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Chapter 3

Composition, optimization, and preparation of culture media for microbiological and biotechnological applications

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1. Introduction:

In microbiology, media play a pivotal role in cultivating and studying microorganisms in laboratory settings. These media provide a nurturing environment for microbial growth and enable researchers to observe, isolate, and identify various types of microorganisms. There are several types of media used in microbiology, each serving specific purposes based on their composition and characteristics**.**

1. **Nutrient Broth:** A liquid medium made of water, peptone, and beef extract; nutrient broth is comparable to nutrient agar. Large-scale microorganism culture, biochemical testing, and inoculum preparation are among its frequent uses.
2. **Nutrient Agar:** Made of agar, peptone, and beef extract, nutrient agar is a versatile solid medium. It facilitates the growth of a variety of microorganisms and is frequently utilized in standard laboratory procedures like the preservation and separation of bacterial cultures.
3. **Selective Media:** Compounds in selective media prevent some germs from growing while encouraging the growth of others. These media are made to separate particular kinds of fungi or bacteria from mixed cultures. Examples are Sabouraud agar, which selectively cultivates fungi by suppressing bacterial growth, and MacConkey agar, which selects for Gram-negative bacteria by preventing the development of Gram-positive bacteria.
4. **Differential Media:** Materials in differential media enable microorganisms to be distinguished from one another according to their metabolic characteristics. Depending on the metabolic activities of the microorganisms, these media usually result in noticeable changes in the color or appearance of the colonies. Examples are eosin methylene blue agar, which separates lactose-fermenting bacteria from non-lactose-fermenting bacteria, and blood agar, which separates bacteria according to their capacity to hemolyze red blood cells.

5. **Enriched Media:** To assist the growth of picky microorganisms with intricate nutritional needs, enriched media include extra nutrients like blood, serum, or egg yolk. These media are frequently used to recover microorganisms from clinical samples or to isolate and cultivate harmful germs.
6. **Transport Media:** Particularly designed to preserve microbe viability during specimen transportation from the collecting site to the laboratory, transport media are used. These media frequently include nutrients to preserve microbial viability during transit and buffers to maintain pH and prevent desiccation.

2. Media composition and preparation method:

2.1. Nutrient broth (NB):

2.1.1. Introduction:

Nutrient broth media is a liquid culture medium utilized in microbiology for cultivating and growing various microorganisms. It comprises a simple mixture of water, peptone (a protein digest), and beef extract or yeast extract, providing essential nutrients for microbial growth. This versatile medium supports the proliferation of a wide range of bacteria, yeasts, and fungi, making it a fundamental tool in laboratory settings for microbial cultivation and experimentation. Its liquid form allows for easy inoculation and observation of microbial growth, facilitating the study of microbial physiology, metabolism, and other biochemical processes. Nutrient broth media can also serve as a base for the preparation of more specialized media tailored to the specific requirements of particular microorganisms or experimental objectives. Overall, its simplicity, versatility, and efficacy make nutrient broth media a cornerstone in microbiological research and diagnostics.

Table 1: Composition of Nutrient Broth

Component	Function of the Component	Concentration (g/L)
Peptone	Source of Protein and Amino Acid	5–10
Beef Extract	Nutrient source for Vitamin and Organic Nitrogen	3 g
Sodium Chloride	To provide osmotic	5 g
Distilled Water	Solvent	1 L
Optional Additives	To provide Enhances growth	Changeable
pH	pH balance	Adjust to 7.0 ± 0.2

2.1.2. Procedure:

1. Put all the components and dissolve them by heating in a steamer.
2. After cooling, the pH should be adjusted to 7.5-7.6.
3. The autoclave was set at a temperature of 121°C for duration of 15 minutes.

2.2. Nutrient agar (NA):

2.2.1. Introduction:

Nutrient agar media is a solid culture medium widely employed in microbiology for cultivating and isolating diverse microorganisms. It consists of a blend of purified agar, a polysaccharide derived from seaweed, and a nutrient-rich mixture containing peptone (a protein digest), beef extract or yeast extract, and sometimes additional components like salts or carbohydrates. This formulation provides essential nutrients necessary for microbial growth and supports the development of various bacteria, yeasts, and molds.
The solid nature of nutrient agar distinguishes it from liquid media like nutrient broth, allowing for the formation of a gel-like matrix upon cooling after sterilization. This solidity provides a stable surface for microbial growth, enabling researchers to observe and manipulate individual colonies. Nutrient agar plates are commonly used for techniques such as streaking, where a sample containing a mixture of microorganisms is spread thinly over the agar surface. Through successive dilutions and streaking, individual colonies can be isolated and studied separately.

