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Decarboxylase activity of Pseudomonas species found in dairy products

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NOTE TO USERS

DECARBOXYLASE ACTIVITY OF *PSEUDOMONAS* SPECIES FOUND IN DAIRY PRODUCTS

by

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INTRODUCTION

The fact that microorganisms are capable of breaking down protein and utilizing the various constituents has been recognized for some time. In most instances the products of these metabolic changes have been assumed to be utilized in one form or another by the different microorganisms as a source of nitrogen.

Recently there have been studies which indicate a possible role for the amino acids liberated, that of amine production in an acid medium to cause a shift in the pH toward neutrality during growth or to retard a shift toward an acid reaction. Such a change in pH of the medium probably acts as a protective mechanism which allows growth to continue even though there are accumulations of incompatible products beyond the level of normal metabolism. This protective mechanism or decarboxylation apparently is not present in all microorganisms; however, many genera and species have not been studied. This mechanism may be present in the psychrophilic organisms which are becoming more and more a problem in dairy products. For example, the production of off flavors and aromas in cottage cheese by some Pseudomonas species may be the result of decarboxylase enzymes.

The present study is concerned with some of the factors

responsible for decarboxylation of amino acids by a selected group of species of the genus Pseudomonas, known to be responsible for certain abnormal flavors in dairy products.

STATEMENT OF PROBLEM

This study was undertaken to determine the abilities of Pseudomonas fragi, Pseudomonas fluorescens, Pseudomonas putrefaciens, Pseudomonas aeruginosa, Pseudomonas graveolens, Pseudomonas mucidolens, Pseudomonas cyanogenes, Pseudomonas synxantha, Pseudomonas ichthyosmia and Pseudomonas viscosa to decarboxylate the amino acids L-arginine, L-glutamic acid, L-histidine, L-lysine, L-ornithine and L-tyrosine.

The research problem is concerned with such environmental factors as pH of growth medium, temperature of incubation, length of culturing and presence of the specific substrate in the growth medium which may influence the decarboxylase activity which can be demonstrated for these microorganisms.

REVIEW OF LITERATURE

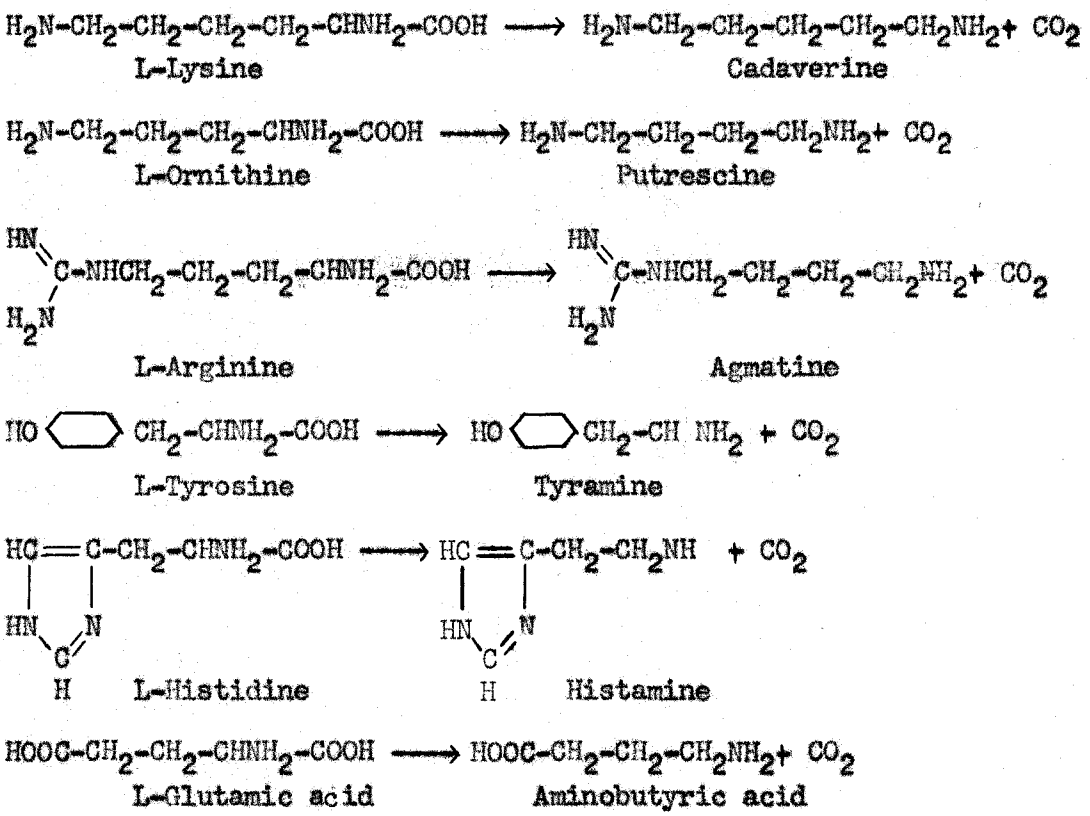
Decarboxylation of Amino Acids by Bacteria

The simple decarboxylation of an amino acid results in the formation of the corresponding amine with the liberation of carbon dioxide. The production of amines as the result of bacterial action was reported some years ago by Barger and Walpole (1909) and Rosenheim (1909). However, Ellinger (1898, 1900) showed that if a synthetic medium containing a specific amino acid was inoculated with putrefying material and examined chemically several weeks later, the corresponding amine could be isolated.

Mellanby and Twort (1912) proved the production of histamine from histidine by the use of pure strains of microorganisms. Hanke and Koessler (1922, 1924) showed that amines were produced by strains of Escherichia coli. Later Kendall and Gebauer (1930) and Eggerth (1939) showed that strains of Clostridium welchii (perfringens) also were capable of producing several amines.

The reaction responsible for the amine production was not known until Gale (1940a, 1940b) showed that specific amino acid decarboxylases are produced by bacteria. These enzymes, according to Gale (1940a, 1940b, 1940c, 1941), are formed within the bacterial cells in response to certain conditions of growth.

All bacterial decarboxylases so far identified react at pH levels between 2.5 and 6.0. Thus, if the reaction takes place in a moderately acid medium, carbon dioxide will be evolved and the reaction can be measured quantitatively by manometric techniques. During recent years bacteria of many genera and species have been tested by these procedures for their ability to decarboxylate the various amino acids. According to Gale (1946) and Lagerborg and Clapper (1952), although nearly all known naturally-occurring amino acids have been tested, only six have been attacked under experimental conditions, the amount of carbon dioxide liberated in the reaction being measured by manometric methods. The six amino acids and the resulting amines are as follows:



Gale (1946) points out that these six amino acids have, in addition to the carboxyl and α-amino groups, the common property of a polar group situated at the end of the molecule far removed from the carboxyl group attacked. Thus it would seem that the presence of the polar group in the right stereochemical relation is necessary for the enzyme to split off the carbon dioxide from the carboxyl group of the amino acid.

