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Growth of Staphylococcus Aureus on Two Selective Media with Special Reference to Cultural Techniques for Growing S. Aureus on Selenite Egg Yolk Agar

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GROWTH OF *STAPHYLOCOCCUS AUREUS* ON TWO SELECTIVE MEDIA WITH SPECIAL REFERENCE TO CULTURAL TECHNIQUES FOR GROWING *S. AUREUS* ON SELENITE EGG YOLK AGAR

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INTRODUCTION

Tellurite and selenite have an inhibitory effect on many microorganisms, thus when added to culture media, they act as selective ingredients for the isolation of resistant microbes.

The ability of certain microorganisms to reduce tellurite and selenite compounds has long been known. Lapage and Bascomb (10) used this ability of selenite reduction in developing a system for differentiating among species of Gram-negative, rod-shaped bacteria. For example, Pseudomonas aeruginosa is more resistant to selenite than is Pseudomonas fluorescens.

Many efforts have been made to determine the final products that result from microbial reduction of selenite and tellurite. By X-ray diffraction analysis, Tucker et al. (19) found that Streptococccus faecalis N83 and Streptococcus faecium K6A reduced tellurite to black tellurium. In a following study Tucker et al. (20) studied the reduction ability of S. faecalis N83 and S. faecium K6A in differentiating between these two species. Tilton et al. (18) have observed that these same two streptococci also reduced selenite, but they did not identify the reduced product that was formed. Woolfolk et al. (22) noted the reduction of selenite and tellurite to their respective elements by extracts of Micrococcus lactilyticus in a hydrogen atmosphere. Ahluwalia et al. (1) observed that selenite metabolism by Escherichia coli involved at least two phases: (i) the reduction of selenite to elemental selenium and

(ii) the incorporation of selenium into organic molecular forms. This was shown by Ahluwalia *et al.* (1) when cells of *E. coli* were washed with carbon disulfide, a selenium solvent, and the red selenium was not completely extracted from the cells. They were able to extract completely the metallic selenium from the cells after enzymatic hydrolysis of the protein material. This gave an indication that at least part of the metallic selenium reduced from selenite by *E. coli* was bound to the protein material of the cell and not incorporated into the protein. Tuve and Williams (21), studying the metabolism of selenite by *E. coli*, found that in a sulfur-deficient medium selenium is incorporated into protein to compensate for the lack of sulfur. Due to the conditions under which Tuve and Williams (21) conducted their investigation, all possibility of deposition of elemental selenium was eliminated; therefore, the characteristic red color, which occurs when elemental selenium is present, did not appear.

Elliott (6) used electron microscopy to determine that tellurite reduction by *S. aureus* Wis. 523 took place in the cytoplasm of cells grown in a broth culture containing tellurite. This was further substantiated by activation analysis studies (8). Identification of the reduced products of selenite and tellurite metabolism by *S. aureus* was made by X-ray diffraction (6). Tellurium oxide (TeO) and TeO_2 were identified as the reduced products of tellurite metabolism after an 8-hr incubation period. The red reduction product from selenite metabolism could not be identified until after a 5-day incubation period, and it was then identified as metallic selenium. McCready *et al.* (11) determined by X-ray diffraction analysis that the red deposit collected from cells of

Salmonella heidelberg was composed almost entirely of metallic selenium. These authors reported that selenite reduction involved two steps: Se^{+4} to Se^{+2} appeared to involve an enzymatic reaction, but the second step Se^{+2} to Se^{o} was spontaneous due to the instability of Se^{+2} at the alkaline pH under which the experiment was conducted.

Partial purification of the tellurite-reducing enzyme obtained from Mycobacterium avium was accomplished by Terai et al. (17). This enzyme, referred to as tellurite reductase, was located in the soluble fraction of the cell. Other efforts have been made to determine the sites of tellurite reduction. Mudd et al. (13), working with members of the genus Mycobacterium, noted that upon reduction of tellurite to metallic tellurium the black crystals appeared to be concentrated around areas assumed to be the reductive sites. This was interpreted as indicating that energy-yielding reactions occur throughout the cytoplasm but in particular in circumscribed organelles that are functionally equivalent to the mitochondria of higher forms. Cooper and Few (3), investigating uptake of radioactive tellurite by E. coli, noted that uptake varied with time, temperature, and pH and that the uptake had the properties of enzymatic action.

Fungi also reduce selenite, as noted by Falcone and Nickerson (9) who studied this property of intact cells and cell-free preparations of Candida albicans. Nickerson et al. (14) noted that Candida albicans 806 growing on an agar medium containing high concentrations of sodium selenite developed small red colonies indicating that the selenite was being reduced. The strongly reducing cells were entirely of the yeast type and failed to become filamentous.

Some investigators, as mentioned above, have noted the development of a red and black color within the cells of microorganisms when grown in the presence of selenite and tellurite. Various investigators (1, 6, 11, 13, 19, 22) have determined that the red and black colors were due to the deposition of selenium and tellurium within the cells.

Because the production by S. aureus of black colonies on Baird-Parker's tellurite egg yolk agar and red colonies on selenite egg yolk agar is not well understood, studies were conducted to determine (i) the chemical nature of the product(s) accumulated in cells grown on these media and (ii) factors influencing yield of accumulated product(s) of cells grown on selenite egg yolk medium. These influencing factors are temperature, pH, aerobic vs anaerobic conditions, age of egg yolks, egg yolk volumes, and hen egg yolk vs prepared egg yolk.

MATERIALS AND METHODS

Media

Baird-Parker's (BP) medium (2) was made and used according to specifications of Difco Laboratories (5).

Selenite egg yolk (SeEy) agar was prepared and used according to the recommendation by Elliott (7). Hen egg yolk was used unless otherwise stated.

Brain Heart Infusion (BHI) broth (Difco) was used to prepare the inocula and BHI agar (Difco) was used to maintain the stock cultures.

Plate Count Agar (PCA) (Difco) was used to determine the total colony count tabulated in results as colony forming units (CFU) of S. aureus in the inoculum as compared to total colony count obtained on SeEy agar.

Bacterial Strains and Their Maintenance

Various strains of Staphylococcus aureus were used in this study. They were obtained from the stock culture collections of Western Kentucky University. They consisted of S. aureus strains Wis. 523, ATCC 12600, 19B, 56D, ATCC 8096, and Dack 9.

At 3-month intervals the stock cultures were transferred to fresh BHI agar slants, incubated for 24 hr at 35 C, then stored at 4 to 8 C between transfers. One loopful was transferred from these stored cultures to BHI broth, incubated at 35 C for 24 hr, and then used to inoculate the BP or SeEy medium. Such 24-hr BHI broth cultures will be referred to as a standard culture throughout the remainder of this thesis.

