THE CLASSIFICATION OF WHITE AND SLIGHTLY PIGMENTED STAPHYLOCOCCI ACCORDING TO SUGARS

By

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Introduction

Staphylococci were first found in pus by Pasteur (1880). Ogston confined Pasteur's work a year later (1881), and in 1883 Becker was able to isolate staphylococci in pure culture. Rosenback (1884) described staphylococci pyogenes, dividing it into two varieties due to the orange and white pigmentation, calling them var. aureus and var. albus. In 1908 the Winslows based their classification upon growth, pigment production and liquefaction of gelatin.

Dudgeon (1908) found staphylococcus albus commonly in normal tissue while staphylococcus aureus was usually obtained from pathogenic sources. He was interested in the inter-changeability of these two varieties and worked upon a classification of these organisms using glucose, lactose, maltose, glycerin, cane sugar, raffinose, erythrite, salicin, litmus milk and neutral red. He finally concluded that they all belonged to the same species.

Winslow, Rothberg and Parsons (1920) studied 180 cultures in their action upon the sugars, glucose, lactose, sucrose, maltose, raffinose, mannitol, dul-

citol, salicin and inulin. They used two different media, the dehydrated bacto nutrient broth prepared by the Digestive Ferments Company and the peptone media of Clark and Lubs. They found that: -- "The action of the staphylococci upon glucose, maltose, sucrose and lactose would seem to offer a possible basis of classification, altho the marked differences due to the effect of the medium would suggest the use of this property as a differential test might prove of doubtful value."

They were able to divide the organisms into three main groups. Group I, Organism fermenting all four sugars. Group II, Organism fermenting glucose, maltose and sucrose, but not lactose. In Group III they classified all the rest of the strains and stated that it was a "highly heterogeneous agglomeration."

They found that "gelatin liquefaction was slightly but distinctly more common among the active fermenters," and that "white and orange pigments were fairly evenly divided among the various fermentative groups with a slightly greater preponderance of vigorous fermenters in the orange than in the white group. Their tests for peptone were all negative and nitrates gave almost uniformally positive results.

Winslow, Rothberg and Parsons, after this extensive work upon various sugars, nitrates, indol chromogenesis and gelatin liquefaction, state that:

"Fundamentally we are inclined to agree with Dudgeon in considering the whole group a reasonably homogeneous one; and it seems clear the central type of the whole genus is the orange-pigment forming vigorously-fermenting, gelatin liquefying, somewhat actively pathogenic St. aureus. As we depart from this type there is a progressive weakening of the various biochemical activities of this more vigorous form. The loss of one characteristic of the St. aureus type tends in some degree to be associated with the loss of others. Thus the white chromogens are less actively pathogenic than the orange forms, less actively gelatinolytic and slightly less vigorous in fermentative action. The forms which fail to liquefy gelatin also tend to be less active fermenters than the liquefiers."

The purpose of this work was to obtain forty-six different strains of staphylococci and to observe their reaction upon sugars and to note any correlation between their ability to ferment sugars and the action upon other media.

Strains were isolated from as many and as widely different sources as were available. Organisms were obtained from the animal body from both normal and

pathological conditions, from the air and from various foods.

TECHNIQUE

All organisms used in this work were freshly isolated. Organisms obtained from non-pathogenic sources were first grown upon ½ l% agar to phenolphthalein but organism from pathogenic sources were first grown upon neutral blood agar or upon serum agar. In most cases it was soon possible to obtain a good growth upon plain agar but in a few cases of organisms isolated from the throat and first grown upon neutral blood agar, this transfer was impossible and they were somewhat difficult to grow upon serum agar.

Organisms were selected for study when after twenty-four hours growth in dextrose broth, the methylene blue morphology stain showed cocci in which the division was in two planes giving rise to flat sheets of cells or irregular masses.

The organisms were inoculated into one percent sugar broth solution of dextrose, lactose, sacharose and mannite and tested in 48 to 72 hours with litmus.

Checks were run upon the majority of the organisms. They were finally tested by making stab inoculations upon semisolids made up with the dextrose, lactose, sacharose and mannite sugars with Andrade' as an indicator. This made it possible to distinguish the slightly acid organism from the neutral or alkaline.

It is upon the combined results of these tests that the classification is based. Later one percent sugar broth solutions of maltose, salicin, dulcite, inulin, raffinose, glyderin, galactose, and xylose were used as tests.

