Effect of Nicotine and Sugars on the Growth and Biofilm Formation of Pseudomonas aeruginosa

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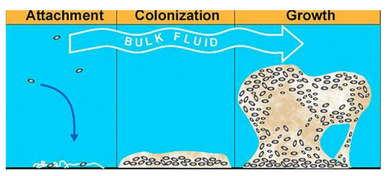
Abstract

Nicotine is the addictive chemical found in tobacco and is consumed by millions of people everyday. It has been proven that smokers experience more bacterial infections than non-smokers. Sugars are also a health risk and can lead to obesity and tooth decay. Pseudomonas aeruginosa (PA) is a gram-negative opportunistic bacterium that is found in the human body, primarily in the gut, as well as the oral cavity. The ability of PA to form biofilms allows the bacteria to survive in adverse conditions and to be more tolerant to antibiotics than stationary-phase planktonic bacteria. The presence of polysaccharide capsules helps the bacteria to stick together, thus promoting biofilm formation. The objective of this study is to examine how nicotine and various sugars affect the growth and biofilm formation of PA. To determine the effect of nicotine on the growth, varying concentrations of nicotine were added to a flat bottom 96-well microtiter plate containing a fresh culture of PA in M63 liquid. After 48 hours of incubation, the growth of the PA was observed using a spectrophotometer. The results were then analyzed using a sample t-test to observe for significant differences ($p<0.05$). To study the effect of nicotine and sugars on biofilm formation, the crystal violet biofilm assay was used. After 48 hours of incubation of the fresh culture PA, the non-adhered PA bacteria were washed and decanted and the adhered PA bacteria were stained with crystal violet. The amount of stain in the bacteria was released with 30% acetic acid and was then measured and analyzed using a sample t-test for significance ($p<0.05$). All data represented quadruplicate runs and the results indicated that nicotine at the concentrations used had no significant effect on the growth of PA, but significantly decreased the amount of biofilms formed, with higher concentrations of nicotine having a greater suppressing effect ($p<0.05$). A capsule stain was carried out to observe the effect of nicotine on the capsules. The observance of decreased capsule size confirmed the fact that nicotine significantly suppresses biofilm formation. The results also showed that of the sugars tested, sucrose, maltose and galactose all significantly suppressed biofilm formation of PA, while glucose had no significant effect. When combined with nicotine at a concentration of $1x10^{-3}$ M, the results showed that the suppressing effect of nicotine with sugar was not summative.

Introduction

Pseudomonas aeruginosa (PA) is a gram-negative opportunistic bacterium found in the human body, including the gut and oral cavity. It is known for its ability to form biofilms, which can lead to various chronic infections such as pneumonia in cystic fibrosis patients, chronic wounds and catheter-associated infections. It affects millions of people each year and can consequently lead to death (Bjarnsholt, 2013). Nicotine and sugars have also been shown to increase the susceptibility of bacterial infections (Bajaitkar, 2008). This study was designed to examine if nicotine has an effect on the growth and biofilm formation of PA in the absence and presence of various sugars.



Objectives

1. To evaluate the effect of nicotine on the growth of PA
2. To observe the effect of nicotine on the biofilm formation of PA
3. To determine the effect of various sugars on the biofilm formation of PA with and without nicotine
1. To observe the effect of nicotine on capsule size

Procedure

Effect of Nicotine on Growth of *Pseudomonas aeruginosa*

A loopful of fresh PA was inoculated into sterile LB broth and incubated at 37oC overnight. The bacteria was then standardized to an OD of 0.1. The bacteria, M63 media, and varying concentrations of nicotine ranging from 1x10-1 M to 1x10-11 M in increments of 1x10-1 M were then added to the wells of a 96-well flat bottom microtiter plate to give eight replicates of each concentration. The control wells were created by adding the bacterial dilution and the M63 media. The microtiter well plate containing the bacterial dilution was incubated for 48 hours at 37oC and the turbidity in each well was measured using a SpectraMax 190. The data was then analyzed using a sample t-test to test for significant differences ($p < 0.05$).

