

The Effect of Laboratory Water Type on Bacterial Growth in Prepared Media

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ABSTRACT

For life on earth, water serves as a reactant in metabolic pathways and as the universal solvent. In its role as a solvent, the use of distilled or even ultrapure water is often specified when preparing growth media. In preparation instructions, the rationale for this choice is rarely given. We wondered if water could be too pure, lacking essential trace elements not provided by the medium powders. Conversely, could tap water still retain compounds that would inhibit microbial growth? We hypothesized that, with current water purification methods, tap water would be suitable for preparing media in the microbiology laboratory. To test this hypothesis, we prepared two commonly used media, Nutrient Broth and Tryptic Soy Broth, in water from three sources: tap, deionized, and ultrapure. We then measured growth of three bacteria commonly used in the microbiology teaching laboratory. Growth was observed in all media preparations. There was no inhibition of growth when tap water was used to prepare the media. For *Escherichia coli* grown in Nutrient Broth, use of tap water appeared to lead to increased growth. Growth in media prepared with ultrapure water did not significantly differ from that in media prepared with tap water, with the one exception, or deionized water. The use of high purity water will remain important when the exact composition of a medium is required. However, our results suggest that tap water is adequate when preparing media for general purposes such as establishing working cultures for short-term use.

Keywords: growth medium preparation, bacterial growth, ASTM water type, water quality, microbial growth

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INTRODUCTION

Microbial cultures in a laboratory setting are supported by a growth medium – a liquid or solid material that contains all the necessary nutrients for growth to occur (25). Ideally, these nutrients are provided in both the type and form in which they are biologically available to the microorganism of interest. Determining the correct form and type of nutrient is not always simple. In the early days of microbiology, bacteria were grown in liquid culture using meat infusions, such as would be used to prepare bouillon, or other extracts of biological material. Pasteur describes attempts to culture the causative agent of anthrax in meat broth, beer yeast extract, and even urine (11).

These complex and rich media typically supported good growth, but they impeded efforts to understand the details of microbial metabolism because their specific composition was unknown. Pasteur formulated a semi-defined growth medium composed of sugar, ammonium salts, and yeast ash (18, 24). Shifts to even more chemically defined media then led to identification of specific nutritional requirements. For example, the opportunistic fungal pathogen *Candida albicans* requires biotin (2, 3). *Haemophilus influenzae*, a bacterial cause of otitis media and meningitis, requires two factors named V and X (19, 23), later identified as nicotinamide adenine dinucleotide (NAD) and heme, respectively (7, 12).

Beyond the nutrients needed for metabolism, an important component of all growth media is water. Water can be considered a nutrient itself in that it can be a reactant in metabolic processes such as hydrolysis of polysaccharides into their monosaccharide subunits. However, it could be argued that water is most important as a solvent. One aspect of biological availability mentioned above is solubility and water is the solvent for living things on Earth. Thus, all cells require water. Vegetative cells of bacteria, fungi, and protozoa are susceptible to dehydration and die unless they have some way to retain water.

ASTM International (formerly the American Society for Testing and Materials) classifies reagent water into four types, with each type designation based on allowable limits of factors such as total organic carbon, bacterial counts, sodium and chloride concentration, and pH (1). Type I is the most highly purified. Dissolved salts are present at the lowest concentrations of any water type so that conductivity is very low (0.056 $\mu\text{S}/\text{cm}$) and resistivity is very high (18 $\text{M}\Omega\cdot\text{cm}$). Although tap water does not fall into any of the designated reagent water types, it is tested for constituents that might affect human health.

Just as water quality affects human health, so it may affect microbial growth. Water that is too pure may lack essential trace elements. However, water that is not pure enough may contain impurities that, while within safe levels for human health, may nevertheless be toxic to microbes. For example, water may retain residual antimicrobial compounds used during water treatment (9). With respect to the presence of toxins, many of the studies highlighting the importance of water purity in culture medium were done in the mid-20th century and before passage of the US Safe Drinking Water Act in 1974. Since then, water treatment approaches have improved (21) leading to the possibility that current water supplies are sufficiently pure to lack inhibitory agents.

