How to use auxanograms to identify nutritional requirements.

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Background

Auxanography (Beijerinck 1889) offers a quick, economical, highly efficient method of determining the growth requirements of auxotrophic mutants. Conidia or mycelial fragments are plated into minimal agar with or without sorbose and the surface is spotted with substances being tested. Cloudy growth in the region of a spot indicates response to the nutrient or nutrients. The precedure need not be completely aseptic.

The method has many advantages." (1) Many growth factors can be tested on one plate, and each plate serves as its own control. (2) Back mutations appear as discrete colonies. (3) If high concentrations of growth factor are toxic, it will not fail to elicit a response because a gradient in concentration is set up by diffusion. (4) Double requirements may be identified by a zone of growth between two supplements. (5) Inhibitions by other supplements are identified by the failure of spreading growth in their vicinity." (Ryan 1950). (6) Substances difficult to sterilize can be tested (Lederberg 1946).

Auxanography can be used to identify new single and multiple requirements, or to confirm and identify genotypes of segregants from crosses. The method has been widely used. For photographic examples see Perkins 1949 (Ustilago), Pontecorvo 1949 (Aspergillus).

Procedure

Plate ~1 ml of a conidial suspension in minimal medium. With nonconidiating strains, a suspension of macerated mycelial fragments can be used. Add substances to be tested to the solidified agar at marked spots. Responses can be seen in as little as 12 hours at 34º. A supply of sterile water blanks in 75 mm tubes is convenient for preparing suspensions. Mycelia can be fragmented by using a 1 ml pipette and grinding against the wall of the small tube. The 1 ml suspension can be poured into the plate without use of a pipette.

To test solid substances, a small quantity of the test mixture is placed on surface of the agar using a flamed, flattened platinum-iridium blade as a microspatula and taking the substance direct from the reagent bottle. For liquids, a small drop is delivered from a Pasteur pipette. Test substances need not be sterile.

If the strain being tested has an already-known requirement in addition to the unknown requirement, that substance can be supplied by adding it to the minimal medium used for plating.

The requirements of unknown auxotrophs can be keyed out stepwise, using two or three successive plates. All three may be prepared at the same time. Those not used in the first step are refrigerated until they are needed.

Step 1: Spot with solid casein hydrolysate, DNA or RNA, liquid vitamin stock solution, and Tween 40 or Tween 80.

Step 2: (a) If the response was to nucleic acid, spot a second plate with adenine and uracil.
(b) If the response was to vitamins, spot with individual vitamins.
(c) If the response was to Tween, spot with acetate and individual fatty acids.
(d) If the response was to caseine hydrolysate, spot with each of six pooled testers made up with mixtures of solid amino acids as shown in the table. The mixtures are formulated to provide for known multiple requirements and to avoid known cross-inhibitions. A third plate can be spotted with a single substance or substances to confirm results with the pooled testers.

References

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TESTER SET FOR AMINO ACID REQUIREMENTS

	1	**2**	**3**	**4**	**5**	**6**
1	histidine	glycine	proline	anthranilic acid	glutamine	isoleucine
2		serine	arginine	phenylalanine	threonine	valine
3			homoserine	tyrosine	methionine	glutamic acid
4				leucine + PABA	cysteine	tryptophan
5					lysine	putrescine
6						asparagine + aspartic acid

Each test mixture contains the substances found in the column beneath the number and the row to the right of the same number. Mixtures are conveniently kept as solids in small screwcap vials, with 20 mg of each component except anthranilic acid (10 mg) and PABA (para-aminobenzoic acid) (5 mg). Ryan (1950) credits K. C. Atwood for devising this testing scheme . The choice of substances can be varied as desired.

Step 3: Confirm the requirement indicated in Step 2 by spotting individual amino acids.

Notes: Difco NZ is an acid hydrolysate that lacks tryptophan and provides acetate. Tryptophan is not destroyed in enzymatic digests of casein such as NZ-case.

Guanine auxotrophs are inhibited by adenine. Histidine, homoserine, and asparagine auxotrophs are inhibited by complex organic medium and by amino acids that compete for uptake. See the Neurospora Compendium (Perkins *et al.* 2001) for these and other inhibitions.

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