Virtanen and Laine (1937) and Virtanen, Rintala and Laine (1938) have demonstrated the formation of β-alanine from aspartic acid by the action of Rhizobium leguminosarum. A three-week incubation period was used and the resulting amine was measured chemically by isolation as a picrate.

Many genera and species of microorganisms are capable, when grown under certain conditions, of producing decarboxylases that are active on the above amino acids. All bacteria are not capable of producing enzymes which decarboxylate all six amino acids; in fact, Gale (1943) stated that many bacteria are unable to produce any enzymes of this type. Possibly bacteria produce decarboxylases which will act on other amino acids than those reported above, but the production rate may be so slow that the activity cannot be measured by manometric techniques.

Hanke and Koessler (1924b) found a number of strains of E. coli possessed strong tyrosine decarboxylase activity; strains of Aerobacter aerogenes also were capable of producing

measurable amounts of this enzyme. Gale (1946) reported that of the 151 strains of E. coli studied, 114 possessed arginine decarboxylase, 142 lysine decarboxylase, 130 glutamic acid decarboxylase, 14 histidine decarboxylase and 6 tyrosine decarboxylase, but 90 per cent of the strains tested were found to be positive.

In summarizing a study of some 800 strains of streptococci examined for decarboxylase activity, Gale (1946) stated that approximately 500 strains of Streptococcus faecalis or streptococci of the Lancefield group D produced tyrosine decarboxylase to some degree. Under the conditions employed, the 500 strains produced only tyrosine decarboxylase. Lagerborg and Clapper (1952), using the established techniques, found S. faecalis, as well as sulfa-resistant strains of Streptococcus mitis, to be capable of producing appreciable quantities of tyrosine decarboxylase.

Gale (1941) reported that seven strains of Cl. welchii (perfringens) produced variable amounts of histidine and glutamic acid decarboxylases. Later, this investigator (1946) stated that nine of ten Cl. welchii (perfringens) strains produce decarboxylases. Other Clostridium species which were reported by Gale (1941) as being capable of producing various amino acid decarboxylases were: Cl. septique, Cl. aerofoetideum, Cl. fallax and Cl. bifermentans.

Three strains of Proteus vulgaris and two strains of Pr.

morganii were tested by Gale (1941) and found to produce decarboxylases for ornithine and glutamic acid.

Lagerborg and Clapper (1952) reported that in testing 31 strains of lactobacilli isolated from saliva, 12 produced arginine decarboxylase, 3 tyrosine and glutamic acid decarboxylases, 1 histidine and ornithine decarboxylases and none acted upon lysine. These authors stated that Lactobacillus casei and Lactobacillus arabinosus showed no activity for any of the six amino acids tested (L-arginine, L-tyrosine, L-lysine, L-histidine, L-ornithine or L-glutamic acid).

Bacillus subtilis and Bacillus mesentericus were reported by Gale (1946) to possess very weak decarboxylase activity for some of the amino acids. The author stated further that no decarboxylase enzymes have been found in organisms belonging to genera Staphylococcus or Pseudomonas. However, Gale and Epps (1944) reported that Pseudomonas aeruginosa was a very good source for the coenzyme (codecarboxylase) for lysine and tyrosine activity.

Factors Affecting Amino Acid Decarboxylase Production

pH of growth medium

The pH greatly affects the cell crop as well as the production of the enzyme. Generally the microorganisms grown on acid medium produce enzymes which attack amino acids by decarboxylation, according to Gale and Epps (1942), while those

on alkaline medium attack amino acids by deamination, as stated by Stephenson and Gale (1937). The lower the pH during microorganism growth, within the range of pH 4.5 to 6.0, the greater the formation of the decarboxylases; according to Gale (1940b, 1941), this is true even though maximum growth of the cells does not occur. Since the greatest enzyme concentration occurs at or near the limits of acid tolerance for the organism concerned, Kocholaty and Weil (1938) suggested that glucose be added to the medium, thus causing a drop in pH during growth. Addition of glucose to the medium results in a heavy crop of cells which possess a high concentration of active enzymes. Gale (1940a) stated that the presence of glucose in the medium had no effect on decarboxylase activity that could not be attributed to the change in pH resulting from fermentation. Bellamy and Gunsalus (1944) found that S. faecalis produced optimum tyrosine decarboxylase activity when grown in a medium containing enough glucose to cause the pH to fall below 5.0 during growth.

Wood and Gunsalus (1942) used 0.067 M phosphate buffer to facilitate the maintenance of pH and at the same time increase growth of the microorganisms. Bellamy and Gunsalus (1944) showed that slightly higher activity could be obtained in unbuffered tryptone medium than with buffered tryptone medium; the difference was so small that the phosphate buffer was retained, since the increased growth obtained with buffer facili-

tated the study of other factors.

Temperature of incubation

In many studies of enzyme production, the temperature of growth is not mentioned as a factor which may affect the activity. However, Eggerth (1939) found that the temperature of cultivation regulated the rate of formation of histidine by various species of bacteria. Gale (1940a), in studying amino acid decarboxylases produced by E. coli, found that several strains possessed more enzyme activity when grown at 20 and 26° C. than when grown at 37° C. Later, Gale (1940b, 1941) showed that S. faecalis and Cl. welchii (perfringens) formed greater quantities of amino acid decarboxylase at 37° C. than at 25° C. Using S. faecalis for several studies, Bellamy and Gunsalus (1944) and Lagerborg and Clapper (1952) found greater activity at 37° C. than at lower temperatures. Gale (1943, 1946) suggested that the temperature effect is characteristic of the particular microorganism's ability to produce the enzyme concerned rather than the effect of thermolability.

Length of incubation

In general "young" cultures have little enzyme activity; however, when growth takes place under optimum conditions, the formation of amino acid decarboxylase occurs late in the logarithmic phase of growth, according to the work of Wooldridge and Glass (1937). In the many experiments of Gale and Stephen-

son (1938), Gale (1940a) and Woods and Trim (1942) it has been shown that cultures possess little activity at the beginning of the logarithmic growth phase, but this activity increases linearly until the initial growth stops, after which the activity may remain steady or, as in most cases, decrease. Winslow and Walker (1939), in their review on variations of metabolic activity with age of the culture, showed that higher activity occurs during the early logarithmic phase and then falls off before the end of the period of active growth.

Bellamy and Gunsalus (1944) and Gale (1946) stated that, in general, incubation of the culture for 14 to 16 hours produced the highest enzyme activity. However, there were some slight variations noted by Gale when the various microorganisms were studied. For example, Cl. welchii (perfringens) produced the highest histidine decarboxylase level in 10 hours, while E. coli required 16 hours for maximum production of arginine, lysine and glutamic acid decarboxylases. Lagerborg and Clapper (1952), working with S. faecalis, compared cells harvested at 16 and 24 hours and found that the higher tyrosine decarboxylase activity occurred with the cells which were 24 hours old.

