

Determination of Unknown Cell Wall Thickness Using β -Lactam Antibiotics

Microbiology Independent Project
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11/17/00

Introduction

Peptidoglycan is a major constituent of the gram-positive bacterial cell wall and “provides rigid mechanical stability by virtue of its highly cross-linked latticework structure” (Mandell, 1980). [18--be careful of the number of quotes you use . . . better to paraphrase] Bacteria rely on their cell walls to maintain normal growth and development. A disturbance to the processes involved in cell wall synthesis can either halt development or even kill the organism. Antibiotics, such as Penicillin, Ampicillin, and Cephalothin act as bacteristatics by inhibiting the completion of the peptidoglycan cross-linkage [4?]. In order to accomplish this, antibiotics must first penetrate the cell wall, acylate the membrane-bound transpeptidase, [and render them useless?] thereby rendering it useless in linking the peptidoglycan subunits. Since it is imperative for an antibiotic to first cross the cell wall of a bacterium [4?], the success of an antibiotic in inhibiting the growth of a colony should be dependent upon, among other factors, the relative cell wall thickness of a species. This study aims to utilize what understanding exists of the underlying mechanisms of β -lactam antibiotics action to investigate [determine?] the cell wall thickness of several [why several . . . I thought I just gave you one and you compared it to knows?] gram-positive, rod bacteria. Using published cell wall thickness from known bacterial species, and assessing the bacterial antibiotic sensitivity in the form of inhibition [15--first time use of inhibition . . . might be good idea to describe to the reader what these are] zones, this study intends to elucidate the cell wall thickness of an unidentified bacterium, X.x [I, what's up with the Xes? Why not “unkown bacterium?”].

The [Our? . . or we hypothesize that . . .] hypothesis states that bacterium X.x. will have quantitatively different inhibition zone radii than either *Bacillus subtilis* (B.s.) or

Bacillus megaterium (*B.m.*). If [delete this] is true then [we . . or who is this it character?] it will be expected that a) Bacterium *X.x.* will have significantly thicker (or thinner) cell wall than either *B.s.* or *B.m.* [12-Is this not implicit from your hypothesis?] b) A reasonable [estimate of the]cell wall thickness for bacterium *X.x.* [could?] should be obtained from the multiple linear regression analysis of all antibiotic treatments [15--this is a bit unclear to me, perhaps it would be better to tell me what it is that you will measure and then leave the information as to how you plan to measure (i.e., regression analyses) for the M & M.]and c) Another gram-positive, rod bacterium of a different cell wall thickness could be plotted on the multiple regression line produced by this experiment.

Methods

Three T-soy broth tubes were inoculated with *Bacillus subtilis*, *Bacillus megaterium*, or *X.x.* bacteria and incubated at 37°C for 24 hours. [spell out when starting a sentence] 4.5-5.0 mm deep T-soy agar plates were prepared and inoculated with 0.1ml of broth culture [of one of the three species?]. Four different antibiotic discs [6, not enough info . . .size, concentration?, manufacturer?] were placed on each plate: Bacitracin, Ampicillin, Penicillin G, and Cephalothin. Discs were secured to the media by pressing them with flamed forceps [makes it sound like the forceps were still on fire . . .that would be a neat magic trick, but misrepresent your methods]. The plates were then inverted and placed in the 37°C incubator for another 24 hours. After incubation, plates were removed and inhibition zones were measured to the nearest millimeter using calipers (Fig.1).



Figure 1. Calipers were used to measure the inhibition zones that resulted after the 24 hour incubation period[nicely done with the picture!]

Single linear regression lines for each antibiotic treatment were generated using data collected by measuring the inhibition zone radii [6--Thus far, this is the part of the paper that lacks detail. It would be helpful if you were to put down the dependent and independent variables for you regression analyses first. As is, I am not quite sure what you did or how the analyses were used.]. The means [means for what? Of what?] and R-values for each group were calculated from raw data. Literature values for the cell wall thickness of *Bacillus subtilis* equal to 35 nm (Graham, 1993) and 21 nm for *Bacillus megaterium* (Doetsch, 1973) were used as independent variables while inhibition zone measurements were used as dependent variables. Multiple linear regression analysis was carried out using SigmaStat software.

Results

The Bacitracin treatment resulted in no measurable inhibition zones and was removed from the subsequent analyses [1-- good—but why did you tell me a different reason in the first sentence of the discussion?]. R-values for the remaining antibiotic treatments were calculated using linear regression statistics [sounds like this sentence belongs in the materials and methods] (Table 1). An R-value of 0.974 was obtained from

a multiple linear regression analysis. This [why not put the equation here?] regression analysis equation produced a value of 87 nm for the cell wall thickness of *X.x* [Figure 2]. The antibiotic of effectiveness trend for each bacterium is shown in Figure 2 [I don't think this sentence is needed].