The utilization of nutrient agar media presents numerous benefits within the realm of microbiological research. The composition of this substance offers a diverse array of nutrients, facilitating the proliferation of a broad spectrum of microorganisms (Reed and Chudek, 1993).

2.2.2. Procedure:

1. Combine 20 grams of agar with the other ingredients of nutrient broth to prepare one liter of nutrient broth.
2. Ensure thorough dissolution by mixing.
3. Sterilize the mixture by autoclaving at 121°C for 15 minutes.

2.3. Alkaline Peptone Water (APW):

Alkaline peptone water (APW) is a specialized liquid medium employed in the field of microbiology for the purpose of enriching and isolating particular bacterial species, with a particular focus on *Vibrio cholerae* and other Vibrio species. It establishes a favorable environment for the proliferation of these bacteria while impeding the growth of rival microbes. APW commonly consists of peptone, which supplies amino acids and nitrogen, as well as sodium chloride to maintain osmotic equilibrium. Raising the pH to around 8.6 improves its specific characteristics, while numerous other bacteria face

difficulties in flourishing in alkaline environments. Preparation involves dissolving peptone and sodium chloride in distilled water, adjusting the pH with an alkaline agent, and sterilizing through autoclaving. Inoculating APW with a sample suspected of containing Vibrio species allows for their selective growth. Subsequent plating on selective solid media enables the isolation and identification of Vibrio colonies based on their characteristic properties. APW is essential in microbiological laboratories for detecting and studying Vibrio species in diverse samples, from food and environmental sources to clinical specimens. It serves not only in bacterial isolation and identification but also in maintaining and transporting Vibrio cultures, ensuring their viability during storage and transit. Overall, APW is a valuable tool for microbiologists investigating Vibrio related infections and environmental contamination (Bergey and Holt, 2000)

2.3.1. Procedure:

1. Take 10g of peptone and 10 grams of sodium chloride should be dissolved in a volume of one liter of purified water.
2. Set the pH to 8.6 and decontaminate by subjecting it to autoclaving at 121°C for duration of 15 minutes.

2.4. MacConkey Agar:

It is a specialized media for the isolation and identification of Gram-negative bacteria, namely those belonging to the Enterobacteriaceae family. It is selective and differential in nature. The agar medium, which was created by Alfred Theodore MacConkey during the latter part of the 19th century, has gained significant recognition in the field of clinical microbiology for its efficacy in differentiating between bacteria that digest lactose and those that do not. MacConkey agar is comprised of peptone, lactose, bile salts, neutral red dye, and agar substrate. Peptone acts as a nitrogen source and a vital nutrient for the proliferation of bacteria, whilst lactose functions as the fermentable carbohydrate substrate. Bile salts possess the ability to impede the proliferation of Gram-positive bacteria, hence augmenting the medium's selectivity towards Gram-negative species. The neutral red dye functions as a pH indicator, exhibiting a pink coloration upon the occurrence of lactose fermentation. The preparation process entails the dissolution of the materials in water, followed by the adjustment of the pH to roughly 7.1, and finally, sterilization using autoclaving.

Once the MacConkey agar plates have solidified, they exhibit a light pink coloration and a translucent appearance. When lactose-fermenting bacteria are introduced, they generate acid, resulting in the agar surrounding their colonies exhibiting a pink or red coloration, which serves as an indication of fermentation. Non-lactose-fermenting bacteria commonly manifest as colonies that lack color or exhibit a pale appearance. The differential nature of this component facilitates the prompt detection of enteric

pathogens, hence assisting in the diagnosis of gastrointestinal infections. The versatility and reliability of MacConkey agar render it an essential instrument in the field of clinical microbiology, serving both research and diagnostic objectives.

