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The Influence of Chemicals on the Growth of Bacteria

Alice J. Burke
Loyola University Chicago

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THE INFLUENCE OF CHEMICALS ON THE GROWTH OF BACTERIA

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THESIS

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ALICE J. BURKE

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INTRODUCTION.

In presenting the paper as a resume of an investigation with regards to the influence of chemicals on the selective growth of bacteria I wish to acknowledge my great indebtedness to Dr. Emil Weiss, Professor of Bacteriology at Loyola University School of Medicine, who suggested the subject. I am also indebted to Dr. Weiss for his constant interest and kind supervision of this work. This project, as a whole, is a continuation of an earlier study of the same subject made by various authors and limited to a small number of chemicals.

We had, at first, intentions of a thorough investigation covering a large number of chemicals with special regard to their influence on the growth of bacteria. As the work progressed it was found that it would be necessary to limit the study to those chemicals which promised to give the best results. During the progress of our work we observed that the alkali metals were the most important and of the most interest. Of these the sodium and potassium salts were of the most value. For this reason the larger percentage of the chemicals used were salts of these two metals. Special consideration had to be given to the solubility in water (H_2O) and the saturation points of these salts. Many salts, especially those of calcium, had to be eliminated because of their poor solubility in water.

The general objects of the experiment can be listed as follows:

1. Whether chemicals can be used for isolation of bacteria.

2. Whether chemicals can be used for identification of bacteria.

3. Whether chemicals can be used for differentiation of related strains.

4. Whether such properties (1, 2, 3) are characteristic of certain chemical groups and not of others.

5. The possibility of whether chemicals could be made useful in a way similar to the carbohydrates.

6. The influence of the hydrogen ion concentration of these chemicals in regard to growth.

7. The relationship of the molecular weights with the usefulness for this purpose.

8. Whether the osmotic pressure produced by the various chemicals (in broth) has any effect on the growth of the bacteria.

No literature pertaining to this experiment has been published heretofore.

MATERIAL AND METHODS.

The medium most extensively employed for this work was nutrient agar. This medium was used in two forms; i.e. agar slants and agar plates. In a few cases nutrient broth was substituted for the agar.

Nutrient Agar. 10 grams of peptone, 5 grams of NaCl, 3 grams of Liebigs beef extract, and 40 grams of agar were dissolved in one liter of distilled water by auto-

claving for 30 minutes at a pressure of 15 pounds. The medium was then filtered and was ready for the addition of the chemicals.

Nutrient Broth. 10 grams of peptone, 5 grams of NaCl, and 3 grams of Liebigs beef extract were dissolved in one liter of distilled water by heating. The medium was filtered and was ready for the addition of the chemicals.

Procedure of adding the chemicals to the media. To 100cc. portions of the medium 1 gram, 5 grams, 10 grams and 15 grams of the chemical to be tested were added to make up 1%, 5%, 10% and 15% solutions respectively. These portions were then autoclaved for half an hour at 15 pounds pressure to aid the dissolving of the chemicals and sterilized the medium. The medium was then poured into sterile petri dishes or cottoned test tubes and allowed to solidify or cool. With those chemicals that precipitated the medium, the sterilization was shortened by bringing the pressure up to 15 pounds only. Where there was no inhibition of growth with the 15% solutions the amount of chemical used was increased to 20%.

The chemicals used for this work were obtained from the stock rooms of the Bacteriology and Chemistry departments as well as the general stock room of the school.

The solubility and usefulness of the ammonium salts and those of the alkali metals were quickly grasped and the major portion of the chemicals used are of these groups. The ammonium

salts used were: ammonium bicarbonate ($NH_4 HCO_3$), ammonium bichromate ($(NH_4)_2Cr_2O_7$), ammonium bromide (NH_4Br), ammonium chloride (NH_4Cl), ammonium chromate ($(NH_4)_2CrO_4$), ammonium iodide (NH_4I), ammonium Oxalate ($(NH_4)_2C_2O_4$), ammonium phosphate ($(NH_4)_2HPO_4$), ammonium sulfate ($(NH_4)_2SO_4$), ammonium sulfocyanide (NH_4SCN), ammonium tartarate($(NH_4)_2C_4H_4O_6$), and ammonium molybdate ($(NH_4)_6Mo_7O_{24}$).

