DECOMPOSITION OF TARTRATES BY THE COLIFORM BACTERIA

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Tartrate decomposition has been reported at least since 1841 when Nöllner first published on the end products of calcium tartrate decomposition. The chemists in the period 1841 to 1858 were not aware of the microbial cause of the decomposition of the tartrates whose end products they analyzed. Even the influence of Pasteur, who first recognized and recorded microbial decomposition of tartrates (1858) and decomposition by the mold *Penicillium glaucum* (1860) and by anaerobic bacteria (1863), was not enough to overcome the tendency of analysts to ignore the microbiological problems and content themselves with the study of the spontaneous decomposition of tartrates as late as 1911.

Even today, the majority of the bacteria responsible for the various types of tartrate decomposition are not well known. Only two groups of bacteria whose taxonomic positions are known have been studied in some detail with respect to their ability to decompose tartrates: the coliform bacteria and certain of the lactic acid bacteria found in wines. Although decomposition of tartrates was used by Pasteur for the first proof of anaerobiosis, the anaerobic sporeforming bacteria causing the decomposition were not studied in pure culture until recently and must yet be completely classified (Vaughn and Marsh, 1943*a*, 1943*b*).

The first conclusive evidence of tartrate decomposition by pure cultures of coliform bacteria was furnished by Grimbert and Ficquet (1897, 1898) and Grimbert (1899). The organism used by Grimbert and Ficquet was named *Bacillus tartricus*, which, fortunately, was described in enough detail to be recognizable as a species of *Aerobacter*. Nijdam (1907) accumulated further supporting evidence with his study of tartrate decomposition by a bacterium of the coliform group which he named *Aerobacter tartarivorum*. More recently Barker (1936) and Sakaguchi and Tada (1940) have confirmed these earlier observations by experiments on tartrate decomposition by two different species. Barker worked with *Aerobacter aerogenes*. Sakaguchi and Tada named their bacterium *Bacterium succinicum*, but it is recognizable as a species of *Escherichia*.

Completely irrefutable evidence for tartrate decomposition by *Escherichia coli* and species of the *Enterobacteriaceae* other than those mentioned above is lacking, although many qualitative experiments have been reported, and this qualitative evidence points to the fact that many of these bacteria probably do have the ability to attack tartrates.

The recovery of tartrates, particularly calcium tartrate, as a major by-product of the wine industry in California has developed only recently. Before the war tartrate recovery was incidental and, with almost no recovery of the neutral calcium tartrate, little attention was paid to losses by microbial decomposition.

With the marked increase in tartrate recovery because of the war, losses caused by bacteria and molds became acute. The general aspects of the spoilage problem were discussed by Vaughn and Marsh (1943*b*), who pointed out that the coliform bacteria, particularly *Aerobacter aerogenes*, caused most of the losses.

It was the purpose of the present investigation to determine the extent to which strains of the various recognized species of the coliform bacteria may be involved in tartrate decomposition, to study some of the factors which influence the decomposition of tartrates by coliform bacteria, and to evaluate the possible use of tartrate decomposition as a characteristic for differentiation of the species of these bacteria.

SOURCE OF CULTURES

The cultures isolated were obtained by direct plating on Levine's eosine methylene blue agar. Samples from which the cultures were isolated included crude calcium tartrate of good quality, calcium tartrate of inferior quality suspected of incipient deterioration, spoiled calcium tartrate, tartrate recovery liquors, tartrate wash liquors and water supplies used in the recovery process, residue sludge from the recovery process, soil impregnated with spoiled calcium tartrate, and grape pomace. A total of 26 different isolates were recovered from these sources.

In addition 79 cultures of *Escherichia* and 100 cultures of *Aerobacter* were used as test cultures for some of the studies. These cultures, from the Division of Food Technology collection, originally were isolated from a wide variety of sources including various waters and soils, human and animal feces, olives, chicken eggs, milk, sawdust, oysters, dehydrated and frozen vegetables, food processing equipment, air, etc.

METHODS

All cultures, regardless of source, were subjected to serial replating on Levine's eosine methylene blue agar for purification. Well-isolated colonies were picked, and purified by repeated plating from lactose broth (Am. Pub. Health Assoc., 1936).

Generic differentiation as well as specific allocation of the cultures was made according to the methods suggested by Levine (1921), Levine *et al.* (1934), and Vaughn and Levine (1942). In the taxonomic study all tartrate cultures were incubated at 30 C because of the increasing evidence that 30 C is a more nearly optimum incubation temperature for the coliform bacteria than 37 C, which is generally specified.

