Bird Medium: an alternative to Vogel Medium

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This medium was designed to circumvent some problems that arise in the use of Medium N (Vogel 1964 Am. Naturalist 98:435-446). These are, among others, the presence of high levels of citrate, a chelator which leaves the concentration of calcium and trace elements uncertain; the use of ammonium nitrate, which leaves the actual source of nitrogen ambiguous; the use of $MgSO_4$, which does not allow the experimenter to vary the concentration of magnesium and sulfur independently; the high activity coefficient for the pK_a values of citrate, which makes the pH unnecessarily sensitive to ionic strength; the use of sucrose, which leaves uncertain the nature and relative amounts of the hexose(s) being used at any particular moment; the need to use chloroform as a preservative, which results in the gradual depletion of the aqueous phase of complexes of trace elements. Molybdate ion is excluded from the trace elements used for Solution 1 because, in concentrated stock solutions, it forms water-insoluble complexes with phosphate plus ammonium ion; instead, it is included in Solution 2, where its presence is genign. Finally, concentrations are expressed in moles rather than in grams, which eases the experimenter's task of thinking in terms of stoichiometry and biomass yield. Bird Medium is not meant to supplant Vogel Medium for routine auxanography, stock-keeping, searches for mutants, or growth of Neurospora for preparing DNA, mitochondria, etc. However, it should be seriously considered for critical applications such as preparation of samples for microarrays and for analysis of subtle phenotypes of new mutants.

Bird Medium supports rapid germination of conidiospores and rapid growth of mycelium in good yield, with apparently normal morphology. It appears at least equal to, or better than, Vogel Medium in this regard. It should be noted, however, that mycelial pads harvested from Bird Medium have a subtly different texture from those grown on Vogel Medium, being somehow more slippery to the touch. It is not evident that more slippery is less "normal" for Neurospora, nor more so, than less slippery.

Solution 1

INGREDIENT	CONCENTRATION AT 1X	
	g/liter	mM
water 45 ml		
MES(Sigma M-8250)	4.85 g	22.75
K_2HPO_4	1.74 g	10
NH_4Cl	1.34 g	25
K_2SO_4	0.174 g	1.
NaCl	0.058 g	1.
trace elements without molybdate, 10,000x *, 0.1 ml.		

Solution 2

INGREDIENT	CONCENTRATION AT 1X	
	g/liter	mM
water 38 ml.		
$MgCl_2.6H_2O$	0.203 g	1.
$CaCl_2.2H_2O$	0.074 g	0.5
glucose	18 g	100
biotin, 10,000x solution *, 0.1 ml.		
sodium molybdate, 10,000x *, 0.1 ml.		

Make up MES, K_2HPO_4, NH_4Cl, K_2SO_4, NaCl, and trace elements solution without molybdate in 45 ml. of warm water, which will produce a volume of 50 ml. (**"Solution 1; 20X final strength"**). There is no utilizable carbon source in this solution. It should be stored at room temperature, without chloroform.

Make up $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, biotin, molybdate solution, and glucose in 38 ml. of warm water, which will produce a volume of 50 ml. (**"Solution 2; 20X final strength"**). Store at room temperature over a few ml. of chloroform.

Obviously, it will usually be convenient to make up these two solutions on at least ten times the above scale.

Solutions 1 and 2 can be autoclaved separately in their concentrated form, if desired, and diluted into sterile water, or each can be diluted to 2X, autoclaved, and then combined. The pH of the diluted medium, about 5.8, equals that of Vogel medium, and should not be adjusted. Solution 1 can instead be made up to 20 ml. of 50X instead of 50 ml. of 20X by dissolving the ingredients in 15 ml. of warm water rather than in 45 ml. In practice, however, there are likely to be fewer calculational errors in medium-making if both stock solutions are kept as 20X stock solutions for dilution to 2X, rather than as 50X and 20X. The minor savings in space for Solution 1 are probably not be worth the increased risk of error.

*** BIOTIN, 10,000X**

	mg/100 ml	mM
water	100 ml	
biotin	6.1	0.25

*** TRACE ELEMENTS WITHOUT MOLYBDATE, 10,000X**

INGREDIENT	CONCENTRATION AT 10,000X	
	mg/100 ml	mM
water	95 ml	
citric acid·H_2O	4200	20
$ZnSO_4 \cdot 7H_2O$	5750	20
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	980	2.5
$CuSO_4 \cdot 5H_2O$	250	1.0
H_3BO_3	62	1.0
$MnSO_4 \cdot H_2O$	33.3	0.2

*** SODIUM MOLYBDATE, 10,000X**

	mg/100 ml	mM
water	100 ml	
$Na_2MoO_4 \cdot 2H_2O$	48.3	0.2