THE EFFECTS OF MEDIA CONSTITUENTS UPON THE GROWTH AND PIGMENT PRODUCTION OF MICROCOCCUS FLAVUS, MICROCOCCUS ROSEUS, MICROCOCCUS SUBCITREUS, AND SARCINA CITREA

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THESIS

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CHAPTER I

INTRODUCTION

Bacterial pigments have attracted the attention of various individuals down through the annals of history. However, it has been only during the last two hundred years or more that these colorations have been accredited to their proper source. In early days the determination of the source of these unusual colorings, such as blues and greens in perspiration and in wounds, pinks, blues, greens, and reds in milk, and the so called blood drops of bread, was attempted in the realm of the supernatural. Today, it is apparent that these colorings are truly of bacterial origin and have served as bases for interesting and significant studies for many observers and actual scientific investigators.

By enabling early workers to develop a systematic bacteriological nomenclature, pigment production had a definite influence upon the development of the science of bacteriology. The work of Pasteur did not define systematically these different kinds. However, he used various indefinite ways and means for describing them. With the work of Pasteur and his followers, interest grew in the study of microorganisms, and many new types were found; hence the need for an orderly classification.

Schroeter[1] found that on pieces of cooked potato exposed to the air, there often appeared little drops of red, blue, yellow, and orange color. By transferring a small amount of these various colored drops individually to other substances he produced the same growth. From this he drew the logical conclusion that although many of the bacteria producing the various colors looked alike, he was actually dealing with a number of different kinds. Koch somewhat revolutionized the study of bacteriology in that he improved upon the work of Schroeter and eventually devised a simple method by which pure cultures of bacteria could be obtained. Since pure cultures have been isolated, many investigators have tried to explain the nature and the meaning of pigments. Experiments concerning the purpose of pigment formation within the cell have been without satisfactory results. However, all seem to agree generally that pigments have been found to be of some value in the identification and classification of some bacterial organisms.

Bacterial pigments vary not only in color and intensity among the various species, but also within a species depending upon the environment of the organisms concerned. Interest in the causes of bacterial pigment production, particularly from a nutritive standpoint, has been responsible for this investigation. The organisms used in this study represent the

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[1] H. W. Conn and H. J. Conn, Bacteriology, fourth edition, p. 35.

two genera Micrococcus and Sarcina of the family Micrococcaceae. These are found widely distributed in nature in soil, dust, and water.

Review of Literature

The literature reveals that somewhat limited investigations of the causes of bacterial growth and pigment production, from the nutritive standpoint, have been made. However, this phase of bacterial pigment study had its real beginning during the early part of the present century. Consistent progress has been made in this field, the most significant contributions having been made during the past decade.

Sandor and Rougebief[2] (1933) found that the red pigmented acid fast bacilli of Grassberger are strongly pigmented red when grown on cultures of synthetic liquid media (asparagin, glycerol, and dipotassium phosphate) with the pH adjusted to a slightly acid reaction (pH 6.5). The bacilli grown on slightly alkaline or neutral media produce only nonpigmented cultures.

Grootten and Bezssonoff[3] (1934) found that on agar

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[2] G. Sandor et. G. Rougebief, "Sur la Chromogenese des Bacilles Acido-resistants. L'effet du pH sur la Chromogenese", Bull. Soc. Chim. Biol., XV (1933), 415-417, cited by M. Hefferan, Biological Abstracts, X, (1936), entry 16513.

[3] Mlle. O. Grootten et.N. Bezssonoff, "Sur les Facteurs qui Arrentent la Synthese d'un Pigment Bacterien", Compt. Rend. Acad. Sci. (Paris), LXLVIII, (1934), 987-989, cited by W. Burrows, Biological Abstracts, IX, (1935), entry 7576.

peptone medium with pH 7.0 salicylic acid, guiacol, and orcinol stimulate growth but inhibit pigment production in Bacillus balticus. Amidon, acrolein hydroquinone, and formaldehyde have no effect on pigment production or growth.

Hoffs[4] (1934) found that carbon and nitrogen must be present in a nutritive medium in order for Bacterium pyocyaneum to grow and form pigments. He found further that phosphorus and magnesium enhance both growth and pigment formation.

Kharasch, Conway, Bloom[5] (1936) report that large concentrations of biologically important catalytic metals (Mn^{++}, Cu^{++}, and Fe^{+++}) inhibit growth of microorganisms whereas smaller concentrations, in some cases, cause the loss of pigment production. They reported, further, that pigment formation in Staphylococcus aureus, Pseudomonas pyocyanea, Serratia marcescens, Torula rosea, Sarcina lutea, and Sprillum rubrum are inhibited by diphenylamine when present in culture media.

Clark and Smith[6] (1939) found that Bacillus niger

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[4]Max Hoffs, "Uber Wachstums und Fermentverhaltnisse des Bakterium pyocyaneum auf einigen Eiweissfreien Nahrboden," Tierarztliche Hochschule, (Berlin), (1934), cited by L. S. Butcher, Biological Abstracts, X, (1936), entry 6404.

[5]M. S. Kharasch, E. A. Conway, and W. Bloom, "Some Chemical Factors Influencing Growth and Pigmentation of Certain Microorganisms", Journal of Bacteriology, XXXII, (July-December, 1936), 539-540.

[6]Francis E. Clark and Nathan R. Smith, "Cultural Requirements for the Production of Black Pigments of Bacilli", Journal of Bacteriology, XXXVII, (1939), 277-284.

produces a black pigment only upon media which contain free or metabolically available tyrosine. He found, also, that Bacillus aterrimus, Bacillus tyrosinogenes, and Bacillus nigrificans produced pigment only in the presence of carbohydrates, and that Bacillus betanigrificans requires metallic iron for pigmentation.

Cicconi[7] (1942) found that Bacillus pyocyaneus does not form pigment if cultured on a medium containing extract of rice bran, which is ordinarily an excellent medium for growth of this organism. He found, also, that when large amounts of alanine are added to rice bran, the inhibiting action is reversed. He found, further, that on a synthetic medium containing ammonium succinate, potassium phosphate, magnesium phosphate, calcium gluconate, and sodium chloride, Bacillus pyocyaneus forms pigment readily.

Dewey and Poe[8] (1943) determined that the minimum essentials for pigment production of Serratia organisms are magnesium, a sulfate, and a phosphate. They found maximum pigment production is obtained when the medium contains 0.001 per cent magnesium chloride, 0.1 per cent potassium sulphate, 0.1 per cent dipotassium phosphate, 0.5 per cent asparagine, and 0.1 per cent ammonium citrate at a pH of 6.5.

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[7] M. Cicconi (Oppedale Civile "Umberto I", Ancona, Italy), "Fehlende Pigmentbildung von Pyocyaneuskulturen auf einem Neven Naehrboden aus Reisklèie," Zentralbl. Bkt. I., Abt. Orig., LXCIX, (1942), 102-110, cited by Ivan Saphra, Biological Abstracts, XVIII, (1944), entry 19689.

[8] Bartlett T. Dewey and Charles F. Poe, "A Simple Artificial Medium for Pigment Production by Members of the Genus Serratia", Journal of Bacteriology, XCV, (1943), 495-498.