Various other media were used in making tests. Di potassium phosphate broth was tested with ten drops of methyl red.

One percent peptone lead acetate agar was inoculated both by stabbing and by streaking upon the slant in testing for the production of hydrogen sulphide.

Litmus milk was tested for acidity, peptonization and coagulation.

Deep stab inoculations were made upon one percent gelatin in testing for liquefaction.

One percent peptone broth was used in testing for indol. To seven c. c. of the culture were added ten drops sulphuric acid, the tube was shaken and then 4 c.c. of 1-1000 sodium nitrate solution were added slowly.

Fresh nitrate broth made up of one percent peptone and two-hundredths percent (.02) of nitrate was tested four days after inoculation by adding ten drops of a solution of sulphanilic acid and acetic acid then ten drops of a solution of naphthylamin chloride, distilled water and acetic acid.

Milk plates were used in testing for proteolytic action. Neutral blood agar plates were used to promote

growth of organisms and in testing for hemolysis.

Gram stains were made from cultures after twenty-four hours growth upon an agar slant. A loopful of water was inoculated with a small portion of culture which was then spread, dried and fixed, covered with carbol gentian violet for three minutes, washed in water, covered with Grams iodin solution for two minutes, washed in alcohol and then water and counter stained with aqueous bismark brown for one minute then washed in water.

The chromogenic power was determined by spreading a portion of culture two weeks old upon white paper.

The color chart indicates the various shades referred to in this work as White, Orange White, Orange, Yellow White and Light Yellow.

COLOR CHART

WHITE

ORANGE WHITE

ORANGE

YELLOW WHITE

LIGHT YELLOW

DATA

The following tables were made to show the reaction of forty-six strains of staphylococci upon dextrose, lactose, saccharose, mannite, galactose, maltose, salicin, litmus milk, gelatin, di potassium phosphate broth, one percent peptone lead acetate agar and milk plates. They also indicate the source and pigmentation.

Media upon which all strains were negative thruout are not mentioned in the tables.

Class 1

No.	Source	Pigm.	Dex.	Lac.	Sach.	Man.	Malt.	Gal.	Xyl.	Sal.	Glyc.	Milk*	Milk**	Gel.	K_2PO_4 Broth	Nitr.	Lead Acct.	Milk plates	Gram stain
5	Pus from ear	White	+	+	+	+	+	+	-	+	+	+	pep	+	+	-	-	-	+
30	Boil (Acalia)	(Orange White)	±	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+
31	Boil (Johnson)	O.White	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+
38	Rabbit sore	White	+	+	+	+	+	+	+	-	-	+	+	+	+	+		-	+
48	Oyster	Clear	+	+	+	+	+	+	-	+	-	+	pep	+	+	+	-	+	+
17	H.Urine(Flu)	White	+	+	+	+	+	+	+	+	-	+	pep	-	+	-	-	-	+
49	Oyster	Clear	+	+	+	+	+	+	-	+	-	+	pep	+	+	-	-	+	+
16	L. Urine(Flu)	White	+	+	+	+	+	+	+		-	+	pep	+	-	-	-	+	+
6	Milk	Y.White	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+
12	Milk	White	+	+	+	+	+	-	+	-	-	+	Cog.	+	-		+	+	+
20	Rabbit Abscess	White	+	+	+	+	+	-	+	-	-	+	Cog.	+	-	+	-	-	+
27	Throat	White	+	+	+	+	+	-	-	+	-	+	+	+	+	+	-	-	+
39	T.B.Infection	O.White	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+
40	Infected Tooth	White	+	+	+	+	+	+	+	-	-	+	+	+	-	+	-	-	+
57	Sore Throat	O.White	+	+	+	+	+	+	±	-	-	+	+	+		+	-	-	+
7	Milk	Y.White	+	+	+	+	+	=	+	-	-	+	Cog.	+	-	+	-		+
3	Air	White	±	+	+	+	+	-	-	-	-	+	+	-	-	+	-	-	+
8	Milk	White	+	+	+	+	+	-	-	-	-	+	pep	+	-	+	-	+	+
9	Milk	O.White	+	+	+	+	+	-	-	-	-	+	pep	+	+	+	+	-	+
55	Chronic Eye Infection	Orange	+	+	+	+	+	-	-	-	-	+	pep	-	+	+	±	+	+

O.White - White with orange tint

Y.White - White with yellow tint

* _ Three days

** _ Two days

The twenty organisms in Class I show a high percentage of acidity, dextrose, lactose, sacharose, mannite, maltose and milk were acidified by all organisms. Galactose was acidified by 12 strains and xylose was acidified by 11 strains, but the two sugars were not always acidified by the same organism. Five organisms acidified salicin and the only organism that acidified glycerine was in this class.

