Vancomycin Protocol and Selection Guide

Background

Vancomycin inhibits growth of many Gram positive bacteria including the antibiotic resistant superbug, MRSA (Methicillin resistant *Staphylococcus aureus*). Vancomcyin is effective for treating MRSA infections because it inhibits cell wall synthesis through a different mechanism than β-lactam antibiotics.

Vancomycin sensitivity test

I. Principle:
The vancomycin test is performed in a fashion similar to the bacitracin sensitivity test. Resistance to vancomycin can be used to differentiate a few of the catalase-negative gram-positve genera.

II. Inoculum
Strains are grown on blood agar plates overnight at 35°C in CO. More than 1 day of incubation may be necessary for more fastidious genera such as the gemellae, alloiococci, and helcococci.

III. Reagents and Materials
1. 30µg vancomycin disk The disks are stored according to the manufacturer's instructions.
2. trypticase soy sheep blood agar plates IV.

IV. Procedure
1. Transfer several colonies of the strain in question to one-half of a blood agar plate and steak heavily.
2. Place the vancomycin susceptibility testing disk (30 µg) in the heavy part of the streak.
3. Incubated the plate in a CO^2enhanced atmosphere at 37 C overnight. Some strains (alloiococci, gemellae, helcococci) may require 48 h or more to show sufficient growth to interpret the test.

V. Reading and Interpretation
Any zone of growth inhibition is considered positive (sensitive). The test is interpreted as resistant (negative) only if there is growth right up to the edge of the disk. This is not a susceptibility test, it is a sensitivity test for identification.

Genetic Transformation of Mature Citrus:

1. Seed germination medium (SGM): 4.3 g/L of Murashige and Skoog (MS) salts (10)(see Note 2), 10 g/L of agar (Bacto-agar, Difco, Detroit, MI), pH 5.7.

2. Inoculation medium (IM): 4.3 g/L of MS salts, 10 mL/L of vitamin stock solution, 30 g/L of sucrose, pH 5.7.

3. Cocultivation medium (CM): IM plus 2 mg/L of 2,4-dichlorophenoxyacetic acid (2,4-D), 2 mg/L of indole-3-acetic acid (IAA), 1 mg/L of 2-isopentenyl-adenine (2,i-P), 8 g/L of agar, pH 5.7.

4. Shoot regeneration medium (SRM): IM plus 3 mg/L of 6-benzylaminopurinem (BAP). Medium is semisolidified using 10 g/L of agar at pH 5.7, supplemented with 100 mg/L of kanamycin sulfate, 500 mg/L of cefotaxime, and 250 mg/L of vancomycin.

5. Shoot-tip grafting medium: 4.3 g/L of MS salts, 10 mL/L of vitamin stock, 75 g/L of sucrose, pH 5.7.

6. Vitamin stock: 10 g/L of myo-inositol, 20µg/L of thiamine-HCl, 100µg/L pyridoxine-HCl, 100 µg/Lof nicotinic acid.

7. 2,4-D stock solution: 5 mg/100 mL. Prepare by dissolving the powder in a few drops of dimethyl sulfoxide (DMSO). Adjust volume with double-distilled water. Store at 4°C.

8. IAA stock solution: 5 mg/100 mL. Prepare as for 2,4-D and store at 4°C.

9. 2,i-P stock solution: 5 mg/100 mL. Prepare as for 2,4-D and store at 4°C.

10. BAP stock solution: 5 mg/100 mL. Prepare by dissolving the powder in a few drops of 1 NNaOH. Complete final volume with double-distilled water. Store at 4°C

11. Kanamycin sulfate stock solution: 100 mg/mL. Prepare by dissolving 1 g of powder in 10 mL of double-distilled water. Sterilize by filtration through a 0.2-µm membrane, make 1-mL aliquots in sterile Eppendorf tubes and store at –20°C.

12. Cefotaxime stock solution: 250 mg/mL. Prepare by dissolving 1 g of powder in 4 mL of double distilled water. Sterilize by filtration through a 0.2µm membrane, make 1-mL aliquots in sterile Eppendorf tubes and store at –20°C.

13. Vancomycin stock solution: 250 mg/mL. Prepare as for cefotaxime, aliquote, and store at –20°C.

General Selection Table

Cell-Line	Species	Tissue	Media	Vancomycin
293	Human	Kidney; fetal	DMEM	400 (µg/ml)

References:

Courvalin, Patrice. "Vancomycin Resistance in Gram-Positive Cocci." *Oxford Journals*(2006): 25-34. *Clinical Infectious Diseaes*. Web. 21 Aug. 2012.

Pollock, H.M., Holt J., and Murray C., Comparison of susceptibilities of anaerobic bacteria to cefemenoxime, ceftriaxone and other antimicrobial compounds, Antimicrob. Agents Chemother., Vol. 23, pp.780-783, 1983

Silva J.A. and Fukai S., The impact of carbenicillin, cefotaxime and vancomycin on chrysanthemum and tobacco TCL morphogenesis and Agrobacterium growth, J. Appl. Hort., Vol. 3(1), pp. 3-12, 2001.

Joshi, S., Ray, P., Manchanda, V., Bajaj, J., Chitnis, D. S., Gautam, V., &...Balaji, V. (2013). Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence & susceptibility pattern. *Indian Journal Of Medical Research*, 137(2), 363-369

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