figure 4: Illustrating the industrial applications of AMPs

Although AMPs are antibacterial, they may also be employed to create edible coatings and packaging materials extending food freshness for more. These coatings may prevent moisture, air, and microorganisms from proliferating, therefore preserving goods fresh and of quality.

CHALLENGES AND LIMITATIONS

A. Scaling up production

Although producing more antimicrobial peptides (AMPs) from microbial biofactories offers advantages, there are several challenges with this process. Microbial systems readily in the lab that may produce AMPs include Escherichia coli, Pichia pastoris, and Bacillus subtilis. But when they go to industrial-scale manufacturing, issues sometimes surface that must be delicately resolved. Getting high rates while maintaining peptide quality is one of the toughest aspects of increasing AMP. Small-scale fermentation techniques in the lab help to more precisely control parameters like temperature, pH, and nutrient availability. Conversely, when huge bioreactors are employed in industries, maintaining conditions steady becomes more difficult. Variations in external elements may lead to reduced output and unequal AMP quality produced. For large-scale fermentation, for example, oxygen transfer rates might be a challenge as they can slow down the development of microorganisms and peptide synthesis—particularly in aerobic bacteria. Low oxygen levels may lead to metabolic alterations that either inhibit the synthesis of AMPs or result in the accumulation of byproducts harming output. Additional issue is cell damage. Many AMPs are detrimental to bacteria; when present in large quantities, they may prevent the host germs from proliferating and producing food. This is favorable for the killing power of the peptides against germs, but it might be challenging when large-scale fermentation is conducted. To fix this, scientists need to find the best ways to ferment things so that the amount of AMPs is balanced with the cells' ability to stay alive. Some methods, like controlled induction, which starts the production of AMP at certain stages of bacteria growth, can help solve this issue. To make sure that high-yielding, non-toxic types are always being made, genetic engineering methods that lower cell toxicity or increase resistance to AMPs may also be needed.

B. Economic feasibility

How economically practical it is to produce antimicrobial peptides (AMPs) using microbial biofactories determines whether they may be used broadly for medicinal, agricultural, and industrial applications. Although microbial systems offer significant advantages—lower production costs than chemical synthesis—the whole economics of AMP manufacture still present major issues that must be resolved before the technique can be used in the actual world. One of the key factors influencing AMP cost of manufacture is raw materials. Microbiological fermentation requires certain nutrients—carbon sources (such as glucose and glycerol), nitrogen sources (such as ammonium salts and peptone), and key minerals. Particularly in large numbers of AMPs, some of these components may be somewhat costly. For instance, the complex medium needed to grow bacteria and produce AMP often contain expensive components that increase the cost. Maintaining the proper circumstances for bacterial development in large bioreactors—that is, managing temperature, pH, and air levels—also increases manufacturing costs. Another major factor influencing the economic viability of AMP is the cost of fermentation. Quick growth and abundance of AMPs make microorganisms such as Escherichia coli, Bacillus subtilis, and Pichia pastoris appealing. But for large-scale fermentation to take place, bioreactor systems must be very sophisticated and the microbiological environment must be continuously monitored.

Setting up, running, and managing these systems comes with a lot of expenses that compound manufacturing costs. Large-scale fermenting techniques may sometimes have significant energy expenses. This is so because, particularly for stirring, providing air, and temperature regulation, maintaining large bioreactors in the ideal conditions for development requires a lot of energy. Cleaning and filtering AMPs farther down the line adds even another significant cost element. Getting AMPs out of the fermenting broth and cleaning them is usually a time-consuming and costly task after fermentation. For instance, while they are somewhat common techniques, chromatography and filtering need certain chemicals and equipment to operate. This makes

AMP's manufacturing greatly influenced by cleaning expenses. Furthermore crucial is ensuring that the purification techniques generate high-purity peptides devoid of much loss of their activity. This may be challenging, particularly with reference to complex or large-scale fermenting systems. Getting the market to embrace it and competing with already-available therapies provide the remaining challenges. Although AMPs offer great promise as substitutes for conventional antibiotics, their manufacturing costs must be around the same as those of well-known antibiotics like produced antibiotics. The great expenses of creating AMPs, particularly in relation to mass-produced antibiotics, might discourage individuals from utilising them unless these expenses could be reduced. Conversely, if the market for AMPs expands, economies of scale might enable over time a reduction in manufacturing costs, therefore making the process more rational.

