R. solanacearum / Culture media

Find here a selection of recipes for *Ralstonia solanacearum* culture media

Original webpage and more recipes (see links at the end of the document)

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Non-selective media

Non-selective media are generally used for growth of pure culture of *R. solanacearum*. (e.g. to retrieve cultures from frozen stocks or for successive plating of cells). These media can also be used for isolation of R. *solanacearum* from fresh, symptomatic plants, due to the high density of the pathogen in the tissues.

- Casamino acid-Peptone-Glucose (CPG) medium

See reference at the end of the document

	Per liter
Casamino acid (casein hydrolysate)	1 g
Peptone	10 g
Glucose	5 g
For solid media (plates) add:	
Agar	17 g

Adjust pH to 6.5-7.0 if necessary. Autoclave at 121˚C for 20 minutes.

On solid medium, colonies of *R. solanacearum* usually are visible after 48-72 hours of incubation at 28ºC. Colonies of the normal (or virulent type) are white or cream-colored, irregularly-round, fluidal, and opaque; and colonies of the mutant (or non-virulent) type are uniformly round, smaller, and butyrous (dry). This shift from virulent to non-virulent bacterial cells occurs during storage or under oxygen stress in liquid media.

- Tripheny tetrazolium chloride (TTC or TZC) medium

See reference at the end of the document

Prepare 1 liter of CPG medium:

	Per liter
Casamino acid (casein hydrolysate)	1 g
Peptone	10 g
Glucose	5 g
For solid media (plates) add:	
Agar	17 g

Adjust pH to 6.5-7.0 if necessary. Autoclave at 121˚C for 20 minutes.

After autoclaving cool the medium to 55˚C and **add 5 ml of a 1% stock solution of 2, 3, 5-tripheny tetrazolium chloride**. The stock can be filter sterilized or autoclaved for 5 minutes at 121˚C, and stored at 4˚C or frozen.

On solid medium, colonies of *R. solanacearum* usually are visible after 48-72 hours of incubation at 28ºC.
This medium was developed to differentiate between the two colony types: virulent colonies appear white with pink centers and non-virulent colonies appear dark red.

Semi-selective media

Semi-selective media can be used for isolation of the pathogen from nonsymptomatic plants or from water and soil samples. Although widely used, they do not support growth of all *R. solanacearum* strains and/or do not suppress growth of all related or unrelated Gram-negative bacteria.

- SM-1 medium
See reference at the end of the document

Prepare 1 liter of TTC (or TZC) medium:

	Per liter
Casamino acid (casein hydrolysate)	1 g
Peptone	10 g
Glucose	5 g
For solid media (plates) add:	
Agar	17 g

Adjust pH to 6.5-7.0 if necessary. Autoclave at 121˚C for 20 minutes.

After autoclaving cool the medium to 55˚C and **add 5 ml of a 1% stock solution of 2, 3, 5-tripheny tetrazolium chloride**. The stock can be filter sterilized or autoclaved for 5 minutes at 121˚C, and stored at 4˚C or frozen.

Add:	**Per liter**
Merthiolate tincture *	5 to 50 µl
Crystal violet **	50 mg
Polymyxin ß sulfate **	100 mg
Tyrothricin **	20 mg
Chloromycetin **	5 mg
Cycloheximide **	50 mg

* Merthiolate tincture contains 1 part merthiolate per 1000 parts of 50% alcohol. Determine the best concentration to suppress local microflora, as suggested (See reference at the end of the document).

** Dissolve in 5 ml of 70% ethanol 30 minutes prior to use.

On solid medium, colonies of *R. solanacearum* usually are visible after 2-5 days of incubation at 28ºC.
Typical bacterial colonies appear fluidal, irregular in shape, and white with pink centers.

- Modified SMSA medium
See reference at the end of the document

Prepare 1 liter of TTC (or TZC) medium, except substitute glycerol (5 ml per liter) for the glucose:

	Per liter
Casamino acid (casein hydrolysate)	1 g
Peptone	10 g
Glycerol	**5 ml**
For solid media (plates) add:	
Agar	17 g

Adjust pH to 6.5-7.0 if necessary. Autoclave at 121˚C for 20 minutes.

After autoclaving cool the medium to 55˚C and **add 5 ml of a 1% stock solution of 2, 3, 5-tripheny tetrazolium chloride**. The stock can be filter sterilized or autoclaved for 5 minutes at 121˚C, and stored at 4˚C or frozen.

Add:	**Per liter**
Crystal violet *	5 mg
Polymyxin ß sulfate *	100 mg
Bacitracin *	25 mg
Chloromycetin *	5 mg
Penicillin *	0.5 mg
When inhibition of fungal contaminants id desirable, add:	
Cycloheximide *	100 mg

* Dissolve in 5 ml of 70% ethanol 30 minutes prior to use.

On solid medium, colonies of *R. solanacearum* usually are visible after 2-5 days of incubation at 28ºC.
Typical bacterial colonies appear fluidal, irregular in shape, and white with pink centers.

References

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- Tripheny tetrazolium chloride (TTC or TZC) medium
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- SM-1 medium
Granada, GA; Sequeira, L. 1983. A new selective medium for *Pseudomonas solanacearum*. Plant Disease 67:1084-1088.

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French, ER; Gutarra, L; Aley, P; Elphinstone, J. 1995. Culture media for *Pseudomonas solanacearum* isolation, identification and maintenance. Fitopatologia 30:126-130.

Internet links

***R. solanacearum* / Culture media original webpage:**
http://plantpath.ifas.ufl.edu/rsol/Culturemedia.html

More recipes from out *Ralstonia solanacearum*/Brown rot-Bacterial wilt website:
http://plantpath.ifas.ufl.edu/rsol/Publications/Publi_DiagnosticsProtocols.html
http://plantpath.ifas.ufl.edu/rsol/Publications/Publi_PDInfoManagement.html