2.4.1. Material required:

1. Peptone: 20g
2. Lactose: 10g
3. Bile salts: 1.5g
4. Neutral red: 0.03g
5. Crystal violet: 0.001g
6. Agar: 13.5g
7. Distilled water: 1 liter
8. pH meter and adjuster
9. Autoclave

2.4.2. Procedure:

1. Weigh out the specified amounts of peptone, lactose, bile salts, neutral red, crystal violet, and agar.
2. Dissolve the peptone, lactose, bile salts, neutral red, and crystal violet in about 900ml of distilled water in a large flask or beaker.
3. Stir the mixture until all components are dissolved.
4. Check the pH of the solution using a pH meter. The pH should be approximately 7.1.
5. Adjust the pH, if necessary, by NaOH and HCl.
6. Adjust the volume to 1 liter with distilled water.
7. Add the specified amount of agar to the solution.
8. Mix the solution thoroughly to ensure even distribution of agar.
9. Heat the mixture while stirring to dissolve the agar completely. Avoid boiling.
10. Once the agar is dissolved and the mixture is homogeneous, sterilize the solution by autoclaving at 121°C for 15 minutes.
11. After autoclaving, allow the agar to cool to approximately 45-50°C, ensuring it is still liquid but not solidifying.
12. Pour the agar into sterile petri dishes to a depth of about 4-5mm.
13. Allow the agar to solidify at room temperature.
14. Store the prepared MacConkey agar plates inverted at 2-8°C until ready to use. Avoid exposure to light as it may degrade the selective agents.

2.5. Blood agar:

2.5.1. Introduction:

In microbiology laboratories, blood agar media, alternatively referred to as trypticase soy agar with 5% sheep blood is a highly adaptable and extensively employed medium. This enriched medium serves both as a general-purpose growth medium and as a differential medium for the characterization of various bacterial species, especially those that need additional nutrients for growth. The composition of blood agar includes trypticase soy agar containing 5% sheep blood. The blood serves as an enriched nutrient source, providing essential growth factors, vitamins, and minerals necessary for the growth of fastidious microorganisms. Preparation involves mixing sterile sheep blood gently with melted and cooled trypticase soy agar, ensuring thorough dispersion while preventing the formation of bubbles or froth.

After pouring the mixture into petri dishes, it solidifies to form a translucent medium with visible blood components. Blood agar's differential properties stem from its ability to detect hemolysis, the breakdown of red blood cells by certain bacterial enzymes. Based on their hemolytic activity, bacteria can produce different types of hemolysis patterns on blood agar plates, including alpha, beta, and gamma hemolysis. This characteristic allows for the rapid identification of pathogenic bacteria and aids in the diagnosis of infections, particularly those caused by streptococci and staphylococci species.

2.5.2. Materials required:

1. Peptone
2. Yeast extract
3. Sterile sheep blood (5% concentration)
4. NaCl
5. Distilled water
6. Autoclave
7. Sterile glassware (flasks, pipettes)
8. Bunsen burner or hot plate
9. Petri dishes

Table 2: Composition of Blood Agar

S. No.	Ingredient	Concentration
1.	Peptone	0.5%
2.	Yeast Extract	0.3%
3.	Agar	1.5%

4.	NaCl	0.5%
5.	Distilled Water	1L
6.	Sheep Blood	5%

2.5.3. Procedure:

1. Start with suspending 28 grams of nutritional agar powder in one litre of distilled water.
2. To be sure all components dissolve completely, heat the mixture while stirring constantly.
3. To get sterility, autoclave the dissolved liquid for fifteen minutes at 121 degrees Celsius.
4. Let the nutritional agar solution cool down enough after autoclaving so that it stays liquid.
5. Once the temperature reaches 45–50°C, carefully stir into the agar solution 5% (vol/vol) of previously room temperature sterile blood. Stir thoroughly but gently.
6. When mixing, take care not to let air bubbles to form.
7. To enable equal distribution, pour the agar mixture into sterile plates while it is still liquid.

2.6. Chocolate agar:

2.6.1. Introduction:

Chocolate agar, alternatively referred to as heated blood agar or enhanced chocolate agar, is a specific growth medium that is extensively employed in the field of microbiology for the purpose of isolating and cultivating fastidious bacteria, with a particular focus on *Haemophilus influenzae* and Neisseria species. In contrast to conventional blood agar, chocolate agar is developed through the application of heat to sheep blood agar, resulting in the lysis of red blood cells. This process facilitates the release of heme and NAD (Nicotinamide Adenine Dinucleotide), both of which are crucial growth factors for the bacteria present in chocolate agar. The preparation of chocolate agar involves the addition of sterile sheep blood to melted and cooled trypticase soy agar or another suitable nutrient agar base. The mixture is then heated to around 80°C to lyse the red blood cells, releasing the necessary growth factors. After solidification, the agar plates exhibit a chocolate-brown color, hence the name.