The objective of this study was to determine if the quality of water used to rehydrate dry growth medium powders would affect bacterial growth. We hypothesized that tap water and deionized water would provide good growth, but that Type I water might result in decreased growth due to the lack of essential trace elements. We further hypothesized that, given current, improved, water treatment techniques, tap water would be sufficiently pure for use in typical laboratory cultures.

METHODS

To test our predictions, we prepared two types of complex media, Nutrient Broth and Tryptic Soy Broth using water from three different sources: tap, house deionized, and ultra-pure (Tap, DI, and MQ, respectively). We inoculated each growth medium with working stock cultures of one of three bacterial species commonly used in microbiology teaching laboratories (*E. coli*, *B. subtilis*, or *S. aureus*). We determined bacterial growth, as measured by turbidity of the culture, after 24 hours of incubation. Growth of each species in each medium was compared using ANOVA.

BACTERIA ISOLATES AND CULTURE CONDITIONS

Growth media were commercially obtained (Neogen, Lansing, MI) as premixed powders. Nutrient Broth (NB, Acumedia NCM0110A) contains beef extract (3 g/L) and an enzymatic digest of gelatin (5 g/L). Powders from two different lots (103093B and 106127A) were used for comparison. Tryptic Soy Broth (TSB, Acumedia NCM0004A, lot 109771B) contains an enzymatic digest of casein (17 g/L), an enzymatic digest of soybean meal (3 g/L), sodium chloride (5 g/L), dipotassium phosphate (2.5 g/L), and glucose (2.5 g/L). Tryptic Soy Agar (Tryptic Soy Broth with 15 g/L agar) was used to culture working stocks of each isolate. Sodium chlo-

ride (S671-500) was obtained from Thermo Fisher Scientific (Fair Lawn, NJ).

Three water sources were used to prepare the growth media: Milli-Q (MQ), house deionized (DI), and tap (T). Milli-Q (Milli-Q⁵⁰ system, MilliporeSigma, Burlington, MA) provides ultrapure, or Type 1 water (1). The house deionized water system (Suez NA, Paramus, NJ) provides Type 2 water (Mike Ancy, SUEZ Water Technologies & Solutions, personal communication).

Growth media were prepared according to the manufacturer's specifications. Experimental broth media were prepared by dissolving growth medium powder in one of the three water sources, then transferred as 4 mL aliquots to glass culture tubes (13 × 100 mm, Thermo Fisher), capped, and sterilized by autoclaving (15 minutes, 121 °C, 15 psi, Priorclave, London, UK). TSB tubes for working stock cultures were prepared with DI water. Tryptic Soy Agar (TSA) plates were prepared by adding the powder to house DI water, heating to dissolve the agar, then autoclaving. Following sterilization, the medium was tempered to 50 °C in a water bath and then aseptically transferred to plastic Petri dishes (15 × 100 mm, MIDSCI, Valley Park, MO) and left to solidify.