Giese[9] (1943) found a brilliant variant occurring in liquid cultures of Achromobacter fisheri grown on glycerol-peptone in sea water. He found, also, that this variant produced a bright yellow-brown pigment on ordinary agar cultures buffered with calcium carbonate, whereas the original strain does not. He found, further, that both strains develop pigment when the media are buffered with phosphates at pH 8.

Gililland and Vaughn[10] (1943) report that temperature is a vital factor in bacterial pigment production. In a study of fifteen chromogenic coliform bacteria on the basis of both qualitative and quantitative data, it was found that, although three cultures produce pigments at all temperatures where growth occurs, the other twelve require incubation at low temperatures. They observed that at 19° centigrade pigments of various shades of yellow are obtained from the twelve cultures, while at 30°-37° centigrade (optimum temperature) no pigment is produced.

Haas and Bushnell[11] (1944) isolated a strain of Mycobacterium lacticola from mud in contact with crude oil.

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[9] Arthur C. Giese, "Studies on the Nutrition of Dim and Bright Variants of a Species of Luminous Bacteria", Journal of Bacteriology, XCVI, (1943), 323-331.

[10] Richard J. Gililland and Reese H. Vaughn, "Biochemical Characteristics of Pigmented Coliform Bacteria", Journal of Bacteriology, XCV, (1943) 499-507.

[11] H. F. Haas and L. D. Bushnell, "The Production of Carotenoid Pigments from Mineral Oil by Bacteria", Journal of Bacteriology, XCVIII, (1944), 219-231.

They found that this organism produces carotenoid pigments when cultured in a mineral salt medium with mineral oil as the sole organic substrate. They found, further, that the addition of astacin to culture media causes a decrease in carotenoid production by this organism.

Lasseur, Dupaix-Lasseur, and Gavolille[12] (1942-1944) found that racemic histidine enhances pigment production of the Rb type of Bacillus aurantiacus tingitanus, and that l-histidine enhances pigment production of the S type of this organism. They found, also, that the Rb and S types of Bacillus aurantiacus tingitanus on Hoffman-LaRoche glycine medium show more pigmentation than on Paulenc glycine medium under sunlight, whereas the opposite is obtained under ultra-violet light. They found, further, that the Ra type produces a pigmentation apparently similar on both glycines when examined under daylight, but under ultra-violet light the cultures on Paulenc-LaRoche glycine are more fluorescent than those on Hoffman-LaRoche glycine.

Lasseur, Dupaix-Lasseur, and Bonnefoy[13] (1942-1944)

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[12] Ph. Lasseur, A. Dupaix-Lasseur, et M. Th. Gavoille, "Culture de Differents Types Dissocies Dans des Milieux Renfermant des Amides ou des Acides Amines Comme Source d'Azote Culture en Millieu Synthetique a Base d-Histidine ou de Glycocolle", Trav. Lab. Microbiol. Fac. Pharm. Nancy, XIII, (1942-1944), 63-83, cited by H. L. Berard, Biological Abstracts, XX, (January, 1946), entry 959.

[13] Ph. Lasseur, A. Dupaix-Lasseur, et Ch. Bonnefoy, "Culture de Differents Types Dissocies Dans les Milieux Synthetiques a base d-Alanine et de Valine", Trav. Lab. Microbiol. Fac. Pharm. Nancy, XIII, (1942-44), 83-97, cited by H. L. Berard, Biological Abstracts, XX, (January 1946), entry 960.

found that the use of amino acids containing more carbon atoms does not favor the growth, nor the chromogenesis of Bacillus aurantiacus tingitanus. They[14] (1942-1944) found also that the amino acids growing the largest amount of orange pigment are not necessarily the ones permitting the maximum production of red fluorescence. These listed in descending order from the most to the least effective for growth are l-histidine, dl-histidine, glycine, dl-alanine, dl-valine, tyrosine, and d-histidine; for chromogenesis (orange pigment) dl-histidine, glycine, l-histidine, dl-alanine, dl-valine, tyrosine, cystine, and d-histidine; for red fluorescence glycine, dl-alanine, dl-histidine, l-histidine, dl-valine, cystine, tyrosine, and d-histidine.

Lasseur, Dupaix-Lasseur, and Montaigu[15] (1942-1944) studied the various dissociated types of Serratia marcescens and Serratia kiliensis on synthetic media containing l-histidine, glycocoll, ammonium succinate, dl-alanine, and cystine. They found that l-histidine medium is excellent

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[14] Ph. Lasseur, A. Dupaix-Lasseur, et Ch. Bonnefoy, "Culture de Differents Types Dissocies de Bacillus aurantiacus tingitanus en Milieu a de Tyrosin, Histidine, et Cystine" Trav. Lab. Microbiol. Fac. Pharm. Nancy, XIII, (1942-1944), 98-113, cited by H. L. Berard, Biological Abstracts, XX, (January, 1946), entry 961.

[15] Ph. Lasseur, A. Dupaix-Lasseur, et R. Montaigu, "Culture de Differents Serratia en Milieu a Base d'acide amine", Trav. Lab. Microbiol. Fac. Pharm. Nancy, XIII, (1942-1944), 122-125, cited by H. L. Berard, Biological Abstracts, XX, (January, 1946), entry 963.

for growth of both species, but that pH 8.2 is more favorable for chromogenesis than pH 7.2. They found also that glycine is a poor nutrient for Serratia kiliensis and is inferior to l-histidine for Serratia marcescens. They found further that ammonium succinate is an excellent nutrient for Serratia kiliensis and the presence of glucose greatly favors chromogenesis, whereas this material, with or without glucose, does not favor the various types of Serratia marcescens. They found, in addition, that dl-alanine is inferior to l-histidine as a nutrient for Serratia marcescens but is a good nutrient for the growth and pigment production for Serratia kiliensis; and that the cystine medium produces only achromogenic cultures in both species of Serratia.

Sevag and Green[16] (1944) showed that Staphylococcus aureus grown on extract agar without glucose does not produce pigment. They found, however, that upon transplantation to glucose agar, colonies of this organism exhibit the typical golden yellow pigment. They concluded that certain carbohydrates, or some unknown product of their intermediary metabolism, are required for pigment production by Staphylococcus aureus.

Holmes and Wilson[17] (1945) reported that Micrococcus moricolor, isolated from contaminated wounds, produces a

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[16] M. G. Sevag and Morris N. Green, "The Role of Carbohydrates in the Development of Pigment by Staphylococcus aureus", Journal of Bacteriology, XCVIII, (July-December, 1944), 496.

[17] Lida F. Holmes and Mary E. Wilson, "A Micrococcus Producing a Mulberry-colored Pigment", Journal of Bacteriology, XCIX, (1945), 311-312.

mulberry-colored growth on potato medium. They found, also, that this organism produces no pigment when cultured on ordinary nutrient agar.

The Problem

The literature reviewed above shows diversity in opinion among investigators as to the various medium constituents influencing the growth and pigment production of various strains of chromogenic bacteria. The significance of this diversity of opinion, regarding the solution of the complex bacterial growth—pigment production problem, is that the solution thereof has not yet been conclusively demonstrated. The results of additional studies are offered here in the hope that they may add further evidence of some value bearing upon the problem. This investigation has for its aim the explanation of growth and pigment production of Micrococcus flavus, Micrococcus roseus, Micrococcus subcitreus, and Sarcina citrea by the addition of various nutrient test materials to a standard culture medium. The problem has restricted itself; first, to the growth and pigment production of these organisms on plain nutrient agar; second, to their growth and pigment production upon nutrient agar, plus some fifty test materials, each added separately; and third, to an attempt to evaluate the findings.