C. Regulatory concerns and safety considerations

Particularly the food and pharmaceutical industries have several safety issues and regulations that complicate the development and marketing of antimicrobial peptides (AMPs) produced by microbial biofactories. Making sure AMPs are safe, efficient, and meet all legal guidelines is highly crucial as they are under consideration for prospective applications in medicine including treating bacterial infections, fungal disorders, and even chronic wounds. Getting regulatory permission for pharmaceutical usage is a difficult and protracted procedure. AMPs have to be extensively tested if they are to be safe and effective in individuals. After pharmacology and immunology, animal tests to look for toxicity, follow-up research to examine how effectively the peptides cure ailments. Approval of novel therapy agents is governed strictly by regulatory agencies such the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA). AMPs have to do a lot of study if they are to satisfy these criteria. Particularly in microbiological systems, it is somewhat difficult to guarantee that AMPs are always of great quality and purity. Close observation of the manufacturing process is necessary to prevent microorganisms or other contaminants that can cause illness from ruining it.

Making AMPs in microbial systems may also provide challenges as minute variations in microbial strains or fermentation conditions might affect the amount and quality of the resultant product batch to batch. Microbial systems must be developed to satisfy government criteria so they may be repeatedly employed and throughout the full manufacturing process fresh quality control procedures must be implemented. To keep the product safe and consistent, it will be necessary to follow Good Manufacturing Practice (GMP) rules. When AMPs are approved by the government, safety is also a very important factor. Even though AMPs are found naturally and are usually thought to be safe, they need to be carefully studied for any side effects that might happen when they are used as medicines. These effects could include cytotoxicity and inflammation. Some AMPs can be harmful to human cells at high levels, and being exposed to them for a long time may cause unwanted side effects like swelling or allergic responses. Because of this, full safety tests are needed to figure out what risks might be involved with AMP-based medicines. In particular, chronic toxicity studies need to be done to look at the long-term effects of AMP treatments, especially if they are meant to be used over and over to treat cuts or infections that don't heal. A similar level of strict rules apply to food and farming uses. Safety criteria for AMPs used as preservatives or in animal feed are established by the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA). These AMP criteria must be satisfied. Safety issues centre on the possibility of allergenicity, consumer injury, and environmental damage. Although most people consider AMPs to be harmless, they must undergo extensive testing to ensure they are so before they can be certified by regulatory authorities. These tests include search for allergic reactions, development of antibiotic tolerance, and environmental chemical longevity.

D. Resistance development against AMPs

Using antimicrobial peptides (AMPs) causes one of the primary concerns about possible resistance of bacteria to them. Regular antibiotics have become useless to bacteria; they may also develop resistance to AMPs. AMP resistance may manifest itself in very different ways than those of resistance to conventional antibiotics. Still, these variations make it difficult for AMP-based therapies to be long-term viable. Several mechanisms exist how bacteria could develop resistance to AMPs. Many AMPs have as their primary target the bacterial cell membrane, which is sometimes modified as a tactic. Reducing the negative charge or adding more stiff lipids among other modifications to the composition of the membrane might make it more difficult for AMPs to enter the membrane and disrupt the cell. Using this resistance mechanism have been seen pathogens including Staphylococcus aureus and Pseudomonas aeruginosa. Their membranes may be shaped so that AMP cannot link and activate them. Another method resistance operates is by the breakdown of AMPs by means of enzymes. Some bacteria produce peptidases or proteases that cleave the peptide bonds in AMPs, therefore preventing their activity. Enterococcus faecium and other Gram-positive bacteria, for example, produce proteases that target and breakdown AMPs including defensins and cathelicidins.

This breakdown by enzymes may make it difficult to employ AMPs efficiently, particularly in hospitals where vast quantities are required to cure illnesses. Bacteria often use efflux pumps to get rid of AMPs that have already entered the cell. A lot of bacteria have special systems called efflux systems that move different types of antimicrobials, like AMPs, out of the cell. This stops the peptides from reaching their targets. This process is especially bad for Gram-negative bacteria, whose cell walls are more complicated and which have efflux pumps that can get rid of AMPs well. Combination treatments are being looked into as a way to lower the chance of resistance. Using AMPs along with regular antibiotics or other germ-killing substances might help stop or slow the development of tolerance. Combination medicines make it less likely that bacteria will become resistant to all parts of the treatment by going after different parts of the bacterial cell. A lot of work is also being done on logical design of AMPs, which includes changing their structure to make them less vulnerable to bacterial defence mechanisms. The goal is to find ways to get around resistant problems.