Three bacterial species commonly used in the microbiol-

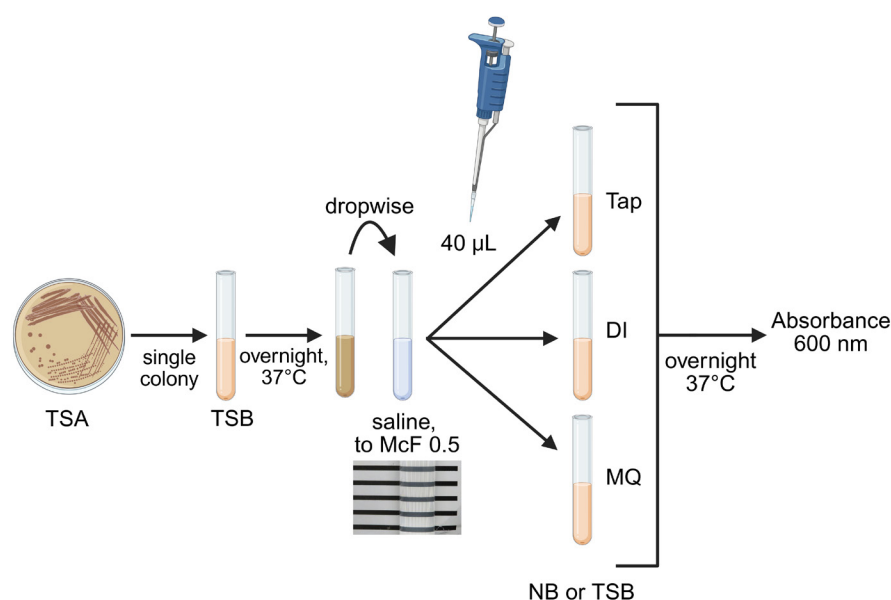
ogy teaching laboratory were used in this study: *Escherichia coli* (ATCC 35218), *Bacillus subtilis* (ATCC 6051), and *Staphylococcus aureus* (ATCC 29213). All were obtained from the American Type Culture Collection (Manassas, VA). *E. coli* is a Gram-negative rod. *B. subtilis* and *S. aureus* are both Gram-positive, with *B. subtilis* having a rod morphology and *S. aureus* being a coccus. We reasoned that use of multiple species would decrease the possibility of species-specific results being observed. Bacterial isolates are maintained as frozen stocks (CryoCare™, Key Scientific, Stamford, TX) at -80 °C. Prior to use in experiments, each isolate was grown from frozen stocks on TSA plates.

EXPERIMENTAL DESIGN

For each organism, an isolated colony from the Tryptic Soy Agar stock plate was used to inoculate a TSB working stock tube. These working stock cultures were incubated overnight at 37 °C. The overnight culture suspension was transferred dropwise to 4 mL of sterile saline (0.85% w/v NaCl in DI water) until the turbidity matched a 0.5 McFarland Turbidity Standard (14). From this 0.5 McFarland saline suspension, 40 µL was used to inoculate each experimental TSB tube (Figure 1). All cultures were prepared in triplicate.

FIGURE 1.

Experimental design.



Overnight cultures in Tryptic Soy Broth (TSB) were prepared for each bacterial isolate by inoculating with an isolated colony from the Tryptic Soy Agar (TSA) working stock plate and incubating at 37 °C. The culture suspension was transferred dropwise to 4 mL of sterile saline until the turbidity matched a 0.5 McFarland Turbidity Standard (inset). Each growth medium, prepared in each water type, was inoculated with 40 µL of the saline suspension. Figure created with BioRender.com.

TABLE 1.

Turbidity measurements (600 nm) for bacterial cultures grown in Nutrient Broth (NB) or Tryptic Soy Broth (TSB) prepared in tap (T), deionized (DI), or MilliQ™ (MQ) water. Data are presented as the arithmetic mean ± sample standard deviation.

	<i>E. coli</i>			<i>B. subtilis</i>			<i>S. aureus</i>		
	T	DI	MQ	T	DI	MQ	T	DI	MQ
NB 1	0.262 ± 0.025	0.230 ± 0.017	0.240 ± 0.024	0.201 ± 0.032	0.111 ± 0.032	0.121 ± 0.054	0.488 ± 0.053	0.439 ± 0.018	0.432 ± 0.016
NB 2	0.284 ± 0.025	0.199 ± 0.016	0.207 ± 0.036	0.141 ± 0.066	0.102 ± 0.019	0.114 ± 0.024	0.422 ± 0.071	0.389 ± 0.073	0.390 ± 0.058
NB-C ^a	0.273 ± 0.025	0.215 ± 0.022	0.223 ± 0.033	0.171 ± 0.057	0.106 ± 0.024	0.117 ± 0.038	0.455 ± 0.066	0.414 ± 0.055	0.411 ± 0.044
TSB	1.039 ± 0.047	1.030 ± 0.041	1.032 ± 0.055	0.412 ± 0.034	0.374 ± 0.057	0.387 ± 0.049	1.152 ± 0.037	1.230 ± 0.051	1.184 ± 0.025