Dilutions and Plating Techniques

The BP and SeEy plates for collection of unknown tellurium and selenium products were inoculated with 0.1 ml of the standard culture of S. aureus Wis. 523. The culture was spread over the surface of the medium by using a bent glass rod sterilized by flaming after being dipped into 95% ethyl alcohol.

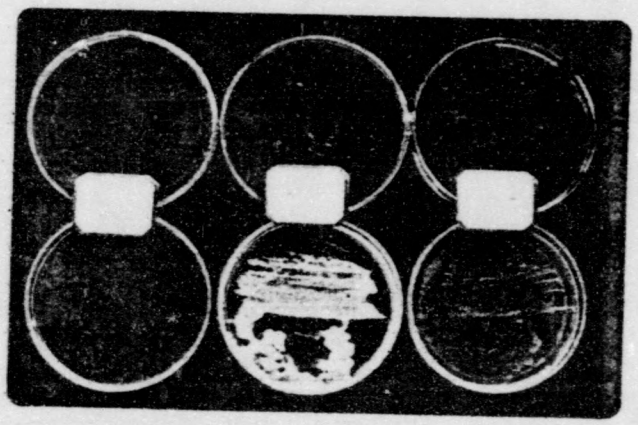
For the growth studies a standard culture of the staphylococci being used was serially, logarithmically diluted in 99-ml sterile, deionized water blanks to a concentration of 10^{-7}. Dilutions of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} were inoculated onto the surface of SeEy agar. These dilutions will be known as the standard dilutions throughout the remainder of this thesis. These standard dilutions were spread-inoculated onto PCA to determine the number of cells present in the inoculum.

Counting Technique

The total colony counts were made for each dilution and were recorded for the dilution giving a countable plate (i.e., having between 30 and 300 colonies present). The colony sizes on the countable plates were measured, averaged, and recorded as follows: + = visible to 0.4mm, + + = 0.4 to 0.5mm, + + + = larger than 0.5mm. The colony color was recorded as follows: - = no color, + = red-centered colonies, + + = overall red colony, and + + + = dark red colony. The egg yolk reaction was recorded as it compared to the egg yolk reaction on the center plate in Figure 1, + + + being approximately that of the egg yolk reaction on the center plate, + + would be less, and + would be only a slight but still noticeable egg yolk reaction.

Figure 1

Growth of an egg yolk-positive S. aureus on SeEy medium.



Inoculation of Selective Media and Harvesting of the Products Produced by Growth of S. aureus on the Media

Tellurite Product(s). Twelve petri dishes of BP medium (20 ml per dish) were inoculated with the standard culture of S. aureus Wis. 523. The plates were inverted and incubated at 45 C for 24 hr.

After the incubation period 5 ml of 0.85% saline solution were added to each of the 12 plates, the black colonies of S. aureus Wis. 523 were suspended with a loop, and then removed with a medicine dropper. The removed liquid containing the bacterial growth was centrifuged, and the clearer supernatant decanted. The heavier precipitate was washed thrice with deionized water by alternate suspension and centrifugation to remove any material that might be attached to the outside of the bacterial cells. The cells were next placed in a cooled, weighed mortar, and the cell walls were broken according to the method of McIlwain (12). The cell-alumina mixture was ground with a cooled pestle for 10 min in a 4 C incubator to insure maximum cell wall breakage. After the 10-min breakage time, the alumina-broken cell mixture was placed in a culture tube containing deionized water and stirred. The suspended materials were next centrifuged at 3000 rpm for 2 min to sediment the heavier alumina from the tellurium product in the suspension. The supernatant was decanted and extraction of the precipitate was by centrifugation at 3000 rpm. This procedure was continued until all visible white alumina was removed. The black product was removed from the tube and placed in a petri dish to allow excess water to evaporate. After complete air-dry evaporation of the water from the product, the remaining solid was scraped from the petri dish, ground to a powder in a mortar and returned to the petri dish to be kept until X-ray analysis could be performed.

In later experiments, breakage of the staphylococcal cell walls after growth on BP medium was performed with a sonifier according to the procedure that will be described for the breakage of S. aureus Wis. 523 cells grown on SeEy agar.

Selenite Product(s). The production of cells and reduced selenite was very similar to that described for tellurite, except that 44 plates of SeEy agar were used, incubation was at 45 C for 48 hr, and cells were broken by sonification. Cells harvested from 44 plates were suspended in 15 ml of deionized water and sonified for 10 min in a plastic centrifuge tube cooled to -10 C in a continuously stirred alcohol-ice mixture. Sonification was done with a Sonifier Cell Disrupter Model W140D (Heat Systems-Ultrasonics, Inc.). The sonifier was operated at 6 output and 50 ma. The precipitate was dried and kept in a petri dish for X-ray analysis.

Chemical Analysis of the Products Produced by S. aureus Wis. 523 Growth on SeEy and BP Agar

The crystals produced from selenite and tellurite uptake were ground into powder. X-ray diffraction analysis with a G. E. powder camera having a diameter of 143.2mm was performed on both the selenite and tellurite products at Western Kentucky University. Copper K radiation with an exposure time ranging from 30 min to 2 hr was used for the tellurite product(s). Exposure time for the selenium product(s) ranged from 45 min to 20 hr. The X-ray tube was operated at 50 kv and 15 ma.

X-ray emission analysis with platinum radiation was performed on both selenite and tellurite products at Western Kentucky University using the G. E. X-ray XRD-5 Spectrometer. The operating parameters for the

emission work were:

Radiation: Pt
Mounting: Al tray
Pulse Height Selector: E_{α} = 2.00 ΔE = 4.00
Counter Tube High Voltage: 1050 Volts
Crystal: LiF
Collimator: 0.010mm
K_V: 50
Ma: 15

Chemical analysis of the ground tellurite product(s) which had been collected by breaking cell walls of S. aureus Wis. 523 with alumina was attempted with the use of a LKB-Gas Chromatograph-Mass Spectrometer Type 9000 at Vanderbilt University, Nashville, Tennessee. The results were recorded by an oscillograph 5-124 (Consolidated Electrodynamics).

Chemical analysis of the tellurite product(s) was performed by X-ray emission semi-quantitative analysis using a ratio of intensity measurements. The ratios used were TeK_{α} : background. The background intensity was taken at ± 0.5 degrees from the TeK_{α} line.