FUTURE PROSPECTS AND INNOVATIONS

A. Advanced biotechnological methods for AMP production

Making more improved biotechnology methods that are more efficient, scalable, and cost-effective is very important for the future of making antibacterial peptides (AMPs). As the need for AMPs grows because they can help fight antibiotic resistance (AMR) and are used in many fields, biotechnology innovations are needed to meet this demand and get past the problems that come up when making a lot of them. Using engineering concepts to create and build new living systems is what synthetic biology tries to do. It is a hopeful area of research. Synthetic biology lets scientists make new AMP biosynthesis pathways, which lets them make peptides with specific qualities. For instance, genes may be altered in bacteria to produce more stable AMPs with higher antibiotic properties or less prone to break down. Synthetic biology allows genetic circuits to be tweaked to produce AMP and sets up on-demand translation systems to generate peptides only when needed. This approach lowers the energy burden on the microbial house while nevertheless increasing production. Another field with great potential to improve AMP production is metabolic engineering. By altering the metabolic routes of the host, scientists may enhance the flow of precursor molecules towards AMP synthesis.

For example, targeted modifications in the metabolic network of the host may enable it to generate too many amino acids and cofactor required for peptide synthesis. Finding the optimum methods for microorganisms to utilise carbon and nitrogen is another aspect of metabolic engineering; this directly influences the generation of AMPs. This is particularly crucial as industrial demands call for more output to fulfil. Computational technologies like as flux balance analysis (FBA) allow researchers to simulate and estimate how certain genetic modifications may impact the overall metabolic flow. AMP manufacturing may therefore be much more efficient. Still another significant advance are cell-free protein synthesis systems. These technologies generate AMP outside of living cells in an environment rich in amino acids, tRNAs, and ribosomes, therefore facilitating protein synthesis. This approach solves the issues related to maintaining live microbe cultures alive in terms of nutrients, rate of development, and contamination possibility. Systems devoid of cells might enable quicker and simpler manufacturing of AMPs. Furthermore, these systems may be adjusted to prevent issues such as product damage to host cells, therefore reducing the yield in normal bacterial fermentation. High-throughput screening techniques are also supporting the hunt for novel AMPs.

B. Emerging microbial strains and synthetic biology approaches

New opportunities for producing antimicrobial peptides (AMPs) arise when synthetic biology techniques are combined with evolving new forms of bacteria. Conventional methods of producing AMPs still suffer with scaling up and producing sufficient. New microbial hosts and cutting edge synthetic biology methods, however, are poised to fundamentally alter industrial production of AMPs. Studying new kinds of bacteria—especially ones that naturally produce antibiotic peptides—is become more fascinating. Two long-standing examples of microorganisms employed in recombinant protein synthesis are Escherichia coli and Bacillus subtilis. Scientists are now investigating the alternatives for less prevalent, other kinds of AMP generation. Large-scale AMPs are being produced by researchers employing microbes such as Lactococcus lactis and Lactobacillus species. Natural environments are known to produce antibacterial peptides by these microorganisms. For food-grade applications particularly, this is crucial. Moreover, particularly for peptides that must be altered and folded in intricate ways, filamentous fungus such as Aspergillus niger and novel varieties from the actinomycetes family are being considered as suitable choices for producing AMP. One of the nicest features of emerging bacterial forms is their inherent resistance to environmental stress. For example, certain extremophiles microorganisms that thrive in hostile conditions are being investigated to determine if they may produce AMPs in conditions not suitable for typical forms. These extremophiles may survive in acidic environments, great salinity, or high temperatures. In manufacturing AMP, they might provide particular advantages like increased stability and strength in industrial fermentation environments. Improving species of bacteria that produce AMP will depend much on synthetic biology. Mixing genetic engineering with biological design ideas allows synthetic biology to create original metabolic pathways for AMPs.

Part of this is creating microbial strains capable of producing novel or altered peptides with improved antibacterial action or stability. Pathway rebuilding, in which full AMP biosynthesis pathways are transferred from one organism to another for example, may enable bacteria produce peptides not found in their native hosts. This approach allows us to create microbial biofactories producing more AMPs, despite difficultly releasing peptides for conventional hosts. Gene synthesis and pathway assembly methods in synthetic biology enable modular creation of AMP gene clusters. This implies that one may modify or add certain genes engaged in peptide synthesis to increase output. This allows scientists to modify certain components of the biochemical process, therefore improving the efficacy of the peptides and raising their production. Direct evolution may also be used to produce artificial enzymes or metabolic pathways that increase the stability, solubility, and decreased likelihood of breakdown of AMPs, hence improving their efficacy. Synthetic biology allows one to modify microbial strains with a degree of precision never seen previously when coupled with the capacity to alter microbial genomes utilizing CRISpen-Cas and other gene-editing technologies. Using these technologies helps us to regulate AMP generation, therefore optimising the metabolism of the microbe and preventing the host from being ill from excessive levels of AMPs.

