BT512 – MICROBIAL BIOTECHNOLOGY

MID TERM MOST IMPORTANT QUESTIONS

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GROUP LINK: https://chat.whatsapp.com/BjC6W0jZx8o9yaC2ISh1Gu

1. How bacteria play an important role to promote plant growth?

Bacteria play a crucial role in promoting plant growth through various direct and indirect mechanisms. Here are the main ways they help:

1. Nitrogen Fixation

Some bacteria, like *Rhizobium*, *Azotobacter*, and *Frankia*, convert atmospheric nitrogen (N_2) into forms plants can absorb, such as ammonium (NH_4^+). This is especially vital because nitrogen is a major nutrient for plant growth.

- ***Symbiotic nitrogen fixers*** (e.g., *Rhizobium*) form nodules on legume roots.
- ***Free-living nitrogen fixers*** (e.g., *Azotobacter*) live in the soil.

2. Phosphate Solubilization

Certain bacteria (*Pseudomonas, Bacillus, Rhizobium*) can solubilize insoluble forms of phosphate in the soil, making it available to plants. Phosphorus is essential for energy transfer and root development.

3. Production of Growth Hormones

Many plant growth-promoting rhizobacteria (PGPR) produce phytohormones like:

- *Auxins* – stimulate root elongation.
- *Gibberellins* – promote stem elongation and seed germination.
- *Cytokinins* – help in cell division and shoot formation.

4. Disease Suppression

Some bacteria protect plants from pathogens by:

- Producing antibiotics and siderophores (iron-chelating compounds).
- Competing with harmful microbes.
- Inducing systemic resistance in plants.

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5. Decomposition and Nutrient Cycling

Decomposer bacteria break down organic matter in the soil, releasing nutrients like nitrogen, phosphorus, and sulfur in forms plants can absorb.

6. Enhanced Stress Tolerance

Certain PGPR help plants cope with:

- Drought
- Salinity
- Heavy metals

7. Biofertilizers

Bacterial inoculants are used as biofertilizers in sustainable agriculture. These improve soil fertility and reduce the need for chemical fertilizers.

2. What are physiological factors of strain improvement?

Physiological factors of strain improvement refer to the internal biological characteristics and conditions of microbial strains (like bacteria or fungi) that can be optimized or modified to increase their productivity, growth rate, or resistance for industrial, agricultural, or pharmaceutical applications.

Here are the key physiological factors considered during strain improvement:

1. Growth Rate

Faster-growing strains can produce desired products more quickly. Strains are selected or engineered to have shorter lag phases and faster exponential growth.

2. Temperature and pH Tolerance

Improved strains can tolerate wider temperature or pH ranges, making them more robust in industrial conditions. This allows for consistent production in varying environments.

3. Nutrient Utilization Efficiency

Some strains are improved to use a broader range of carbon or nitrogen sources, such as waste materials. Efficient nutrient uptake increases biomass and product yield.

4. Metabolic Pathway Optimization

Key metabolic pathways are enhanced or rerouted to increase product formation (e.g., antibiotics, enzymes) and decrease by-product formation that may inhibit growth.

5. Genetic Stability

Stable strains maintain their improved traits over multiple generations without mutation or loss. Ensures consistent performance in large-scale applications.

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6. Product Yield and Productivity

Strains are selected or engineered to produce higher quantities of target compounds, like: Antibiotics, Amino acids, Organic acids and Enzymes.

7. Resistance to Toxins or Inhibitors

Improved strains can resist their own metabolic by-products (e.g., ethanol, lactic acid) and external stresses like antibiotics, salts, or solvents.

8. Sporulation and Morphology

In fungi or spore-forming bacteria, morphology (e.g., pellet vs. filamentous form) affects Nutrient uptake, Aeration in fermenters and Ease of downstream processing.

9. Regulation of Gene Expression

Strains can be modified to Overexpress beneficial genes and repress undesired gene expression to avoid wasteful energy use.