Requirement of specific substrate

Addition of the specific substrate, in this case the amino acids, to the growth medium results in production of the corresponding decarboxylase, provided that the enzyme potential is present in the microorganism studied. The need for the presence

of the specific substrate in the growth medium in order that enzyme production may result is very adequately reviewed by Dubos (1940). Gale (1940a) showed a 2.5-fold stimulation of tyrosine decarboxylase activity by the addition of tyrosine to a deficient medium, thus indicating the adaptive nature of the enzyme system. Later, Gale (1940b) found that strains of S. faecalis possess a very active tyrosine decarboxylase when grown in casein digest-glucose medium, but when grown in a salt-glucose-marmite medium containing 1 per cent tyrosine, the enzyme activity was only about 10 per cent as high. Bellamy and Gunsalus (1944), studying the same type of enzyme system, found that addition of tyrosine to a peptone medium increased the activity six-fold. These same authors stated that tyrosine decarboxylase production on an acid hydrolyzed casein medium was not improved by the addition of tyrosine. Use of casein digest-glucose medium did not, for all practical purposes, require the addition of the specific substrate or amino acid for optimum production, as shown by Gale (1946). This author, using a casein digest-glucose medium fortified with 1 per cent lysine, obtained results for lysine decarboxylase activity of 210 (Q_{CO_2}) compared with 194 (Q_{CO_2}) for the control casein digest-glucose medium. However, in the same study, higher glutamic acid decarboxylase activity was obtained with the control medium than with the casein digest-glucose medium fortified with 1 per cent glutamic acid. From these experiments Gale concluded that the addition

of the specific amino acid to the casein digest-glucose medium was not necessary since adequate amounts of the substrate already were present for development of decarboxylase activity. Later, Lagerborg and Clapper (1952), working with S. faecalis, were able to get good tyrosine decarboxylase activity by using a medium containing trypticase, a peptone derived from casein by pancreatic digestion.

Codecarboxylase requirement.

Gale (1943) suggested that the organism is unable to synthesize certain factors other than the specific substrate involved in enzyme production. Following this suggestion, Bellamy and Gunsalus (1944) tested the effect of eight vitamin B compounds on the decarboxylase activity of S. faecalis. These authors concluded that addition of pyridoxine or nicotinic acid had a marked stimulatory effect on decarboxylase activity, but further studies revealed that both vitamins together developed the full activity with this microorganism. However, addition of these two factors to a simplified medium will not increase the arginine decarboxylase production by E. coli under the conditions studied by Gale (1946), although growth on casein digest-glucose medium gives full enzyme activity. Apparently a coenzyme is required for production of full decarboxylase activity, but the codecarboxylase varies with the particular microorganisms to be studied. In all cases the co-

decarboxylase has been present in the casein digest-glucose medium, since full enzyme activity has been obtained when compared to the simple salt-glucose medium fortified with the vitamin B factors.

Gale and Epps (1944) reported that the codecarboxylase is distributed widely in nature, being found in bacteria, yeasts and animal or plant tissues.

Gunsalus, Bellamy and Umbreit (1944) postulated that the coenzyme of tyrosine decarboxylase is a phosphorylated derivative of pyridoxal. Later Gunsalus, Umbreit, Bellamy and Foust (1945) described the preparation of a synthetic codecarboxylase which yielded full decarboxylase activity. This preparation contained 6.2 per cent organic phosphorus, 32 per cent pyridoxal and about 50 per cent barium.

Bellamy, Umbreit and Gunsalus (1945) presented data which show that organisms capable of growing without vitamin B_6 are able to synthesize codecarboxylase. The synthesis of the codecarboxylase activity was obtained on the casein digest-glucose medium and not on the simple fortified medium described by Bellamy and Gunsalus (1944).

Biological Function of Amino Acid Decarboxylases

Hanke and Koessler (1924a) have suggested that bacteria produce amines in an acid environment as "a protective mechanism resorted to when the accumulation of H ions within the organism's protoplasm is incompatible with its normal life pro-

cesses." Gale and Epps (1942) showed that when bacteria with active amino acid decarboxylases are grown in an acid medium there is a constant shift of the pH toward neutrality. Thus, production of decarboxylases under such conditions may be a mechanism used by the cells in attempting to neutralize acid condition within the cells against unfavorable changes which may occur. Such a mechanism would help enable the cells to exist in an environment which otherwise would be made unfavorable by the high acid content.

Using streptococci belonging to Lancefield group D, Sharp (1948) was able to measure tyrosine decarboxylase activity by pH variations, since formation of the amine caused a rise in pH. Cultures possessing a strong tyrosine decarboxylase activity were able by tyramine formation to bring about a rise in pH of as much as 2.5 units.

It is thus suggested by the above investigators that these decarboxylase enzymes have no direct function during active growth, but formation of the various decarboxylases by the microorganisms is a means by which the cells are able to survive under what otherwise would be adverse acidic conditions.

EXPERIMENTAL

Methods

Cultures

Of the ten cultures used, nine were selected from the stock culture collection of the Dairy Industry Department, Iowa State College. The remaining culture (Ps. viscosa) was obtained from the culture collection of the Department of Dairy Husbandry, University of Missouri. All cultures chosen for study possessed typical characteristics of the various species and in all cases rapid growing strains were used. All stock cultures were carried on tryptone-glucose-extract-milk agar slants, whereas casamino acids-glucose-phosphate medium was used for all experimental trials.

Determination of plate counts

Tryptone-glucose-extract-milk agar was used to pour plates for standard plate counts (American Public Health Association, 1948). All plates for enumeration of Ps. aeruginosa were incubated at 37° C. for 48 hours, while all other species were counted after incubation for 48 hours at 21° C.

Determination of pH

The pH determinations were made with a Beckman model G glass-electrode potentiometer.

Selection of growth medium

Several preliminary trials with certain test organisms indicated that production of the enzyme is greater on a more complex nutrient medium than on a simple synthetic medium containing only the essential growth factors and specific substrates. The medium used for all Pseudomonas species consisted of:

Casamino acids (technical)	10 g.
Glucose	10 g.
Phosphate buffer (0.2 M, pH 6.0)	100 ml.
Distilled water	900 ml.

The medium was dispersed in 100 ml. quantities and sterilized in an autoclave at 250° F. for 15 minutes.

The composition of the medium was derived from recommendations of Burton, Eagles and Campbell (1947) on amino acids and Robinson (1932) on use of phosphate. Likewise, a phosphate-containing casein medium of the approximate composition above was used by Gale (1943) and Bellamy and Gunsalus (1944). The pH of the medium after sterilization ranged from 5.36 to 5.65.

Preparation of cell suspensions

The cells for experimental trials were propagated in casamino acids-glucose-phosphate medium for 24 hours at 18° C. for all species except Ps. aeruginosa, which was incubated at 37° C. for 24 hours. Fifty ml. of the medium was placed in 32-ounce medicinal ovals, inoculated and incubated in a hori-

zontal position, with the flat side down. The surface of the medium for growth under these conditions was approximately 8.5 X 18 cm., with the depth of the liquid medium being approximately 3 mm.