Chocolate agar provides a rich and nutritious environment for the growth of fastidious bacteria, enabling their isolation and identification in clinical specimens, particularly those obtained from sites such as the respiratory tract and genitourinary tract. Its selective and differential properties make it a valuable tool in clinical microbiology for

diagnosing infections caused by *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and other fastidious bacteria, contributing to improved patient care and treatment outcomes (Pelczar et al., 1993).

2.6.2. Material required:

1. Peptone: 10g
2. Proteose peptone: 10g
3. Sodium chloride (NaCl): 5g
4. Agar: 20g
5. Defibrinated blood (sheep or horse): 50-100mL
6. Distilled water: 1 liter
7. pH meter and adjuster
8. Autoclave

2.6.3. Procedure:

1. Weigh out the specified amounts of peptone, proteose peptone, NaCl, and agar.
2. Dissolve the peptone, proteose peptone, NaCl, and agar in about 900ml of distilled water in a large flask.
3. Stir the mixture until all components are dissolved.
4. Check the pH of the solution using a pH meter. The pH should be approximately 7.4.
5. Adjust the pH if required, using NaOH and HCl.
6. Adjust the volume to 1 liter with distilled water.
7. Once the medium is prepared, autoclave at 121°C for 15 minutes to sterilize.
8. Allow the medium to cool to around 50°C but ensure it remains liquid.
9. Aseptically add 50-100mL of sterile defibrinated blood (sheep or horse) to the medium.
10. Mix gently but thoroughly to ensure even distribution of blood.
11. After blood addition, pour the agar into sterile petri dishes to a depth of about 4-5mm.
12. Allow the agar to solidify at room temperature.
13. Store the prepared chocolate agar plates inverted at 2-8°C until ready to use. Avoid exposure to light as it may degrade the hemolytic properties of the blood.

2.7. BG-11 media (Allen and Arnon, 1955):

2.7.1. Introduction:

In 1968, Allen and Stanier developed BG 11, a growth medium specifically designed for photoautotrophic blue green algae. This medium is ideal for supporting cyanobacteria from various habitats, including fresh water, soil, and marine environments (Yadav et al., 2016; Tripathi et al., 2018). It provides a rich source of nitrate and phosphate,

essential nutrients for these organisms. Since cyanobacteria are capable of fixing nitrogen, they contribute a significant amount of nitrogen to the ecosystem. This medium also contains a variety of micro-nutrients, including boron, as well as macro-nutrients and trace metals.

EDTA, also known as Ethylenediamine tetraacetic acid, can be found in the media where it forms bonds with metal ions like $MgNO_2$ EDTA. The stock solution, formed by combining ferric citrate and citric acid, is then added to the main medium after autoclaving. Ammonium citrate is utilized in BG agar as a carbon source, which has an impact on the production of CO_2 (Tripathi et al., 2013a, b; Reddy et al.,2019; Yadav et al., 2021).

2.7.2. Material required:

Table 3: Composition of BG11 Media

Stock	Components	Amount (g/l)
Stock 1 (1L)	Na_2Mg EDTA	0.1
	(NH_4) $Fe(C_6H_4O_7)_2$	0.6
	Citric acid.$1H_2O$	0.6
	$CaCl_2.2H_2O$	3.6
Stock 2 (1L)	$MgSO_4.$ H_2O	7.5
Stock 3 (1L)	K_2HPO_4	3.05
Stock 4 (Trace	H_3BO_3	2.86
Metal Solution(1L)	$MnCl_2.4$ H_2O	1.81
	$ZnSO_4.7$ H_2O	0.222
	$CuSO_4.5$ H_2O	0.079
	$COCl_2.6$ H_2O	0.050
	Na_2MoO_4 .2 H_2O	0.391
	or MoO_4 (85%)	0.018

2.7.3. Procedure:

2.7.3.1. Preparation of Stock Solutions:

1. Begin by sequentially dissolving the specified components for stocks 1 to 4 in 900 ml of distilled water, ensuring the prescribed order.
2. Utilize a magnetic stirrer for gentle mixing during this process.

