MEDIA FOR MUTANTS EXPERIMENT

CZAPEK GLUCOSE 2 %	1 liter	1.2 liter
NaNO ₃	3 g	3.6 g
$K_2HPO_4*3H_2O$	1.31 g	1.572 g
MgSO _{4*} 7H ₂ O	0.5 g	0.6 g
FeSO _{4*} 7H2O	0.01 g	0.012 g
KCl	0.5 g	0.6 g
glucose	20 g	24 g
For liquid medium only: MES 20 mM	3.9 g	4.68 g
2-(N-Morpholino) ethane sulphonic acid		
Distilled H ₂ O	to 11	to 1.21
Agar 1%	10 g	12 g

Starting pH 8.3 Final pH 6 (with HCl 1M) After autoclaving pH 5,08

ACM	1 liter	1.2 liter
NaNO ₃	6 g	7.2 g
MgSO _{4*} 7H ₂ O	0.52 g	0.625 g
KCl	0.52 g	0.625 g
KH ₂ PO ₄	1.52 g	1.824 g
FeSO _{4*} 7H ₂ O	traces	traces
ZnSO _{4*} 7H ₂ O	traces	traces
casamino acids	1.5 g	1.8 g
yeast extract	1.5 g	1.8 g
bacteriological peptone	2 g	2.4 g
glucose	2 g	2.4 g
Distilled H ₂ O	to 11	to 1.21
Agar 1%	10 g	12 g

Starting pH 5,52 Final pH 5,52

After autoclaving pH 5.1

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CZAPEK-DOX	
(CZAPEK SUCROSE 3 %)	
NaNO ₃	2 g
Potassium sulphate	1.31 g
Magnesium glycerophosphate	0.5 g
FeSO _{4*} 7H2O	0.01 g
KCl	0.5 g
Sucrose	30 g
For liquid medium only: MES 20 mM	3.9 g
2-(N-Morpholino) ethane sulphonic acid	
Distilled H ₂ O	to 11
Agar 1%	10 g

33.4~g in 1~l distilled H_2O , 40.08 in 1.2~l Starting pH 6.8 Final pH 6 (with HCl 1M) After autoclaving pH 5,47

CZAPEK PECTIN 0.8%	1 liter
NaNO ₃	3 g
K ₂ HPO _{4*} 3H ₂ O	1.31 g
MgSO _{4*} 7H ₂ O	0.5 g
FeSO _{4*} 7H2O	0.01 g
KCl	0.5 g
Pectin	8 g
For liquid medium only: MES 20 mM	3.9 g
2-(N-Morpholino) ethane sulphonic acid	
Ethanol	13 ml
Distilled H ₂ O	to 11
For solid medium: Agar 1%	10 g

Starting pH 4.3

Final pH 5.5 (with NaOH 1M)

After autoclaving pH 5.41 (+ZnSO₄ 10 mM pH 5.19)

Procedure:

- 1. In a glass beaker dissolve salts and MES in distilled water bringing it at a temperature between 50°C and 60°C
- 2. In the mean time dissolve pectin in ethanol in a separate beaker (subtract ethanol ml from the water ml required to bring to volume)
- 3. When salts and MES are completely dissolved and the temperature is between 50°C and 60°C add pectin suspension
- 4. Leave the medium on the magnetic stirrer until pectin will be perfectly dissolved
- 5. Leave it to cool
- 6. Measure the pH bringing it to 5.5