The cells were harvested by centrifuging the 24 hour culture medium in an International type SB trunnion-head centrifuge operated at 4,200 rpm. for 15-18 minutes. The supernatant fluid was decanted off, the remaining cells washed twice by resuspending in 30 ml. of saline and centrifuged as with the original medium. The cells were prepared for final study by diluting with saline until a 1 to 100 dilution of the prepared cells gave a reading of 50 on the Klett-Summerson photoelectric colorimeter, using a No. 60 filter. This modification of the method of Price (1947) gave a cell population in the final suspension of approximately 5,000,000,000 per ml.

Selection of buffer

The selection of a buffer was based partially on the work of Gale (1941) and on the fact that Pseudomonas species apparently require phosphorus for normal growth and activity. Thus a 0.2 M phosphate buffer of pH 6.0 (Clark, 1928) was chosen for all measurements. A 0.02 M phosphate buffer was used for culturing purposes, since it was assumed that a more concentrated phosphate medium would be detrimental to the growth of the cells. The use of the 0.02 M phosphate buffer gave a KH_2PO_4 concentration of 0.068 per cent, which is in

close agreement with the 0.05 per cent phosphate requirement for Pseudomonas as reported by Sullivan (1905) and Robinson (1932).

Selection of substrates

The substrates used in the side arm of the Warburg flasks were amino acids which had been standardized to various pH levels with sodium hydroxide or lactic acid. The substrates selected were L-arginine, L-glutamic acid, L-histidine, L-lysine, L-ornithine and L-tyrosine. These amino acids were reported by Gale (1943) as the only ones possessing structures which could be attacked by bacterial decarboxylases. The stock amino acids were prepared as 0.067 M solutions and the pH adjusted to 3.0 for L-arginine, 4.0 for L-glutamic acid, 3.0 for L-histidine, 3.0 for L-lysine, 3.2 for L-ornithine and 3.5 for L-tyrosine. The substrate concentration with the substrate-cell suspension-buffer in the reaction flask was 0.011 M.

Decarboxylase measurements

The decarboxylase activity was measured by the amount of carbon dioxide given off when cell suspensions were allowed to react with specific amino acid substrate in the Warburg constant-volume respirometer. The methods employed were those described by Dixon (1943) and Umbreit, Burris and Stauffer (1949). The single side arm cell respiration flasks and manometers were calibrated using mercury, following the method

described by Umbreit, Burris and Stauffer (1949).

The flasks used had a total volume of approximately 13 ml.; however, the contents during manometric measurements were 2.0 ml. of phosphate buffer (pH 6.0), 0.5 ml. of cell suspension and 0.5 ml. of amino acid substrate, giving a total of 3.0 ml. of reaction mixture.

Endogenous carbon dioxide production was measured by substituting 0.5 ml. of acidified distilled water for the amino acid in the side arm of the Warburg flask. No measurements were deemed necessary for "bound carbon dioxide" due to the reaction between the phosphate buffer and the carbon dioxide, since the flask contents were held at a pH of 5.0 or below.

The temperature of the water bath was maintained at 30±0.02° C. The shaker of the Warburg apparatus was operated through a distance of 4 cm. at approximately 140 strokes per minute. The flasks were shaken for 15 minutes to allow for equilibration, then the stopcocks were closed; thus atmospheric air was used as the gas phase. Readings of the manometers were made at 5 minute intervals throughout the test period. After tipping the contents of the side arm, interval readings were made for at least 1 hour and the data reported as microliters of carbon dioxide liberated per hour.

Results

The results reported in this study are based on single strains of ten Pseudomonas species. Decarboxylase activity was determined on the following species: Ps. fragi, Ps. fluorescens, Ps. putrefaciens, Ps. aeruginosa, Ps. graveolens, Ps. mucidolens, Ps. cyanogenes, Ps. synxantha, Ps. ichthyosmia and Ps. viscosa. The strains used, where a preference could be made, were those which showed the most rapid growth when streaked on tryptose-glucose-extract-milk agar.

Effect of specific substrate on activity of cells

The preliminary trials with Ps. fragi indicated that adequate growth could be obtained with a 1 per cent ammonium phosphate-glucose medium. Measurement of the change in pH during growth did not show a trend toward neutrality, regardless of the length of incubation up to 42 hours. However, the addition of an amino acid (histidine) to the ammonium salt-glucose medium caused a rise in pH, as well as a slightly higher cell count after 24 hours of incubation. After these qualitative measurements, more complete data were obtained by quantitative methods.

The media used for specific substrate trials were as follows: (a) 1 per cent $NH_4H_2PO_4$, 1 per cent glucose plus 0.1 per cent liquid salt mixture (salt mixture consisted of 8.0 g. $MgSO_4$, 0.4 g. $FeSO_4$ and 1.2 g. $MnCl_2$ per 100 ml. water), (b)

0.5 per cent tryptone, 0.1 per cent glucose, 0.3 per cent beef extract and 1.5 per cent agar and (c) 1 per cent casamino acids, 1 per cent glucose and 0.068 per cent phosphate. Each of the three media was made up in 100 ml. quantities, sterilized at 250° F. for 15 minutes and 50 ml. portions dispensed into 32-ounce bottles. One 50 ml. portion of each medium was fortified with 20 mg. of L-histidine. All media were inoculated with 5 ml. of a 1 to 100 dilution of a culture of Ps. fragi. The medium containing agar was solidified first and then inoculation was accomplished by flooding the surface with the dilution of culture. After incubation for 24 hours at 21° C., the harvesting of cells, preparation of cell suspension and standardization of the cell concentrations were carried out as outlined in "Methods".

The standardized cell suspensions were tested for decarboxylase activity against histidine at pH 4.0 by Warburg manometric techniques using flask volumes as described in "Methods". The reaction pH was not determined, since Gale (1940a) reported the use of histidine at pH 4.0 to be optimum under similar conditions for decarboxylase of this amino acid. The quantitative results obtained are presented in Table 1.

The data show that the decarboxylase activity is highest in cells from the media containing the specific amino acid. The ammonium salt-glucose medium failed to show any activity unless fortified with the amino acid under study, although

Table 1

The effect of the specific substrate in the growth medium upon the decarboxylase activity of Pseudomonas fragi cells. (L-histidine adjusted to pH 4.0 used in reaction flasks)

Growth media	pH of the growth media		Klett-Summerson reading of the suspended cells	CO_2 liberated (µl./hr.)
	Initial	Final		
$NH_4H_2PO_4$ -glucose medium	5.53	5.55	47	0
$NH_4H_2PO_4$-glucose medium + 200 mg. histidine/liter	5.55	4.96	43	14
Tryptose-glucose-extract agar	5.51	--[a]	50	3
Tryptose-glucose-extract agar + 200 mg. histidine/liter	5.54	--[a]	51	13
Casamino acids-glucose-phosphate medium	5.47	4.60	51	32
Casamino acids-glucose-phosphate medium + 200 mg. histidine/liter	5.56	4.66	50	34

[a]pH of the agar not determined.

there was an adequate growth of cells on this basal medium. The casamino acids-glucose-phosphate medium, when fortified with the histidine, produced the highest histidine decarboxylase activity. However, the increased amount of activity produced by Ps. fragi on the fortified casamino acids medium when compared to the unfortified medium does not appear great enough to justify addition of the specific decarboxylase substrate. Apparently the basal casamino acids-glucose-phosphate medium contains enough of the specific amino acid for the production of the decarboxylase.