The sodium compounds that were tested were: sodium acetate ($NaC_2H_3O_2$), sodium ammonium phosphate ($Na_2NH_4PO_4$), sodium borate ($Na_2B_4O_7$), sodium bitartarate ($NaHC_4H_4O_6$), sodium calcium hydrate ($NaOH + Ca(OH)_2$), sodium carbonate (Na_2CO_3), sodium chloride (NaCl), sodium choleate ($C_{23}H_{39}O_3COONa$), sodium chromate ($Na_2Cr\ O_4$), sodium cyanide (NaCN), sodium citrate ($Na_3C_6H_5O_7$), sodium hyposulfite ($Na_2S_2O_3$), sodium iodide (NaI), sodium nitrate ($NaNO_3$), sodium oxalate ($Na_2C_2O_4$), mono-sodium phosphate(NaH_2PO_4), di-sodium phosphate (Na_2HPO_4), tri-sodium phosphate(Na_3PO_4), sodium potassium tartarate ($KNaC_4H_4O_6$), sodium sulfate (Na_2SO_4), sodium sulfite (Na_2SO_3), sodium sulfocarbolate ($C_6H_4OHSO_3Na$), sodium sulfocyanate (NaCNS), sodium thiosulfate ($Na_2S_2O_3$), sodium tungstate (Na_2WO), sodium nitro-prusside ($Na_2Fe(CN)_5NO$).

The potassium salts included were: potassium acetate ($KC_2H_3O_2$), potassium bichromate ($K_2Cr_2O_7$), potassium biniodate ($KH(IO_3)_2$), potassium bromide (KBr), potassium carbonate (K_2CO_3), potassium chloride (KCl), potassium chlorate ($KClO_3$), potassium chromate (K_2CrO_4), potassium citrate ($K_3C_6H_5O_7$), potassium cyanate (KCNO), potassium cyanide (KCN), potassium ferricyanide

($K_3Fe(CN)_6$), potassium ferrocyanide ($K_4Fe(CN)_6$), potassium hydroxide (KOH), potassium iodate (KIO_3), potassium iodide (KI), potassium molybdate (K_2MoO_4), potassium nitrate (KNO_3), potassium nitrite (KNO_2), potassium oxalate ($K_2C_2O_4$), potassium phosphate (K_2HPO_4), potassium sulphate (K_2SO_4), potassium sulfide (K_2S), potassium tartarate ($K_2C_4H_4O_6$), potassium thiocyanate (KCNS), and potassium thiosulphate ($K_2S_2O_3$).

Of the alkaline earth metals only the following salts of barium were used: barium acetate ($Ba(C_2H_3O_2)_2$), barium carbonate ($BaCO_3$), barium chloride ($BaCl_2$), barium hydroxide ($Ba(OH)_2$), barium iodide (BaI_2), barium nitrate ($Ba(NO_3)_2$), barium sulfate ($BaSO_4$), barium sulfide (BaS).

The number and strains of bacteria used were:

1. Staphylococcus albus.
2. Staphylococcus aureus.
3. Bacillus anthracis.
4. Bacillus subtilis.
5. Eberthella typhi.
6. Salmonella paratyphi.
7. Pseudomonas aeruginosa.
8. Sarcina lutea.
9. Escherichia coli.
10. Proteus vulgaris.

These cultures were obtained from the collections of the Bacteriological Departments of the Loyola University and the University of Illinois Medical Schools. A few of the strains

were obtained from the American Type Collection at McCormack Institute. These cultures were transferred daily to plain agar slants or broth and in turn were used to inoculate the medium containing the chemicals.

The method used for inoculating the prepared medium was as follows: After the medium containing the chemical to be tested had solidified and cooled, one loop of a 24 hour culture was placed on the center of the agar. This amount of the culture was then carefully and uniformly spread over the surface of the medium with a sterile glass spreader. The cover was replaced on the petri dish and was placed cover-side down in the incubator.

The first incubation was for 24 hours at 37½° Centigrade. If growth of the organism used was absent or only slight in character, an additional incubation period was given them at the same temperature and time. The results were then taken as final. Those cases that gave results of doubtful character after the prolonged incubation period were repeated on new media. The number of repetitions and recheckings that were done depended only upon parallel results, so that no doubt of the findings of a particular experiment could be had.

DISCUSSION OF RESULTS

As the work progressed it became evident that the sodium and potassium salts would be the most useful, and the majority of the chemicals that give differential results are compounds of these elements.

Of the ammonium group only one compound of value was found,

namely, the sulphate($(NH_4)_2SO_4$). In this case a 5% solution of the salt entirely eliminates the growth of Pseudomonas aeruginosa, but has no noticeable effect on the remaining organisms that were tested.

Barium nitrate ($Ba(NO_3)_2$), hinders the growth of Escherichia coli and Pseudomonas aeruginosa in a 10% solution and does not hinder the growth of the other strains used. This salt is the only useful one found of this metal.