Table 1. Mean radii of kill zones?, standard error and single linear regression R-values for varied antibiotic treatments.

	<i>Bacillus Subtilis</i>		<i>Bacillus Megaterium</i>		<i>X.x.</i>		R Value
	Mean (mm)	Std. Error	Mean (mm)	Std. Error	Mean (mm)	Std. Error	
Cephalothin	15.6	0.227	16.7	0.214	5	0.367	0.815
Ampicillin	9.2	0.306	10.1	0.332	3.1	0.135	0.652
Penicillin	7.6	0.227	9.45	0.166	1.17	0.24	0.937
All							0.974

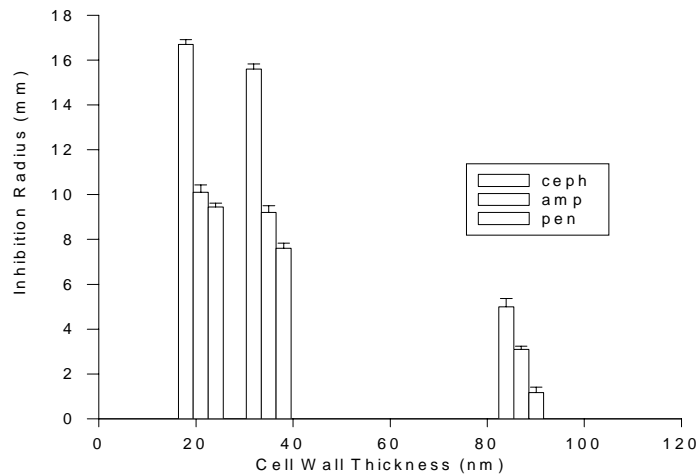


Figure 2. Dependence of inhibition radii on cell wall thickness is supported by the trends in each antibiotic treatment. [spell out antibiotics] . . . nicely done otherwise

Discussion

Bacitracin treatment results were disregarded after it was ascertained that it is derived from *Bacillus subtilis* cultures and would therefore have no effect on this species.

Linear regression data for penicillin G, cephalothin, and ampicillin yielded slightly varied R-values yet showed there could be a linear relationship between the inhibition radii and cell wall thickness. The combined statistical analysis of all treatments resulted in an R-value of 0.974, which demonstrates a strong correlation between the different data sets in response to antibiotic treatment. Due to this apparent association, the cell wall thickness of *X.x.* [we estimated?] was determined to be 87 nm. This result falls within literature values [4?] for gram-positive bacterial cell walls and supports our hypothesis that this bacterium has a quantitatively thicker cell wall than either *B.s.* or *B.m.*

Comparisons of cell wall ultra-structure and composition could lead to further insight into the results obtained in this experiment, which could either strengthen or weaken the correlation established [what does this sentence say? . . is it needed or would the example you use in the subsequent sentence be more useful]. For example, the relative abundance of transport proteins in the peptidoglycan layer or the size and polarity of the antibiotic molecule could influence the rate of penetration for different antibiotics. This study does not demonstrate that the multiple linear regression line equation holds true for other gram-positive bacteria, although the 0.974 R-value indicates this [9-] to be feasible.

Bibliography

Doetsch R.N., Cook T.M. Introduction to Bacteria and their Eco-Biology. University Park Press, Baltimore. 1973, p 42.

Graham L.L., Beveridge T.J. Journal of Bacteriology. “Structural Differentiation of the *Bacillus subtilis* 168 Cell Wall.” 1994 March. 176(5): 1413-21.

Mandell G.L., Sande M.A. Goodman and Gilman’s The Pharmacological Basis of Therapeutics. “Antimicrobial Agents: Penicillins and Cephalosporins.” Macmillan Publishing Company, New York. 1980, p. 1126-1161.

Any more?

Good job you guys. I thought your grammar and sentence structure were very appropriate for the task. Your sentences were generally very clear, but I suspect that the manuscript could have been improved if you had worked a little on describing the regression analyses . . . which, as it turned out, were a large part of your paper.

As it turns out, and as you might know, the cell walls of gram negative bacteria are about 1-2nm thick and those of gram positives range from 15-35nm depending on the species. The species which I gave you (*Bacillus cereus*) is often cited on the thick end of the spectrum. You value correctly determined that *B. cereus* contained a thicker cell wall. However, I suspect that your estimate of 87 nm is a bit large. It is known that *B. cereus* commonly produces endospores and this may account for some of the large thickness of your estimate (although this is rare for 24-hour cultures). Nevertheless, I think your study accurately approximates the direction (if not the actual thickness) of the cell and is worthy of a good grade.

Grade: 94—thanks for putting in so much work.