Media for study of tartrate decomposition. Tartrates chosen for investigation included ammonium tartrate ($(NH_4)_2\ C_4H_4O_6$), barium tartrate ($BaC_4H_4O_6$), calcium tartrate ($CaC_4H_4O_6 \cdot 4H_20$), copper tartrate ($CuC_4H_4O_6 \cdot 3H_2O$), magnesium tartrate ($MgC_4H_4O_6 \cdot 4H_2O$), sodium tartrate ($Na_2C_4H_4O_6 \cdot 2H_2O$), potassium tartrate ($K_2C_4H_4O_6 \cdot \frac{1}{2}\ H_2O$), sodium potassium tartrate ($NaKC_4H_4O_6 \cdot 4H_2O$), and potassium acid tartrate ($KHC_4H_4O_6$). All were made from dextrorotatory tartaric acid ($C_4H_6O_6$). These salts were used in concentrations of

approximately 1 per cent (10 g per liter) in a medium which also contained 5 g of bacto tryptone and 1 g of di-basic potassium phosphate ($K_2HPO_4 \cdot 3H_2O$) per liter. When a solid medium was desirable 15 g of agar were added per liter. For the sake of convenience and accurate duplication, the calcium tartrate medium was prepared by mixing 2 volumes of a double strength basal solution (10 g bacto tryptone and 2 g $K_2HPO_4 \cdot 3H_2O$ per liter) with 1 volume of 0.154 M potassium tartrate solution and 1 volume of 0.154 M calcium chloride solution to give a finished medium containing 1 per cent calcium tartrate.

Later, when the insoluble calcium tartrate made rate studies difficult, an ammonium tartrate medium was used. This medium was prepared by mixing equal portions of 0.135 M ammonium tartrate solution and the double strength tryptone basal medium already described. The resulting medium contained more than 1 per cent of the salt, but the tartrate-ion concentration was about 1 per cent. The method of using a fixed concentration of tartrate ion was considered more advantageous for use with the colorimetric method employed for analysis.

For study of the effect of hydrogen-ion concentration (pH) on the decomposition of tartrates, the media were prepared by mixing ammonium tartrate and tartaric acid solutions with the basal double strength tryptone solution. One volume of the basal medium was mixed with one volume of a mixture of 0.135 M ammonium tartrate and 0.13 M tartaric acid solutions which previous testing showed would give the desired reaction after sterilization. The percentage of each tartrate component for any given pH value was taken from curves prepared for this purpose. These volumetric methods for preparing tartrate-containing media not only reduced errors arising from weighing small individual samples of the tartrate salts but also saved appreciable time. The media were sterilized at 15 pounds' steam pressure for 20 minutes unless the pH value was less than 5.0, in which case 15 minutes was considered sufficient time.

Quantitative determination of tartrate. The procedure used for tartrate determination is based on a modification of the method described by Underhill *et al.* (1931) as modified to photoelectric colorimetry by Matchett *et al.* (1944).[1] The method makes use of the characteristic color developed through the interaction of sodium metavanadate and the tartrate ion in dilute aqueous acetic acid solution.

Before the determination of tartrate content, cloudy or colored samples require clarification and decolorization. In both operations, particularly the latter, tartrate may be lost by absorption and adsorption. Matchett *et al.* prevented this by using an extremely acid reaction. The filtered and decolorized solution was then neutralized before testing. Nearly all activated carbons work satisfactorily under the conditions employed by Matchett *et al.*, but the method becomes highly empirical. This requires that the unknown be determined by steps identical to the standard curve or equation in all respects. Use of a carbon which adsorbs tartrate, even in small amounts, requires careful weight or volume measurement of this material for purposes of duplication. Therefore, it was

[1] Also see Western Regional Research Laboratory (1943).

considered preferable to use a carbon without this property if such existed or could be prepared.

Extensive testing proved that carbon could be prepared with its tartrate-adsorbing property fully satisfied. Treatment of the carbon by the usual acid washing procedure with the addition of 5 g of tartaric acid per liter of acid solution used accomplished this goal. The carbon must be dried before use. Drying is an essential step, but what it actually accomplishes is not known.

Only a single calibration curve need be established for all tartrate-containing compounds provided such a curve is based upon the tartrate-ion concentration. Multiplication of the tartrate-ion concentration obtained by analysis by the appropriate factor converts it to that of the specific compound being determined. The tartrate-vanadate color complex obeys Beer's law when the concentration of tartrate ion in the solution under immediate testing varies from 0.004 to 0.02 per cent, using a 520 filter, and from 0.004 to 0.032 per cent, using a 540 filter in an Evelyn photoelectric colorimeter. The formula for the standard curve with the 540 filter is $\text{Log } G = 0.76 \times X + 2.021$, in which X equals mg of tartrate ion in the 25 ml of reaction mixture.