Since the decarboxylase activity was highest for Ps. fragi when grown on the casamino acids-glucose-phosphate medium, this medium also was used for the other species subsequently studied.

The enzyme was not produced unless the specific amino acid was present in the growth medium, thus indicating that the decarboxylase mechanism is adaptive in nature.

Effect of various acids used for adjusting pH on decarboxylase activity

When Ps. fragi cells were found to possess good enzyme activity for histidine at pH 4.0, the question arose as to whether the carbon dioxide liberated was due to action of the decarboxylase or to the acid used for adjusting the pH. Manometric measurements were made where approximately 0.02 M

lactic and phosphoric acid solutions adjusted at the various pH levels were substituted for the amino acid substrates. The Ps. fragi cells used were grown on casamino acids-glucose-phosphate medium for 24 hours at 21° C. The cells were harvested and suspensions for testing were made according to "Methods".

The results presented in Table 2 clearly indicate that the changes in manometric pressures were not due to presence of lactic or phosphoric acids and that the activity previously measured must have been due to the action of the decarboxylase on the amino acid substrate in the Warburg flasks.

For further proof that the gas liberated was carbon dioxide, trials as shown in Table 3 were repeated, except that 0.2 ml. of 10 per cent KOH was placed in the center well of the reaction flasks. Under these conditions there was no measurable liberation of gas, indicating that the gas was absorbed by the added base. Thus it was concluded that the gas liberated was carbon dioxide and the presence of such was due to the action of the decarboxylase produced by bacteria.

However, results with lactic and phosphoric acids as shown in Table 2 did not give a measure of the desirability of one acid over the other for adjusting the pH of the amino acid substrates. Following the above procedures with Ps. fragi cell suspension, measurements were repeated using lactic and phosphoric acids for adjusting the pH of the histidine. Table 3 shows the amounts of carbon dioxide liberated from

Table 2

The effect of lactic and phosphoric acids on carbon dioxide liberation in the presence of Pseudomonas fragi cells. (0.2 M phosphate buffer used in reaction flasks)

Acids used	Trial 1			Trial 2		
	pH of reaction		Gas liberated[a] (µl./hr.)	pH of reaction		Gas liberated[a] (µl./hr.)
	Initial	Final		Initial	Final	
Lactic acid	2.61	2.69	2	2.65	2.73	3
	2.82	2.84	6	2.82	2.85	0
	3.20	3.18	-2[b]	3.15	3.16	1
	3.72	3.67	-4[b]	3.60	3.62	1
	4.03	4.05	1	4.03	4.07	2
	4.80	L.A.[c]	-1	4.80	4.80	-6[b]
Phosphoric acid	2.71	2.88	4	2.70	2.80	2
	3.00	3.07	2	2.95	3.07	0
	4.23	4.30	2	4.30	4.42	-1[b]
	4.98	5.12	-3[b]	5.02	5.20	-2[b]
	5.60	5.53	1	5.50	5.52	-2[b]
	5.60	5.58	0	5.52	5.60	-5[b]

[a]No amino acid used in the reaction flasks. Results corrected for thermobarometer.

[b]Negative results obtained, indicating oxygen uptake.

[c]Laboratory accident.

Table 3

The effect of lactic and phosphoric acids used for adjusting the pH of histidine on the rate of carbon dioxide liberated by Pseudomonas fragi cells. (0.067 M Histidine used in side arm and 0.2 M phosphate buffer used in reaction flasks)

Acids used	pH of histidine used	pH of reaction		CO_2 liberated[a] (µl./hr.)
		Initial	Final	
Lactic acid	2.0	2.60	2.62	79
	2.5	2.87	2.90	85
	3.0	3.56	3.60	90
	3.5	3.92	4.05	76
	4.0	4.79	5.52	31
	4.5	5.25	5.67	27
Phosphoric acid	2.0	2.99	3.12	88
	2.5	4.85	4.95	81
	3.0	5.22	5.35	60
	3.5	5.31	5.47	58
	4.0	5.37	5.70	55
	4.5	5.37	5.72	36

[a]Results corrected for thermobarometer.

histidine adjusted to various pH levels with each acid. Neither acid apparently has any appreciable effect on the amount of carbon dioxide liberated and neither induces greater activity than the other. The lactic acid appeared to hold the pH at a more constant level during the reaction. Thus lactic acid was used for adjusting the pH of all subsequent substrates in this study, since phosphoric acid failed to buffer at the desired pH, based on the lactic acid results.

Effect of the age of the cells on decarboxylase activity

The casamino acids-glucose-phosphate medium was inoculated with Ps. fragi and incubated at 21° C. for 18, 24, 30, 36, and 42 hours. The cells were harvested, resuspended and standardized as described in "Methods" for use in the Warburg flasks. The results obtained from three trials with histidine and glutamic acid are presented in Table 4.

These results show that in all cases the highest decarboxylase activity was present in the cells cultured for 24 hours. No activity measurements were made on the 18 hour culture because of the insufficient cell harvest, as indicated by the very low cell count and turbidity. The decarboxylase activity decreased as the age of the cells advanced beyond 24 hours. The greatest amount of decrease in activity occurred between the 30 and 36 hour growth periods. The slight increase in cell count between 24 and 30 hours of culturing

Table 4

The effect of the age of culture upon the decarboxylase activity of Pseudomonas fragi cells.
(0.067 M Glutamic acid and histidine adjusted to pH 4.0.
All cells grown on casamino acids-glucose-phosphate medium.)

Hours of incubation at 21° C.	Standard plate count[a]	pH of the growth media		Cell concentration (Klett-Summerson)		CO_2 liberated (µl./hr.)	
		Initial	Final	Initial[b]	Adjusted	Glutamic acid	Histidine
Trial 1							
18	430,000	5.25	5.08	7	-	-[c]	-[c]
24	16,000,000	5.25	4.59	55	50	33	53
30	21,000,000	5.25	4.33	59	51	18	47
36	19,000,000	5.25	4.30	58	50	14	21
42	16,000,000	5.25	4.37	62	51	4	21
Trial 2							
18	320,000	5.25	5.18	4	-	-[c]	-[c]
24	11,000,000	5.25	4.85	50	50	27	46
30	15,000,000	5.25	4.65	64	50	17	38
36	17,000,000	5.25	4.24	64	51	16	29
42	15,000,000	5.25	4.15	63	48	4	20
Trial 3							
18	580,000	5.56	5.34	4	-	-[c]	-[c]
24	14,000,000	5.56	3.90	63	50	33	67
30	17,000,000	5.56	4.50	64	50	20	58
36	17,000,000	5.56	4.81	64	49	18	44
42	18,000,000	5.56	4.70	72	50	16	43

[a]Per ml. of broth culture.
[b]Reading when total cell population was diluted to 4 ml. with saline.
[c]Insufficient cells harvested to make a determination.

shows that the stationary growth phase of Ps. fragi had been reached. The decline in carbon dioxide liberated for the same test period indicated that decarboxylase activity apparently is greatest near the end of the logarithmic growth phase.