Effect of Nicotine and Sugars on Biofilm Formation of *Pseudomonas aeruginosa*

A 24 hour culture of PA grown in LB broth was added to each well of a 96-well round bottom microtiter plate for all tests. To test the effect of nicotine only, varying final concentrations ranging from 1x10-1 M to 1x10-11 M in increments of 1x10-1 M were added to each well in addition to of M63 media. To evaluate the effect of different sugars, each sugar at a final concentration 0.1% and 0.2% were added to each well in addition to M63 media. And to observe the effect of nicotine and varying concentrations of sugar together, nicotine kept at a constant final concentration of 1x10-3 M, sugar at final concentrations of 0.1% and 0.2% and M63 media were added to each well. The plate was then incubated for 48 hours at 37oC. After incubation, the cells were dumped out by turning the plate over and shaking out the liquid. The plate was then gently submerged in a tub of water twice to help remove the unattached cells. 0.1% solution of crystal violet was then added to each well and left to incubate at room temperature for 15 minutes. The plate was then rinsed 4 times with water and left upside down to dry for a few hours. To quantify the biofilm, 30% acetic acid was added to each well to solubilize the crystal violet and was left to incubate at room temperature for 15 minutes. Then the solubilized crystal violet was transferred to a new 96-well flat bottom microtiter plate. The absorbance was then read using a SpectraMax 190 at 550 nm to measure the amount of biofilm present. The data was analyzed using a sample t-test to test for significant differences ($p < 0.05$).

Results

Nicotine Concentration	Mean ± SD[A]
$1x10^{-1}$ M	0.18 ± 0.08
$1x10^{-2}$ M	0.17 ± 0.04
$1x10^{-3}$ M	0.19 ± 0.05
$1x10^{-4}$ M	0.23 ± 0.06
$1x10^{-5}$ M	0.26 ± 0.10
$1x10^{-6}$ M	0.26 ± 0.09
$1x10^{-7}$ M	0.26 ± 0.06
$1x10^{-8}$ M	0.31 ± 0.09
$1x10^{-9}$ M	0.28 ± 0.15
$1x10^{-10}$ M	0.25 ± 0.11
$1x10^{-11}$M	0.27 ± 0.07
Control	0.21 ± 0.09

Table 1. Effect of Nicotine on Growth of PA for 48 hours. [A]Triplicate runs (8 wells of each concentration/run. Control = No nicotine. * Significant difference from control using sample t-test at p = < 0.05.

Nicotine Concentration	Mean ± SD[A]
$1x10^{-1}$ M	0.06 ± 0.01*
$1x10^{-2}$ M	0.07 ± 0.01*
$1x10^{-3}$ M	0.07 ± 0.01*
$1x10^{-4}$ M	0.10 ± 0.03*
$1x10^{-5}$ M	0.11 ± 0.05*
$1x10^{-6}$ M	0.10 ± 0.01*
$1x10^{-7}$ M	0.10 ± 0.01*
$1x10^{-8}$ M	0.12 ± 0.03*
$1x10^{-9}$ M	0.13 ± 0.03*
$1x10^{-10}$ M	0.11 ± 0.01*
$1x10^{-11}$M	0.12 ± 0.04*
Control	0.31 ± 0.03*

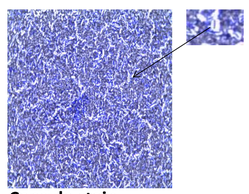
Table 2. Effect of Nicotine on the Biofilm Formation of PA for 48 hours. [A] Triplicate runs (8 wells of each concentration/run. Control = No nicotine. * Significant difference from control using sample t-test at p = < 0.05.