CHAPTER II

LABORATORY WORK

Preparation of glassware.----All glassware was washed in warm soapy water, rinsed in tap water, and then rinsed through two washings of distilled water. The two washings in distilled water insured that no materials, foreign to the experiment, were present. All glassware was allowed to dry in air. Two hundred petri dishes, six cotton-stoppered flasks, filter papers, spatulas, and cotton-stoppered test tubes were sterilized at one time in the hot air oven at 156°-160° centigrade for two hours.

Preparation of stock cultures and broth cultures.---- Stock cultures of Micrococcus flavus, Micrococcus roseus, Micrococcus subcitreus, and Sarcina citrea were prepared on nutrient agar slants, incubated for seventy-two hours and placed in the ice box. New stock cultures were made in the same manner each week. Broth cultures for inoculations were prepared from the stock cultures, incubated for seventy-two hours, and then placed in the ice box. These were prepared three days prior to the time of using.

Preparation of media.----Six sterile flasks with 250 cc. of nutrient agar each were prepared, sterilized, and cooled to approximately 45°-50° centigrade before the test materials

were added. Five hundred milligrams of each test material, except three which came in commercially prepared weights, were transferred by sterile spatulas to sterile filter paper, weighed out, and wrapped in sterile filter paper until ready for use. Mol-iron (Table 33), choline dihydrogen citrate (Table 14), and the sodium salt of folic acid (Table 19) test materials weighed 198 milligrams, 250 milligrams, and fifteen milligrams respectively in the commercial preparations. Many of the test materials, because of their physical and chemical nature, decompose if subjected to autoclave sterilization. Therefore, these were not added to the nutrient agar prior to sterilization. Five of the flasks were prepared by using 250 cc. of nutrient agar and the previously weighed test materials, whereas one flask contained only the 250 cc. of nutrient agar.

Preparation of plates.----All plates were poured and allowed to solidify by cooling. Each plate was then inverted to prevent the formation of water drops on the surface of the medium. Five test materials with four organisms, three plates per test material per organism, were run simultaneously, making a total of sixty plates for each run. A total of six hundred plates were used for the fifty test materials. For each run sixty plates were set up in four groups, one group for each organism, of fifteen plates. In the fifteen plate grouping, two of the three plates per test

contained the test material and one contained only nutrient agar. All plates were inoculated from seventy-two hour broth cultures.

One plate for each organism for each test material was incubated for seventy-two hours at 37° centigrade and one was incubated for seventy-two hours at room temperature. Ten plates for each organism on nutrient agar alone were incubated for seventy-two hours at 37° centigrade and room temperature for each run of sixty plates.

Observation.----After seventy-two hours of incubation, individual colonies were observed for presence and intensity or absence of pigment, and for growth quality. The measurement of the effects of the medium constituents on pigment production and growth were made by visually comparing the colonies on the test medium with those on nutrient agar. Pigmentation results were tabulated according to (1) normal pigmentation indicated by stating the color produced, (2) sub-normal pigmentation indicated by using the word "fair" before the color produced, (3) above-normal pigmentation indicated by using the words "good" or "dark" before the color produced, and (4) no pigmentation indicated by the word "none". Growth was described using the terms "none", "poor", "fair", "good", and "abundant". All plates were observed by daylight, or under fluorescent lights with the light source coming across the plate.

Test Materials Used

1. Nutrient Agar (Difco)
   - Bacto-Beef Extract . . . . . 3 grams
   - Bacto-Peptone . . . . . 5 grams
   - Bacto-Agar . . . . . 15 grams

2. Adenine Sulphate (Eastman Kodak Company)

3. dl-Alpha-alanine (Merck)

4. Aminoacetic Acid (Glycine) (Pfanstiehl)

5. Amino-Vibex (Park-Davis & Company)
   Each sixty grams represents:
   - Thiamine Hydrochloride (B_1) . . . . 1 mg.
   - Riboflavin (B_2 or G) . . . . . 3 mg.
   - Niacin . . . . . 30 mg.
   - Pantothentic Acid . . . . . 4 mg.
   - Pyridoxine Hydrochloride (B_6) . . . 1 mg.
   - Folic Acid . . . . . 1 mg.
   - Choline . . . . . 100 mg.
   - Iron . . . . . 20 mg.
   - Phosphorus . . . . . 375 mg.

6. Am-Vatine (Smith Dorsey)
   Each thirty grams represent:
   - Protein Hydrolysate (72% Protein) ... 15 g.
   - Food Yeast (45% Protein) . . . . . 15 g.

7. l-Arabinose (Pfanstiehl)

8. l(+)-Arginine Monohydrochloride (Merck)

9. dl-Aspartic Acid (Merck)

10. Bacto-Asparagine (Difco)

11. Brewers' Yeast Powder (Mead)
    Each ten grams represent:
    - Thiamine Hydrochloride (B_1) . . . . 350 units
    - Riboflavin (B_2) . . . . . 500 micrograms
    - Pyridoxine (B_6) . . . . . 450 micrograms
    - Pantothentic Acid . . . . . 2.8 mg.
    - Protein . . . . . 3.7 mg.
    - Carbohydrate . . . . . 5.5 mg.
    - Fat . . . . . 0.25 mg.
    - Minerals (Ash) . . . . . 0.55 mg.

12. Casein Hydrolysate (Squibb)
    Approximate Analysis:

Total Nitrogen	13.5%
Amino Nitrogen	3.4%
Moisture	4.0%
Minerals (Ash)	6.0%

13. Cholesterol (Pfanstiehl)

14. Choline Dihydrogen Citrate (Walker)

15. l(+)-Cystine (Pfanstiehl)

16. Delcos Granules (Sharp and Dohme)
    100 grams contain:

Protein (NX 6.25) derived from milk	50 gms.
Carbohydrate derived from cane, milk, and grain sugars	30 gms.
Vanillin and Coumarin	

17. Dulcitol (Pfanstiehl)

18. Essenamine (Stearns and Company)
    Contains all the essential amino acids

19. Sodium Salt of Folic Acid (Lederle)

20. Gerilac (Borden)
    Approximate Analysis: 11 level tbs. (82 g.) and 1 pint water

Milk fat	1.5%
Milk Protein	5.0%
Milk Sugar	7.0%
Minerals	1.0%
Water	85.0%
Calcium	830 mg.
Phosphorus	800 mg.
Iron	16 mg.
Vitamin A	6500 U.S.P. Units
Vitamin B_1	2.2 mg.
Vitamin B_2	2.7 mg.
Vitamin C	85 mg.
Vitamin D	700 U.S.P. Units
Calories	4.1 per gram

21. l(-)-Glutamic Acid (Pfanstiehl)

22. Glutathione (Eastman Kodak Company)

23. l-Histidine Monohydrochloride (Eastman Kodak Company)

24. i-Inositol (Pfanstiehl)

25. Inulin (Pfanstiehl)

26. dl-Isoleucine (Merck)

27. Ledinac (Lederle)
Each 30 grams represent:

Thiamine Hydrochloride	1 mg.
Riboflavin	2 mg.
Niacinamide	6.6 mg.
Pantothentic Acid	2.3 mg.
Pyridoxine Hydrochloride (B_6)	0.24 mg.
Biotin (H)	2.70 microgms.
Inositol	23.00 mg.
Choline	120.00 mg.
Calcium	106.00 mg.
Phosphorus	297.00 mg.
Iron	4.80 mg.
Calories	103.8

28. l(-)-Leucine (Pfanstiehl)

29. d-Levulose (Pfanstiehl)

30. d-Lysine Monohydrochloride (Eastman Kodak Company)

31. Maltose (Difco)

32. dl-Methionine (Merck)

33. Mol-Iron (White)
Each tablet represents:

Ferrous Sulphate (3 grains)	195 mg.
Molybdenum Oxide ($\frac{1}{20}$ grains)	3 mg.

