The effects of herbs traditionally used to treat respiratory illnesses by the Pacific Islanders on the quorum-sensing activity of Pseudomonas aeruginosa

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Abstract

Management of Cystic Fibrosis (CF) lung infection due to biofilm-mediated colonization of Pseudomonas aeruginosa is a challenge. Plants traditionally used in the Pacific Islands (Piper methysticum Hibiscus tiliaceus, and Waltheria americana) to treat respiratory illnesses were tested to measure their biofilm and quorum-sensing inhibitory properties against P. aeruginosa. Water extracts of the root, and both water and methanol extracts of the stem of P. methysticum were found to inhibit biofilm formation (p < 0.05), with the methanol extract inhibiting biofilm formation to the largest degree (89.7% inhibition), even compared with the tobramycin control (84.6% inhibition).

Introduction

Cystic Fibrosis (CF) is an autosomal recessive disorder resulting from mutations in the CFTR protein (Derichs 2013). This leads to an overproduction of mucus leaving patients susceptible to bacterial colonization and life-threatening infections (Twomey et al. 2013). Pseudomonas aeruginosa is a gram-negative opportunistic pathogen that relies on quorum-sensing and biofilm formation. The biofilms they produce cause a down regulation of flagellin-mediated Toll-like receptors, inhibiting the ability of phagocytes to move and recognize the flagellar components of P. aeruginosa (Amiel et al. 2010). To date, infection by P. aeruginosa is the most frequent pathogenic cause of death in CF patients (Govan et al. 2007). As treatment of chronic P. aeruginosa infection remains a concern, this research aims to screen plants traditionally used in the Pacific Islands to treat respiratory illnesses for their ability to disrupt the quorum-sensing activity of P. aeruginosa.

Methods

1. Plants were collected from Oahu, Hawaii
2. Plants were frozen with liquid nitrogen and ground into a powder to prepare methanol and water extracts
3. Extracts were filtered, dried, and resuspended in dimethyl-sulfoxide
4. Pseudomonas aeruginosa was grown on Nutrient Agar and isolates were added to Nutrient Broth amended with plant extracts to 1% (v/v)
5. Water and tobramycin were used as negative and positive controls, respectively
6. Tubes were emptied and stained with safranin, then acetone was added to record absorbance readings at 518 nm
7. Results were analyzed using one-way ANOVA with post-hoc Tukey Pairwise comparisons

Results

• All plant extracts, except water extracts from H. tiliaceus, significantly reduced biofilm formation (p < 0.05)
• Water extracts of the root, and both water and methanol extracts of the stem from P. methysticum were statistically similar to the tobramycin positive control
• P. methysticum root extract in water, and stem extracts in both water and methanol reduced biofilm formation by 71.0%, 79.9%, and 89.7%, respectively
• The tobramycin positive control reduced biofilm formation by 84.6%

Figure 1: The 95% CI of the absorbance reading means for each plant extract and control. The samples in red squares denote the samples that were not significantly different from the negative control, while the samples in blue circles denote extracts that were significantly different from the water negative control. Asterisks denote the plant extracts that were not statistically different (p > 0.05) than the positive control. Abbreviations are: NC = water negative control, PC = tobramycin positive control, WAW = W. americana water extract, WAM = W. americana methanol extract, PMSW = P. methysticum stem water extract, PMSM = P. methysticum stem methanol extract extract, PMRW = P. methysticum root water extract, PMRM = P. methysticum root methanol extract, HTW = H. tiliaceus water extract, HTM = H. tiliaceus methanol extract

Discussion

Through this research the historic use of P. methysticum to treat respiratory illnesses in the Pacific Islands was validated. Because methysticum showed the ability to inhibit biofilms when extracted with both water and methanol, there is likely more than one chemically active component in P. methysticum that can inhibit biofilm formation. Piper methysticum was observed to inhibit biofilm formation to a greater degree than tobramycin, the antibiotic commonly used to treat infection in CF patients. A crucial step now is to obtain P. aeruginosa isolates from CF patients and to repeat the study. There is great potential for P. methysticum to be used in modern medicine upon fractionation of extracts to determine the specific compounds responsible for inhibiting biofilm formation.

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References