Determination of Factors That Might Influence the Growth of S. aureus on SeEy Medium

Temperature. Twenty-five plates of SeEy medium for each of the standard dilutions of S. aureus Wis. 523 were spread-inoculated and five plates of each dilution were incubated at each of the temperatures 25, 30, 35, 45, and 48 C. These plates were observed after 48 hr incubation. A comparison of the total number of colonies present on the plates at each temperature was made. The size of the egg yolk reaction was noted and recorded as well as the degree of colony redness.

pH. Either sterile 1N HCl or 1N NaOH was used as necessary to make trial adjustments of different lots of 250 ml of SeEy agar to pH 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0.

After the volumes of acid or base needed to adjust the medium to the six pH's had been measured, six 250-ml lots of SeEy medium were prepared in Erylenmeyer flasks. The appropriate amounts of acid or base needed to obtain the desired pH's were measured and added to separate flasks. The flasks of medium and flasks of acid and base were autoclaved at the same time and temperature. At the time the medium was to be poured, the acid or base was added along with the sodium selenite and egg yolk. Triplicates for each pH were used. Inoculation of the plates was similar to that for the temperatures except dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were used as well as the standard dilutions. A control of SeEy agar to which neither acid nor base had been added (pH 7.6) was maintained. A total colony count was made for each dilution on PCA.

Aerobic vs Anaerobic Growth Conditions. The standard dilutions of S. aureus Wis. 523 were inoculated onto 16 plates of SeEy medium. Two plates of each dilution were placed in an Anaero-Jar (Case Laboratories, Chicago, Illinois). Each of the other two plates was incubated aerobically. Anaerobiosis was established in the Anaero-Jar by 3 consecutive flushings with a mixture of 90% N_2 and 10% CO_2. The anaerobic and aerobic plates were incubated at 45 C for 48 hr. Total plate counts were made, and the redness of the colonies and the amounts of egg yolk reaction obtained in each environment were compared.

Age of Egg Yolk Added to SeEy Agar. To one 500-ml volume of SeEy agar a yolk from a newly purchased egg was added. To another 500-ml volume of SeEy agar an egg yolk was added from an egg that had been kept at refrigerator temperature (4 C) for 4 weeks. The media were poured into petri plates, and the standard dilutions of S. aureus Wis. 523 were spread-inoculated onto the surface of the medium. The plates were incubated at 45 C for 48 hr.

Measurement of Egg Yolk Volumes. The yolks of 25 grade A large hen eggs were separated from the whites by breaking the shells and passing the yolks from one half of the shell to the other; thus allowing the white to fall from the yolk. The volume of each yolk was measured in a 100-ml graduated cylinder. The graduated cylinder was washed and allowed to drain before each measurement.

Optimum Egg Yolk and Colbeck EY Broth Volume for Growth of S. aureus on SeEy. Standard dilutions of six strains of S. aureus were plated in triplicate on SeEy medium. Lots of SeEy agar had 15, 20, 25, or 30 ml of egg yolk added per 500 ml of medium. Plate count agar was inoculated with the standard dilutions of each strain. All plates were incubated at 45 C for 48 hr. Six strains of S. aureus were inoculated onto SeEy agar containing 15, 20, 25, or 30 ml per 500 ml of medium of Bacto-Colbeck EY broth (Difco).

Hen Egg Yolk vs Prepared Egg Yolk. A comparison was made between the growth of these six strains of S. aureus on SeEy agar containing the above volumes of EY broth and their growth on SeEy agar containing the same volumes of hen egg yolk. All plates were spread-inoculated with the standard dilutions of the various strains. The plates were incubated at 45 C for 48 hr, and the results were recorded after this time.

RESULTS AND DISCUSSION

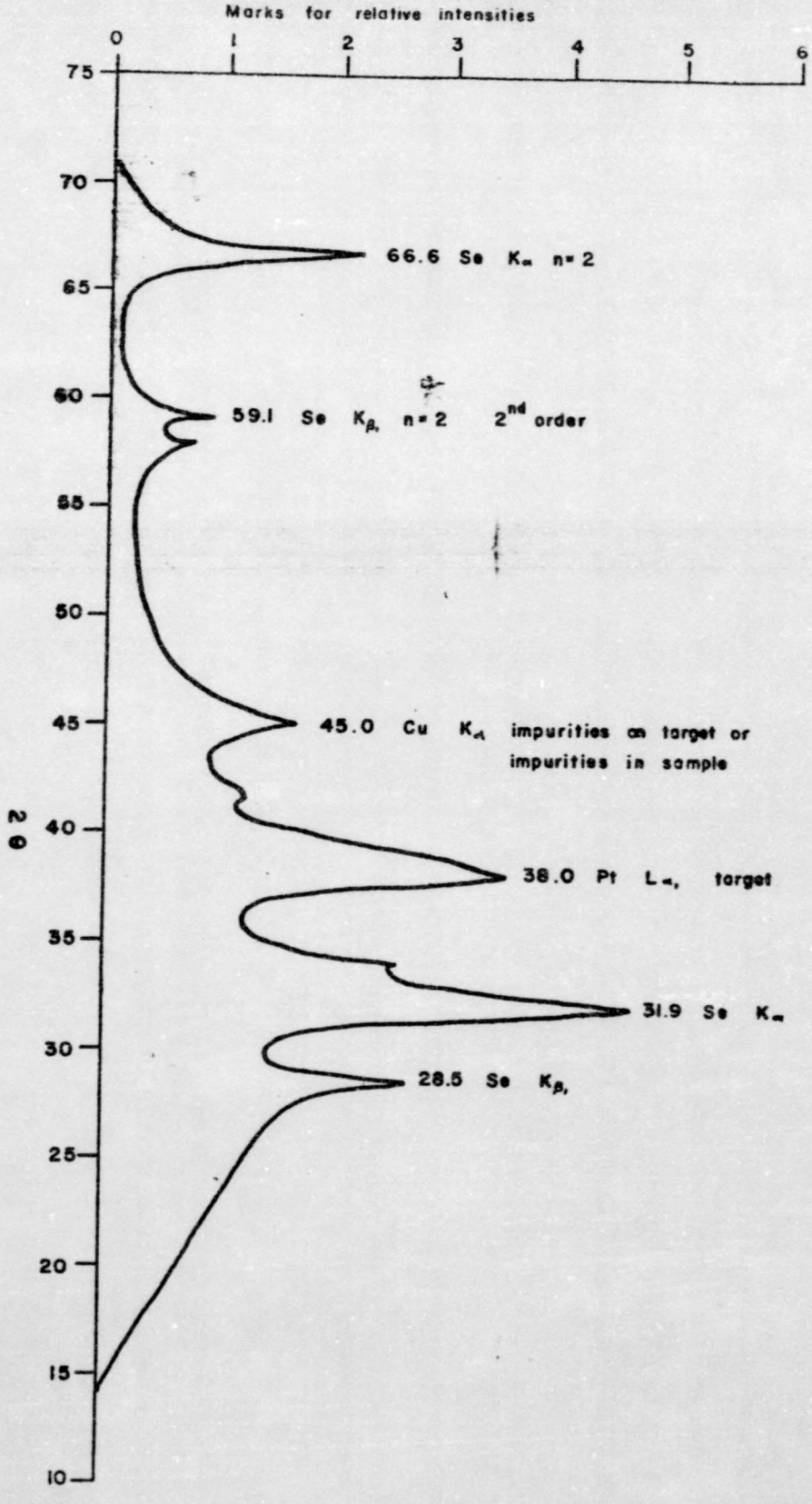
Chemical Analysis of the Products Produced by S. aureus Wis. 523 Grown on SeEy and BP Agar