3. Write importance of microbial biotechnology.

Importance of Microbial Biotechnology (Short & Bullet Format)

- ***Agriculture***
    - Produces biofertilizers (e.g. *Rhizobium*) for soil fertility.
    - Develops biopesticides (e.g. *Bt*) to control pests naturally.
    - Promotes plant growth through beneficial microbes.

- ***Industry***
    - Microbes produce enzymes used in food, textiles, and detergents.
    - Used in fermentation to produce alcohol, acids, and vitamins.
    - Involved in bioplastics and biodegradable materials production.

- ***Environment***
    - Used in bioremediation to clean oil spills and toxic waste.
    - Helps in wastewater treatment and recycling processes.

- ***Healthcare***
    - Produces antibiotics, vaccines, and probiotics.
    - Used in gene therapy and the development of diagnostic tools.

- ***Energy***
    - Produces biofuels like ethanol and biogas from organic waste.

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4. Explain importance of microbes.

Importance of Microbes (in Bullet Points)

Microbes (bacteria, fungi, viruses, protozoa, algae) are tiny organisms that play essential roles in nature, health, and industry.

1. In Agriculture

- Nitrogen fixation by bacteria (e.g. *Rhizobium*) improves soil fertility.
- Decompose organic matter**,** recycling nutrients for plant growth.
- Control pests naturally through microbial biopesticides.

2. In Industry

- Used in fermentation to make bread, yogurt, cheese, alcohol.
- Produce enzymes**,** antibiotics**,** vitamins, and other bio-products.
- Help in bioplastic and biofuel production.

3. In Medicine

- Source of antibiotics (e.g. *Penicillium* for penicillin).
- Used to make vaccines and probiotics.
- Help in genetic engineering and biotechnology research**.**

4. In the Environment

- Essential for decomposition and nutrient cycling.
- Used in bioremediation to clean oil spills and pollutants.
- Maintain ecological balance in soil and water.

5. In Research & Biotechnology

- Help study genetics**,** evolution**,** and disease mechanisms.
- Used in DNA technology and synthetic biology.

5. Write a brief note on subunit vaccine.

Brief Note on Subunit Vaccine

A subunit vaccine is a type of vaccine that contains only specific parts (subunits) of a virus or bacterium—usually proteins or sugars—that trigger an immune response.

Key Features:

- Contains no live pathogens, making it very safe, especially for people with weak immune systems.
- Targets only the essential antigens, reducing the risk of side effects.
- May require adjuvants (boosters) and multiple doses to enhance immunity.

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Examples:

- Hepatitis B vaccine
- Human papillomavirus (HPV) vaccine
- Acellular pertussis vaccine (part of DTaP)

Advantages:

- Safe and stable
- Low risk of causing disease
- Can be produced using genetic engineering

6. What is lactic acid fermentation?

Lactic acid fermentation is an anaerobic process in which certain bacteria, such as *Lactobacillus*, or animal muscle cells convert glucose into lactic acid in the absence of oxygen. This process generates a small amount of energy (ATP) and is essential for organisms to survive in low-oxygen environments. It is widely used in the food industry to produce fermented products like yogurt, cheese, pickles, and sauerkraut. The lactic acid produced also acts as a natural preservative by lowering the pH and inhibiting the growth of spoilage microbes. Lactic acid fermentation is important in both biological energy production and food preservation.

7. How yeast causes bread to rise?

Yeast, a type of fungus, helps bread rise through a process called fermentation. When mixed with flour and water, yeast consumes the sugars in the dough and produces carbon dioxide (CO_2) and alcohol. The CO_2 gas gets trapped in the dough's gluten network, forming tiny bubbles that make the dough expand and rise. The alcohol mostly evaporates during baking, while the heat solidifies the dough structure, giving bread its soft, airy texture. In short, yeast releases gas that inflates the dough, making it light and fluffy.