^aNB-C: combined NB trials

Normalizing the saline suspension to a turbidity standard ensured that each experimental culture tube for a given isolate received an equivalent inoculum. Cultures were incubated at 37 °C. After 24 hours of incubation, turbidity was measured spectrophotometrically (Spectronic 20D+, Model 333183, Thermo Electron Corporation). Absorbance at 600 nm was measured and recorded, using a tube of uninoculated medium as a reagent blank.

For each day's measurements, the mean and standard error of the mean were calculated. Each trial with each organism grown in each medium prepared with water from each of the three different sources, was done in triplicate. Data sets were compared by two-way ANOVA using SPSS[®] (10). Pair-wise comparisons were performed using Bonferroni post-hoc tests. For all comparisons, $\alpha = 0.05$. Box plots were generated using BoxPlotR (20).

RESULTS

On first inspection, each organism appeared to grow equally well regardless of which type of water was used to prepare the medium (Table 1). Closer inspection of the results revealed three additional trends. First, in the NB trials, it appeared that turbidity was slightly higher in the Tap water tests than in the DI and MQ water tests. This trend was less apparent in the TSB trials. Second, all absorbances in TSB were considerably higher than those in NB. Depending on the water source and organism, this increase was 2.5 – 4.8-fold. Finally, there appeared to be differences among the organisms. *B. subtilis* tended to exhibit less growth in both media and in all three water types when compared to the other two organisms. *S. aureus* exhibited the greatest amount of growth in comparison to both other bacteria in each medium and for each water type.

To determine if the observed growth reflected a specific medium formulation (i.e., was lot-specific), growth in two

separate lots of Nutrient Broth was measured and compared. Individual cases involving each organism in each medium in each water type were analyzed. (e.g., *E. coli* NB-1 and NB-2 in tap water; *E. coli* NB-1 and NB-2 in deionized water; etc.) An F-test and a two-tailed t-test were conducted for each pair to compare the variance and means ($\alpha = 0.05$), respectively. Results of these tests indicated that variances were the same ($p > 0.05$ for all cases) and means were the same ($p > 0.05$ for all cases). Since the variances were equivalent, data from the six NB trials were combined (Table 1, NB-C).

Two-way ANOVA analysis of the data sets supported this conclusion. For each organism, a two-way ANOVA was performed using the three water sources and the three media (two NB lots and the TSB trials). This type of analysis allows us to compare all cases without the increased probability of Type I error (rejecting the null hypothesis that means are equivalent when it is actually true) that would result from performing multiple *t*-tests. Results of this analysis showed that variances were equal for all groups, and that the means of the two NB lots were not different ($p = 1.0, 0.703, 0.102$ for *E. coli*, *B. subtilis* and *S. aureus*, respectively).

The two-way ANOVA analysis was repeated using the combined NB data in the medium comparison. The tests of between-subjects effects resulted in a low *p*-value and a high partial eta squared (η^2_p) value for each organism (Table 2). This suggests a large effect due to the medium (NB vs TSB). When comparing water sources, the results were less clear. For all organisms, no significance was associated with water source ($p > 0.05$). However, for effect size estimates, suggested benchmarks are given as 0.01 (small effect), 0.06 (medium effect), 0.14 (large effect) (5). Our estimate calculations all exceed the 0.01 benchmark, suggesting some effect due to water.

Based on these results, we wondered if the suggested water effect was influenced by including the medium comparisons. Thus, we performed one-way ANOVAs comparing

TABLE 2.