CONTROL AND ZINC

	0	5mM	10mM	15mM	20mM	25mM	30 mM	35 mM	40 mM	45 mM
Media	U									
		Zn	Zn	Zn	Zn	Zn	Zn	Zn	Zn	Zn
	1 flask	1 flask	1 flask	1 flask						
Czapek	with	with	with	with						
glucose	100 ml	100 ml	100 ml	100 ml						
~ .		+500 µl	+1 ml	+1.5 ml	+2 ml	+2.5 ml	+3 ml	+3.5 ml	+4 ml	+4.5 ml
flasks		stock.sol	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.
with 100 ml	2 -1-4	1 M	1 M	1 M	1 M	1 M	1 M	1 M	1 M	1 M
	3 plates 6 wells	3 plates	3 plates	3 plates	3 plates 3 wells					
+ 1g agar	5.08	3 wells	3 wells	3 wells	5 wells					
pH after	(pH before	4.76	4.61	4.36	4.16	4.09				
autoclave	autoclave:	4.70	7.01	4.50	4.10	4.02				
	6)									
	1 flask	1 flask	1 flask	1 flask						
Czapek	with 100	with	with	with	with	with	with	with	with	with
dox	ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml
~ .		+500 µl	+1 ml	+1.5 ml	+2 ml	+2.5 ml	+3 ml	+3.5 ml	+4 ml	+4.5 ml
flasks		stock.sol	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.
with	2.1.	1 M	1 M	1 M	1 M	1 M	1 M	1 M	1 M	1 M
100 ml	3 plates	3 plates 3 wells	3 plates	3 plates 3 wells	3 plates 3 wells					
+ 1g agar	6 wells 5.47	5 wells	3 wells	5 wells	5 wells					
pH after	(pH before	3.88	3.80	3.81	3.81	3.79				
autoclave	autoclave:	3.00	3.00	3.01	3.01	3.17				
	6)									
	1 flask	1 flask	1 flask	1 flask						
ACM	with 100	with	with	with	with	with	with	with	with	with
	ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml
flasks		+500 µl	+1 ml	+1.5 ml	+2 ml	+2.5 ml	+3 ml	+3.5 ml	+4 ml	+4.5 ml
with		stock.sol	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.
100 ml	2.1.	1 M	1 M	1 M	1 M	1 M	1 M	1 M	1 M	1 M
+ 1g agar	3 plates	3 plates	3 plates	3 plates						
	6 wells 5.1	3 wells	3 wells	3 wells	3 wells					
pH after	(pH before	4.53	4.39	4.33	4.41	4.39				
autoclave	autoclave:	4.33	7.33	7.33	7,71	7.33				
	5.52)									
TOTAL	9 plates	9 plates	9 plates	9 plates						
PLATES	18 wells	9 wells	9 wells	9 wells	9 wells	9 wells	9 wells	9 wells	9 wells	9 wells
WELLS		2220					,	,	,	,

CADMIUM

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	0.01mM	0.05mM	0.10mM	0.15mM	0,25mM	0,30mM	0,35mM	0,40mM		
Media	Cd	Cd	Cd	Cd	Cd	Cd	Cd	Cd	Cd	
Czapek glucose	1 flask with 100 ml +100 μl	1 flask with 100 ml +500 μl	1 flask with 100 ml +1 ml	1 flask with 100 ml +1.5 ml	1 flask with 100 ml +2 ml	1 flask with 100 ml +2,5 ml	1 flask with 100 ml + 3 ml	1 flask with 100 ml +3,5 ml	1 flask with 100 ml + 4 ml	
flasks with	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	
100 ml + 1g agar	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	
pH after autoclave	5.27	5.21	5.24	5.17	5.16					
Czapek dox	1 flask with 100 ml +100 μl	1 flask with 100 ml +500 μl	1 flask with 100 ml +1 ml	1 flask with 100 ml +1.5 ml	1 flask with 100 ml + 2 ml	1 flask with 100 ml +2,5 ml	1 flask with 100 ml +3 ml	1 flask with 100 ml +3,5 ml	1 flask with 100 ml + 4 ml	
flasks with	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	stock.sol.	
100 ml + 1g agar	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	
pH after autoclave	5.58	5.56	5.61	5.38	5.34					
ACM flasks with 100 ml	1 flask with 100 ml +100 µl stock.sol.	1 flask with 100 ml +500 μl stock.sol.	1 flask with 100 ml +1 ml stock.sol.	1 flask with 100 ml +1.5 ml stock.sol.	1 flask with 100 ml +2 ml stock.sol.	1 flask with 100 ml +2,5 ml stock.sol.	1 flask with 100 ml +3 ml stock.sol.	1 flask with 100 ml +3,5 ml stock.sol.	1 flask with 100 ml +4 ml stock.sol.	
+ 1g agar	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	3 plates 3 wells	
pH after autoclave	5.15	5.16	5.14	5.13	5.12					
TOTAL PLATES WELLS	9 plates 9 wells	9 plates 9 wells	9 plates 9 wells	9 plates 9 wells	9 plates 9 wells	9 plates 9 wells	9 plates 9 wells	9 plates 9 wells	9 plates 9 wells	