8. Differentiate between conjugate vaccine, DNA vaccine and recombinant vaccine.

Conjugate Vaccine

A conjugate vaccine is created by chemically linking (conjugating) a weak antigen, usually a polysaccharide from the surface of bacteria, to a stronger protein carrier. This combination helps the immune system recognize the polysaccharide better, especially in young children whose immune responses to polysaccharides alone are weak. Conjugate vaccines are commonly used against bacteria like *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae*. The protein carrier boosts the immune response, making the vaccine more effective.

DNA Vaccine

DNA vaccines involve directly injecting a piece of genetic material (DNA) that encodes an antigen from a pathogen into the body's cells. Once inside, the host cells use this DNA to produce the antigen protein, which stimulates the immune system to recognize and fight the real pathogen. DNA vaccines are relatively new and offer advantages like easy production and stability. They are being researched for infectious diseases and some cancers but are not yet widely used in humans.

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Recombinant Vaccine

Recombinant vaccines are produced by inserting the gene for a specific antigen from a pathogen into a harmless organism, like yeast or bacteria, which then produces the antigen protein in large amounts. This purified protein is used as the vaccine. Because they contain only specific antigen parts, recombinant vaccines are very safe and well-tolerated. Examples include the Hepatitis B vaccine and the human papillomavirus (HPV) vaccine.

9. What is Live Attenuated vaccine? Give example.

Live Attenuated Vaccine

A live attenuated vaccine contains a version of the living microorganism (virus or bacterium) that has been weakened (attenuated) so it can't cause disease in healthy people but still stimulates a strong immune response. Because the vaccine mimics a natural infection, it usually provides long-lasting immunity with fewer doses.

Example:

- Measles, Mumps, and Rubella (MMR) vaccine
- BCG vaccine for tuberculosis
- Oral Polio Vaccine (OPV)

10. What is DNA vaccine? Give example.

DNA Vaccine

A DNA vaccine uses a small, circular piece of DNA called a plasmid that contains the gene encoding an antigen from a pathogen. When injected into the body, this DNA enters host cells, which then produce the antigen protein themselves. This triggers the immune system to recognize and build protection against the real pathogen. DNA vaccines are stable, easy to produce, and can induce both antibody and cellular immune responses. DNA vaccines are still an emerging technology but show great promise for various infectious diseases and cancer.

Example:

- **ZyCoV-D**, a DNA vaccine developed for **COVID-19** in India.

11. Write two names of pharmaceutical drugs and how it produced from microbes?

1. Penicillin

- ***Produced by:*** *Penicillium* mold (a fungus)
- ***How:*** The *Penicillium* mold secretes penicillin as a secondary metabolite during fermentation. This antibiotic inhibits bacterial cell wall synthesis and is widely used to treat bacterial infections.

2. Streptomycin

- ***Produced by:*** *Streptomyces griseus* (a soil bacterium)
- ***How:*** *Streptomyces* species produce streptomycin during their growth in fermentation tanks. Streptomycin is an antibiotic that targets bacterial protein synthesis and is used to treat tuberculosis and other infections.

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12. What bacteria are used in food production?

Bacteria Used in Food Production

- **Lactobacillus**
  Used in yogurt, cheese, sauerkraut, pickles, and sourdough bread fermentation. It helps ferment sugars into lactic acid, preserving food and enhancing flavor.

- **Streptococcus thermophilus**
  Used along with *Lactobacillus* in yogurt and cheese making to ferment milk.

- **Leuconostoc**
  Involved in the fermentation of vegetables like sauerkraut and kimchi, contributing to flavor and texture.

- **Propionibacterium**
  Used in Swiss cheese production, producing carbon dioxide bubbles that create characteristic holes and contribute to flavor.

- **Acetobacter**
  Converts alcohol into acetic acid in vinegar production.

13. Name the microbes used in food industry.

Microbes Used in Food Industry

- ***Bacteria:***
  - *Lactobacillus* (yogurt, cheese, pickles)
  - *Streptococcus thermophilus* (yogurt, cheese)
  - *Leuconostoc* (fermented vegetables)
  - *Propionibacterium* (Swiss cheese)
  - *Acetobacter* (vinegar production)

- ***Fungi (Molds):***
  - *Penicillium* (blue cheese, Camembert)
  - *Aspergillus oryzae* (soy sauce, sake)

- ***Yeasts:***
  - *Saccharomyces cerevisiae* (bread, beer, wine fermentation)
  - *Candida* species (some fermented foods)

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14. What is alcoholic fermentation?

