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GROWTH AND PIGMENT PRODUCTION OF PSEUDOMONAS JANTHINA.

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The study here presented was undertaken to determine the growth characters of *Pseudomonas janthina* and the relations between color production and composition of medium.

The culture used was derived from the water of a farm well in northwestern Iowa. From its morphology and growth characters it was determined to be *Pseudomonas janthina* (Zopf.) Chester.

MORPHOLOGY.—Bacillus, 0.5 to 0.8 x 1.5 to 5 microns, ends rounded, motile by means of one or two polar flagellæ. Stains well using Gram's method. No spore production, stains readily with ordinary analine dyes.

CULTURAL CHARACTERS, *Gelatin Plates.*—Colonies appear first as small yellowish, or whitish dots, liquefying the the gelatin, and eventually with greenish purple centers and violet borders. Microscopically fragmental, grumose.

Gelatin Stab.—Gelatin liquefied after some time, growth at first whitish, becoming violet.

Agar Slant.—Growth smooth, spreading, thin at first and whitish yellow, soon becoming deep violet or even violet black and very much crumpled, tough.

Potato.—Growth on the potato at first whitish or whitish yellow, soon developing small purple points.

Bouillon.—At first turbid, but soon developing a membrane at first whitish, soon turning violet.

Milk.—Milk not coagulated, upper portion violet.

PHYSIOLOGICAL CHARACTERS.—In the cultivation on the above media great variations in color production were

noticed. Cultures growing under seemingly identical conditions in some cases produced very little, if any, color, in other cases producing a large amount. An effort was made to determine the cause, or some of the causes of this variation.

Effect of the Acidity and Alkalinity of the Culture Medium.—To test tubes containing 10c.c. of bouillon, varying amounts of normal hydrochloric acid, and sodium hydrate were added, i.e., $\frac{1}{50}$ c.c., $\frac{1}{20}$ c.c., $\frac{3}{50}$ c.c., $\frac{1}{10}$ c.c., $\frac{1}{10}$ c.c., and by tenths of c.c., up to nine-tenths c.c. These together with neutral bouillion were inoculated with this organism. The limits of growth were found to lie between 0.1c.c. sodium hydrate, and a little less than 0.1c.c. of normal hodrochloric acid, the maximum at about $\frac{3}{50}$ c.c. hydrochloric acid. The organism is quite sensitive in the variations of the reactions of the medium in which it is grown, but reaches its maximum development in a medium which is very slightly acid. The amount of growth in all cases and the amount of color produced were similar. In no case was there growth without color production.

Effect of Sunlight.—Agar slants of the organism, freshly inoculated, were exposed to the direct rays of the sun for periods ranging from five minutes to four hours. In no case could any difference be detected between the growth of cultures so exposed, and those which were kept in a dark chamber. The organism seems to be unusually resistant to the antiseptic properties of light.

The Relation of Free Oxygen to Growth and Color Production.—The absence of oxygen practically prevents the growth of the organism, and no color, therefore, is produced.

Effect of Variations in the Composition of Medium on the Growth and Color Production.—Various solutions and media were tested to determine, if possible, what were the sources of carbon and nitrogen that would produce the maximum growth and production of color.

As a basis for these solutions the Stickstoffreie Mineralische Nährlösung (M. nährlösung) of Meyer* was used.

*Arthur Meyer. Mikrokopisches Practicum. II. 15.

KH_2PO_4, 1.00 g., $CaCl_2$, 1.00 g., $MgSO_4+7H_2O$, 0.3 g., NaCl, 0.1 g., Fe_2Cl_6, .01 g., Water, 1000 g. The various solutions made from this and their effect upon the growth and color production of this organism are given below.

Dextrose, 0.5 g., cane sugar, 0.5 g., glycerin, 0.5 g., M. nährlösung, 100.00 g. A very slight membranous sediment was formed.

Potassium nitrate, 1.0 g., dextrose, 1.0 g., M. nährlösung, 100 g. Slight growth but no color was produced.

Potassium nitrate, 1.0 g., glycerin, 1.0 g., M. nährlösung, 100 g. Some growth, but no color.

Potassium nitrate, 1.0 g., cane sugar, 0.5 g.,glycerin,1.0 g., M. nährlösung, 100 g. Decided growth, but no color. It seems that this organism is capable of using its nitrogen in the form of nitrates, but can not produce color.