Between-subject effects test from a two-way ANOVA comparing growth in two different media (NB and TSB) prepared in water from three sources (tap, DI, MQ) ($\alpha = 0.05$).

Organism	Medium		Water	
	Significance (<i>p</i>)	Effect Estimate (η^2_p) ^a	Significance (<i>p</i>)	Effect Estimate (η^2_p)
<i>E. coli</i>	> 0.001	0.993	0.131	0.176
<i>B. subtilis</i>	> 0.001	0.910	0.068	0.226
<i>S. aureus</i>	> 0.001	0.984	0.620	0.044

^aEffect size rule of thumb: 0.01 (small), 0.06 (medium), 0.14 (large)

only the water groups – that is, a separate ANOVA for each medium. Post-hoc comparisons of the one-way ANOVAs showed that when the bacteria were grown in TSB, water had no effect (no difference in mean turbidity, Table 3). This was also true when *B. subtilis* and *S. aureus* were grown in NB (Table 4). When *E. coli* was grown in NB, mean turbidity for Tap water was significantly different from that in DI ($p = 0.006$) and MQ ($p = 0.02$). These results are graphically presented as box plots (Figure 2).

To determine if there was an actual growth difference between the organisms, we performed a 2-way ANOVA for each medium, looking at the effect of water and organism. For TSB, there was an effect associated with the organism ($p > 0.001$, $\eta^2_p = 0.989$). This analysis indicated no effect due to water ($p = 0.163$, $\eta^2_p = 0.018$), which is consistent with the ANOVAs described above. For NB, there was also an effect associated with the organisms ($p > 0.001$, $\eta^2_p = 0.905$). The effect of water noted above was seen again in this analysis ($p = 0.001$, $\eta^2_p = 0.279$).

DISCUSSION

We originally hypothesized that water type (tap, deionized (DI), or ultrapure (MQ)) would affect growth of bacteria in two media typically used in microbiology teaching laboratories. Specifically, media prepared with tap and DI could provide good growth, while media prepared with ultrapure

water might show less growth due to missing trace elements not supplied by the medium. We also considered, while formulating our hypothesis, that growth in tap water would be lower due to some residual presence of a growth inhibitor. Taken together, our results suggest that the water types tested did not significantly affect bacterial growth.

Water type did not significantly affect growth of the test bacteria. Several previous studies identified negative effects of water type on bacterial growth. Butterfield (4) suspended bacteria in distilled water or water containing sodium bicarbonate (0.5 g/L) for 15 or 30 minutes. They reported a decrease in growth relative to control when bacteria were subsequently inoculated onto standard growth medium. They observed that growth returned to baseline when the suspension water was supplemented with potassium phosphate or with a combination of potassium phosphate, calcium chloride, magnesium sulphate, and ferric chloride. The authors surmised that the cells were damaged by the hypotonic environment, similar to the rupturing of protozoa in the same conditions. Price & Gare (16) reported the presence of an inhibitor of bacteria growth in distilled water. In this case, the water tested was used to rinse tubes prior to the addition of growth medium. The authors concluded that the rinse water left the inhibiting compound on the glass. This inhibitor was not identified but was evidently removed by degassing the water or by passing the water through a mixed bed ion-exchange column. Hoadley & Cheng (9) studied the survival of

TABLE 3.

Post-hoc tests of significance (Bonferroni) comparing Tryptic Soy Broth medium prepared in the three water sources ($\alpha = 0.05$).