34. Nicotinamide (Eastman Kodak Company)

35. Nutragest (Burroughs-Wellcome and Company)
Each 100 grams represent:

Protein Hydrolysate	45 g.
Carbohydrate	48.5 g.
Vitamin A	5000 Units
Vitamin D	500 Units
Thiamine Hydrochloride	1 mg.
Riboflavin (B_2)	2 mg.
Nicotinamide	10 mg.
Ascorbic Acid (C)	50 mg.
Calcium Gluconate	1.12 g.
Iron (Ferrous Gluconate)	28 mg.
Magnesium Sulfate	0.39 g.

36. Nutrose (Pfanstiehl)

37. dl-Phenylalanine (Merck)

38. Pimelic Acid (Eastman Kodak Company)

39. l(-)-Proline (Merck)

40. Protein Hydrolysate (Walker)
Each ounce supplies:
Amino Acids;
Alanine, Arginine, Aspartic Acid, Cystine, Glutamic Acid, Glycine, Histidine, Hydroxyproline, Isoleucine, Leucine, Lycine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine, and their peptides as contained in approximately sixteen grams of protein hydrolysate (enzymatic digest of casein).
Vitamins;

Vitamin A (From Fish Liver Oils) . .	5000	U.S.P. Units
Vitamin D (Irradiated Ergosterol) .	500	U.S.P. Units
Thiamine Hydrochloride	10	mg.
Riboflavin (B_2).	6	mg.
Pyridoxine (B_6).	1	mg.
Calcium Pantothenate	1	mg.
Niacinamide	50	mg.
Ascorbic Acid (C)	500	mg.

Minerals;

Iron Peptonate	0.1	g.
Calcium Phosphate, Tribasic	1.0	g.

Analysis;
Protein 60%, Fat 15%, Carbohydrates 4%, Flavored with Bouillon, Hydrolized Vegetable and Yeast Protein, and Vegetable Concentrates. Available calories per ounce is seventy-eight.

41. Raffinose (Pfanstiehl)

42. l-Rhamnose (Pfanstiehl)

43. Riboflavin (Eastman Kodak Company)

44. dl-Serine (Merck)

45. Thiamine Hydrochloride (Eastman Kodak Company)

46. dl-Threonine (Eastman Kodak Company)

47. Trypsin 1:110 (Difco)

48. l(-)-Tyrosine (Pfanstiehl)

49. Uracil (Eastman Kodak Company)

50. dl-Valine (Merck)

51. d-Xylose (Pfanstiehl)

Experimental Data

Table 1 contains data of the average pigment production and growth for Micrococcus flavus, Micrococcus roseus, Micrococcus subcitreus, and Sarcina citrea. The averages in Table 1 are the results of one hundred nutrient agar plates incubated at room temperature and one hundred nutrient agar plates incubated at 37° centigrade for seventy-two hours. Tables 2-51 contain data showing the effects of the individual fifty test materials, added to nutrient agar, upon the pigment production and growth of Micrococcus flavus, Micrococcus roseus, Micrococcus subcitreus, and Sarcina citrea after seventy-two hours of incubation at 37° centigrade and room temperature.

The tables which follow are similar in nature and are considered as a series. For this reason, there is no discussion following each table, and these are listed, one following the other, without interruption.

TABLE I

NUTRIENT AGAR

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	good	yellow	yellow
Micrococcus roseus	fair	fair	white to rose pink	white to rose pink
Micrococcus subcitreus	good	good	yellow	yellow
Sarcina citrea	good	good	yellow	yellow

TABLE 2

NUTRIENT AGAR WITH ADENINE SULPHATE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	none	none	———	———
Micrococcus roseus	fair	fair	white	white
Micrococcus subcitreus	none	none	———	———
Sarcina citrea	none	none	———	———

TABLE 3

NUTRIENT AGAR WITH dl-ALPHA-ALANINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	fair	yellow	yellow
Micrococcus roseus	good	fair	white	white
Micrococcus subcitreus	good	good	yellow	yellow
Sarcina citrea	fair	good	yellow	yellow

TABLE 4

NUTRIENT AGAR WITH AMINOACETIC ACID (GLYCINE)

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37°C.
Micrococcus flavus	poor	poor	yellow and white	light yellow
Micrococcus roseus	good	good	white	white
Micrococcus subcitreus	good	good	yellow	yellow
Sarcina citrea	fair	abundant	yellow	yellow

TABLE 5

NUTRIENT AGAR WITH AMINO-VIBEX

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	poor	yellow	yellow
Micrococcus roseus	fair	contaminated	white	———
Micrococcus subcitreus	fair	good	light yellow	yellow
Sarcina citrea	fair	good	yellow	deep yellow

TABLE 6

NUTRIENT AGAR WITH AM-VATINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	poor	yellow	yellow
Micrococcus roseus	fair	fair	white	white
Micrococcus subcitreus	fair	fair	yellow	yellow
Sarcina citrea	fair	fair	yellow	yellow

TABLE 7

NUTRIENT AGAR WITH l-ARABINOSE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	fair	yellow	yellow
Micrococcus roseus	fair	fair	white	white
Micrococcus subcitreus	good	good	yellow	yellow
Sarcina citrea	good	good	yellow	yellow

TABLE 8

NUTRIENT AGAR WITH l(+) ARGININE MONOHYDROCHLORIDE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	good	light yellow	deep yellow
Micrococcus roseus	good	good	white	white
Micrococcus subcitreus	very abun-dant	abun-dant	deep yellow	deep yellow
Sarcina citrea	abun-dant	abun-dant	deep yellow	yellow

TABLE 9

NUTRIENT AGAR WITH dl-ASPARTIC ACID

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	poor	none	light yellow	———
Micrococcus roseus	none	none	———	———
Micrococcus subcitreus	none	poor	———	light yellow
Sarcina citrea	none	none	———	———

TABLE 10

NUTRIENT AGAR WITH BACTO-ASPARGINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	poor	poor	yellow	yellow
Micrococcus roseus	good	fair	white	white
Micrococcus subcitreus	fair	good	yellow	yellow
Sarcina citrea	fair	good	yellow	yellow

TABLE 11

NUTRIENT AGAR WITH BREWERS' YEAST POWDER

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	poor	light yellow	deep yellow
Micrococcus roseus	abun-dant	good to abun-dant	white	deep white
Micrococcus subcitreus	abun-dant	fair	deep yellow	deep yellow
Sarcina citrea	good	good	yellow	yellow

TABLE 12

NUTRIENT AGAR WITH CASEIN HYDROLYSATE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	good	light yellow	yellow
Micrococcus roseus	good	good	white	white
Micrococcus subcitreus	good	abun-dant	light yellow	yellow
Sarcina citrea	fair	good	light yellow	yellow