In the tartrate determination, the preliminary treatment employed depended upon the tartrate media used and the factors under investigation. In all cases except rate studies, the entire contents (100 ml) of the culture flask were transferred to a 250-ml volumetric flask and made to volume with distilled water. With calcium tartrate media, exactly 1.5 ml of concentrated HCl were added to effect solution of the insoluble residue before making this transfer. In the case of rate studies conducted with soluble tartrate salts, a portion of the medium was withdrawn aseptically by pipette from the culture flask. From 10 to 50 ml of solution, depending upon the amount of decomposition judged to have occurred, were taken for determination. After being adjusted to approximately 50 ml with water, the solution was decolorized by boiling for 2 to 3 minutes with about one-quarter teaspoon of the acid-treated activated carbon, allowed to cool, and transferred to a 100-ml volumetric flask. After being adjusted to volume, a portion of the solution was filtered through no. 2 Whatman paper into clean, dry beakers and from 2 to 20 ml were used for colorimetric estimation. Care was taken to remove any bacterial turbidity during this step.

The colorimetric estimation was carried out as follows: The estimated quantity of the foregoing solution was transferred by pipette to 25-ml glass-stoppered mixing cylinders. Distilled water was added to a volume of 20 ml. Then 0.5 ml of glacial acetic acid, 2 ml of a 5.0 per cent sodium metavanadate ($NaVO_3$) solution, and enough distilled water were added to adjust the final volume to 25 ml. After thorough mixing the sample was set aside in subdued light for 30 minutes to allow for color development. The transmittancy of the reaction mixture was then determined by an Evelyn photocolorimeter using the 540 filter. From the galvanometer reading thus obtained and the formula for the standardization curve the residual tartrate content of the original medium was readily computed.

DIFFERENTIATION OF THE BACTERIA

The 26 cultures isolated from the tartrate samples were typical coliform bacteria in that they all produced acid and gas from lactose; were gram-negative, short rods; did not form endospores; and, if motile, possessed peritrichous flagella. Generic differentiation of these cultures was made on the basis of the Voges-Proskauer reaction and Koser's citrate test. Specific allocation was accomplished by investigating the decomposition of sucrose, starch, aesculin, salicin, and glycerol, and the production of hydrogen sulfide and indole in

TABLE 1

Characteristics of coliform bacteria isolated from tartrates and tartrate-containing materials

NUMBER OF CULTURES*	GENERIC CHARACTERS		SPECIFIC CHARACTERS							SUGGESTED ALLOCATION
	V.-P. test	Growth in Koser's citrate	Decomposition of: Sucrose	Starch	Aesculin	Salicin	Glycerol	H_2S	Indole	
	Number of cultures showing positive reactions†									
10	10	10	10	10	10	10	10	0	2	*Aerobacter aerogenes*
5	0	0	0	3	5	5	5	0	5	*Escherichia coli*
2	0	0	2	2	2	2	2	0	2	*Escherichia neapolitana*
1	0	0	1	1	0	0	1	0	1	*Escherichia communior*
1	0	0	0	1	0	0	1	0	1	*Escherichia acidilactici*
4	0	4	1	0	1	2	4	4	2	*Escherichia freundii*
3	0	3	3	3	3	3	3	0	0	*Escherichia intermedium*

* All the cultures decomposed calcium tartrate in amounts ranging from 25 to 100 per cent of that contained in the media after 7 days at 37 C.

† Based on incubation at 30 C for a maximum period of 4 days.

suitable media. The differential characteristics of the bacteria together with their suggested taxonomic allocations are shown in table 1.

It is particularly interesting that with the exception of *Aerobacter cloacae* all widely recognized species of *Aerobacter* and *Escherichia* were isolated from the tartrate-containing samples. The most abundant single species was *Aerobacter aerogenes* (10 cultures). It will be noted, however, that the total number belonging to the genus *Escherichia* was greater (16 cultures). The isolation of so many different species from so few samples may perhaps be considered to be fortuitous. Nonetheless, in view of some of the following experimental evidence it is reasonable to believe that representatives of all the species of the coliform

bacteria may be involved in tartrate decomposition rather than chiefly species of *Aerobacter* as inferred in the literature already cited; for, in several instances, cultures of *Escherichia* and *Aerobacter* were isolated from the same sample and in others only cultures of *Escherichia* were recovered.

ABILITY OF COLIFORM BACTERIA TO DECOMPOSE TARTRATES

The observation that species of *Escherichia* as well as *Aerobacter aerogenes* were involved in the decomposition of tartrates prompted an investigation of the incidence of tartrate decomposition among a larger collection of coliform bacteria as well as other studies on the decomposition of tartrates.

Incidence of the ability to decompose calcium tartrate. Pure cultures of *Escherichia* and *Aerobacter* from the laboratory collection were used to ascertain the

TABLE 2

*The decomposition of calcium tartrate by coliform bacteria**

GENUS	ESCHERICHIA						AEROBACTER	
Species	*coli*	*communior*	*acidilactici*	*neapolitana*	*freundii*	*intermedium*	*aerogenes*	*cloacae*
Number	13	8	9	8	24	17	50	50
DECOMPOSITION OF CALCIUM TARTRATE	Number of cultures and range of decomposition							
per cent								
0–9.9†	2	0	0	3	6	9	27	46
10.0–19.9	0	0	4	1	0	0	1	1
20.0–39.9	6	5	1	4	6	5	3	2
40.0–59.9	5	3	4	0	11	3	1	1
60.0–79.9	0	0	0	0	0	0	0	0
80.0–99.9	0	0	0	0	1	0	18	0

* Incubated at 30 C for 7 days.