Table 3: Stock solution per liter of medium

Stock	Per liter of medium
Stock 1	10 ml
Stock 2	10 ml

Stock 3	10 ml
Na_2CO_3	0.02 g
Stock 4	1.0 ml
$NaNO_3$	1.5 g

2.7.3.2. Adjustment and Sterilization:

1. Upon complete dissolution of components, adjust the volume to 1 liter by adding distilled water.
2. For sterilization, set the autoclave to a pressure of 15 psi and maintain a temperature of 121°C for duration of 30 minutes.

2.7.3.3. Combination and pH Adjustment:

1. Combine the prepared solutions while maintaining sterility precautions.
2. Carefully adjust the pH to 7.5 using either 1.0M HCl or NaOH, as necessary, ensuring meticulous precision in this step.

2.7.3.4. For solid media preparation:

1. **Agar Incorporation:** Introduce 1.5% agar into the liquid BG11 media, ensuring thorough mixing to achieve uniform dispersion.
2. **Flask Preparation:** Transfer the agar-containing solution into flasks, utilizing either 50 ml or 125 ml flasks based on the desired volume. Seal the flasks securely with cotton stoppers to prevent contamination.
3. **Autoclaving Process:** Place the flasks in an autoclave and subject them to a pressure of 15 psi for 30 minutes at a temperature of 121°C, ensuring proper sterilization.
4. **pH Adjustment:** Post-autoclaving and cooling, carefully monitor the pH as it may have experienced alterations. Make any necessary adjustments to ensure optimal pH levels for the growth medium.

2.8. Murashige and Skoog (MS) media:

2.8.1. Introduction:

Understanding the composition of the growth medium is pivotal in any experiment involving living organisms. The importance of this phenomenon became evident during the initial phases of hydroponic cultivation and the development of plant tissue cultures. The origins of plant tissue cultures can be attributed to prominent individuals such as Hildebrandt and other notable contributors (e.g., Hildebrandt et al., 1946). The 1960s and 1970s witnessed notable research, particularly Murashige and Skoog's groundbreaking study in 1962, which greatly enhanced our understanding of plant media.

While initially designed for *Nicotiana tabacum* (Tobacco), Murashige and Skoog's medium has found broad adaptation, including in the study of *Arabidopsis thaliana*. As biologists, we understand that different plant species have specific media requirements. However, MS-based media have gained popularity across various plant species. Despite this, numerous variations exist due to plants' diverse characteristics. For instance, Knudson media, developed for orchids by Knudson in 1951 and commercially available through Sigma-Aldrich, represents one such variation. We will explore these variations in composition compared to other common alternatives. In essence, differences in macronutrient levels, such as nitrogen or phosphorus, and the presence of micronutrients can significantly impact the suitability of media for short-term cultivation.

In discussing the composition of the media, we categorize the compounds into four main groups:

1. **Basal salts,** encompassing both macro and micronutrients.
2. **Vitamins,** including organic essentials and beneficial elements.
3. **Buffering substances** and **a carbon source,** typically sucrose.
4. **A reinforcing agent,** absent in liquid media but present in solid media like agar or phytagel.

The overall composition of the medium can be created using basic ingredients or enhanced with commercial products. At times, there may be a blend of mediums, such as combining Murashige-Skoog medium with Gamborg vitamins, showcasing a crossover approach. This breakdown provides a comprehensive understanding of the components involved in formulating the medium, highlighting the versatility in crafting media tailored to specific research needs.

The following table presents a comparison of MS medium with other commonly used media for *in-vitro* plant/tissue cultivation, adapted from Misawa (1994).