An X-ray diffraction powder photograph shows a series of lines for each of which a measurement can be made and transformed into the interplanar d value (15). Lines were obtained by the analysis of precipitates from BP plates, but no lines could be obtained from the precipitate collected from SeEy plates even when the sample was exposed to the X-rays for a 20-hr period. The lines from the tellurite sample provided d values that corresponded to neither any known tellurium compound nor to elemental tellurium. These results do not agree with those of many investigators (1, 6, 11, 19, 22) who were able to identify the reduced products produced by microorganisms grown in the presence of tellurite and selenite as being metallic tellurium and selenium.

X-ray emission analysis was performed to determine if any selenium or tellurium products had actually been taken up by the bacterial cells. Peaks were obtained for selenium as shown in Figure 2. This demonstrated that selenium is present in the precipitate obtained from disrupted cells of S. aureus Wis. 523 grown on SeEy medium. X-ray emission analysis of tellurite product could not conclusively prove that tellurium was in the precipitate since the tellurium peaks were so small that they could not be separated from the background scattering. X-ray emission-semiquantitative analysis was performed on the tellurite product. At TeK_{α} the number of counts per second was 566; at TeK_{α} +0.5 degrees, 548; and at

Figure 2

X-ray emission scan of the product of selenite reduction by S. aureus Wis. 523 grown on SeEy agar.


Marks for relative intensities
0
1
2
3
4
5
6
75
70
65
60
55
50
45
40
35
30
25
20
15
10
2θ
66.6 Se Kα n=2
59.1 Se Kβ, n=2 2nd order
45.0 Cu Kα impurities on target or impurities in sample
38.0 Pt Lα, target
31.9 Se Kα
28.5 Se Kβ,

TeK_{α_1} -0.5 degrees, 552. This indicates a trace amount of tellurium. Although these results did not identify the selenite and tellurite precipitates as to whether they were organic or inorganic forms, they did establish that the red precipitate was composed of some form(s) of selenium, and the black precipitate was composed of some form(s) of tellurium.

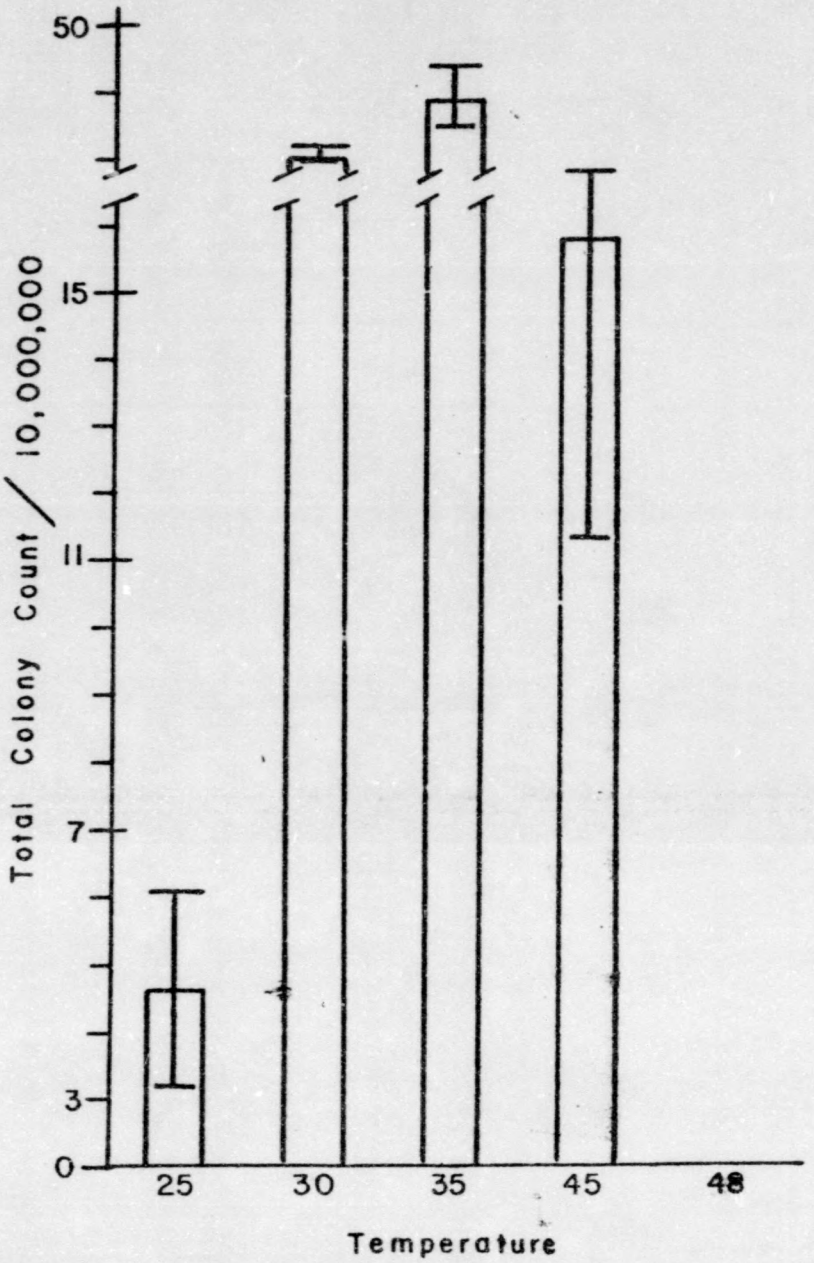
Analysis of the tellurite product by mass spectrometric analysis gave no results that could help in identifying the black product. No peaks were obtained for tellurium or any tellurium product, perhaps because the mass spectrometer did not have the capacity to vaporize tellurium.