† None of these cultures attacked the tartrate.

ability of the various species of coliform bacteria to decompose calcium tartrate in the medium already described.

A total of 179 cultures including 79 strains of *Escherichia* and 100 strains of *Aerobacter* were tested. Inoculations were made from 24-hour nutrient agar slant cultures using one loopful of inoculum. The inoculated medium was incubated at 30 C for 7 days before the amount of calcium tartrate decomposition was determined. The results of this investigation are shown in table 2.

These data show that representatives of all species of *Escherichia* as well as *Aerobacter* possess the ability to decompose calcium tartrate. The general lack of ability of cultures of *Aerobacter cloacae* used in this experiment to decompose calcium tartrate may explain why cultures of this species were not recovered from the spoiled tartrates.

Decomposition of various tartrate salts. Several tartrate salts as well as the insoluble calcium tartrate have been used by others who have studied tartrate

decomposition by bacteria. It was thought advisable to determine whether the kind of tartrate salt might influence the activity of the coliform bacteria.

The *d*-tartrate salts listed previously were used under the conditions prescribed. All were soluble in 1 per cent concentration (10 g per liter) in the medium with the exception of potassium acid tartrate which was used in a concentration of 0.5 per cent. The species of *Escherichia* and *Aerobacter* isolated from the tartrate-containing materials were used as test cultures. Copper tartrate and tartaric acid were not attacked. Potassium acid tartrate was decomposed by some of the more acid-tolerant cultures. The pH of the medium was low in the case of the tartaric acid (pH 2.6) and potassium acid tartrate (pH 4.1). These low pH values retard or entirely prevent the growth of most coliform bacteria. The

TABLE 3

Decomposition of isomeric forms of ammonium tartrate by coliform bacteria

ORGANISM	ISOMERIC FORM OF AMMONIUM TARTRATE*					
	d		*dl*		*l*	
	Percentage of decomposition at 30 C after					
	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks
E. coli	62.9	94.5	61.6	68.2	35.0	82.7
E. communior	66.0	93.3	66.9	69.4	30.0	40.8
E. acidilactici	43.0	91.9	52.1	52.1	24.6	52.3
E. neapolitana	39.3	88.3	20.5	62.5	19.8	59.6
E. freundii	61.7	92.2	45.4	54.7	10.2	32.6
E. intermedium	92.1	94.1	48.6	67.2	29.2	56.5
A. aerogenes	92.8	93.3	86.4	94.1	89.6	95.3
A. cloacae	68.1	96.1	52.1	63.8	14.6	30.4

* Contained in the basal tryptone medium in concentrations of approximately 1 per cent.

amount of copper contained in the copper tartrate medium apparently was responsible for the failure of the test organisms to attack this salt.

All the other neutral *d*-tartrate salts were decomposed by all the cultures tested. Since they were attacked at approximately the same rate and to the same extent, it was decided to use ammonium *d*-tartrate for the studies in which a soluble salt was essential. Other investigators previously had used the ammonium salt. Furthermore, circumstances prevailing at the initiation of these studies dictated the choice. Supplies of chemically pure neutral salts were low, and ammonium *d*-tartrate could be prepared in the laboratory with ease.

The tartrate isomers investigated included the ammonium salts of *dl*(racemic) and *l*(levo) tartaric acid. These salts were used in the basal tryptone medium in concentrations of approximately 1 per cent. Test cultures included the seven species of *Escherichia* and *Aerobacter* commonly recognized. Tartrate decomposition was determined quantitatively after 1 and 2 weeks of incubation at 30 C. The results of this experiment are shown in table 3.

Although all the test cultures could decompose the three tartrate isomers,

Aerobacter aerogenes was the most active species tested. It also will be noted that the dextrorotatory form in general was attacked more vigorously by all test organisms than either the inactive or levorotatory isomers, even after 2 weeks of incubation, indicating a distinct preference for the *d* form.

Meso-tartaric acid was not investigated. This compound does not occur naturally. Furthermore, the sodium metavanadate method is of no value for determination of *meso*-tartrate.

SOME FACTORS AFFECTING THE DECOMPOSITION OF D-TARTRATE SALTS

The factors showing the greatest influence on the decomposition of *d*-tartrate salts were the temperature of incubation, the reaction (pH) of the medium, and the length of the incubation period.