The potassium compounds responded more readily, and gave several useful possibilities of differentiation of the bacteria used. The acetate ($KC_2H_3O_2$) prevents the Staphylococcus aureus from growing in a 5% solution though it does not influence the growth of the other organisms. The chromate (K_2CrO_4) in a 5% solution does not allow Bacillus subtilis or Pseudomonas aeruginosa to grow yet it does not materially hinder the growth of the rest. The citrate of this metal ($K_3C_6H_5O_7$) prevents the growth of Staphylococcus albus in a 10% solution. A 1% solution of the cyanide (KCN) inhibits the growth of both Bacillus subtilis and Bacillus anthrax while still allowing reproduction of the remaining organisms used. Potassium hydroxide (KOH) in as low a concentration as 1% prevents Bacillus subtilis, Pseudomonas aeruginosa and Eberthella typhi from growing, though allowing the remaining organisms to live. Potassium Iodide (KI) in 5% solution allows the organisms to grow with the exception of Escherichia coli which it inhibits entirely. The Bacillus subtilis was prevented fromgrowing by a 10% solution of potassium phosphate (K_2HPO_4) though there was no appreciable effect on the other strains used.

The sodium salts were equally as useful for differential purposes. Sodium borate ($Na_2B_4O_7$)for example, in 1% solution allowed all of the organisms to grow heartily but subtilis which it totally eliminated. The chloride of this metal (NaCl) prevented only Escherichia coli from growing. Sodium chromate (Na_2CrO_4) was found to prevent not only Escherichia coli from growing but also Proteus vulgaris, Staphylococcus aureus and Bacillus subtilis. Sodium citrate ($Na_3C_6H_5O_7$) prevents Staphylococcus albus fromgrowing in a 15% solution. In concentrations of10% sodium nitrate ($NaNO_3$) prevents Escherichia coli from growing as well as Pseudomonas aerugihosa. The growth of Bacillus anthrax, Eberthella typhi, and Salmonella paratyphi was retarded, but no appreciable difference was exhibited in the growth of Staphylococcus albus, Staphylococcus aureus and Sarcina lutea and Proteus vulgaris. Sodium phosphate (Na_3PO_4) in 5% solution prevents the growth of Staphylococcus aureus, Escherichia coli, and Bacillus subtilis. It was observed that in the case of the phosphates of this metal (mono- di- tri-) that the growth of these organisms decreased inratio to the basic strength of the compound. Escherichia coli, Bacillus subtilis, and Eberthella typhi are inhibited by 7.5% sodium sulfite (Na_2SO_3). Sodium sulpho carbolate ($C_6H_4OHSO_3Na$) in 5% solution does not allow Escherichia coli to grow but has no apparent effect on the other organisms that were tested.

SUMMARY AND GENERAL CONSIDERATIONS

From the scope of this work, there is not enough evidence

to show that there is a possibility of using chemicals for the isolation of bacteria. It is of only a limited value as the chemicals do not respond to the growth of the bacteria in such a way as will eliminate all but one or two desired strains. This could only be accomplished by raising the concentration of the compounds used to a point that would impair the nutritive value of the medium used.

The use of chemicals for the identification of bacteria is of no apparent value as concluded from this work. There was no case where a reaction of any chemical tested with any strain gave a positive identification.

The greatest value of the use of chemicals in media is in differentiation of related strains. This is not only a more logical but more desirable use, as the related strains once differentiated on a chemical medium can easily be identified by other means.

The compounds found to be useful in regard to this work were in the majority those of sodium and potassium. However many compounds of these two metals could not be used and raises the question of whether or not the negative radicals were responsible. A conclusion in this direction seems to be a favorable one, as the useful compounds of these metals, i.e.: chromate citrate, cyanide, phosphate, are in agreement. Ammonium and potassium iodide, ammonium and sodium sulphates, barium and sodium nitrates also point to this conclusion. The value of the potassium and sodium apparently lies in the greater solubility and

and ionization of their compounds.

Chemicals,(with exception of the use of NaCl for osmotic pressure,) have apparently no nutritive value and cannot be used with the organisms tested to split them up and thus cause a difference in pH to change indicators for differential results as in the case of carbohydrates.

The hydrogen ion concentration apparently is not of any great importance as the solid media does not allow the chemicals to remain in the ionized state after solidification and in the case of liquid media the peptone and meat extract are sufficiently strong buffers to allow any appreciable change.

The acidity or alkalinity of the compound apparently is no outstanding factor because the useful chemicals were not only acid and basic but neutral as well.

Although there is a predominance of high molecular weights among the more useful chemicals that were used, there are many whose molecular weights are quite low, apparently pointing more to the chemical composition with regard to usefulness than to the number of elements present.

The thesis "The Influence of Chemicals on the Growth of Bacteria", written by Alice J. Burke, has been accepted by the Graduate School of Loyola University, with reference to form, and by the readers whose names appear below, with reference to content. It is, therefore, accepted in partial fulfilment of the requirements for the degree of Master of Arts.