Determination of Factors That Might Influence the Growth of S. aureus on SeEy Medium

Temperature. Growth had occurred on plates after 48 hr of incubation at all temperatures except 48 C. The colony counts on SeEy agar obtained after incubation at the different temperatures are shown in Figure 3. Highest colony counts were obtained at 35 C. At 35 C the staphylococcal colonies were redder and larger than at the other temperatures. There was an egg yolk reaction at 30, 35, and 45 C, but a larger zone of precipitation was noted at 45 C. At 25 C the colonies did not have the usual red color nor did they produce an egg yolk reaction. Seemingly, the best temperature for use of this medium would depend on the purpose of the investigator using it. If one uses the SeEy agar for growth of a pure culture of staphylococcus for collection of cells, then 35 C would be the most suitable temperature. But if the medium is used as a selective medium for isolation of staphylococci, then it would be better to incubate the inoculated medium at 45 C. The

Figure 3

Effect of temperature on growth of S. aureus Wis. 523 on SeEy agar. The bar shows the mean, and I shows the range of the colony counts for two trials.


50
15
11
7
3
0
Total Colony Count / 10,000,000
25
30
35
45
48
Temperature

combination of sodium selenite, sodium azide, 7.5% sodium chloride, and 45 C would eliminate contaminating microorganisms such as E. coli and Proteus (6). Since a more distinct egg yolk reaction was noted at 45 C, the investigator would have a diagnostic advantage since virulent strains of S. aureus tend to be egg yolk-positive (16).

pH. Figure 4 shows the effect of pH on the number of colonies produced on SeEy agar during incubation at 45 C for 48 hr. Selenite egg yolk agar at pH 7.0 and 7.5 gave higher colony counts than did SeEy agar having a more acidic pH, but SeEy agar at pH 7.6, which is the unadjusted pH of the medium, gave a higher colony count than did any other pH. The colonies at pH 7.6 showed greater egg yolk precipitation, were redder, and were larger than those at the other pH's. These results indicate that it is not advantageous to change the pH of the medium.

Aerobic vs Anaerobic Growth Conditions. The results of the colony counts made after 48 hr incubation under the two environmental conditions are shown in Table 1. Colonies grown aerobically were larger, deeper red, and had a larger ring of egg yolk precipitation than those grown anaerobically. De Waart et al. (4) grew S. aureus on BP medium anaerobically in an attempt to eliminate contaminating microorganisms, but the technique was discontinued because it interferred with egg yolk clearing. Since the number of colonies on the anaerobic plates was reduced as compared to those on the aerobic plates, the plates should be incubated in an aerobic environment.

Age of Egg Yolk. The results of growth of S. aureus Wis. 523 on SeEy medium containing an egg yolk that was newly purchased and SeEy medium containing an egg yolk that had been kept at refrigerator temperature for 4 weeks are shown in Table 2. Although the colony counts differed,

Figure 4

Effect of pH on growth of S. aureus Wis. 523 on SeEy agar. The bar shows the mean, and I shows the range of the colony counts for two trials.

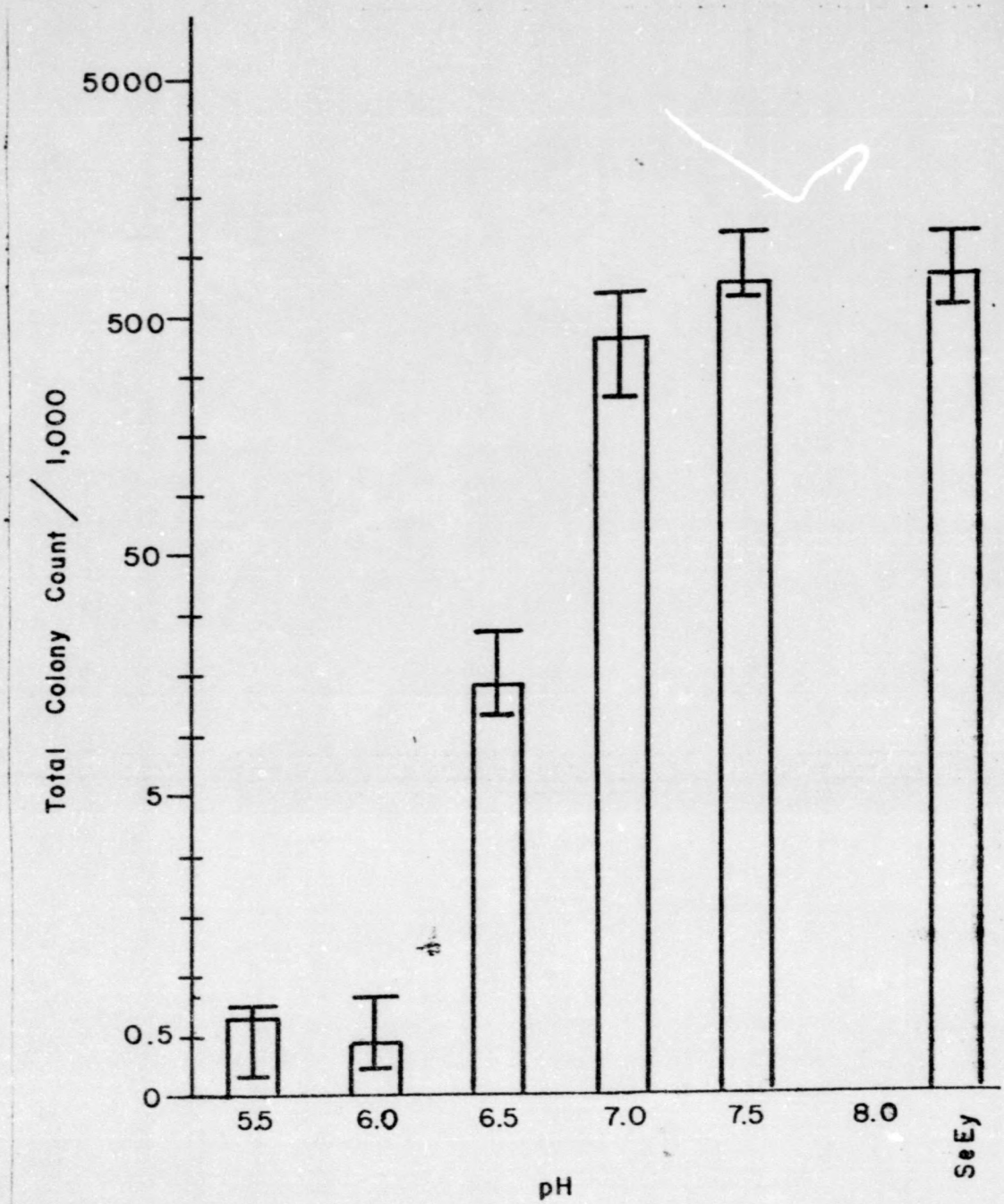

5000
500
50
5
0.5
0
Total Colony Count / 1,000
5.5
6.0
6.5
7.0
7.5
8.0
pH
SeEy

TABLE 1

Comparison of aerobic and anaerobic growth of S. aureus Wis. 523 on SeEy agar

Conditions	Trial	CFU per ml/10^5	Egg yolk reaction	Colony	
				Size	Color
Aerobic	I	170[a]	++	++	++
Aerobic	II	174[a]	++	++	++
Anaerobic	I	237	+	+	+
Anaerobic	II	210	+	+	+