TABLE 13

NUTRIENT AGAR WITH CHOLESTEROL

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	good	yellow	yellow
Micrococcus roseus	good	poor	white	white
Micrococcus subcitreus	good	good	yellow	yellow
Sarcina citrea	poor	poor	white	yellow

TABLE 14

NUTRIENT AGAR WITH CHOLINE DIHYDROGEN CITRATE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	none	none	———	———
Micrococcus roseus	good	fair	white	white
Micrococcus subcitreus	none	fair	———	dark yellow
Sarcina citrea	none	none	———	———

TABLE 15

NUTRIENT AGAR WITH l-(-)-CYSTINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	good	light yellow	yellow
Micrococcus roseus	good	good	white	white
Micrococcus subcitreus	good	good to abundant	deep yellow	yellow
Sarcina citrea	good	abundant	yellow	yellow

TABLE 16

NUTRIENT AGAR WITH DELCOS GRANULES

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	poor (part. contam.)	contaminated	light yellow	———
Micrococcus roseus	poor (part. contam.)	contaminated	white	———
Micrococcus subcitreus	contaminated	contaminated	———	———
Sarcina citrea	contaminated	contaminated	———	———

TABLE 17

NUTRIENT AGAR WITH DULCITOL

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	fair	yellow	yellow
Micrococcus roseus	fair	good	white	white
Micrococcus subcitreus	fair	abun-dant	yellow	yellow
Sarcina citrea	fair	good	yellow	yellow

TABLE 18

NUTRIENT AGAR WITH ESSENAMINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	fair	light yellow	yellow
Micrococcus roseus	abun-dant	good	white	white
Micrococcus subcitreus	abun-dant	abun-dant	yellow	deep yellow
Sarcina citrea	good	abun-dant	yellow	yellow

TABLE 19

NUTRIENT AGAR WITH SODIUM SALT OF FOLIC ACID

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	fair	light yellow	yellow
Micrococcus roseus	fair	good	light yellow	white yellow
Micrococcus subcitreus	good	good	light yellow	deep
Sarcina citrea	good	good	deep yellow	yellow

TABLE 20

NUTRIENT AGAR WITH GERILAC

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	good	yellow	yellow
Micrococcus roseus	good	good	white	white
Micrococcus subcitreus	good	fair	deep yellow	deep yellow
Sarcina citrea	fair	good	deep yellow	yellow

TABLE 21

NUTRIENT AGAR WITH l(+) GLUTAMIC ACID

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	none	none	———	———
Micrococcus roseus	none	none	———	———
Micrococcus subcitreus	none	none	———	———
Sarcina citrea	none	none	———	———

TABLE 22

NUTRIENT AGAR WITH GLUTATHIONE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	none	none	———	———
Micrococcus roseus	fair to good	fair to good	white	white
Micrococcus subcitreus	none	none	———	———
Sarcina citrea	none	none	———	———

TABLE 23

NUTRIENT AGAR WITH l-HISTIDINE MONOHYDROCHLORIDE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	none	none	———	———
Micrococcus roseus	good	abundant	white	white
Micrococcus subcitreus	none	none	———	———
Sarcina citrea	none	none	———	———

TABLE 24

NUTRIENT AGAR WITH l-INOSITOL

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	fair	yellow	yellow
Micrococcus roseus	good	good	white & creamy pink	white
Micrococcus subcitreus	abundant	good	yellow	yellow
Sarcina citrea	good	good	yellow	yellow

TABLE 25

NUTRIENT AGAR WITH INULIN

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	poor	fair	light yellow	yellow
Micrococcus roseus	good	fair	white	white
Micrococcus subcitreus	good	good	yellow	deep yellow
Sarcina citrea	fair	good	yellow	yellow

TABLE 26

NUTRIENT AGAR WITH dl-ISOLEUCINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	poor	poor	yellow	white
Micrococcus roseus	fair	fair	white	white
Micrococcus subcitreus	good to abundant	good	yellow	yellow
Sarcina citrea	fair	good	yellow	yellow

TABLE 27

NUTRIENT AGAR WITH LEDINAC

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	good	yellow	yellow
Micrococcus roseus	good	fair	white	white
Micrococcus subcitreus	fair	good	yellow	yellow
Sarcina citrea	good	good	white	yellow

TABLE 28

NUTRIENT AGAR WITH l(-)-LEUCINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	fair	yellow	yellow
Micrococcus roseus	fair	fair	white	white
Micrococcus subcitreus	abun-dant	abun-dant	yellow	yellow
Sarcina citrea	good	good to abun-dant	yellow	yellow

TABLE 29

NUTRIENT AGAR WITH d-LEVULOSE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	fair	yellow	yellow
Micrococcus roseus	good	fair	white	yellow center white halo
Micrococcus subcitreus	abundant	abundant	yellow	yellow
Sarcina citrea	abundant	good	yellow	yellow

TABLE 30

NUTRIENT AGAR WITH d-LYSINE MONOHYDROCHLORIDE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	good	yellow	yellow
Micrococcus roseus	good	good	white	white
Micrococcus subcitreus	abundant	abundant	yellow	yellow
Sarcina citrea	fair	fair	yellow	yellow

TABLE 31

NUTRIENT AGAR WITH MALTOSE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	abundant	fair	yellow	yellow
Micrococcus roseus	contaminated (poor)	contaminated (poor)	white	white
Micrococcus subcitreus	abundant	good	yellow	yellow
Sarcina citrea	good	good	yellow	yellow

TABLE 32

NUTRIENT AGAR WITH dl-METHIONINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	good	light yellow	yellow
Micrococcus roseus	fair	fair	white	white
Micrococcus subcitreus	good	abundant	yellow	yellow
Sarcina citrea	good	abundant	deep yellow	yellow

TABLE 33

NUTRIENT AGAR WITH MOL-IRON

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	none	none	———	———
Micrococcus roseus	abun-dant	good	good, rose pink	good, rose pink
Micrococcus subcitreus	abun-dant	good	yellow	yellow
Sarcina citrea	fair	good	yellow	deep yellow

TABLE 34

NUTRIENT AGAR WITH NICOTINAMIDE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	abun-dant	yellow	yellow
Micrococcus roseus	fair	good	white	white
Micrococcus subcitreus	abun-dant	abun-dant	yellow	yellow
Sarcina citrea	good	good	yellow	yellow

TABLE 35

NUTRIENT AGAR WITH NUTRAGEST

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	contaminated	yellow	———
Micrococcus roseus	fair	contaminated	white	———
Micrococcus subcitreus	good	contaminated	yellow	———
Sarcina citrea	fair	contaminated	yellow	———

TABLE 36

NUTRIENT AGAR WITH NUTROSE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcua flavus	abundant	good	yellow	yellow
Micrococcus roseus	good	contaminated (poor)	white	(white)
Micrococcus subcitreus	good	good	yellow	yellow
Sarcina citrea	abundant	good	light yellow	white

TABLE 37

NUTRIENT AGAR WITH dl-PHENYLALANINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	contaminated	yellow	———
Micrococcus roseus	fair	contaminated	white	———
Micrococcus subcitreus	abundant	good	yellow	yellow
Sarcina citrea	abundant	abundant	yellow	yellow