Effect of incubation temperature on decarboxylase activity

Temperature is known to have a marked effect on the growth rate of bacteria; therefore, it is to be expected that the temperature at which the culture is grown likewise will affect the activity of the cells. Previous trials with Ps. fragi were made at 21° C., the reported optimum growth temperature, and a decarboxylase activity for histidine and glutamic acid was shown. However, all of the Pseudomonas species to be studied do not have the same temperature requirement for optimum growth. Therefore, in order to study this problem of the effect of temperature on the cell activity, Ps. fragi and Ps. aeruginosa which have optimum growth temperatures of 21 and 37° C., respectively, were selected. The temperatures selected were 15, 18, 21 and 24° C. for Ps. fragi and 25, 30, 37 and 45° C. for Ps. aeruginosa. Casamino acids-glucose-phosphate medium was inoculated with Ps. fragi or Ps. aeruginosa and incubated at the different temperatures for 24 hours. The cells were harvested, washed, resuspended and standardized according to "Methods". The results for the various temperatures studied are presented in Table 5.

Table 5

The effect of growth temperature upon the decarboxylase activity of Pseudomonas fragi and Pseudomonas aeruginosa cells. (0.067 M Histidine and glutamic acid adjusted to pH 4.0)

Organism	Incubation temperature °C[a]	pH of the growth media		Cell concentration (Klett-Summerson)		CO_2 liberated (µl./hr.)	
		Initial	Final	Initial[b]	Adjusted	Histidine	Glutamic acid
Ps. fragi (Trial 1)	15	5.56	5.19	48	48	37	23
	18	5.56	4.66	53	50	82	34
	21	5.56	4.60	63	51	60	26
	24	5.56	4.59	60	50	55	15
Ps. fragi (Trial 2)	15	5.56	5.04	44	44	49	18
	18	5.56	4.59	61	50	70	26
	21	5.56	4.33	69	50	63	26
	24	5.56	4.37	68	50	52	18
Ps. aeruginosa (Trial 1)	25	5.65	6.01	52	50	69	61
	30	5.65	6.10	56	50	77	65
	37	5.65	6.50	61	51	85	85
	45	5.65	6.32	47	47	61	47
Ps. aeruginosa (Trial 2)	25	5.65	6.08	49	49	65	66
	30	5.65	6.22	59	51	85	72
	37	5.65	6.37	64	51	89	78
	45	5.65	6.30	54	50	58	54

[a]All cultures incubated for 24 hours in casamino acid-glucose-phosphate medium.

[b]Reading when total cell population was diluted to 4 ml. with saline.

In trial 1, the highest activity occurred for those cells of Ps. fragi incubated at 18° C. In trial 2, histidine showed the highest activity at 18° C., but glutamic acid activity was equal at 18 and 21° C. In averaging the two trials, there would be a small difference between the two temperatures in favor of the 18° C. incubation. Further examination of the data shows that at the time of harvest, a higher cell population occurred at 21° and 24° C. than at 18° C., indicating that decarboxylase production is not entirely dependent on number of cells but also depends upon other factors. For an explanation of why higher activity occurred with Ps. fragi cells grown at 18° C., rather than at the optimum of 21° C., Gale (1946) states that certain growth temperatures may act as an inhibitor for the production of the amino acid decarboxylases.

The results show that the optimum temperature for the production of decarboxylase activity by Ps. aeruginosa is 37° C. Maximum activity as well as maximum cell population occurred at the same temperature. This temperature effect on production of amino acid decarboxylases apparently is characteristic of the organism rather than of the enzyme, as shown by the above two organisms.

Effect of buffer composition on the activity of the decarboxylases

All preliminary trials on specific substrate, adjustment of pH of the reaction mixture, variation in temperature and

length of incubation were carried out by using cells which were grown in a medium containing a phosphate buffer originally at pH 6.0 (Clark, 1928). To determine the effect of the buffer composition on growth of the organism and production of decarboxylase, three lots of casamino acids-glucose-phosphate medium were made according to "Methods", except that a different buffer was used in each medium. The buffers used were 0.2 M Clark and Lubs phosphate (Clark, 1928), 0.2 M Clark and Lubs phthalate (Clark, 1928) and McIlvaine citrate-phosphate (Hodgman, 1949), all at pH 6.0. The 0.2 M buffers were added at the rate of 100 ml. per 900 ml. of distilled water, thus making a 0.02 M buffer concentration in the final medium. The various media and controls were inoculated with Ps. fragi and incubated at 13° C. for 24 hours. After incubation the cells were prepared for measurement of the decarboxylase activity as described in "Methods". The results for decarboxylase activity on histidine and glutamic acid are reported in Table 6.

The three media containing the different buffers gave higher activity than the controls; however, the amount of cells harvested from the control was smaller, so that if the same quantity of cells were used, the difference in enzyme activity probably would have been very small. The cells grown in the presence of phosphate buffer gave the higher histidine decarboxylase value. The results on glutamic acid were slightly higher when the citrate-phosphate buffer was used, but the difference was not as great as with histidine. Another factor

Table 6

The effect of buffer composition in the growth medium upon the rate of cell growth and production of decarboxylases by Pseudomonas fragi. (0.067 M Histidine and glutamic acid adjusted to pH 4.0)

Buffer used[a]	pH of the growth media		Klett-Summerson reading of the initial cells	CO_2 liberated (µl./hr.)	
	Initial	Final		Histidine	Glutamic acid
Trial 1					
Clark and Lubs phosphate	5.27	4.47	48	50	18
Clark and Lubs phthalate	5.31	4.65	48	30	10
McIlvaine citrate-phosphate	5.64	4.60	42	31	22
Control (no buffer used)	5.00	4.40	36	31	16
Trial 2					
Clark and Lubs phosphate	5.25	4.90	49	53	19
Clark and Lubs phthalate	5.34	4.93	45	29	13
McIlvaine citrate-phosphate	5.60	5.20	48	34	19
Control (no buffer used)	5.10	4.71	40	28	16

[a]All buffers of pH 6.0 used in casamino acid-glucose medium.

which may be of importance is that the citrate-phosphate buffer had the smallest amount of variation between the initial and final pH during growth. This could be because the citrate-phosphate buffer exercises a greater buffering effect against metabolic products produced by the bacteria or because slightly slower growth may not have produced conditions which would lower the pH as with the other buffers. The phosphate buffer not only gave the highest histidine decarboxylase activity, but also had the largest cell population at the end of 24 hours. This slight increase in cell population may be due to the role of phosphate in the metabolism of certain Pseudomonas species. Gunsalus, Bellamy and Umbreit (1944) stated that a codecarboxylase, consisting of a phosphorylated derivative of pyridoxal, was required for tyrosine decarboxylase activity. It may be that the medium containing the phosphate buffer presented conditions more favorable for the microorganisms to synthesize the phosphorylated coenzyme. According to Burton, Eagles and Campbell (1947), Ps. aeruginosa required phosphate for optimum function of the cells; however, these authors did not state the role of phosphorus during growth.

