BOTANY [Hons.]
Fourth Semester
C8P (Practical)

Molecular Biology

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Study 1: Preparation of LB medium

Introduction:

LB Broth is the most extensively used nutritionally rich medium, for the culture and growth of bacteria specially *E. coli*. It is commonly known as lysogeny broth, Luria broth, Lennox broth, LB Agar or Luria-Bertani medium. It is frequently utilized during cloning experiments in order to amplify competent bacteria. Bertani G. first created the media to culture *Shigella* indicator strain. There are numerous diverse formulations of LB broth, but the most common composition is made of peptides and casein peptones, trace elements, mineral and vitamins. LB media can also be used to create LB agar plates after the addition of agar which assist the growth of bacteria on a solid medium.

Materials required:

Chemicals:

1. **Tryptone**
2. **Yeast extract**
3. **NaCl**
4. **Bacto agar (optional)**
5. **NaOH**
6. **Selectable component *e.g.*, antibiotics (optional)**
7. **Deionised water [dH_2O]**

Glasswares & Plastic Wares:

1. Beakers / media Bottle
2. Petridishes
3. Conical flasks
4. Glass rods
5. Stirrer
6. Spatula
7. Measuring cylinder

Equipments:

1. Measuring balance
2. Autoclave
3. pH meter

Procedure:

1. Approximately ~80% of the final volume of dH2O [i.e. 800ml] to be added to a sterile media bottle.
2. A volumetric flask or measuring cylinder must be used to measure the desired final volume.
3. The final volume to be adjusted as in the table given after the addition of the calculated amounts of tryptone, yeast extract, and NaCl to the dH2O.
4. The cap of the media bottle should be sealed and shaken until components are completely dissolved.

TABLE 1: COMPONENT COMPOSITION

FINAL VOLUME	1000ml
Component	*Weight [g]*
Tryptone	1
Yeast extract	0.5
NaCl	1
bacto agar (optional)	1.5

Procedure:

1. The solution should be then neutralized (pH = 7.0) using NaOH. ~ 1mL of 1N NaOH should be expected.
2. After adjusting the pH the solution should then be transferred to a sterile volumetric flask/measuring cylinder and the final volume adjusted using dH_2O.
3. The solution is to be well mixed before returning it to the media bottle.
4. The appropriate amount of agar to be added to the solution only for solid media.
5. The media bottle is then to be sealed propoerly and autoclaved on liquids cycle.
6. After autoclaving, the solution to be cooled to 55°C.
7. For solid medium only, approximately 15mL of the media to be poured into each labeled petri dish and allowed to cool to room temperature.
8. The liquid sterilized media needs to be stored at 4°C.

Precautions:

1. **Generally, the capacity of the media bottle should be greater than the final volume of media that is being prepared.**
2. **When using culture media one should always label or identify the container with the specimen details before inoculation.**
3. **The culture medium has to be inoculated using aseptic techniques and incubated under the appropriate conditions.**
4. **Any unused media needs to autoclaved before disposal.**
5. **The media bottle should be handled only after sufficient cooling following autoclaving to protect the hands from burning.**
6. **Protective gloves and face mask are advised.**

Students please Note:

The relevant photographs will be sent to all of your email-IDs.

References/sources used:

1. Sambrook, J. & Russell, D. W. (2001). Molecular Cloning: A Laboratory Manual, 3 edn. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press. pp. A2.2

Further reading/ Viewing:

1. https://www.youtube.com/watch?v=NhnOsvmv2AY
2. http://www.cabri.org/guidelines/micro-organisms/M203Ap1.html