TABLE 38

NUTRIENT AGAR WITH PIMELIC ACID

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	none	none	———	———
Micrococcus roseus	none	none	———	———
Micrococcus subcitreus	none	none	———	———
Sarcina citrea	none	none	———	———

TABLE 39

NUTRIENT AGAR WITH l(-)-PROLINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	good	good	yellow	yellow
Micrococcus roseus	fair	fair	white	white
Micrococcus subcitreus	good	abun-dant	yellow	yellow
Sarcina citrea	fair	fair	yellow	yellow

TABLE 40

NUTRIENT AGAR WITH PROTEIN HYDROLYSATE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	contam-inated	yellow	———
Micrococcus roseus	good	fair	white	white
Micrococcus subcitreus	good	fair	deep yellow	deep yellow
Sarcina citrea	fair	fair	yellow	deep yellow

TABLE 41

NUTRIENT AGAR WITH RAFFINOSE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	poor	light yellow	yellow
Micrococcus roseus	good	fair	white	white
Micrococcus subcitreus	good	good to abundant	yellow	deep yellow
Sarcina citrea	good	good	yellow	yellow

TABLE 42

NUTRIENT AGAR WITH l-RHAMNOSE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	abundant	good	yellow	yellow
Micrococcus roseus	none	good	———	white
Micrococcus subcitreus	abundant	abundant	yellow	yellow
Sarcina citrea	abundant	very abundant	yellow	yellow

TABLE 43

NUTRIENT AGAR WITH RIBOFLAVIN

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	poor	fair	yellow	yellow
Micrococcus roseus	abun-dant	abun-dant	white	light pink
Micrococcus subcitreus	good	abun-dant	yellow	yellow
Sarcina citrea	poor	abun-dant	yellow	yellow

TABLE 44

NUTRIENT AGAR WITH dl-SERINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	poor	yellow	light yellow
Micrococcus roseus	fair	poor	white	white
Micrococcus subcitreus	good	good	dark yellow	yellow
Sarcina citrea	poor	good	yellow	yellow

TABLE 45

NUTRIENT AGAR WITH THIAMINE HYDROCHLORIDE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	none	none	———	———
Micrococcus roseus	good	fair	white	white
Micrococcus subcitreus	none	none	———	———
Sarcina citrea	none	none	———	———

TABLE 46

NUTRIENT AGAR WITH dl-THREONINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	abun-dant	abun-dant	yellow	yellow
Micrococcus roseus	good	abun-dant	white	white
Micrococcus subcitreus	good	abun-dant	yellow	yellow
Sarcina citrea	fair	fair	light yellow	yellow

TABLE 47

NUTRIENT AGAR WITH TRYPSIN 1:110

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	fair	yellow	yellow
Micrococcus roseus	abun-dant	abun-dant	white	white
Micrococcus subcitreus	good	good	yellow	yellow
Sarcina citrea	good	fair	yellow	yellow

TABLE 48

NUTRIENT AGAR WITH l(-)-TYROSINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	fair	yellow	yellow
Micrococcus roseus	good	good	white	white
Micrococcus subcitreus	abun-dant	very abun-dant	yellow	deep yellow
Sarcina citrea	good	abun-dant	deep yellow	deep yellow

TABLE 49

NUTRIENT AGAR WITH URACIL

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	poor	fair	yellow	yellow
Micrococcus roseus	fair	fair	white	white
Micrococcus subcitreus	good	good	yellow	yellow
Sarcina citrea	fair	good	yellow	yellow

TABLE 50

NUTRIENT AGAR WITH dl-VALINE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	good	light yellow	yellow
Micrococcus roseus	fair	abun-dant	white	white
Micrococcus subcitreus	abun-dant	abun-dant	yellow	yellow
Sarcina citrea	fair	abun-dant	white	yellow

TABLE 51

NUTRIENT AGAR WITH d-XYLOSE

Organism	Growth		Pigment	
	72 hrs. room temp.	72 hrs. 37° C.	72 hrs. room temp.	72 hrs. 37° C.
Micrococcus flavus	fair	fair	yellow	yellow
Micrococcus roseus	poor	fair	white	white
Micrococcus subcitreus	good	good	yellow	yellow
Sarcina citrea	good	good	yellow	yellow

CHAPTER III

DISCUSSION, SUMMARY AND CONCLUSIONS

Discussion

Table 1 shows an average of the pigment production and growth of each Micrococcus flavus, Micrococcus roseus, Micrococcus subcitreus, and Sarcina citrea grown on nutrient agar. Data contained in this table were obtained from one hundred nutrient agar plates incubated for seventy-two hours at each room temperature and 37° centigrade. These serve as a comparative standard for results obtained, under the same conditions, when each of the fifty test materials was added to nutrient agar. The characteristic color of pigment for Micrococcus roseus was white to pink. Each Micrococcus flavus, Micrococcus subcitreus, and Sarcina citrea was characterized by a yellow pigment. Micrococcus roseus produced only fair growth on nutrient agar while Micrococcus flavus, Micrococcus subcitreus, and Sarcina citrea produced good growth at both room temperature and 37° centigrade.

Micrococcus flavus did not grow, as is shown in Tables 2, 14, 21, 22, 23, 33, 38, and 45 at either 37° centigrade or at room temperature when each adenine sulphate, choline dihydrogen citrate, l(+)-glutamic acid, glutathione, l-histidine monohydrochloride, Mol-iron, pimelic acid, and

thiamine hydrochloride was added to nutrient agar. Table 9 shows that this organism did not grow on dl-aspartic acid at 37° centigrade, and that growth was poor at room temperature with retarded pigmentation. Growth at 37° centigrade and room temperature was inferior to that on nutrient agar, as shown by Tables 3, 4, 5, 6, 7, 10, 11, 17, 19, 24, 25, 26, 28, 29, 41, 43, 44, 47, 48, 49, and 51, when each dl-alpha-alanine, aminoacetic acid, Amino-vibex, Am-vatine, l-arabinose, Bacto-asparagine, brewers' yeast powder, dulcitol, sodium salt of folic acid, i-inositol, inulin, dl-isoleucine, l(-)-leucine, d-levulose, raffinose, riboflavin, dl-serine, trypsin 1:110, l(-)-tyrosine, uracil, and d-xylose was added to nutrient agar. According to Table 18, Essenamine produced fair growth at 37° centigrade but at room temperature produced similar growth to that on nutrient agar alone. Gerilac and dl-valine, Tables 20 and 50 respectively, produced only fair growth at room temperature but good growth at 37° centigrade. Table 46 shows that dl-threonine stimulated growth at 37° centigrade and at room temperature. Nutrose and l-rhamnose according to Tables 36 and 42, respectively, stimulated growth at room temperature but produced no visible change at 37° centigrade. Table 34 shows that nicotinamide stimulated growth at 37° centigrade but produced normal growth at room temperature. Tables 35 and 37 show normal growth and pigmentation produced by each Nutragest and dl-phenylalanine at room temperature. Table

40 shows that protein hydrolysate retarded growth of Micrococcus flavus at room temperature. All other test materials accounted for normal growth.