Comparison	Significance (p)		
	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>
tap → DI	1.000	1.000	0.144
tap → MQ	1.000	1.000	1.000
DI → MQ	1.000	1.000	0.593

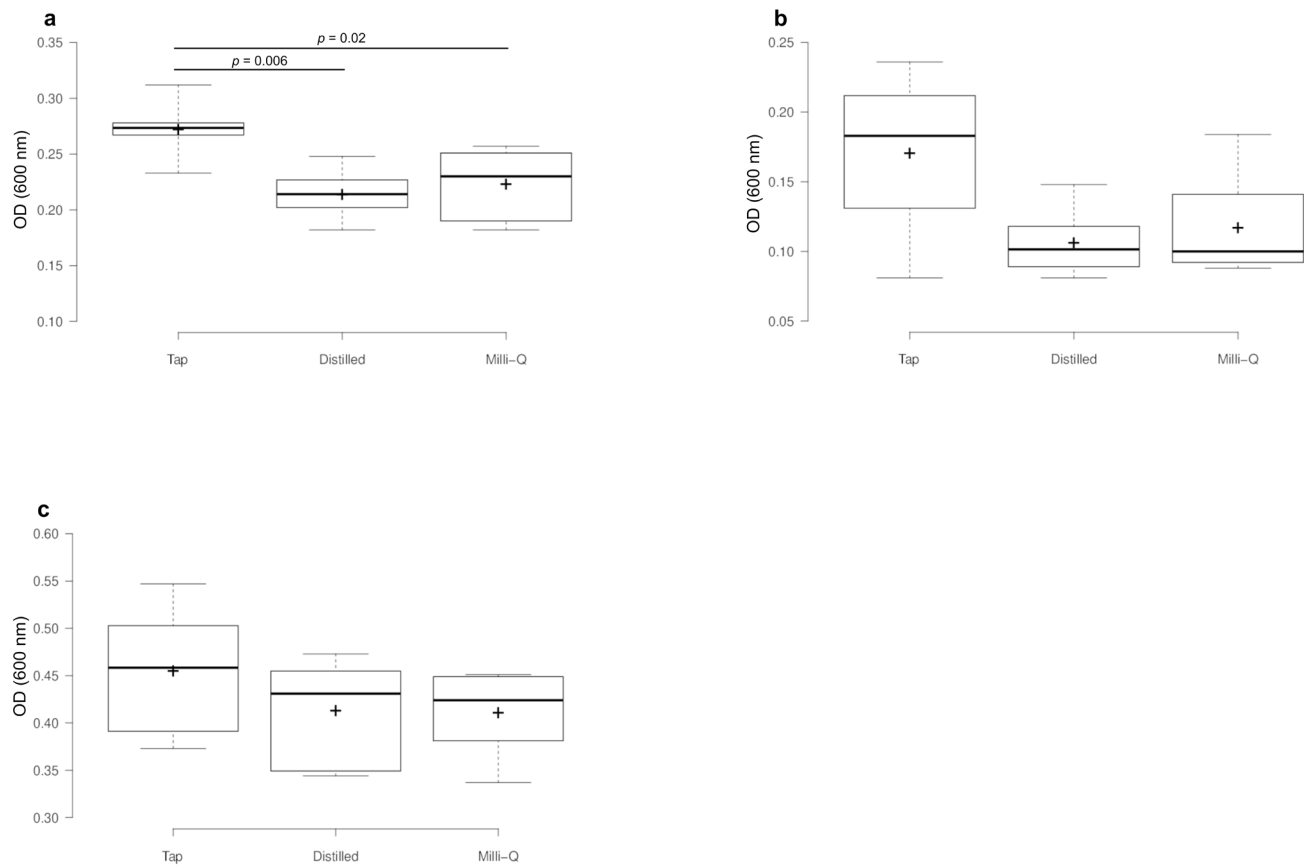
TABLE 4.

Post-hoc tests of significance (Bonferroni) comparing Nutrient Broth medium prepared in the three water sources ($\alpha = 0.05$).

Comparison	Significance (p)		
	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>
tap → DI	0.006	0.051	0.661
tap → MQ	0.020	0.125	0.575
DI → MQ	1.000	1.000	1.000

FIGURE 2.

