

## MEDIA FOR MUTANTS EXPERIMENT

<b>CZAPEK GLUCOSE 2 %</b>	<b>1 liter</b>	<b>1.2 liter</b>
NaNO <sub>3</sub>	3 g	3.6 g
K <sub>2</sub> HPO <sub>4</sub> *3H <sub>2</sub> O	1.31 g	1.572 g
MgSO <sub>4</sub> *7H <sub>2</sub> O	0.5 g	0.6 g
FeSO <sub>4</sub> *7H <sub>2</sub> O	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 2-(N-Morpholino) ethane sulphonic acid	3.9 g	4.68 g
Distilled H <sub>2</sub> O	to 1 l	to 1.2 l
Agar 1%	10 g	12 g

**Starting pH 8.3    Final pH 6 (with HCl 1M)**

**After autoclaving pH 5,08**

<b>ACM</b>	<b>1 liter</b>	<b>1.2 liter</b>
NaNO <sub>3</sub>	6 g	7.2 g
MgSO <sub>4</sub> *7H <sub>2</sub> O	0.52 g	0.625 g
KCl	0.52 g	0.625 g
KH <sub>2</sub> PO <sub>4</sub>	1.52 g	1.824 g
FeSO <sub>4</sub> *7H <sub>2</sub> O	traces	traces
ZnSO <sub>4</sub> *7H <sub>2</sub> 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 <sub>2</sub> O	to 1 l	to 1.2 l
Agar 1%	10 g	12 g

**Starting pH 5,52    Final pH 5,52**

**After autoclaving pH 5,1**

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

**33.4 g in 1 l distilled H<sub>2</sub>O, 40.08 in 1.2 l**

**Starting pH 6.8    Final pH 6 (with HCl 1M)**

**After autoclaving pH 5,47**

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

**Starting pH 4.3**

**Final pH 5.5 (with NaOH 1M)**

**After autoclaving pH 5.41 (+ZnSO<sub>4</sub> 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

[illegible]

# CADMIUM

[illegible]

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 flasks with 100 ml + 1g agar	1 flask with 100 ml +10 µl stock.sol. 3 plates	1 flask with 100 ml +50 µl stock.sol. 3 plates	1 flask with 100 ml +100 µl stock.sol. 3 plates	1 flask with 100 ml +250 µl stock.sol. 3 plates	1 flask with 100 ml +500 µl stock.sol. 3 plates	1 flask with 100 ml +1 ml stock.sol. 3 plates	1 flask with 100 ml +2 ml stock.sol. 3 plates	1 flask with 100 ml +4 ml stock.sol. 3 plates

## STOCK SOLUTIONS

**Zn as ZnSO<sub>4</sub>·7H<sub>2</sub>O (PM 287.54 g/mol)**

stock solution 1 M (28.752 g in 100 ml)

**Cd as 3CdSO<sub>4</sub>·8H<sub>2</sub>O (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)

**1<sup>st</sup> 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, 0.15, 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

**2<sup>nd</sup> 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:

**3<sup>rd</sup> 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