Micrococcus flavus in the presence of each l(+)-arginine monohydrochloride and brewers' yeast powder, according to Tables 8 and 11 respectively, showed increased pigment production at 37° centigrade and retarded pigment production at room temperature. Aminoacetic acid, as shown in Table 4, retarded pigment production at 37° centigrade and produced white colonies interspersed among the light yellow colonies of this organism at room temperature. Table 26 shows that dl-isoleucine influenced the formation of normal yellow pigment at room temperature but produced only colorless colonies at 37° centigrade. Table 44 shows that dl-serine retarded pigment production at 37° centigrade but produced normal pigment at room temperature. Tables 12, 15, 18, 19, 25, 32, 41, and 50 indicate that pigment production was retarded at room temperature but apparently remained normal at 37° centigrade upon the addition to nutrient agar of each casein hydrolysate, l(-)-cystine, Essenamine, sodium salt of folic acid, inulin, dl-methionine, raffinose, and dl-valine. All other test constituents used produced normal pigmentation at both 37° centigrade and at room temperature.

Micrococcus roseus did not grow at room temperature or at 37° centigrade, shown by Tables 9, 21, and 38, when each

dl-aspartic acid, l(+)-glutamic acid, and pimelic acid were used. Table 42 shows that the medium with l-rhamnose did not permit growth at room temperature but showed stimulated growth with normal pigment production at 37° centigrade. Table 44 shows that dl-serine retarded growth at 37° centigrade but permitted normal growth at room temperature. Table 13 shows that cholesterol retarded growth at 37° centigrade but stimulated growth at room temperature. Stimulated growth at both room temperature and at 37° centigrade is shown in each of Tables 4, 8, 11, 12, 15, 18, 20, 22, 23, 24, 30, 33, 43, 46, 47, and 48 respectively, when each aminoacetic acid, l(+)-arginine monohydrochloride, brewers' yeast powder, casein hydrolysate, l(+)-cystine, Essenamine, Gerilac, glutathione, l-histidine monohydrochloride, i-inositol, d-lysine monohydrochloride, Mol-iron, riboflavin, dl-threonine, trypsin, and l(-)-tyrosine was added to nutrient agar. Tables 2, 17, 19, 34, and 50 show stimulated growth at 37° centigrade and normal growth at room temperature when tests were made on each adenine sulphate, dulcitol, sodium salt of folic acid, nicotinamide, and dl-valine. Tables 3, 10, 14, 25, 27, 29, 40, 41, and 45 show stimulated growth at room temperature and normal growth at 37° centigrade upon the addition of each dl-alpha-alanine, Bacto-asparagine, choline dihydrogen citrate, inulin, Ledinac, d-levulose, protein hydrolysate, raffinose, and thiamine hydrochloride to the nutrient agar base. Tables 5, 35, and 37 show normal

growth at room temperature when Amino-vibex, Nutragest, and dl-phenylalanine were used. Table 36 shows that nutrose stimulated growth at room temperature. All other test materials produced normal growth at both 37° centigrade and at room temperature.

Table 19 shows that the sodium salt of folic acid retarded pigment production of Micrococcus roseus at room temperature but produced normal pigment at 37° centigrade. Table 11 shows that brewers' yeast powder stimulated pigment production at 37° centigrade but produced normal pigment at room temperature. Table 29 shows that the d-levulose medium contained some interspersed white colonies with yellow centers among the normally pigmented ones. Table 24 shows that i-inositol produced both white and creamy pink pigmentation at room temperature but only white pigmentation at 37° centigrade. Table 43 shows that the riboflavin medium produced light pink colonies at 37° centigrade and white colonies at room temperature. The Mol-iron medium, according to Table 33, stimulated pigment production and the formation of rose pink colonies at both room temperature and 37° centigrade. All other test constituents produced normal pigmentation at both 37° centigrade and room temperature.

Micrococcus subcitreus failed to grow at either 37° centigrade or at room temperature, according to Tables 2,

21, 22, 23, 38, and 45, when media were used containing each adenine sulphate, l(+)-glutamic acid, glutathione, l-histidine monohydrochloride, pimelic acid, and thiamine hydrochloride. Tables 9 and 14 show that dl-aspartic acid and choline dihydrogen citrate inhibited growth at room temperature but merely retarded growth at 37° centigrade. The dl-aspartic acid retarded pigmentation and the dihydrogen citrate medium stimulated pigmentation at 37° centigrade. Table 6 shows that Am-vatine retarded growth at both room temperature and 37° centigrade. Table 11 shows that brewers' yeast retarded growth at 37° centigrade and stimulated growth at room temperature. Gerilac and protein hydrolysate, as shown in Tables 20 and 40, retarded growth at 37° centigrade and effected normal growth at room temperature. Tables 5, 10, and 27 show that the growth of Micrococcus subcitreus was retarded at room temperature, but grew normally at 37° centigrade in the presence of Aminovibex, Bacto-asparagine, and Ledinac. Table 17 shows that dulcitol retarded growth at room temperature and stimulated growth at 37° centigrade. Tables 8, 18, 28, 29, 30, 34, 42, 48, and 50 show that growth was stimulated at both 37° centigrade and at room temperature when each l(+)-arginine monohydrochloride, Essenamine, l(-)-leucine, d-levulose, d-lysine monohydrochloride, nicotinamide, l-rhamnose, l(-)-tyrosine, and dl-valine was added to the test medium. Tables 12, 15, 32, 39, 41, 43, and 46 show that growth was

stimulated at 37° centigrade, and normal growth was effected at room temperature in the presence of casein hydrolysate, l(-)-cystine, dl-methionine, l(-)-proline, raffinose, riboflavin, and dl-threonine. Tables 24, 26, 31, 33 and 37 show that growth was stimulated at room temperature and normal growth was characteristic at 37° centigrade upon the addition of each i-inositol, dl-isoleucine, maltose, Mol-iron, and dl-phenylalanine to the nutrient agar. Table 35, nutrient agar with Nutragest, shows normal growth and pigment production at room temperature. Other test constituents produced normal growth at room temperature and at 37° centigrade.

Amino-vibex and casein hydrolysate, according to Tables 5 and 12, respectively, retarded pigment production of Micrococcus subcitreus at room temperature but permitted normal pigmentation at 37° centigrade. The sodium salt of folic acid, as shown in Table 19, retarded pigmentation at room temperature; while it stimulated pigment production at 37° centigrade. Tables 8, 11, 20, and 40 show stimulation of pigmentation at both room temperature and 37° centigrade on nutrient agar with the addition of l(+)-arginine monohydrochloride, brewers' yeast powder, Gerilac, and protein hydrolysate. Essenamine, inulin, raffinose, and l(-)-tyrosine, as shown in Tables 18, 25, 41, and 43, stimulated pigmentation at 37° centigrade and effected normal pigment

at room temperature. As shown in Tables 15 and 44, l(-)-cystine and dl-serine stimulated pigmentation at room temperature and produced normal pigment at 37° centigrade. All other test constituents produced normal pigmentation in Micrococcus subcitreus at both 37° centigrade and room temperature.