Comparison of growth in Nutrient Broth prepared with tap, deionized (DI) or Milli-QTM (MQ) water. (a) *Escherichia coli*, (b) *Bacillus subtilis*, (c) *Staphylococcus aureus*.



Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend to minimum and maximum values; crosses represent sample means. $n = 6$.

bacteria in a water environment and observed a rapid loss of viable bacteria when suspended in tap water. They were unable to recover viable bacteria after 4 – 8 hours. However, in all of these reports, researchers used water as a sample diluent and not to prepare the growth medium, distinguishing them from this study.

Our results provide no evidence that tap water contains anything toxic to the test bacteria. The statistical analyses suggested that differences in growth, as measured by turbidity, were an effect of the medium or the organism, but not of the water type used to prepare the medium. The exception to this was for *E. coli* grown in Nutrient Broth, in which growth in NB prepared with tap water differed from that in NB prepared with deionized water or ultrapure water (Table 1, Table 4, Figure 2). Visualization of the data by boxplot suggests this

difference relates to the spread of data points for the *E. coli* trials (Figure 2, see Factors for considerations section below).

TSB supported higher growth than NB did. We observed that, for the bacteria tested, growth was higher in TSB than in NB. As water had no significant effect on growth, the difference between growth in NB and TSB likely stems from differences in composition. NB and TSB are both complex, meaning that the exact chemical composition is not known due to the inclusion of extracts from plants, animals, or yeasts. NB contains beef extract and enzymatic digest of gelatin. TSB contains enzymatic digest of casein (a primary protein derived from milk), enzymatic digest of Soybean meal, NaCl, K_2HPO_4 , and glucose.

The addition of K_2HPO_4 to TSB may provide some buffering capacity for the medium. Static cultures used in this

study leads to fermentative metabolism, which could lower culture pH below optimum levels. However, amino acids and other extract components can also provide some buffering. Thus, systematic measurement of culture pH before inoculation and after incubation would be needed to determine a growth-culture pH correlation. A more likely explanation is the inclusion of glucose (0.25%) in TSB. Because it can enter glycolysis at the first step, glucose as a catabolic substrate may serve as a preferred substrate for these species and provide an initial head start to microbial growth. Further experiments, adding glucose to NB to determine effects on growth, would test this hypothesis.

***Bacillus subtilis* grew less than *E. coli* or *S. aureus* regardless of water type or medium.** A final observation from the data was that *B. subtilis* had lower amounts of growth than *E. coli* or *S. aureus* regardless of medium or water type (Table 1). One possible explanation for this is lack of a critical nutrient. Although they did not specifically test *B. subtilis*, Proom & Knight (17) reported a requirement for thiamine for various *Bacillus* species, when using ammonium as a nitrogen source and amino acids as carbon sources. However, since both media used in this study were complex, composition analysis would be needed to determine if either was thiamine deficient or if thiamine supplementation would improve *B. subtilis* growth. Another explanation is the growth temperature used in this study. According to ATCC, the recommended growth temperature for *B. subtilis* is 30 °C, while that for both *E. coli* and *S. aureus* is 37 °C. Thus, it is possible being above optimal temperature was sufficient to inhibit growth. Replicating the *B. subtilis* trials when grown at 30 °C would test this possibility.

Factors for consideration. Our results raised several factors for consideration regarding our study. These include the sample sizes, testing conditions, the types of media and organisms used in the study.

Sample size and testing conditions. Each trial was performed in triplicate over different days and working cultures. This was done to normalize results over potential changes in laboratory conditions and was sufficient to conduct statistical analysis of the results. However, additional replicates could help clarify suggested differences, such as *E. coli* growth in NB prepared in tap water compared to DI and MQ (Table 1, 4). Further, we tested only one strain for each species. Extension of the experiments to involve other strains are needed to demonstrate that results are not strain-specific. Additionally, we performed all experiments using a single preparation of each growth medium. Although we would not expect to see differences in batches of media prepared using the same stock bottle of powder, running these experiments would confirm

that expectation. Finally, we did not measure water parameters such as conductivity, pH, chlorine content, dissolved solids, etc. While these values are reported by the city water treatment facility, values may differ between the testing facility and the tap. Conductivity is measured by the Milli-Q⁵⁰ system, but no other parameters are reported by that system. These water parameters were included in our discussions during experimental design, and we reasoned that any differences in the water itself would be eliminated after the addition of the medium. Systematically tracking these parameters both before and after addition of medium in future studies would confirm this reasoning and identify possible variation over time.