Sugar	Sugar (0.1%)[A,B]	Sugar (0.2%)[A,B]
Sucrose	0.55 ± 0.15*	0.65 ± 0.10*
Maltose	0.80 ± 0.14*	0.65 ± 0.09*
Galactose	0.61 ± 0.09*	0.61 ± 0.05*
Glucose	0.89 ± 0.12*	0.87 ± 0.13*
Control	0.95 ± 0.12*	0.95 ± 0.12*

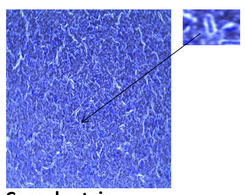
Table 3. Effect of Different Sugars on the Biofilm Formation of PA. [A] Quadruplicate runs (8 wells of each concentration/run). [B] Mean ± Standard Deviation. Control (no sugar): M ± SD= 0.95 ± 0.12. * Significant difference from control (no sugar) using sample t-test at p=<0.05.

	Sugar (0.1%)		Sugar (0.2%)	
	Sugar Only[A,B]	Sugar + Nicotine[A,B]	Sugar Only[A,B]	Sugar + Nicotine[A,B]
Sucrose	0.55 ± 0.15*	0.64 ± 0.14*	0.65 ± 0.10*	0.67 ± 0.09*
Maltose	0.80 ± 0.14*	0.81 ± 0.10*	0.65 ± 0.09*	0.69 ± 0.08*
Glucose	0.89 ± 0.12*	0.82 ± 0.09*	0.87 ± 0.13*	0.76 ± 0.08*
Galactose	0.61 ± 0.09*	0.62 ± 0.12*	0.61 ± 0.05*	0.63 ± 0.12*
Control	0.95 ± 0.12*	0.95 ± 0.12*	0.95 ± 0.12*	0.95 ± 0.12*

Table 4. Effect of Different Sugars in the Presence of Nicotine on the Biofilm Formation of PA. [A] Quadruplicate runs (8 wells of each concentration/run). **B** Mean ± Standard Deviation. Control (no sugar): 0.95 ± 0.12, Nicotine Control: 0.59 ± 0.09, Nicotine = $1x10^{-3}$ M.* Significant comparing sugar to sugar + nicotine using student t-test at p=<0.05.



Capsule stain
Pseudomonas aeruginosa grown *without nicotine*



Capsule stain
Pseudomonas aeruginosa grown *with nicotine*

Materials

Bacteria:

Pseudomonas aeruginosa (ATCC 10145)

Reagents:

Crystal Violet (Fisher Scientific 229641000)
India Ink (Fisher Scientific R21518)
Acetic Acid (Fisher Scientific MFCD00036152)
Nicotine (Sigma-Aldrich N5260-50G)
Sucrose (Fisher Scientific 57-50-1)
Maltose (Fisher Scientific 6363-53-7)
Glucose (Fisher Scientific 50-99-7)
Galactose (Fisher Scientific 59-23-4)

Media:

Bacto-Tryptone Extract (BD 211701)
Bacto-Yeast Extract (BD 212750)
NaCl (Sigma-Aldrich S9888)
K_2HPO_4(Fisher Scientific P288-500)
KH_2PO_4 (Fisher Scientific P285-500)
$(NH_4)_2SO_4$ (Sigma-Aldrich A5132)

Plates and Equipments:

Microtiter Plates (BD Biosciences 353911)
Microtiter Plate lids (BD Biosciences 353913)
SpectraMax 190

Statystical Analysis:

Statistical analysis conducted with Student t-tests

Conclusions

1. Nicotine had no significant effect on the growth of PA, but significantly decreased biofilm formation in a dosage-dependent manner
2. Sucrose, maltose, and galactose all significantly suppress biofilm formation of PA, while glucose has no effect
3. When the sugars were combined with nicotine at a concentration of $1x10^{-3}$ M, the suppressing effect of nicotine and sugar were not additive
4. A reduction in capsule size was observed with the addition of nicotine to reaffirm the fact that nicotine suppresses biofilm formation

Discussion

At the normal concentration of nicotine in a smoker's body, nicotine significantly suppressed the amount of biofilms formed. This was confirmed by the observance of reduced capsule size of PA. With the amount of nicotine smoked and sugar consumed, our findings show that biofilm formation of PA is suppressed, but other studies have found that other bacteria demonstrate different results

References

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