Gelatin was liquified by all but three organisms. Thirteen organisms reduced nitrates to nitrites. Twelve strains gave a positive reaction upon dipotassium phosphate broth. Lead acetate was blackened by only three organisms and only six showed proteolytic action upon milk plates.

Twelve of these organisms were from pathogenic sources but only organism No. 55 showed much pigmentation and Probably should not be included in this classfcation. The majority were white or only slightly tinted with color.

Class II

No.	Source	Pigment.	Dex.	Lac.	Sach.	Man.	Malt.	Gal.	Xyl.	Sal.	Milk	Milk	Gel.	K_2PO_4	Nitr.	Lead Acct.	Milk plate	Gram stain
29	Acne	White	+	+	+	-	+	+	+	-	+	Cog.	+	+	+	-	+	+
34	Milk	White	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+
37	Arm. Infec.	White	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+
35	Milk	White	+	+	+	-	+	+	+	-	+	+	+	+	-	+	-	+
28	Acne	White	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+
2	Skin of Nose	White	+	+	+	-	+	+	-	-	+	Cog.	+	+	+	-	-	+
41	Butter	White	+	+	+	-	+	+	-	-	+	+	-	-	+	-	-	+
43	Oleomargarine	Y.White	+	+	+	-	+	+	-	-	+	+	-	-	+	-	-	-
46	Butter	Y.White	+	+	+	-	+	+	-	-	+	+	+	-	+	-	-	+
47	Infected Tooth	White	+	+	+	-	+	+	-	-	+	pep.	+	-	+	-	+	+
26	Throat	White	+	+	+	-	+	-	-	-	+	+	-	-	-	-	-	+
52	Tonsil	O.White	+	+	+	-	+	-	-	-	+	pep.	+	-	+			-
33	Sneeze	White	+	+	+	-	+	-	-	-	+	+	-	-	+	-	+	+

The thirteen organisms in Class II showed acidity upon dextrose, lactose, saccharose and litmus milk, but were negative upon mannite and salicin. Ten were acid in galactose and four in xylose. A gradual decline upon the acidity in sugars was noticable within this class. Four organisms did not liquefy gelatin. Nitrates were reduced to nitrites by eleven organisms. Five strains gave a positive reaction upon di potassium phosphate broth with methyl red. Two organisms blackened lead acetate agar. Six strains showed proteolytic action upon milk plates.

The majority of the organisms were white, only a few strains showing slight pigmentation. Five strains may possibly have been pathogenic.

Class III

No.	Source	Pigment.	Dex.	Lac.	Sach.	Man.	Malt.	Gal.	Xyl.	Sal.	Milk	Milk	Gel.	Red	Mit	Lead	Milk plate	Gram stain
21	Faces (Flu)	L.yellow	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+
25	Milk	L.yellow	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+
22	Faces(Flu)	L.yellow	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
54	Infected tooth	White	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
50	Oyster	White	-	-	-	-	-	-	-	-	-	pep		-	-	-	-	+
51	Sore Throat	O.White	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Sub Classes

14	Pus from rabbit	L.yellow	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
45	Air	L.yellow	+	-	-	-	-	-	-	-	-	pep	+	-	-	-	+	+

L.yellow - Light Yellow

Organisms in Class III are negative upon all sugars and upon litmus milk. Only two organisms liquefied gelatin. One gave a positive reaction with methyl red in di potassium phosphate broth. One strain reduced nitrates to nitrites. Three strains were able to blacken lead acetate agar. Three strains produced proteolysis upon milk plates.

More pigmentation is shown by the organisms of this class than by those producing acid in sugars.

The two organisms indicated as a sub class were able to produce a slight acidity in dextrose.