Alcoholic Fermentation

Alcoholic fermentation is an anaerobic process where certain microorganisms, mainly yeasts like *Saccharomyces cerevisiae*, convert sugars (such as glucose) into ethanol (alcohol) and carbon dioxide. This process releases energy that the microbes use to grow and reproduce.

Key points:

- Occurs without oxygen.
- Produces ethanol (used in alcoholic beverages and biofuels).
- Releases CO_2, which helps bread dough rise.
- Widely used in brewing**,** winemaking**,** and baking industries.

15. What are different types of fermentation?

1. Alcoholic Fermentation

- Performed mainly by yeasts (e.g., *Saccharomyces cerevisiae*).
- Converts sugars into ethanol and carbon dioxide.
- Used in making beer**,** wine**,** and bread.

2. Lactic Acid Fermentation

- Performed by lactic acid bacteria (e.g., *Lactobacillus*).
- Converts sugars into lactic acid**.**
- Used in producing yogurt**,** cheese**,** sauerkraut**,** and pickles.
- Occurs in muscle cells during intense exercise (causing muscle fatigue).

3. Butyric Acid Fermentation

- Carried out by Clostridium species.
- Produces butyric acid, carbon dioxide, and hydrogen.
- Involved in spoilage of food and some industrial processes.

4. Propionic Acid Fermentation

- Done by Propionibacterium species.
- Produces propionic acid, acetic acid, and carbon dioxide**.**
- Important in the production of Swiss cheese (the holes are from CO_2).

5. Mixed Acid Fermentation

- Performed by some Enterobacteriaceae bacteria.
- Produces a mixture of acids like lactic, acetic, succinic acids, along with CO_2 and hydrogen.
- Important in gut microbiota and some industrial processes.

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16. What is compost?

Compost is a nutrient-rich, natural fertilizer formed by the decomposition of organic matter such as food scraps, leaves, and garden waste. Microorganisms like bacteria and fungi break down these materials into a dark, soil-like substance called humus. Compost improves soil structure, enhances its ability to retain moisture, and supplies essential nutrients to plants. It is widely used in agriculture and gardening as an eco-friendly alternative to chemical fertilizers. Additionally, composting helps reduce household and agricultural waste, making it an important practice for sustainable environmental management.

17. Write importance of Microbial Biotechnology in economy of Pakistan.

Importance of Microbial Biotechnology in the Economy of Pakistan

Microbial biotechnology plays a vital role in strengthening Pakistan's economy by supporting key sectors such as agriculture, industry, healthcare, and environmental management. In agriculture, the use of biofertilizers and biopesticides made from microbes helps improve crop yield and reduce dependence on costly chemical inputs, benefiting farmers and promoting sustainable farming. In the industrial sector, microbes are used in the production of enzymes, biofuels, and fermented foods, which supports local manufacturing and reduces imports. In healthcare, microbial biotechnology contributes to the development of antibiotics, vaccines, and diagnostics, improving public health and supporting the pharmaceutical industry. Additionally, microbes aid in waste management and bioremediation, helping to clean polluted environments and promote eco-friendly practices. Altogether, microbial biotechnology boosts productivity, reduces costs, and opens up opportunities for export and job creation**,** making it an important contributor to Pakistan's economic development.

18. Write functions of Symbiotics in maintenance of health.

Functions of Symbiotics in Maintenance of Health

Symbiotics are a combination of probiotics (beneficial live bacteria) and prebiotics (non-digestible food ingredients that support the growth of good bacteria). Together, they work synergistically to promote gut health and overall well-being.

