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CORE JOBS · INTERVIEW QUESTIONS

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Plant, Animal and Microbial Biotechnology Interview Questions

INSIDE

- 24 questions with short answers
- 5 numericals with the working
- Grouped by topic

Companies do not publish their interview questions; these are ours.

Online, with links to the notes: **iaeacademy.in/study/plant-animal-microbial-biotechnology/interview-questions**

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Plant tissue culture, regeneration and secondary metabolites

1. What is totipotency, and why does plant tissue culture depend on it?

Totipotency is the ability of a single plant cell to divide and develop into a whole plant. Differentiated cells can dedifferentiate into callus and then restart development when given the right hormones and medium. This is why a small explant can be regenerated into many complete plants in culture.

2. Why does the auxin to cytokinin ratio in the medium decide what an explant forms?

Plant cells respond to the relative level of the two hormones, not to either one alone. A high auxin to cytokinin ratio favours root formation, a high cytokinin to auxin ratio favours shoot formation, and a roughly balanced ratio keeps the cells dividing as undifferentiated callus.

3. What is the difference between organogenesis and somatic embryogenesis?

Organogenesis forms single organs, shoots or roots, from callus or an explant, and the two must be induced separately to make a plant. Somatic embryogenesis forms an embryo-like structure from one somatic cell. It has both a shoot pole and a root pole, is not vascularly joined to the parent tissue, and germinates directly into a plantlet.

4. A plant cell suspension in exponential growth rises from 2×10^5 to 1.6×10^6 cells per mL in 48 hours. What is the doubling time?

The doubling time is 16 h.

> **WORKING**
>
> 1. $N = N_0 \cdot 2^n$ for n doublings
> 2. $2^n = (1.6 \times 10^6)/(2 \times 10^5) = 8$, so $n = 3$
> 3. $t_d = 48\ \text{h}/3 = 16\ \text{h}$

5. What is somaclonal variation, and why does it matter for micropropagation?

Somaclonal variation is genetic or epigenetic change among plants regenerated from cultured cells. It arises during culture, through chromosome rearrangements, point mutations and changes in DNA methylation. It is a problem when true-to-type clones are needed, so axillary bud propagation and short callus phases are preferred. It can also be used to select useful variants.

Protoplast fusion and transgenic plants

6. What do the vir genes do in Agrobacterium-mediated transformation?

The vir genes sit on the Ti plasmid outside the T-DNA and carry out the transfer. Phenolics such as acetosyringone from wounded plant cells activate the VirA and VirG proteins. The vir proteins then nick the T-DNA at its border repeats and move the single-stranded T-DNA into the plant cell. Any gene placed between the borders is transferred.

7. What is the difference between a somatic hybrid and a cybrid?

A somatic hybrid carries the nuclear genomes of both fusion parents. A cybrid has the nucleus of one parent and the cytoplasm, so the mitochondria and chloroplasts, of the other, or a mix of organelles. Cybrids are used to move cytoplasmically inherited traits such as cytoplasmic male sterility.

8. What is the difference between a selectable marker and a reporter gene?

A selectable marker, such as nptII for kanamycin resistance, lets only transformed cells survive or grow on selective medium. A reporter gene, such as gus or gfp, gives a product that can be detected or measured, as a blue colour or fluorescence, and shows where and how strongly a gene is expressed. It does not by itself kill untransformed cells.

9. A transgenic plant carries one copy of a dominant antibiotic resistance gene at a single locus, with no copy on the other chromosome. It is self-pollinated and 200 seeds are germinated on the antibiotic. How many seedlings are expected to survive?

About 150 seedlings are expected to survive.

> **WORKING**
>
> 1. Selfing a hemizygous plant gives TT : T0 : 00 in the ratio $1 : 2 : 1$
> 2. Seedlings with at least one copy of the gene survive, which is $3/4$ of them
> 3. $200 \times 3/4 = 150$

10. Why is plastid transformation used for some transgenic crops?

Plastid genomes are inherited maternally in most crops, so the transgene is not carried in pollen, which lowers the risk of gene flow. Each cell holds many plastid genomes, so expression can be very high. Insertion is by homologous recombination at a chosen site, which avoids position effects. The plastid genome must be made homoplasmic.

Animal cell culture

11. What does serum do in an animal cell culture medium?

Serum, usually fetal bovine serum at 5 to 10%, supplies growth factors, hormones, attachment factors, transport proteins and trace elements that a basal medium lacks. It also binds toxins and adds buffering capacity. Its composition varies between batches and it can carry contaminants, so serum-free media with defined supplements are used for products.

12. What is the difference between a primary culture and a continuous cell line?

A primary culture is started directly from tissue and keeps the properties of that tissue, but divides for only a limited number of generations. A continuous cell line has gained the ability to divide indefinitely, spontaneously or by transformation. It is easy to maintain, but often has an abnormal chromosome number and has drifted from the tissue of origin.