TABLE 4

The effect of temperature of incubation on calcium tartrate decomposition by coliform bacteria

SPECIES	DAYS OF INCUBATION	TEMPERATURE*							
		16 C	19 C	25 C	28 C	30 C	34 C	37 C	43.5 C
		Calcium tartrate decomposition, per cent							
A. aerogenes (CPGI)†	2	71.6	75.8	62.6	84.7	73.3	71.5	84.2	20.0
A. aerogenes (CPGI)†	2		65.4	76.9	83.2	75.4	82.0	92.6	50.0
A. aerogenes (CPGI)†	7	88.5	93.2	94.2	95.9	95.6	95.3	97.2	94.7
A. cloacae (202/2)	7					51.0	46.9	69.3	38.2
E. coli (JVR)	7					36.2	49.5	30.0	72.9
E. acidilactici (CPA)	7					50.1	53.6	60.7	74.5
E. neapolitana (GW-2)	7					50.1	34.6	58.2	66.3
E. freundii (56/5)	7					45.9	98.8	68.5	19.3
E. intermedium (326)	7					53.6	48.5	51.0	24.0

Blank spaces indicate no culture was grown.

* These temperatures refer to the air temperatures of the various incubators.

† Separate experiments repeated at widely separated intervals.

The effect of temperature. Although the influence of temperature on the growth of coliform bacteria is well known, little attention has been paid to its effect on the ability of the coliform bacteria to decompose various substrates. To determine the effect of the temperature of incubation on calcium *d*-tartrate decomposition by the coliform bacteria representative cultures were grown in the calcium *d*-tartrate medium at temperatures (incubator air) ranging from 16 to 43.5 C. Inoculations were made from 18-hour broth cultures of the organisms using 0.5 ml of inoculum per 100 ml of medium. The inoculated flasks were incubated for 7 days at the desired temperatures and then analyzed for tartrate content. The results of this study are shown in table 4.

The culture of *Aerobacter aerogenes* was active over a wide range of temperatures (19 to 43.5 C), whereas the other test cultures tended to exhibit a definite optimum in a narrower range (30 to 43.5 C). It is interesting in particular that the culture of *Aerobacter cloacae* had an optimum temperature of

37 C for calcium *d*-tartrate decomposition, for this particular strain (202/2) was known to decompose lactose and other sugars very poorly at 37 C. It is also noteworthy that three citrate-negative species of *Escherichia* had higher optima (43.5 C) for calcium *d*-tartrate decomposition than the citrate-positive species (30 to 37 C).

The effect of pH of the medium. Preliminary observations indicated that some of the coliform bacteria associated with tartrate spoilage were active at rather low pH values. To test the effect of pH on the ability of the coliform bacteria to grow and decompose *d*-tartrate, liquid media were adjusted to various pH values between 3.8 and 7.3 as previously described. Inoculations were made from 18-hour broth cultures using 0.5 ml per 100 ml of tartrate medium con-

TABLE 5

The effect of pH on tartrate decomposition by coliform bacteria

SPECIES	DAYS INCUBATION	pH VALUE*									
		3.8	4.0	4.25§	4.5	4.75	5.0	5.35	6.15	6.4	7.3
		Tartrate decomposition, per cent									
A. aerogenes (CPGI)†	1½	0	0	18.7	96.2	95.7	95.7	95.4	96.1	95.9	96.2
A. aerogenes (CPGI)	7	0	8.1	93.8	93.8	93.8	94.4	94.9	95.5	96.0	95.6
A. aerogenes (CAT)	7	0	0	2.8	94.5	91.6	94.2	94.8	95.3	96.1	94.7
A. cloacae (202/2)	7	0	0	0	0	77.7	61.2	62.5	60.0	57.2	45.6
E. coli (JVR)	7	0	0	13.5	89.4	91.6	91.6	83.6	78.1	65.2	54.8
E. coli (JVR)	30	0	0	93.2	93.6	93.7	91.9	90.8	80.3	70.5	58.2
E. communior (WD-1)	7	0	0	0	0	60.4	70.4	71.6	70.8	69.7	68.3
E. acidilactici (CPA)	7	0	0	27.2	94.4	91.4	94.2	94.6	94.5	95.0	94.7
E. neapolitana (GW-2)	7	0	0	0	76.6	64.6	48.5	45.3	43.0	36.7	27.8
E. freundii (56/5)	7	0	0	4.7	5.8	93.6	94.0	95.0	82.3	76.7	61.0
E. freundii (B1)	7	0	0	8.0	96.1	90.4	90.9	92.1	91.6	92.4	92.1
E. intermedium (326)	7	0	0	0	13.1	93.5	93.2	90.0	82.8	77.1	68.8

* pH value within ±0.02 after sterilization.

† This strain of *A. aerogenes* decomposed tartrate within 7 days in media with initial pH values of 3.90.

tained in 300-ml Erlenmeyer flasks. The inoculated media were incubated at 37 C for varying lengths of time, but in most cases for 7 days before analysis. The amount of decomposition occurring is shown in table 5.