Table 4: Comparison of MS medium with other commonly used media for *in-vitro* plant/tissue cultivation

Category of Components	Components	Amount per 1 L of Medium (mg)
Macronutrients	$MgSO_4.7\ H_2O$	370
	$CaCl_2.2\ H_2O$	440
	KNO_3	1900
	NH_4NO_3	1650
	KH_2PO_4	170
Micronutrients	$FeSO_4.7\ H_2O$	27.8
	Na_2EDTA	37.3
	$MnSO_4.4\ H_2O$	22.3

	$ZnSO_4.7\ H_2O$	8.6
	$CuSO_4.5\ H_2O$	0.025
	$CoCl_2.6\ H_2O$	0.025
	KI	0.83
	H_3BO_3	6.2
	$Na_2MoO_4.2\ H_2O$	0.25
Vitamins	Pyridoxine HCl	0.5
	Thiamine HCl	0.1-1
	Glycine	2
Carbon Resource	Sucrose	30000
Buffering Substance	MES	500
Reinforcing Agent	Agar	5000-10000
	Phytagel	1500-2500

This comparison provides insights into the composition of different media used for *in-vitro* plant/tissue cultivation, highlighting variations in nutrient and component concentrations.

2.8.2. Buffering substances:

A range of compounds, including Tris, Tricine, MES, HEPES, PB-74, and CAPS, are employed in tissue cultures to serve as buffering agents. These compounds exhibit varying levels of toxicity, depending on the plant species involved. MES stands out as a popular choice due to its robust buffering capacity, particularly in mildly acidic pH environments. Additionally, MES demonstrates minimal nutrient binding and is generally considered low in toxicity for most plants, except for *Brassica napus* protoplasts, where PB-74 is preferred in such instances.

2.8.3 pH

1. In the original study conducted by Murashige and Skoog in 1962, an acidic solution with a pH range of 5.7-5.8 was utilized.
2. Plant tissues typically thrive in an acidic pH range of 5.2-5.8, as observed in various studies (Skirvin et al., 1986).
3. Ensuring stable buffering conditions is crucial for long-term cultivation, as the pH undergoes constant fluctuations throughout the process. One of the reasons MES is frequently used is because it has a good buffering capacity within the pH range of 5-7 (approximately). Additionally, it is non-toxic to the majority of plant species.

2.8.4. Material required:

1. Basal salts
2. Vitamins
3. Buffering substance (MES)
4. Carbon resource (sucrose)
5. Reinforcing agent (agar)
6. NaOH/HCl for pH adjustment
7. Glass beaker
8. Weighing balance
9. Magnetic stirrer and pellet
10. pH meter
11. Measuring cylinders
12. Pipette
13. Bottles for autoclaving

2.8.5. Procedure:

1. Measure precise amounts of basal salts, vitamins, sucrose, MES, and agar (for solid medium) using a weighing balance. To streamline the process, create a stock powder of basal salts and vitamins by combining the indicated masses as per the summary table.
2. Prepare three-fourths of the final volume of distilled water in a glass beaker.
3. Dissolve salts, vitamins, sucrose, and MES (excluding agar) in the prepared water using a magnetic stirrer.
4. After complete dissolution, measure and adjust the pH level as necessary.
5. Use a measuring cylinder to adjust the final volume.
6. Transfer the liquid to bottles for autoclaving, adding agar to each bottle proportionally.
7. Sterilize the media bottles in an autoclave, ensuring proper temperature and pressure settings.
8. Allow the sterilized bottles to cool slightly before pouring the contents onto plates, considering safety and agar solidification temperatures.
9. Maintain sterility by working in a controlled environment, possibly utilizing a flow box to minimize contamination.
10. If desired, add hormones or antibiotics to the cooling medium.
11. Apply the five-second rule to assess the temperature suitability for labile compounds before adding them.
12. Transfer the medium into plates, remembering to shake the bottle before each pour.

2.8.6. Preparation of Solid Media:

1. For solid medium, choose between agar or phytagel, with recommended concentrations of 5-10 g/l for agar and 1.5-2.5 g/l for phytagel.
2. After pouring, allow the plates to cool and solidify, ensuring they are inverted to prevent moisture accumulation.
3. Incubate all plates and tubes for a minimum of 24 hours before use to verify sterility.

2.8.7. Storage and Sterilization:

- Store basal salts, vitamins stock, prepared liquid media, and solid MS plates at 4°C.
- Load the autoclave with the media for sterilization, ensuring proper sealing and setting autoclave parameters (time, temperature, and exhaust rate).
- After sterilization, carefully remove the containers from the autoclave, wearing heat-proof gloves to avoid burns.