Ammonium chloride,1.0 g., dextrose,1.0 g., M. nährlösung, 100 g. Slight sediment, whitish, granular, no color.

Ammonium chloride, 1.0 g., cane sugar, 0.5 g., glycerin, 0.5 g., M. nährlösung, 100 g. Some sediment, whitish granular, more than in the preceding.

Ammonium tartrate, 1.0 g., glycerin, 1.0 g., cane sugar, 0.5 g., M. nährlösung, 100 g., cloudy, granulated, whitish, sediment, no color.

Ammonium tartrate,1.0 g., dextrose,1.0 g., M. nährlösung, 100 g. Cloudy, granulated, white sediment, rather more than in the preceding.

Asparagin, 0.2 g., $MgSO_4$, 0.1 g., K_2HPO_4, 0.1 g. Good growth, and decided color produced on the surface.

Asparagin, 1.0 g., K_2HPO_4, 0.1 g., $MgSO_4$, 0.1 g., glycerin, 2.0 g. Good growth, large amount of color on the suface. The medium below the surface had a decided green tinge. This is to be noted in a large number of solutions in which asparagin was used.

Asparagin, 1.0 g., M. nährlösung, 100 g. Slight purple scum, white sediment, decided green tinge.

Asparagin,1.0 g., dextrose, 3.0 g., M. nährlösung, 100 g. A heavy purple scum and membranous white growth

throughout. At first there was a slight green tinge, but this soon disappeared.

Asparagin, 1.0 g., glycerin, 1.0 g., M. nährlösung 1.00 g., white membranous growth, tinged with purple at the top. Medium with a decided greenish tinge.

Asparagin, 1.0 g., glycerin, 1.0 g., cane sugar, 0.5 g., M. nährlösung, 100 g. A light purple scum, white membranous growth at the base of tube, medium with a green tinge.

As above noted the presence of asparagin is favorable to growth When this organism is grown in a medium containing asparagin, the green tinge is almost invariably present. The color is very similar to that which appears when an alcoholic solution of the violet coloring matter is treated with a solution of sodium hydrate.

Peptone, 1.0 g., cane sugar, 1.0 g., water, 100 g. Light purple scum, considerable light sediment.

Peptone, 1.0 g., beef extract, 1.0 g., cane sugar, 1.0 g., water, 100 g. A very heavy, dark, almost black, purple scum, thick and decidedly wrinkled, was formed. The maximum amount of growth and color obtained, was had in this medium.

Peptone, 1.0 g. beef extract, 1.0 g., dextrose, 1.0 g., water, 100 g. The growth similar to the preceding.

Peptone, 1.0 g., dextrose, 1.0 g., water, 100 g. Color production somewhat less than in the preceding. Beef extract seems to be very favorable to color production.

Peptone, 1.0 g., ammonium sulphate, 1.0 g., potassium nitrate, 1.0 g., M. nährlösung, 500 g. Purple scum similar to the preceding is formed.

Arrowroot Starch was also used as a medium. This is an almost pure starch containing very little proteid. Growth was slight on slant, but well colored.

Potato.—On the potato the growth was not so luxuriant as that which has been described by various authors. It is generally stated that the growth is luxuriant and spreading. In this study it formed a brownish, or yellowish white, glistening, raised growth, which in the course of

time, became spotted here and there with small violet spots.

Rice Flour.—There was a better growth and greater production of color on the rice flour than on any of the other solid media used. The growth is spreading, dark purple, thick at first, and becoming very much wrinkled.

Blood Serum.—Growth good.

Egg Albumen.—Coagulated albumen of egg was also tested and proved to be excellent as a culture medium. Growth very thick and heavy.

Pigment.—The purple pigment is soluble in alcohol, but not in ether, chloroform or xylol. The results obtained by *Schneider were checked as follows: The addition of ammonia changes the color to a blue or blue-green, as is the case when sodium hydrate is added. The addition of normal hydrochloric acid decolorizes the solution. When the solution is evaporated to dryness and the purple residue is treated with sulphuric acid a yellow solution is formed.

A strong alcoholic solution when allowed to stand for a period of three weeks, tightly corked, was found to have lost its purple color and to have become almost transparent, but with a reddish-brown tinge. This solution, when treated with acids and alkalies, bahaved as did the original purple solution.

*Die Bedentung der Bakterienfarbstoffe. Separatabdruck aus den Arbeiten des Bakterial. Instits der Grossh. Hochschule zu Karlsruhe. 25.