Sarcina citrea failed to grow at either 37° centigrade or room temperature, according to Tables 2, 9, 14, 21, 22, 23, 38, and 45 on nutrient agar with the addition of each adenine sulphate, dl-aspartic acid, choline dihydrogen citrate, l(+)-glutamic acid, glutathione, l-histidine monohydrochloride, pimelic acid, and thiamine hydrochloride. Tables 6, 13, 30, 39, 40, and 46 show retarded growth at both 37° centigrade and room temperature upon the addition of each Am-vatine, cholesterol, d-lysine monohydrochloride, l(-)-proline, protein hydrolysate, and dl-threonine to nutrient agar. Trypsin, according to Table 47, retarded growth at 37° centigrade and effected normal growth at room temperature. Tables 3, 5, 10, 12, 17, 20, 25, 26, 33, 44, and 49 indicate retarded growth at room temperature and normal growth at 37° centigrade upon the addition to nutrient agar of dl-alpha-alanine, Amino-vibex, Bacto-asparagine, casein hydrolysate, dulcitol, Gerilac, inulin, dl-isoleucine, Mol-iron dl-serine, and uracil. Tables 4, 43, and 50 show that amino-acetic acid, riboflavin, and dl-valine retarded growth at room temperature and stimulated growth at 37° centigrade.

Table 35 shows that Nutragest retarded growth at room temperature with normal pigmentation. Tables 8, 37, and 42 show that l(+)-arginine monohydrochloride, dl-phenylalanine, and l-rhamnose stimulated growth at both 37° centigrade and room temperature. Tables 15, 18, 28, 32, and 48 show stimulated growth at 37° centigrade and normal growth at room temperature in the presence of l(-)-cystine, Essenamine, l(-)-leucine, dl-methionine, and l(-)-tyrosine. Tables 29 and 36 show that d-levulose and nutrose respectively stimulated growth at room temperature and provided normal growth at 37° centigrade. All other test constituents produced normal growth at both 37° centigrade and room temperature.

Tables 12 and 46 show that the pigment production of Sarcina citrea was stimulated at room temperature and was normal at 37° centigrade on nutrient agar with each casein hydrolysate and dl-threonine. Nutrose, according to Table 36, retarded pigmentation at room temperature and at 37° centigrade. Tables 5, 33, and 40 show stimulated pigmentation at 37° centigrade and normal pigment production at room temperature when this organism was cultivated in the presence of Amino-vibex, Mol-iron, and protein hydrolysate. Tables 8, 19, 20, and 32 show increased pigmentation at room temperature and normal pigment production at 37° centigrade when this organism was grown upon each l(+)-arginine

monohydrochloride, the sodium salt of folic acid, Gerilac, and dl-methionine. Tables 13, 27, and 50 show the lack of pigmentation at room temperature and normal yellow pigmentation at 37° centigrade upon subjection of this organism to cholesterol, Ledinac, and dl-valine. All other test constituents showed normal pigment production at both room temperature and 37° centigrade.

Summary

1. A study of the growth and pigment production of Micrococcus flavus, Micrococcus roseus, Micrococcus subcitreus, and Sarcina citrea was made by the subjection of each of these to various test materials added to nutrient agar. Incubation was at each 37° centigrade and room temperature.

2. The laboratory work consisted of the preparation of glassware, stock cultures, broth cultures, media, plates, and observations. After seventy-two hours of incubation, individual colonies were observed for growth quality and for presence and intensity, or absence of pigment.

3. The results of the effects of the various test materials used were recorded in tabular form in a series of fifty-one tables.

4. No marked difference occurred, in growth or pigmentation, between incubation at 37° centigrade and room temperature (approximately 29° centigrade).

5. Adenine sulphate, choline dihydrogen citrate, l(+)-glutamic acid, glutathione, l-histidine monohydrochloride, Mol-iron, pimelic acid, and thiamine hydrochloride completely inhibited growth of Micrococcus flavus.

6. Growth and pigment production of Micrococcus flavus was retarded by twenty-one of the test materials. l(+)-arginine monohydrochloride and brewers' yeast powder stimulated pigment production at room temperature and produced normal pigmentation at 37° centigrade. dl-Threonine stimulated markedly the growth of Micrococcus flavus.

7. Retarded pigmentation for Micrococcus flavus was effected on media containing each casein hydrolysate, l(-)-cystine, Essenamine, sodium salt of folic acid, inulin, dl-methionine, raffinose, and dl-valine.

8. dl-Aspartic acid, l(+)-glutamic acid, and pimelic acid completely inhibited growth of Micrococcus roseus. Growth of this organism was stimulated by each dl-alpha-alanine, Bacto-asparagine, choline dihydrogen citrate, inulin, Ledinac, d-levulose, protein hydrolysate, raffinose, and thiamine hydrochloride. The sodium salt of folic acid retarded, whereas brewers' yeast powder stimulated pigmentation of Micrococcus roseus. The Mol-iron medium stimulated growth and pigmentation of this organism thereby producing definite rose pink colonies.

9. Micrococcus subcitreus failed to grow when the nutrient agar base contained each adenine sulphate,

l(+)-glutamic acid, glutathione, l-histidine monohydrochloride, pimelic acid, and thiamine hydrochloride. Amvatine, however, retarded growth, whereas l(+)-arginine monohydrochloride, Essenamine, l(-)-leucine, d-levulose, d-lycine monohydrochloride, nicotinamide, l-rhamnose, l(-)-tyrosine, and dl-valine stimulated growth of this organism.

10. Pigment color of Micrococcus subcitreus was not affected by any of the test materials. Quanity production, however, was retarded by l(+)-arginine monohydrochloride, brewers' yeast powder, Gerilac, and protein hydrolysate, and stimulated by Essenamine, inulin, raffinose, and l(-)-tyrosine.

11. Adenine sulphate, dl-aspartic acid, choline dihydrogen citrate, l(+)-glutamic acid, glutathione, l-histidine monohydrochloride, pimelic acid, and thiamine hydrochloride completely inhibited growth of Sarcina citrea. The growth of this organism was retarded by Am-vatine, cholesterol, d-lysine monohydrochloride, l(-)-proline, protein hydrolysate, and dl-threonine. l(+)-Arginine monohydrochloride, dl-phenylalanine, and l-rhamnose stimulated growth of Sarcina citrea. Nutrose retarded, and l(+)-arginine monohydrochloride, sodium salt of folic acid, Gerilac, and dl-methionine stimulated pigment production of this organism.

12. Non-pigmented colonies of Sarcina citrea were formed on the media containing each cholesterol, Ledinac, and dl-valine.

Conclusions

Results of this study show that of all the test materials to which Micrococcus flavus was subjected, forty-seven per cent retarded, six per cent stimulated, seventeen per cent completely inhibited, and thirty per cent permitted normal growth of this organism. Furthermore, thirteen per cent of these materials retarded, two per cent stimulated, and eighty-three per cent did not change materially pigment production of Micrococcus flavus.

Of these same test materials upon which Micrococcus roseus was cultured, three per cent retarded, seven per cent inhibited completely, fifty per cent stimulated, and forty per cent effected normal growth of this organism. In addition, three per cent stimulated pigment formation, one per cent retarded pigmentation, and ninety-six per cent produced pigment comparable to that grown on nutrient agar.

Further observations upon results of this investigation show that eleven per cent of these test materials retarded, fourteen per cent completely inhibited, thirty-two per cent stimulated, and forty-three per cent produced normal growth of Micrococcus subcitreus. Four per cent of these retarded, sixteen per cent stimulated, and eighty per cent effected normal pigment production by this organism.

Cultivation of Sarcina citrea upon these test materials show that, of the number used, forty-eight per cent accounted for normal growth, eight per cent caused an increase in

growth, and sixteen per cent completely inhibited growth of this organism. Of the same materials three per cent retarded, nine per cent enhanced, and four per cent effected color changes in pigmentation of Sarcina citrea.

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