[a]CFU per ml/10^6.

the size of the zone of egg yolk reaction, the color of the colonies, and the size of the colonies were approximately the same. Although age of the egg yolk does give a difference in colony count with the medium having the older egg yolk giving the greater number of colonies, the age of the egg yolk would not be a critical factor in the use of this medium.

Measurement of Egg Yolk Volume. The volumes of the yolks of 25 grade A large hen eggs are shown in Table 3. The volumes ranged from 15 to 23 ml with a mean of 18.6 ml. This variation in volume of egg yolk could possibly account for variation in the growth of staphylococci on this medium and could affect the egg yolk reaction.

Optimum Egg Yolk and Colbeck EY Broth Volume for Growth of S. aureus on SeEy. Table 4 shows the growth of six strains of S. aureus on SeEy agar containing different volumes of egg yolk. Results will be discussed based on the average of the trials for each strain. The highest colony counts for strains 19B and 56D were produced on medium containing 15 ml of egg yolk. For strains Dack 9 and Wis. 523, medium containing 20 ml of egg yolk gave the highest colony counts. For strains ATCC 12600 and ATCC 8096, 25 ml of egg yolk in the medium resulted in the production of the highest colony counts.

For strains Dack 9, ATCC 8096, and 56D, 30 ml of egg yolk added to the medium gave the largest zone of egg yolk precipitation. For strain Wis. 523, 25 ml of egg yolk in the medium resulted in the production of the largest zone of egg yolk reaction. Strain 19B never produced an egg yolk reaction, and strain ATCC 12600 produced an egg yolk reaction in only one trial.

Strains 19B, 56D, and Wis. 523 produced reddest colonies on medium containing 30 ml of egg yolk. Strain Dack 9 produced colonies that were

TABLE 2

Comparison of growth of S. aureus Wis. 523 on SeEy medium containing egg yolks of different ages

Age	Trial	CFU per ml/10^7	Egg yolk reaction	Colony	
				Size	Color
Fresh	I	32	++	++	++
Fresh	II	34	++	++	++
Month	I	62	++	++	++
Month	II	56	++	++	++

TABLE 3

Volumes of yolks from 25 grade A large hen eggs

Egg	Egg yolk volume ml
1	18
2	17
3	21
4	20
5	16
6	17.5
7	18
8	19.5
9	18
10	20
11	15
12	16
13	23
14	19
15	18
16	18
17	20
18	19
19	21
20	20
21	19
22	18
23	18
24	20
25	17
	Average 18.6

the same color on medium containing any one of the volumes within a trial, but this color varied from trial to trial. Strains ATCC 12600 and ATCC 8096 produced reddest colonies on medium containing 25 or 30 ml of egg yolk.

For strains 19B, Dack 9, and ATCC 12600, medium containing either 25 or 30 ml of egg yolk produced the largest colonies. Colonial size for strain ATCC 8096 varied from one set of trials to another but was constant within each set of trials. Strain Wis. 523 produced the largest colonies on medium containing 30 ml of egg yolk. Colonial size for strain 56D was largest on medium containing 15 ml of egg yolk.

These results indicate that within a strain of S. aureus different characteristics and different colony counts on SeEy agar containing different volumes of hen egg yolk were produced. It can also be seen that the growth and characteristics of these six strains on the medium containing the different volumes of egg yolk varied among trials.

Results of the growth of six strains of S. aureus on SeEy medium containing different volumes of Colbeck EY broth are shown in Table 4. Results will be discussed based on the average of the trials for each strain. For strains 19B, ATCC 8096, ATCC 12600, and 56D, medium containing 25 ml of Colbeck EY broth produced larger colony counts than did medium containing the other volumes. Strain Dack 9 had the largest colony count on medium containing 15 ml of Colbeck EY broth. For strain Wis. 523, 30 ml of Colbeck EY broth in the medium produced the largest colony count.

For strains 56D, ATCC 8096, and Dack 9, 30 ml of Colbeck EY broth in the medium gave the largest egg yolk reaction. Strain Wis. 523 produced approximately the same size zone of egg yolk reaction on medium containing 25 or 30 ml of Colbeck EY broth. Strains 19B and ATCC 12600

did not produce egg yolk reactions.

All strains except Dack 9 had reddest colonies on medium containing 30 ml of Colbeck EY broth. Strain Dack 9 produced reddest colonies on medium containing either 25 or 30 ml of the EY broth.

For all strains except ATCC 8096, 30 ml of EY broth in the medium resulted in the production of the largest colonies. For strain ATCC 8096, medium containing 15 ml of Colbeck EY broth produced the largest colonies.

Hen Egg Yolk vs Prepared Egg Yolk. Results of the direct comparison of growth of S. aureus strains on SeEy medium containing different volumes of Colbeck and hen egg yolk are shown in Table 4. Results will be discussed based on the average of the two trials for each strain.

Strains 19B and Dack 9 produced highest colony counts on medium containing Colbeck EY broth. Strain 19B produced highest colony counts on medium containing 20 ml of Colbeck EY broth, and strain Dack 9 produced highest colony counts on medium containing 30 ml of Colbeck EY broth. Strains 56D, ATCC 12600, Wis. 523, and ATCC 8096 produced highest colony counts on medium containing hen egg yolk. Strains ATCC 12600 and Wis. 523 had the largest colony counts on SeEy medium containing 25 ml of hen egg yolk while 56D had the largest colony count on medium containing 30 ml of hen egg yolk. Strain ATCC 8096 had the largest colony count on medium containing 15 ml of hen egg yolk.

No egg yolk reaction was produced by strains 19B and ATCC 12600. Dack 9 produced a larger egg yolk reaction on medium containing either 30 ml of Colbeck EY broth or 30 ml of hen egg yolk than on medium containing the other volumes of either hen egg yolk or Colbeck EY broth. Both strains ATCC 8096 and Wis. 523 produced the largest egg yolk reaction on medium containing 25 or 30 ml of hen egg yolk.