Class IV

No.	Source	Pigment.	Dex.	Lac.	Sach.	Man.	Malt.	Gal.	Xyl.	Sal.	Milk	Milk	Gel.	Red.	Mit.	Lead	Milk plate	Gram stain
4	Air	White	+	-	+	-	+	-	-	-	-	pep	+	-	-	+	+	+
53	Tonsil	O.White	+	-	+	-	+	-	-	-	-	-		-	+	-	+	+
32	Sneeze	Y.White	+	-	+	-	+	-	-	-	-	-	-	--	-	-	-	-
15	Scalp	Y.White	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+

Irregular Organisms

11	Milk	O.White	+	-	+	+	+	-	-	-	-	pep	+	+	+	+	+	+
23	Unknown	White	+	-	+	+	-	-	-	+	-	pep	+	+	+	+	+	+

Class IV is composed of only four organisms these were positive upon dextrose, sacharose and maltose but were negative upon lactose, mannite, galactose, xylose, salicin and litmus milk. One strain liquified gelatin. Di potassium phosphate broth was negative. One strain reduced nitrates to nitrites. One strain blackened lead acetate and two strains showed proteolysis on milk plates.

Two strains were so irregular upon sugars that they were not classified.

The general classification was based upon the action of the various strains upon dextrose, lactose, sacharose and mannite.

Maltose, salicin, galactose and xylose were also of value in checking results but raffinose, dulcite and inulin were consistently negative thruout.

Dudgeon (1908) reported a large percentage of positive results with raffinose, glycerine, inulin and salicin, but in my work they have been negative thruout with the exception of one positive in glycerin and six positive in salicin.

Winslow, Rothberg and Parsons (1920) suggest that salicin, inulin and raffinose are attacked by staphylococci so rarely as to be of no serious diagnostic value.

Blood agar plates showed hemolysis in such a small number of cases that that test was discontinued.

Ten organisms blackened lead acetate agar showing the production of hydrogen sulphide.

Milk plates showed proteolytic fermentation by eighteen organisms, only two of which were not gelatin liquefiers.

Litmus milk was acidified and peptonized most frequently by the organisms in classes one and two. Some organisms showed coagulation without peptonization while a number of organisms were acid in milk without further reaction at the end of three to four weeks.

Gelatin was liquefied by all organisms peptonizing or coagulating milk, with two exceptions. There was no noticable connection between gelatin liquefaction and chromogenisis as so few of the organisms showed pigmentation.

Thirty-one strains liquefied gelatin and twenty-seven of these strains were in classes one and two.

Thirty-three organisms reduced nitrates to nitrites and twenty-seven of these strains were in classes one and two.

Di potassium Phosphate broth tested with methyl red gave positive results in twenty cases, seventeen of these strains were in classes one and two. The negative tests were of a peculiar yellowish green, probably similar to the negative test mentioned by Dudgeon.

Tests made upon peptone broth for the production of indol were negative thruout.

Gram stains were almost uniformally positive, four organisms were gram negative. One was in class one, two were in class two and one was in class four.

None of the cultures used in this work showed deeper pigmentation than is indicated as Cadmium Orange Chromo V in the color chart made by the Winslows in their Systematic Relationship of the Coccaceae.

SUMMARY AND CONCLUSION.

Staphylococci may be roughly divided into at least four classes:

Class I, strains that were acid in dextrose, lactose, sacharose, mannite and maltose.

Class II, strains that were acid in dextrose, lactose, saccharose and maltose but were negative in mannite.

Class III, strains that were negative upon all sugars.

Class IV, strains that were negative upon lactose and mannite with a sub class of organisms that were negative only upon lactose.

Sugars were the only media of any importance in this classification.

However, gelatin is more frequently liquefied, nitrates more frequently reduced to nitrites and di potassium phosphate broth gave the positive methyl red reaction more often with the strains producing acidity in a large percentage of the sugars than did the strains that were unable to ferment sugars so readily.

BIBLIOGRAPHY.

Winslow, C. E. A., 1909. Systematic Relationships of the Coccaceae.

Dudgeon, Lenard S., 1909. The Differentiation of of Staphylococci. Journal of Pathology and Bacteriology. Vol. 12.

Kligler, I. J., 1913. A Systematic Study of the Coccacea in the Collection of the Museum of Natural History. The Journal of Infectious Disease. Vol. 12.

Winslow, C. E. A. Rothberg and Parsons, 1920. Classification of Orange and White Staphylococci. Journal of Bacteriology.