It will be seen (table 5) that the pH of the medium has a marked effect on the ability of the coliform bacteria to decompose *d*-tartrate. Some cultures exhibit a definite optimum pH value for maximum decomposition. Other cultures show comparable activity over a wide range. The most active culture (*A. aerogenes* CPGI) decomposed the tartrate equally well between pH 4.25 and 7.3 after 1 week of incubation although with shorter incubation a retarding effect was noted at pH 4.25. Some of the less active strains exhibited a definite optimum pH value in the range 4.5 to 5.0. Others showed similar activity between pH 4.5 and 7.3. The difference in activity does not appear to be specific, as is illustrated by the two strains of *Escherichia freundii*.

The pH value which prevented growth was somewhat lower than that which prevented decomposition of the tartrate when cultures were incubated at 37 C for 1 week. This was illustrated by growing 5 cultures of each species of *Escherichia* and 15 cultures of each species of *Aerobacter* in the tryptone tartrate broth adjusted to pH values ranging between 3.92 and 4.50. The medium was contained in 6 by 3/4 inch tubes in 10-ml amounts. The inoculum consisted of one loopful (4 mm diam.) of 18-hour nutrient broth cultures.

The cultures of *Aerobacter aerogenes* tolerated a lower pH than any of the other species tested. Three strains grew at pH 3.92 within 7 days.[2] (These 3 cultures can decompose tartrate at this pH value within 7 days if the inoculum contains bacteria to ensure an initial population of 1 to 3 $\times$ 10^6 organisms per ml of medium.) At pH 4.0 there were 10 cultures which grew. Twelve cultures grew at pH 4.12 within 7 days. At pH 4.24 all 15 strains of *Aerobacter aerogenes* grew luxuriantly within 2 days. In comparison, only one strain of *Aerobacter cloacae* grew at pH 4.12. At pH 4.24, 12 of the strains grew; at pH 4.36, 14 of the 15 *Aerobacter cloacae* strains grew; yet all the cultures did not grow until the pH had been increased to pH 4.50.

The species of *Escherichia* exhibited a marked degree of strain variation. None grew in a medium adjusted to pH 4.12. Some cultures of *Escherichia coli* grew in a medium adjusted to pH 4.24. However, until the reaction was adjusted to pH 4.50 a marked degree of inhibition was noted. Even at pH 4.5 some inhibition was observed as 2 strains of *Escherichia communior* and 1 strain of *Escherichia neapolitana* did not grow.

Rate of decomposition of d-tartrate by coliform bacteria. As indicated by some of the data presented in tables 2 and 4, there are two distinct types of coliform bacteria insofar as destruction of *d*-tartrate is concerned. One group, represented by 21 very active strains of *Aerobacter aerogenes*, decomposes the *d*-tartrate rapidly. The other group, represented by all of the other strains of coliform bacteria investigated, decomposes the *d*-tartrate slowly.

A comparison of the rates of ammonium *d*-tartrate decomposition by these two groups was made. Cultures of *Aerobacter aerogenes* (strain CPGI, rapid group) and *Escherichia coli* (strain JVR, slow group) were used. These cultures were grown in ammonium *d*-tartrate broth prepared to give pH values of 4.5, 5.0, and 7.3 after sterilization. The inoculum consisted of 10 loops (4 mm diam.) of a 24-hour calcium tartrate agar slant culture, suspended in 5 ml of lactose broth, per 1,500 ml of medium contained in a 2-liter Erlenmeyer flask. Samples were withdrawn at desired intervals during incubation at 37 C in order to determine the amount of decomposition. Curves which illustrate the marked difference in rate of destruction of ammonium *d*-tartrate by the two groups, grown in the medium with an initial pH value of 7.3, are shown in figure 1.

The time required to decompose most of the tartrate was similar when the cultures were grown in the tartrate broth at pH 4.5 and 5.0. Other test cultures

[2] *Aerobacter aerogenes* has been found in larger numbers in commercial tartrate-bearing solutions with pH values of 3.5 to 3.6.

also showed comparable rates of destruction. The very active group, under favorable conditions, can decompose all the tartrate (1 g per 100 ml) within 36 hours when ammonium *d*-tartrate is supplied, or 48 hours when calcium *d*-tartrate is used. The slow group needs from 5 to 14 days to decompose the tartrate supplied and may never cause complete destruction.