Use of Complex Media. As noted above, both NB and TSB are complex media, with sources of growth factors and trace elements needed by bacteria and other microorganisms. We chose these media because they are commonly used to prepare stock cultures for microbiology teaching and research labs. Defined media, in which the exact chemical composition is known, may be more sensitive to levels of varying levels of solutes, particularly trace metals, due to the water source.

Use of Non-fastidious Organisms. The study organisms were chosen because they are standard cultures grown in a microbiology teaching lab. All are non-fastidious organisms, meaning they do not require special nutritional additives or growth conditions. Fastidious organisms, which have more specialized nutritional requirements, might be more sensitive to the presence or absence of solutes, such as trace metals, depending on the water source.

CONCLUSIONS

To our knowledge, this is the first study looking at the effect of water type (quality) on laboratory growth of bacteria since a study conducted by Geldreich & Clark (8) in 1965, in which they reported on a method for testing the suitability of water produced by various distillation methods. To demonstrate the efficacy of a suitability test they had developed, they compared nine different distilled water samples, using the same minimal growth medium to determine if the respective water samples were toxic to growth of *Aerobacter aerogenes*.

Most papers in the literature specify distilled water for media preparation. In 1966, Ellner et al. (6) specified the use of distilled water for media formulation without further elaboration. In 1974, Neidhardt et al. (15) specified glass-distilled water for preparation of a defined medium, again providing no rationale. The Difco Manual (25) specifies rehydration in “purified water” or water without any “added substance (pg. 5, 22).” Historically, the rationale for using distilled water to

prepare growth medium is not explicitly stated, though it is reasonable to suppose the concern is the presence of environmental toxins remaining in unpurified tap water. A note from Oxoid Technical Support (22) advises against the use of tap water for hydration of culture media given the presence of impurities including calcium, magnesium, and metal ions in addition to chlorine and fluorine, the latter two of which can “alter characteristics of selective media.” They ended the note with a focus on the extremely “variable” ion content present in tap water. However, it is unclear as to when these recommendations were first developed or if they were ever retested. Even in the literature, the advice to use distilled water is not entirely consistent. For example, McClung (13) describes preparation of Nutrient Broth by dissolving powders (peptone & beef extract) in tap water. Furthermore, the paper goes on to note that “distilled water is often listed for this medium [NB] but is unnecessary.”

We do not mean to suggest that water type is unimportant. Neidhardt et al. (15) noted that variation in growth media and conditions hindered the full understanding of *E. coli* and *S. typhimurium* physiology. Given their observations, the conventional wisdom alluded to above of using the highest purity water that is economically feasible (e.g., deionized water for rehydrating growth media) makes sense. Moreover, everyone experiences some changes in tap water quality in times of drought, flooding, or water main damage. Nor are

downstream purification systems immune from water quality fluctuations. Recently, our institution experienced degradation of water from our in-house deionization system because of contamination during building renovations.

Nevertheless, our results suggest that the current-day quality of tap water generally is sufficient for day-to-day lab stock cultures and can be used if the DI system is unavailable. This can be particularly beneficial for resource-limited labs in which purchase and maintenance of water purification systems is cost-prohibitive. Even when such systems are in place, they may fail or otherwise have some interruption of water production. In these types of circumstances, our results demonstrate that useful cultures can still be grown, allowing for students to experience and master basic microbiological concepts and skills.

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