Strain 19B had reddest colonies on medium containing 25 or 30 ml of hen egg yolk. Dack 9 had colonies that were redder on hen egg yolk than on Colbeck EY broth medium. The colonies were approximately the same color on medium containing any one of the volumes of hen egg yolk. For strain ATCC 8096, colony color would probably be redder on medium containing 25 or 30 ml of either Colbeck or hen egg yolk than on medium containing the other volumes of either. Strain 56D had reddest colonies on medium containing 30 ml of hen egg yolk. Strain ATCC 12600 produced colonies that were the same color on medium containing 20, 25, and 30 ml of hen egg yolk. Concentration of hen egg yolk in the medium had no effect on the color of the colonies of strain Wis. 523.

All strains produced largest colonies on medium containing Colbeck EY broth. Colonies of strains 19B, 56D, and Wis. 523 were largest on medium containing 30 ml of Colbeck EY broth. Strain ATCC 8096 had colonies that were approximately the same size on medium containing 15, 25, and 30 ml of Colbeck EY broth. Strain ATCC 12600 had the largest colonies on medium containing 25 or 30 ml of Colbeck EY broth. Strain Dack 9 had the same size colonies on medium containing any one of the volumes of Colbeck EY broth.

In the direct comparison of strains of S. aureus on medium containing different volumes of hen egg yolk or Colbeck EY broth, no one volume of either could be recommended for use, but it appears that both could be used. Cultural characteristics of the staphylococci did vary on the selenite medium plus Colbeck or hen egg yolk.

If this medium is to be used as a selective medium for an unknown strain, it would be left to the investigator as to whether he uses Colbeck EY broth or hen egg yolk and to the volume he chooses.

From a composite of the results as presented in Figures 3 and 4 and Table 4, the greatest amount of precipitate for chemical analysis can be obtained by growing S. aureus 56D at 35 C, pH 7.6, under aerobic conditions, and on SeEy medium containing 30 ml of hen egg yolk.

TABLE 4

Some characteristics of growth of S. aureus strains on SeEy medium having added either Colbeck or hen egg yolk

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
19B	15	A	57	55		-		+		+	
		B	100	57		-		+		+	
		C	186		109		-		++		++
		D	100		66		-		+		+
		E	160	44	103	-	-	++	+	++	+++
		F	172	46	109	-	-	+	+	++	++
		average		51	97						
	20	A	57	43		-		++		++	
		B	100	44		-		+		+	
		C	186		140		-		++		++
		D	100		128		-		+		+
		E	160	57	102	-	-	++	+	+	+++
		F	172	51	121	-	-	+	+	++	++
		average		49	122						

TABLE 4 - Continued

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
19B	25	A	57	31		-		++		++	
		B	100	34		-		+		++	
		C	186		184		-		++		++
		D	110		149		-		+		+
		E	160	40	111	-	-	++	+	++	+++
		F	172	41	67	-	-	++	+	++	++
		average		36	128						
	30	A	57	32		-		++		++	
		B	100	31		-		+++		++	
		C	186		150		-		++		++
		D	100		121		-		++		++
		E	160	55	91	-	-	++	+	++	+++
		F	172	59	116	-	-	++	+	++	+++
		average		44	119						
Dack 9	15	A	142	43		++		++		++	
		B	181	54		++		+		+	

TABLE 4 - Continued

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
Dack 9	15	C	137[c]		263		++		+		+
		D	289[c]		288[c]		++		+		+
		E	81[c]	281	219	-	-	++	++	+	++
		F	54[c]	261	212	+	+	++	+	+	++
		average		160	89[c]						
	20	A	142	105		+		++		++	
		B	181	106		+		+		+	
		C	137[c]		235		+		++		+
		D	289[c]		241[c]		++		+		+
		E	81[c]	229	200	+	+	++	+	+	++
		F	54[c]	241	219	+	+	++	+	+	++
		average		170	77[c]						
	25	A	142	40		++		++		++	
		B	181	45		++		+		++	
		C	137[c]		274		++		+++		++
		D	289[c]		172[c]		+		++		++

TABLE 4 - Continued

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
Dack 9	25	E	81[c]	242	31[c]	++	+	++	+	++	++
		F	54[c]	254	248	++	++	++	++	+	++
		average		145	64[c]						
	30	A	142	63		+++		++		++	
		B	181	62		++		+		++	
		C	137[c]		239		++		+++		+++
		D	289[c]		204[c]		+		++		++
		E	81[c]	250	32[c]	+++	+++	++	+	+	++
		F	54[c]	247	33[c]	+++	+++	++	++	++	++
		average		155	73[c]						
ATCC 8096	15	A	68[c]	157		++		++		++	
		B	102[c]	93		+		+		++	
		C	84[c]		171		-		+		+++
		D	129[c]		30[c]		+		+		++
		E	121[c]	90[c]	33[c]	+	++	+	+	+	++
		F	115	97[c]	39[c]	+	+	+	+	+	++
		average		53[c]	298						

TABLE 4 - Continued

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
ATCC 8096	20	A	68[c]	175		++		++		++	
		B	102[c]	72[c]		+		++		++	
		C	84[c]		245		+		+		++
		D	129[c]		230		++		+		+
		E	121[c]	78[c]	245	+	-	+	+	+	+
		F	115[c]	70[c]	257	+	+	+	+	+	++
		average		59[c]	244						
	25	A	68[c]	141		++		++		++	
		B	102[c]	80[c]		+		++		++	
		C	84[c]		258		+		+		+
		D	129[c]		55[c]		++		++		++
		E	121[c]	86[c]	57[c]	++	+	+	+	+	++
		F	115[c]	58[c]	31[c]	++	+	++	++	+	++
		average		60[c]	42[c]						
	30	A	68[c]	111		+++		++		++	
		B	102[c]	71[c]		++		++		++	

TABLE 4 - Continued

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
ATCC 8096	30	C	84[c]		215		++		++		+
		D	129[c]		59[c]		++		++		++
		E	121[c]	71[c]	234	++	+	+	+	+	++
		F	115[c]	64[c]	252	++	++	++	++	+	++
		average		54[c]	32[c]						
56D	15	A	47	31		-		+		++	
		B	121[c]	63[c]		-		+		++	
		C	40[c]		159		++		++		++
		D	133[c]		119		++		++		+
		E	126[c]	66[c]	38[c]	+	+	++	+	++	++
		F	174[c]	59[c]	44[c]	+	+	++	++	++	++
		average		47[c]	270						
	20	A	47	47		+		+		+	
		B	121[c]	56[c]		+		+		+	
		C	40[c]		136		++		++		++
		D	133[c]		54[c]		+		++		++