Key Functions:

- ***Improve Gut Health:*** Enhance the balance of beneficial gut bacteria, supporting digestion and nutrient absorption.
- ***Boost Immunity:*** Strengthen the immune system by stimulating the production of protective antibodies and regulating immune responses.
- ***Prevent Digestive Disorders:*** Help reduce symptoms of diarrhea, constipation, irritable bowel syndrome (IBS), and inflammatory bowel disease (IBD).
- ***Enhance Nutrient Absorption:*** Improve absorption of essential nutrients like calcium, magnesium, and B-vitamins.
- ***Protect Against Pathogens:*** Inhibit the growth of harmful microbes in the gut by competing for space and nutrients.
- ***Support Mental Health:*** Positively influence the gut-brain axis, potentially reducing stress, anxiety, and depression.

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19. How second generation biofuels are produced?

Second-generation biofuels are produced from non-food biomass such as agricultural residues (e.g., straw, husks), wood chips, and other plant waste. Unlike first-generation biofuels, they do not compete with food crops and are more environmentally sustainable.

Production Steps:

1. ***Biomass Collection***
   Non-edible plant materials like corn stalks, sugarcane bagasse, or forestry waste are gathered.
2. ***Pretreatment***
   The biomass is physically or chemically treated to break down complex structures like lignocellulose (a mix of cellulose, hemicellulose, and lignin).
3. ***Enzymatic Hydrolysis***
   Enzymes are used to convert cellulose and hemicellulose into simple sugars (e.g., glucose).
4. ***Fermentation***
   Microorganisms (like engineered bacteria or yeast) ferment the sugars into bioethanol or other biofuels.
5. ***Purification***
   The biofuel is separated and purified for use as an energy source.

20. Write any two examples of biofertilizers.

Two Examples of Biofertilizers:

1. ***Rhizobium***
   - A nitrogen-fixing bacterium that forms symbiotic relationships with legume roots.
   - Converts atmospheric nitrogen into a usable form for plants.

2. ***Azotobacter***
   - A free-living nitrogen-fixing bacterium found in soil.
   - Enhances soil fertility and promotes plant growth, especially in non-leguminous crops.

21. How probiotics are formed?

Probiotics are live beneficial microorganisms, mainly bacteria and some yeasts that are grown and processed to support gut health and overall well-being.

Steps in Probiotic Formation:

1. ***Selection of Strain:*** Specific strains (e.g., *Lactobacillus*, *Bifidobacterium*) with proven health benefits are selected.
2. ***Cultivation:*** The selected microbes are grown in controlled lab conditions using nutrient-rich media (like milk or broth).
3. ***Fermentation:*** Microorganisms multiply and produce beneficial compounds (like lactic acid) during fermentation.
4. ***Harvesting:*** The microbial culture is separated from the growth medium and concentrated.

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5. ***Stabilization:*** The microbes are freeze-dried or spray-dried to preserve them for storage and use in foods, capsules, or supplements.
6. ***Formulation:*** The probiotics are added to products like yogurt, drinks, or capsules while ensuring the microbes remain alive and effective.

22. Write steps of GMO.

Steps in the Production of a Genetically Modified Organism (GMO)

1. ***Gene Identification:*** Select and isolate the desired gene from a source organism (e.g., a gene for pest resistance, drought tolerance, or vitamin production).

2. ***Gene Cloning:*** The identified gene is copied and inserted into a plasmid (a small circular DNA molecule).

3. ***Gene Insertion:*** The plasmid carrying the gene is introduced into the host organism (plant, animal, or microbe) using techniques like Gene gun, Agrobacterium-mediated transformation, Microinjection and CRISPR/Cas9 (modern gene-editing tool).

4. ***Selection and Screening:*** The modified cells are screened to confirm successful gene transfer and expression. Marker genes (like antibiotic resistance) may be used to identify GM cells.

5. ***Regeneration:*** Modified cells are grown into whole organisms (e.g., plants through tissue culture).

6. ***Testing and Evaluation:*** GMOs are tested for safety, stability, effectiveness, and environmental impact before approval for commercial use.

Disclaimer:

For best results, it is advised NOT to rely on this file only. Go through video lectures, handouts and other sources as well.

Regards,
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