A characteristic which can be used for ready identification of the group of

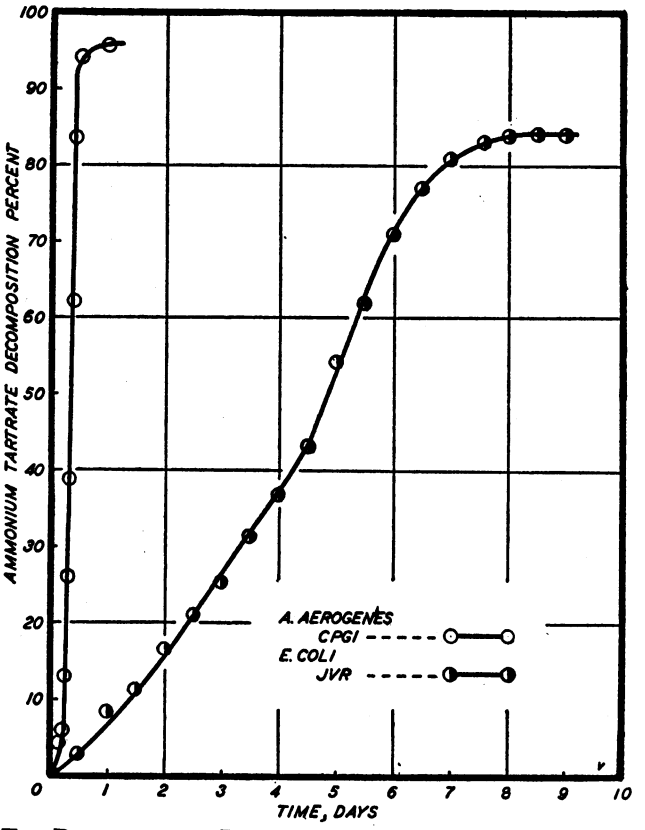

FIG. 1. THE DIFFERENCE IN RATE OF DESTRUCTION OF AMMONIUM TARTRATE BY A. AEROGENES (CPGI) AND E. COLI (JVR)

Aerobacter aerogenes which cause rapid decomposition of *d*-tartrate salts is their ability to produce copious amounts of gas in agar media containing these salts.

GROWTH OF COLIFORM BACTERIA IN DIFFERENTIAL TARTRATE MEDIA

Differentiation of species of *Salmonella* from other *Enterobacteriaceae* by the use of tartrate media has been suggested by numerous investigators including Altobelli (1914), Wagner (1920), Pesch (1921), Brown *et al.* (1924), and Jordan and Harmon (1928). Many strains representing different species of *Escherichia* and *Aerobacter* do not decompose tartrates. It has been claimed that tartrate media may be used for the differentiation of species of *Enterobacteriaceae* including

Escherichia coli. The medium of Jordan and Harmon has been used extensively with *Salmonella* and related bacteria. It was desirable to check the differential value of the two most promising media (Brown *et al.*, and Jordan and Harmon) when used with the cultures under investigation.

The media were prepared, inoculated, and incubated under conditions which duplicated as nearly as possible those prescribed by the original workers. The dehydrated Difco phenol red tartrate agar which duplicates the medium of Jordan and Harmon was also tested. Strains of the recognized species of *Escherichia* and *Aerobacter* which decomposed all or nearly all, about half, from one-fourth to one-tenth, and none of the tartrate, respectively, were tested. When the medium and technique of Brown *et al.* were employed, conflicting results were obtained. With test cultures which caused almost complete destruction of the sodium *d*-tartrate, positive qualitative tests for decomposition were obtained. With cultures which decomposed none or at most 15 to 25 per cent of the tartrate, negative results were obtained. When, however, cultures which had decomposed about half of the *d*-tartrate were used, obscure and inconclusive qualitative tests were observed. The results obtained with the Jordan and Harmon medium showed no significant difference in growth or reaction between strains of species of the two genera. The non-tartrate-decomposing strains could not be distinguished from tartrate-decomposing strains of *Escherichia* and *Aerobacter* when tested on Jordan and Harmon medium prepared from laboratory supplies or Difco dehydrated product.

It is certain that the proposed tartrate media cannot be used for differentiation of cultures of *Escherichia* and *Aerobacter*. The qualitative technique of Brown *et al.* is not delicate enough to detect small amounts of decomposition. The value of the Jordan and Harmon medium depends upon a change in reaction (pH). Tartrate decomposition by coliform bacteria is accompanied by an increase in pH to 8.0 or 8.5. Non-tartrate-decomposing strains liberate enough ammonia from the peptone to give corresponding increases in pH. It is obvious therefore that much of the data in the literature indicating tartrate decomposition by different bacteria is of questionable value.

THE TAXONOMIC STATUS OF BACILLUS TARTRICUS, AEROBACTER TARTARIVORUM, AND BACTERIUM SUCCINICUM

Three species of bacteria have been described whose validity is questionable in the light of our present knowledge of the coliform bacteria. These species are *Bacillus tartricus* (Grimbert and Ficquet, 1897), *Aerobacter tartarivorum* (Nijdam, 1907) and *Bacterium succinicum* (Sakaguchi and Tada, 1940). *Bacillus tartricus* and *Aerobacter tartarivorum* were given specific status primarily because of their ability to decompose *d*-tartrates. *Bacterium succinicum* was so named because it produced larger amounts of succinic acid from *d*-tartaric and other organic acids than did other coliform bacteria with which it was compared. These three species have characteristics which relate them to recognized species of coliform bacteria, as is seen in table 6. Furthermore, their characteristics have been investigated sufficiently to show their very close resemblance to the corresponding

recognized species of the genera *Escherichia* and *Aerobacter*, as shown in the table.