TABLE 4 - Continued

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
56D	20	E	126[c]	53[c]	48[c]	-	+	+	+	+	+
		F	174[c]	56[c]	65[c]	+	+	++	+	+	+
		average		42[c]	45[c]						
	25	A	47	44		++		++		++	
		B	121[c]	47[c]		++		++		++	
		C	40[c]		233		++		++		++
		D	133[c]		99[c]		+		++		+
		E	126[c]	37[c]	34[c]	++	++	++	+	+	++
		F	174[c]	38[c]	41[c]	++	++	++	+	++	++
		average		37[c]	49[c]						
	30	A	47	37		+++		+++		++	
		B	121[c]	56[c]		+++		+++		++	
		C	40[c]		136		++		++		++
		D	133[c]		82[c]		++		+++		++
		E	126[c]	62[c]	35[c]	+++	++	+++	++	+	++
		F	174[c]	64[c]	49[c]	++	++	++	++	++	+++
		average		46[c]	45[c]						

TABLE 4 - Continued

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
ATCC 12600	15	A	142	43		+		++		++	
		B	281	91		-		+		+	
		C	177		31		-		+		+
		D	232		83		-		+		+
		E	83[c]	173	38	-	-	++	++	++	+++
		F	194	172	33	-	-	++	++	++	++
		average		120	47						
	20	A	142	105		+		++		++	
		B	281	106		-		+		+	
		C	177		45		-		+		+
		D	232		53		-		+		+
		E	83[c]	167	43	-	-	+++	++	++	+++
		F	294	156	57	-	-	+++	++	+	++
		average		133	49						
	25	A	142	121		++		++		+++	
		B	281	125		-		++		++	

TABLE 4 - Continued

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
ATCC 12600	25	C	177		57		-		++		++
		D	232		63		-		+		+
		E	83[c]	254	86	-	-	+++	++	++	+++
		F	294	251	69	-	-	+++	++	++	+++
		average		188	69						
	30	A	142	84		++		++		+++	
		B	281	89		-		++		++	
		C	177		66		-		++		++
		D	232		69		-		++		++
		E	83[c]	228	54	-	-	+++	++	++	+++
		F	294	214	50	-	-	+++	++	++	+++
		average		154	60						
Wis. 523	15	A	94	63		+		+		++	
		B	45[c]	154		+		+		++	
		C	119[c]	104[c]		+		+		++	

TABLE 4 - Continued

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
Wisc 523	15	D	141		95		+		+		++
		E	173		33		+		+		+
		F	43[c]		102		-		+		+
		G	37[c]		171		+		+		+
		H	221	103	94	+	+	++	+	+	++
		I	68[c]	217	216	+	+	++	+	+	+
		average		31[c]	118						
	20	A	94	80		+		+		++	
		B	45[c]	34[c]		++		+		+	
		C	119[c]	99[c]		+		+		+	
		D	141		75		+		+		++
		E	173		62		+		+		+
		F	43[c]		151		-		+		+
		G	37[c]		32[c]		++		++		++
		H	221	117	66	+	+	++	+	+	++
		I	68[c]	46[c]	197	+	+	++	+	+	+
		average		40[c]	145						

TABLE 4 - Continued

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
Wis. 523	25	A	94	67		+++		++		++	
		B	45[c]	31[c]		++		++		+	
		C	119[c]	36[c]		++		++		++	
		D	141		90		++		++		++
		E	173		66		+		++		+
		F	43[c]		142		+		+		+
		G	37[c]		31[c]		++		++		++
		H	221	137	104	++	+	++	++	+	++
		I	68[c]	63[c]	251	+	+	++	+	++	++
		average		30[c]	160						
	30	A	94	51		++		+++		++	
		B	45[c]	36[c]		+		+++		+	
		C	119[c]	31[c]		++		+++		++	
		D	141		84		++		++		++
		E	173		102		+		++		+
		F	43[c]		193		+		++		+
		G	37[c]		35[c]		++		++		++

TABLE 4 - Continued

S. aureus strains	Volume of egg yolk	Trial	CFU per ml/10^6 on PCA	CFU per ml/10^6 on SeEy		Egg yolk reaction		Colony color		Colony size	
				H[a]	C[b]	H	C	H	C	H	C
Wis. 523	30	H	221	79	117	++	+	++	++	++	++
		I	68[c]	34[c]	230	+	+	++	++	++	+++
		average		228	179						

[a]Hen egg yolk.

[b]Colbeck EY broth.

[c]CFU per ml/10^7.

SUMMARY

By X-ray diffraction analysis, inorganic forms of selenium and tellurium were not found in the metabolic products formed by S. aureus Wis. 523 grown on SeEy medium and BP medium. X-ray emission analysis did reveal that selenium and tellurium were constituents of the products, although tellurium was present only in trace amounts.

The incubation temperature found to give the highest colony counts was 35 C. The colonies were a more distinctive red at 35 C. Colonies grown at 45 C showed a greater zone of egg yolk precipitation but were somewhat smaller than those grown at 35 C.

The pH of the medium found to yield best growth of S. aureus Wis. 523 was 7.6. This is the pH of the unmodified SeEy agar.

Aerobic conditions were found to be more suitable for growth of S. aureus Wis. 523 on SeEy medium than were anaerobic conditions. Higher colony counts were obtained on the SeEy medium when the bacterium was grown under aerobic conditions, and the colonies were redder and larger.

The volume of egg yolk is not constant from egg to egg. The volume of yolks from 25 grade A large hen eggs varied from 15 ml to 23 ml, with the average volume being 18.6 ml.

It was found that the aging of an egg yolk up to 4 weeks in a refrigerator would probably not influence the growth of S. aureus Wis. 523 on SeEy medium.

Variation of growth of six strains of S. aureus occurred on SeEy medium containing different volumes of hen egg yolk and also on medium

containing different volumes of Colbeck EY broth. The growth of 5 strains of S. aureus was not constant enough from trial to trial in an experiment and from experiment to experiment to determine conclusively that Colbeck EY broth in the medium gave it any advantage over medium containing hen egg yolk. Also, it was not possible to indicate any one volume of hen egg yolk or Colbeck EY broth as being the one to use with SeEy medium.

Staphylococcus aureus 56D was relatively constant in its growth on SeEy agar containing the two different kinds of egg yolk. It was found that S. aureus 56D produces consistently high colony counts, red colonies, large colonies, and a consistent egg yolk reaction when grown on SeEy agar containing 30 ml of hen egg yolk. This strain should be used when collecting the red selenite precipitate from growth on SeEy agar.

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