Figure 1: Pouring of nutrient agar media

2.9. Hoagland media:

2.9.1. Introduction:

Hoagland solution, named after plant physiologist Daniel Israel Arnon and collaborator Hoagland, is a nutrient solution extensively utilized in plant biology and agricultural research to cultivate and study plants under controlled laboratory conditions. This solution offers a balanced and readily available blend of essential mineral nutrients crucial for plant growth and development. Originally formulated with precise combinations of macro- and micronutrients, it includes nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), and trace elements such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo), boron (B), and chlorine (Cl). Hoagland solution mimics natural soil conditions, with nutrient concentrations tailored to meet diverse plant species' specific nutritional requirements (Hoagland, 1950). Typically, it is prepared as a concentrated stock solution, which is diluted to the desired strength with distilled water before use. Adjustments to pH levels are made using acids or bases to optimize nutrient availability for plant uptake. In research, Hoagland solution serves as a staple for investigating plant physiology, nutrient absorption, and responses to environmental stimuli.

It is particularly useful for hydroponic experiments, enabling precise nutrient control and facilitating studies on plant responses to deficiencies, toxicities, and environmental stresses. The scalable solution presented herein addresses the requirements of plant cell, tissue, and organ cultures by offering indispensable macroelements, microelements, and an iron source to facilitate optimal growth and development (Murashige, Skoog,1962).

Table 5: composition of Hogland Media

Components	**Basal Salt Mixture Composition (milligrams/liter)**
Potassium nitrate	505.50
Calcium nitrate	820.75
Magnesium sulphate	240.94
Potassium dihydrogen phosphate	136.10
Manganese chloride.4H_2O	1.81
Boric acid	2.86
Molybdenum trioxide.2 H_2O	2.00
Zinc sulphate.7 H_2O	0.22
Copper sulphate.5 H_2O	0.08
Ferric tartarate	5.00
TOTAL gm/liter	1.71

2.9.2. Preparation:

1. To achieve complete dissolving, 1.71 grams of dehydrated basic salt combination to be dissolved in 600ml of distilled water. To eliminate any residual powder, it is recommended to rinse the media vial with a little quantity of distilled water.
2. Stir the liquids continuously until the powder is fully dissolved. The required heat-stable additives should be added to the solution prior to autoclaving.
   The pH of the medium should be adjusted to the desired level by employing a 1N HCl/NaOH solution.
3. The final volume should be adjusted to 1000ml using distilled water. The medium should be sterilized by subjecting it to autoclaving at a temperature of 15 pounds or 121ºC for duration of 15 minutes.
4. The autoclaved medium should be cooled to a temperature of 45ºC prior to the addition of the filter-sterilized heat-labile additives.
5. The necessary quantity of medium should be poured into sterile culture vessels in an aseptic manner.

6. To maintain optimal storage conditions and extend shelf life, protect the dehydrated plant tissue culture media powder from atmospheric moisture due to its high hygroscopic nature.

2.9.3. Precautions:

1. Ensure all equipment, including glassware, stir bars, and water used in preparation, is properly sterilized. Autoclaving at appropriate temperatures (usually around 121°C) and durations (typically 15-20 minutes) is essential to eliminate contaminants.
2. Accurately measure all ingredients according to the recipe or manufacturer's instructions. Use precise weighing scales and measuring cylinders to avoid inaccuracies in concentrations, which can affect the growth of microorganisms.
3. After sterilization, allow the nutrient broth to cool sufficiently before pouring into sterile containers or Petri dishes. Pouring hot media can cause condensation and subsequent contamination.
4. Work in a sterile environment such as a laminar flow hood or under aseptic conditions to minimize the risk of contamination. Wear appropriate personal protective equipment (PPE) such as gloves and lab coat.
5. Ensure thorough mixing of the ingredients after sterilization to achieve homogeneity. Use sterile stir bars or vigorous swirling to evenly distribute components.
6. Store prepared nutrient broth media in sterile containers or tubes at appropriate temperatures (usually around 4°C for short-term storage or -20°C for long-term storage) to prevent microbial growth.
7. Perform regular quality control checks by incubating a sample of prepared media to ensure sterility before use in microbial culture.
8. Store prepared nutrient agar plates in sealed plastic bags or containers at appropriate temperatures (usually around 4°C for short-term storage or -20°C for long-term storage) to maintain sterility and prevent dehydration.
9. Clearly label agar plates with the date of preparation, contents, and any other relevant information. Proper labeling ensures traceability and helps in monitoring the expiration of the media.

2.10. References:

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