MENADIONE

Media	0.1mM menad	0.5mM menad	1mM menad	2.5 mM menad	5 mM menad	10 mM menad	20 mM menad	40 mM menad
Czapek glucose	1 flask with 100 ml +10 µl	1 flask with 100 ml +50 µl	1 flask with 100 ml +100 μl	1 flask with 100 ml +250 μl	1 flask with 100 ml + 500 μl	1 flask with 100 ml + 1 ml	1 flask with 100 ml + 2 ml	1 flask with 100 ml + 4 ml
flasks with 100 ml + 1g agar	stock.sol. 3 plates	stock.sol. 3 plates	stock.sol. 3 plates	stock.sol. 3 plates	stock.sol. 3 plates	stock.sol. 3 plates	stock.sol. 3 plates	stock.sol. 3 plates

STOCK SOLUTIONS

Zn as ZnSO_{4*}7H₂O (PM 287.54 g/mol)

stock solution 1 M (28.752 g in 100 ml)

Cd as 3CdSO_{4*}8H₂O (PM 769.5 g/mol)

stock solution 10 mM (0.385 g in 50 ml)

MENADIONE (PM 276,24 g/mol) (SIGMA M2518-100G, kept at -20°C)

stock solution 1M (13,812 g in 50 ml)

1st inoculum: strain O. maius Zn

99 Plates utilized: 9 cm

CG 0, 5, 10, 15, 20, 25 mM Zn

CG 0.01, 0.05, 0.10, 015, 0.2 mM Cd

CD 0, 5, 10, 15, 20, 25 mM Zn; 0.01, 0.05, 0.1 mM Cd

CD 0.15, 0.2 mM Cd

ACM 0, 5, 10, 15, 20, 25 mM Zn; 0.01, 0.05, 0.1, 0.15, 0.2 mM Cd

9 Multi-dishes utilized: 12 wells, 2 ml x well:

- 1 CG 0, 5, 10, 15 mM Zn
- 2 CG 20, 25 mM Zn; 0.15, 0.2 mM Cd
- 3 CG 0, 0.01, 0.05, 0.1 mM Cd
- 4 CD 0, 5, 10, 15 mM Zn
- 5 CD 20, 25 mM Zn; 0.15, 0.2 mM Cd
- 6 CD 0, 0.01, 0.05, 0.1 mM Cd
- 7 ACM 0, 5, 10, 15 mM Zn
- 8 ACM 20, 25 mM Zn; 0.15, 0.2 mM Cd
- 9 ACM 0, 0.01, 0.05, 0.1 mM Cd

2nd inoculum: strain O. maius Zn

81 Plates utilized: 9 cm

CG 0, 30, 35, 40, 45 mM Zn

CG 0.25, 0.30, 0.35, 0.40 mM Cd

CD 0, 30, 35, 40, 45 mM Zn

CD 0.25, 0.30, 0.35, 0.40 mM Cd

ACM 0, 30, 35, 40, 45 mM Zn

ACM 0.25, 0.30, 0.35, 0.40 mM Cd

6 Multi-dishes utilized: 12 wells, 2 ml x well:

3rd inoculum: strain O. maius Zn and O. maius A

CG 0, 0.1, 0.5, 1, 2.5, 5, 10, 20, 40 mM menadione strain O. maius Zn

CG 0, 5, 10, 15, 20, 25, 30, 35, mM Zn strain O. maius A

CG 0.01, 0.05, 0.1, 0.15, 0.2, 0.25, 0.30 mM Cd strain O. maius A

CG 0, 0.1, 0.5, 1, 2.5, 5, 10, 20, 40 mM menadione strain O. maius A