13. Why are animal cells frozen slowly with DMSO and stored in liquid nitrogen?

A cryoprotectant such as DMSO, at about 10%, limits ice crystal formation and solute damage. Cooling at about 1 °C per minute lets water leave the cells gradually, so little lethal ice forms inside them. Storage at minus 196 °C stops metabolism and ageing. The cells are thawed quickly and the DMSO is diluted out.

14. Cells in exponential growth double every 24 hours. What is their specific growth rate?

The specific growth rate is $\mu = \ln 2 / t_d$, which holds only while growth is exponential. With a doubling time of 24 hours, $\mu = 0.693/24 \approx 0.029\ \mathrm{h}^{-1}$. Over one day this gives $e^{\mu t} = e^{0.693} = 2$, the doubling.

WORKING

1. $\mu = \ln 2 / t_d$
2. $\mu = 0.693 / 24\ \text{h}$
3. $\mu \approx 0.0289\ \text{h}^{-1}$

15. A cell suspension is mixed 1:1 with trypan blue. A hemocytometer count gives an average of 50 unstained cells in each large square, and each square holds 0.1 mm³, that is 10^{-4} mL. What is the viable cell concentration of the original suspension?

The viable cell concentration is 1×10^6 cells/mL.

WORKING

1. Cells per mL in the mixture: $50 / 10^{-4}\ \text{mL} = 5 \times 10^5$
2. Dilution factor is 2, since the sample was mixed 1:1 with dye
3. $5 \times 10^5 \times 2 = 1 \times 10^6$ cells/mL

Hybridomas, stem cells, cloning and transgenic animals

16. In hybridoma technology, how does HAT medium select the hybrid cells?

Aminopterin blocks the de novo route of nucleotide synthesis, so cells must use the salvage route, which hypoxanthine and thymidine feed. The myeloma partner lacks the enzyme HGPRT, so unfused myeloma cells die. Unfused spleen B cells have the enzyme but die after a short time in culture. Only the fused hybridomas survive.

17. What is the difference between totipotent, pluripotent and multipotent cells?

Totipotent cells, such as the zygote, can form a whole organism including the extra-embryonic tissues. Pluripotent cells, such as embryonic stem cells, can form all cell types of the body but not the placenta, and cannot form an organism alone. Multipotent cells, such as haematopoietic stem cells, are limited to the related cell types of one tissue, here all the blood cells.

18. Why does gene targeting for a knock-out mouse use both neo and HSV-tk markers?

The neo gene gives resistance to G418, so it selects cells that took up the construct anywhere in the genome. HSV-tk lies outside the homology arms. If the whole construct integrates at random, tk comes along and ganciclovir kills the cell. Homologous recombination leaves tk behind, so those cells survive both drugs, which enriches correctly targeted cells.

19. How is an animal cloned by somatic cell nuclear transfer?

The nucleus of an egg is removed, and a nucleus from a somatic donor cell is placed in the egg by fusion or injection. The egg is activated electrically or chemically to start division, grown to an early embryo and transferred to a surrogate mother. The offspring is almost identical to the donor, apart from mitochondrial DNA from the egg.

Microbial products

20. What is the difference between trophophase and idiophase in a fermentation?

Trophophase is the phase of rapid growth, in which primary metabolites such as amino acids, nucleotides and ethanol are made. Idiophase follows when growth slows, often through nutrient limitation, and secondary metabolites such as antibiotics are made, which the cell does not need for growth. Such fermentations first build biomass and then switch to production.

21. In a batch culture, biomass rises from 1.0 g/L to 9.0 g/L while 16 g/L of glucose is consumed. What is the biomass yield coefficient on glucose?

The yield is 0.5 g biomass per g glucose.

WORKING

1. $Y_{X/S} = \Delta X / \Delta S$
2. $\Delta X = 9.0 - 1.0 = 8.0\ \text{g/L}$
3. $Y_{X/S} = 8.0/16 = 0.5\ \text{g/g}$

22. Why is yeast chosen instead of E. coli for some recombinant proteins?

Yeast is a eukaryote that can fold and secrete many proteins and add glycosylation, which E. coli cannot do, and it does not make endotoxin. E. coli grows faster and cheaper and gives high yields of simple proteins, but often forms insoluble inclusion bodies. Yeast glycosylation is of the high mannose type and differs from human patterns.

23. What is the usual order of steps in recovering an intracellular recombinant protein?

Cells are harvested by centrifugation or filtration and broken open, and the debris is removed. The extract is concentrated or partly purified, for example by precipitation. Chromatography follows, with a capture step first, then intermediate steps and a final polishing step. The purified protein is then formulated or dried.

24. How does a soil screen for new antibiotic producers work?

Soil is diluted and plated to give single colonies, often of actinomycetes and fungi. Colonies are tested against test organisms, for example by the cross streak or agar plug method, where a clear zone shows inhibition. Active isolates are grown in liquid culture, and the product is extracted and tested further.

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