It has been shown that the decomposition of *d*-tartrates is a characteristic common to some strains of all the accepted species of coliform bacteria belonging

TABLE 6

Probable synonymy of Bacillus tartricus, Aerobacter tartarivorum, and Bacterium succinicum

DESCRIBED SPECIES	BACILLUS* TARTRICUS	AEROBACTER† TARTARIVORUM	BACTERIUM‡ SUCCINICUM
Morphological characters			
Shape	short rods	short rods	short rods
Size (μ)	1-2 long	±1.2 x ±2.0	0.6-0.8 x 1.3-2.1
Flagella	+++	+++	Peritrichous
Spore formation	−(−)	−(−)	−(−)
Gram stain	−(−)	−(−)	−(−)
Biochemical characters, formation of			
Acetylmethyl carbinol (V.-P.) test	+(+)	+(+)	−(−)
H_2S	0(−)	0(−)	+(+)
Indole	−(−)	+(+)	−(−)
Utilization of citric acid	0(+)	+(+)	+(+)
Methyl red test	0(+)	0(−)	+(+)
Gelatin liquefaction	+(+)	−(−)	−(−)
Acid and gas formation from			
Lactose	+(+)	+(+)	+(+)
Sucrose	+(+)	+(±)	+(+)
Starch	−(−)	+(+)	−(−)
Aesculin	0(+)	0(+)	0(−)
Salicin	0(+)	0(+)	−(−)
Glycerol	−(−)	+(+)	+(+)
Probable synonymy§	*Aerobacter cloacae* Bergey *et al.*	*Aerobacter aerogenes* (Kruze) Beijerinck	*Escherichia freundii* (Braak) Yale

0, not determined; −, negative reaction, + positive reaction, in original descriptions; presently accepted differential characteristics in ( ).

* Grimbert and Ficquet, 1897.

† Nijdam, 1907. +++ Type of flagellation not specified.

‡ Sakaguchi and Tada, 1940.

§ Consult table 1 and Bergey *et al.*, 1939; Levine, 1921; Levine, Epstein, and Vaughn, 1934; West, Gilliland, and Vaughn, 1941; Vaughn and Levine, 1942; and other literature for characteristics of these species.

to the genera *Escherichia* and *Aerobacter*. It is not sufficient to claim specific status for any of the coliform bacteria because one strain produces more succinic acid from organic acids or glucose than other strains. It is well known that succinic acid is an end product arising from the decomposition of organic acids or glucose by members of the coliform bacteria.

Each group of bacteria having similar characteristics must bear only one name: the valid one based on prior use. It is suggested, therefore, that synonymy be created for *Bacillus tartricus*, *Aerobacter tartarivorum*, and *Bacterium succinicum* inasmuch as the possible synonymy of these organisms apparently has not received prior attention, except a suggestion by Barker (1936) on the possible synonymy of *Aerobacter tartarivorum* and *Aerobacter aerogenes*. On the basis of priority it is probable that *Bacillus tartricus* Grimbert and Ficquet is synonymous with *Aerobacter cloacae* (Jordan) Bergey *et al.*; *Aerobacter tartarivorum* Nijdam is synonymous with *Aerobacter aerogenes* (Kruze) Beijerinck; and *Bacterium succinicum* Sakaguchi and Tada is synonymous with *Escherichia freundii* (Braak) Yale in Bergey *et al.*

SUMMARY

The ability to decompose *d*-tartrate salts is possessed by some strains of all the recognized species of coliform bacteria.

The rates of destruction of *d*-tartrates by the coliform bacteria fell into two groups. One group represented by about half the cultures of *Aerobacter aerogenes* decomposed the *d*-tartrate salts rapidly and with vigorous evolution of gas. The rest of the cultures decomposed the *d*-tartrates slowly and with no visible evolution of gas.

Strains of *Aerobacter aerogenes* that cause very rapid destruction of *d*-tartrates bring about the most rapid and most complete destruction of *dl*- and *l*-ammonium tartrates. All species decomposed the *dl*- and *l*-ammonium tartrates less rapidly than the *d* isomer. The *l* form was attacked least rapidly of all.

The optimum temperature of incubation for maximum destruction of *d*-tartrates by the recognized species of coliform bacteria varied with the cultures investigated.

The initial pH of the medium has a marked effect on the ability of recognized species of coliform bacteria to grow and to decompose *d*-tartrate. Some of the species have definite optima ranging from pH 4.5 to 5.0; others have optima ranging between 5.0 and 7.3. Some strains of *Aerobacter aerogenes* decompose *d*-tartrates in media with initial pH values of 3.9.

Tartrate media used for the differentiation of *Salmonella* from other *Enterobacteriaceae* are of no value for distinguishing between cultures of *Escherichia* and *Aerobacter*.

The probable synonymy of *Bacillus tartricus*, *Aerobacter tartarivorum*, and *Bacterium succinicum* with *Aerobacter cloacae*, *Aerobacter aerogenes*, and *Escherichia freundii*, respectively, was discussed.

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