C. Integrating microbial biofactories into sustainable production systems

Including bacterial biofactories in sustainable production systems is a crucial approach to guarantee long-term continuation of antimicrobial peptide (AMP) output. Finding ecologically safe methods to create AMPs is becoming crucial as their applications in farming, food freshness, and health care, as well as their ability to help battle antibiotic resistance, need them. In addition to ensuring that manufacturing AMP is cost-effective and environmentally friendly, a sustainable production technique helps meet more global objectives for sustainability, resource economy, and reduced environmental impact. The fact that bacterium biofactories break down organically and have no effect on the surroundings is among their finest features. Making AMP from bacterial fermentation is healthier for the environment than chemical production techniques, which can call for hazardous chemicals and generate toxic waste products. Green sources of carbon, such as sugars or agricultural waste, allow microorganisms

to avoid depending as heavily on nonrenewable resources such fossil fuels. Moreover, microorganisms may be altered to generate less waste and toxic residues, thereby improving the manufacturing process and reducing the environmental impact. Closed-loop production systems in which waste from one stage of the manufacturing process may be either returned or utilised in another can also be built using microbial biofactories. For other biological operations, such as producing biofuels or bioplastics, the biomass left behind after fermentation might be a raw resource. This circular-based approach promotes resource-wise efficient utilisation and reduces the overall effect of producing AMP on the planet.

Furthermore crucial for sustainable production systems is the ability of microbial biofactories to be scaled up. Once they are tuned for producing AMP, microorganisms may be cultured in bioreactors to satisfy commercial demands. Not much has to be modified about the manufacturing line. Flexible bioreactors that may be added to or removed from depending on production requirements enable flexible and resource-efficient large-scale AMP manufacture. Additionally employed to increase efficiency and reduce the need for batch operations using a lot of energy are continuous fermenting systems. This makes the technique of manufacturing much more ecologically friendly. Furthermore included in sustainability is ensuring that investing in AMP is wise. As metabolic engineering, synthetic biology, and fermentation optimisation make microbial fermentation more efficient and less costly, the cost of producing AMPs on a wide scale will drop. Over time, this will make AMPs more reasonably priced than other antibiotics and simpler to get. Growing environmental and social consequences of chemical pesticide usage as well as antibiotic resistance raise questions regarding by offering a more ethical and sustainable alternative to conventional techniques, sustainable production systems may assist to allay these fears.

CONCLUSION

Made on a large scale in microbial biofactories, antimicrobial peptides (AMPs) might be a long-term, affordable, scalable solution for the escalating antimicrobial resistance (AMR) issue. Given their broad-spectrum antibacterial effect, AMPs offer potential as a substitute for conventional antibiotics, particularly given increasing resistance of bacteria to them. Microbial biofactories are now potent sites to produce AMPs for various sectors; including medicines, agriculture, and food preservation as microorganisms can naturally create these bioactive molecules and because of genetic engineering and synthetic biology have gone a distance. The greatest features of employing microbial systems for AMP production are their flexibility, rapid growth, simplicity of genetic modification, and ability to maximize metabolic pathways to raise output levels. AMP has been produced effectively from bacteria, yeasts, and fungi. Regarding output, handling ease, and the capacity to create complicated peptides with post-translational modifications, every cell has advantages. Also, synthetic biology tools like CRISPR-Cas and metabolic engineering make it possible to make specially engineered microbial types that can make a lot of AMPs, keep them stable, and make them more effective against microbes. But there are still problems that need to be fixed before AMP production can be scaled up to an industrial level. These include improving fermentation, lowering costs, and making sure that the product is processed properly after fermentation. Some important things that can be done to make large-scale AMP production more cost-effective and long-lasting are using modern bioreactor systems, constant fermentation, and combined cleaning methods. Another thing that will help make microbial AMP synthesis more environmentally and economically viable is the creation of long-lasting production systems that use natural resources and reduce trash.

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