The pH of the phosphate medium was lower, which may have been due to the increased growth. At the same time this lower pH probably produced conditions which favored the higher enzyme activity. In a review by Gale (1946), it was shown that within limits, the lower the pH during growth of the cell, the high-

er the decarboxylase activity produced. In general the cells grown in the presence of phosphate had a higher decarboxylase activity as well as the largest population. Based upon these considerations, the phosphate buffer (Clark, 1928) was adopted for inclusion in the culture medium for all further trials.

Effect of buffer composition on carbon dioxide liberation

Since differences in cell population and decarboxylase activity were influenced by the various buffers in the growth media, trials were made to determine if the buffer composition could have additional effect on the decarboxylase activity in the Warburg reaction flasks. The reaction flasks contained 2.0 ml. of the buffer to be tested, 0.5 ml. of cell suspension of Ps. fragi (prepared according to "Methods") and 0.5 ml. of L-histidine or L-glutamic acid at pH 4.0 in the side arm. The buffers tested were Clark and Lubs phosphate (Clark, 1928), Clark and Lubs phthalate (Clark, 1928) and McIlvaine citrate-phosphate (Hodgman, 1949). The results obtained are presented in Table 7.

The carbon dioxide liberated in the presence of the various buffers was highest for the phosphate buffer of pH 6.0, while the phthalate gave results slightly lower and the citrate-phosphate gave the lowest response. Also it should be noted that the phosphate buffer had the lowest final pH, while the citrate-phosphate had the highest. This may be the explanation for the lower activity obtained when using the citrate-

Table 7

The effect of buffer composition in the reaction mixture upon the decarboxylase activity of Pseudomonas fragi. (L-histidine adjusted to pH 4.0. Klett-Summerson reading on cell suspension used was 49)

Buffer used	pH of buffer	pH of reaction		CO_2 liberated from histidine (μl./hr.)
		Initial	Final	
Trial 1				
Clark and Lubs phosphate	6.0	5.03	5.34	40
Clark and Lubs phthalate	6.0	5.41	5.60	32
McIlvaine citrate-phosphate	6.0	5.62	5.76	28
Trial 2				
Clark and Lubs phosphate	6.0	5.10	5.28	44
Clark and Lubs phthalate	6.0	5.44	5.54	35
McIlvaine citrate-phosphate	6.0	5.54	5.66	34

phosphate buffer, since the activity of the decarboxylase seems to be highest in the lower pH range. The decarboxylase activity with the phosphate buffer apparently is higher because of the lower pH during the reaction. Thus it appears that the other two buffers produce a slightly inhibitory effect which may be caused by the higher pH resulting from their greater buffering ability.

Effect of amino acid substrate pH upon the decarboxylase activity

In the previous temperature and time studies, histidine and glutamic acid solutions were adjusted to pH 4.0 as recommended by Gale (1940a, 1940b). However, in certain trials where the pH of reaction was lower, higher enzyme activity resulted, indicating that the pH of the reaction undoubtedly is more important than the initial pH of the amino acid.

The various amino acids were tested, using different species of Pseudomonas to determine the different decarboxylases which may be produced. All six amino acids in the preliminary trials were adjusted to pH 4.0 for testing purposes. The organism that produced the highest decarboxylase activity for amino acids at pH 4.0 was chosen for a more complete investigation of the effect of the pH on the amount of decarboxylase activity. These organisms were grown in the casamino acids-glucose-phosphate medium and the cells harvested and resuspended as in "Methods". The reaction flasks contained 2.0 ml. of

0.2 M phosphate buffer and 0.5 ml. of the cell suspension. To the reaction flask side arm was added 0.5 ml. of the adjusted amino acid solution. The pH of the six amino acid substrate solutions used was adjusted within a range of 2 to 5, using 0.5 unit subdivisions. However, the pH range of 2 to 5 proved to be too great for determining the decarboxylase activity for ornithine and tyrosine. For these two amino acids, additional trials were made and higher enzyme activities were obtained when the pH was adjusted within a range of 3.0 to 4.0, using 0.2 unit subdivisions. Ps. fragi was used for measurement of the histidine and glutamic acid decarboxylase activities; Ps. aeruginosa for lysine, arginine and ornithine; and Ps. viscosa for tyrosine. The Ps. fragi and Ps. viscosa organisms were grown at 18° C. and the Ps. aeruginosa cultured at 37° C. for 24 hours. The results obtained from a representative trial for each amino acid are presented in Tables 8, 9 and 10.

The highest decarboxylase activity for histidine was obtained with an initial reaction pH of 3.21 and the resulting final pH of 3.35. However, good histidine decarboxylase activity was obtained over a much wider reaction range, including pH 2.5 to 3.8. A reaction of pH 4.7 or above caused considerable decrease in enzyme activity.

The optimum activity for glutamic acid was found to be at pH 4.0. A pH above 5.0 or one as low as 3.3 was not conducive

Table 8

The effect of pH of the reaction mixture upon the rate of decarboxylation by Pseudomonas fragi. (Phosphate buffer at pH 6.0 used in the reaction flasks)

Amino acid used	pH of amino acid solution	pH of reaction		CO_2 liberated (µl./hr.)
		Initial	Final	
Histidine	2.0	2.53	2.61	60
	2.5	2.73	2.86	62
	3.0	3.21	3.35	68
	3.5	3.74	3.81	61
	4.0	4.68	4.72	35
	4.5	5.22	5.28	27
Glutamic acid	2.5	2.68	2.75	0
	3.0	3.25	3.30	4
	3.5	3.65	3.72	34
	4.0	3.95	4.10	42
	4.5	4.62	4.68	25
	5.0	5.18	5.23	3

Table 9

The effect of pH of the reaction mixture upon the rate of decarboxylation by Pseudomonas aeruginosa. (Phosphate buffer of pH 6.0 used in the reaction flasks)

Amino acid used	pH of amino acid solution	pH of reaction		liberation (µl./hr.)
		Initial	Final	
Arginine	2.0	2.89	2.96	22
	2.5	3.21	3.30	53
	3.0	3.70	3.78	62
	3.5	5.94	6.04	25
	4.0	6.37	6.44	0
	4.5	6.45	6.46	0
Lysine	2.0	2.72	2.78	29
	2.5	3.10	3.22	60
	3.0	3.71	3.84	84
	3.5	4.14	4.20	51
	4.0	5.60	5.62	6
	4.5	6.34	6.32	0

Table 10

The effect of pH of the reaction mixture upon the rate of decarboxylation of ornithine and tyrosine by Pseudomonas aeruginosa and Pseudomonas viscosa, respectively. (Phosphate buffer of pH 6.0 used in the reaction flasks)

Amino acid used	pH of amino acid solution	pH of reaction		CO_2 liberation (μl./hr.)
		Initial	Final	
Ornithine	3.0	3.60	3.66	37
	3.2	4.22	4.30	48
	3.4	4.85	4.91	33
	3.6	5.65	5.72	10
	3.8	6.05	6.10	3
	4.0	6.22	6.23	0
Tyrosine	3.4	4.04	4.21	26
	3.5	4.21	4.40	30
	3.6	4.62	4.84	19
	3.8	5.85	5.90	2
	4.0	5.91	6.02	0
	4.2	6.08	6.23	0

to strong decarboxylase activity. Activity on glutamic acid seems to be far more sensitive to the various pH levels than does activity of histidine decarboxylase. Apparently certain acid concentrations bring about a slightly inhibitory effect for glutamic acid decarboxylase.

The optimum pH's for activity on arginine and lysine are almost identical, both being within the range of 3.70 to 3.85. It possibly is significant that the amount of decarboxylase activity was approximately 25 per cent greater for lysine than for arginine, even though the final pH levels of the two reactions were the same. This would indicate that under the conditions of these trials, Ps. aeruginosa was capable of greater lysine decarboxylase production, possibly through the adaption of the cells being stimulated by greater concentrations of lysine than arginine in the basal medium. Below pH 3, a considerable decline in lysine or arginine decarboxylase activity was apparent. Lysine decarboxylase activity was somewhat more sensitive to reactions of pH 5.6 and above than was the arginine decarboxylase.

The ornithine decarboxylase was most active at about 4.25. The optimum pH for ornithine could be slightly lower than the 4.25 shown in Table 10, because data between pH's 3.6 and 4.2 were not obtained and decarboxylase activity was strong at pH 3.6. However, additional trials with ornithine adjusted to lower pH levels failed to show greater decarboxyl-

ase activity than was shown at pH 4.25 in Table 10. There was no appreciable activity when the pH of the ornithine reaction mixture was above 6.0.

Ps. viscosa was the only species examined which produced a decarboxylase capable of attacking tyrosine. The tyrosine activity produced by this organism under these experimental conditions was the smallest total enzyme activity value found for any of the six amino acids studied. The pH of the substrate had to be adjusted to a very low level to obtain low pH levels in the reaction mixture. As noted in Table 10, the highest enzyme activity occurred when the pH of the reaction was between 4.21 and 4.40; however, the pH of the tyrosine used in the side arm was 3.5. It should be observed that the pH subdivisions for the tyrosine were close, yet the pH of the reaction was much wider because of poor buffering of the tyrosine as compared to phosphate in this range. Previous investigations by Gale (1940b, 1946) with S. faecalis cultures producing very strong tyrosine decarboxylase activity indicate that the rate of enzyme reaction at pH 6.0 was many times less than at pH 5.0. In discussing glutamic acid decarboxylase activity, Gale (1940a) stated that pH 5.0 was the upper limit of reaction and that any pH higher than 5.0 was insufficiently acid for the enzyme reaction to take place.

Based on the representative data in Tables 8, 9, and 10, the amino acid decarboxylases of several species were found to

be optimally active at the following pH levels: L-arginine, 3.75; L-glutamic acid, 4.0; L-histidine, 3.25; L-lysine, 3.75; L-ornithine, 4.25 and L-tyrosine, 4.25.

Decarboxylase production by the various Pseudomonas species

Many factors affecting enzyme production have been tested and certain conditions for high decarboxylase activity have been established. Using these conditions, the Pseudomonas species were tested to determine their ability to produce the different decarboxylases. When the enzymes were found to be produced by the different species, additional trials were made to measure the amount of enzyme activity against each of the six amino acids.

The experimental conditions employed in this series of trials were as follows: (a) Casamino acids-glucose-phosphate medium was used, (b) all cultures were incubated at 18° C., with the exception of Ps. aeruginosa, which was incubated at 37° C., (c) incubation period was 24 hours, (d) the harvesting, resuspending and standardization of the cells were carried out as described in "Methods", (e) the constituents of the Warburg flasks consisted of 2.0 ml. of 0.2 M phosphate buffer of pH 6.0 (Clark, 1928), 0.5 ml. of cell suspension and 0.5 ml. of the amino acid substrate in the side arm and (f) manometric measurements were made at 30° C. and readings taken at 5 minute intervals for a period of one hour after tipping.

The 0.067 M amino acid solutions were adjusted with lactic acid so that the resulting pH levels of the reaction mixtures were for the most part within the range found to be optimum for decarboxylase activity. The results obtained for arginine decarboxylase activity are shown in Table 11.

The various Pseudomonas species studied for arginine decarboxylase could be divided into those with relatively high activity, those with low activity and those which showed no activity. Based on the average volume of carbon dioxide liberated, Ps. aeruginosa and Ps. viscosa were capable of producing high decarboxylase activity, while Ps. fragi, Ps. mucidolens and Ps. ichthyosmia were very low producers of this enzyme. The other five species were not capable of producing measurable quantities of an enzyme which could decarboxylate arginine.

In all cases the growth of each species was adequate or at least the cell counts were comparable. Thus it would seem that the five species which did not show arginine decarboxylase activity under the conditions of these trials, were not capable of adapting their metabolic processes to produce the specific enzyme. The activity for the most part was higher for the second trial than for the first.

Greater changes occurred between the initial and final reaction pH of the highly active species than with the other two groups. The difference between the pH shifts of the low

Table 11

The ability of some Pseudomonas species to produce L-arginine decarboxylase. (L-arginine solution adjusted to pH 3.0)

Organisms	Trial 1			Trial 2			Average CO_2 liberated
	pH of reaction		CO_2 liberated (μl./hr.)	pH of reaction		CO_2 liberated (μl./hr.)	
	Initial	Final		Initial	Final		
Pseudomonas fragi	3.42	3.48	8	3.40	3.56	16	12
fluorescens	3.42	3.50	0	3.44	3.55	0	0
putrefaciens	3.44	3.50	0	3.42	3.59	0	0
aeruginosa	3.88	3.98	41	3.97	4.06	68	54
graveolens	3.44	3.45	0	3.46	3.48	0	0
mucidolens	3.45	3.47	13	3.46	3.46	20	16
cyanogenes	3.42	3.50	0	3.42	3.56	0	0
synxantha	3.40	3.56	0	3.46	3.45	0	0
ichthyosmia	3.44	3.48	16	3.40	3.55	18	17
viscosa	3.46	3.72	63	3.42	3.76	57	60

activity species and those which were inactive was not of consequence; in fact, in some cases the variation was larger in the inactive group. The initial pH of Ps. aeruginosa cells was much higher than the others, which probably was due to the higher pH of the cells during growth.

The results of the tests for glutamic acid decarboxylase activity of the Pseudomonas species are given in Table 12. The number of species active against glutamic acid is much smaller than with the arginine decarboxylase, only three species being capable of producing this enzyme. The amount of decarboxylase activity produced by Ps. fragi, Ps. putrefaciens and Ps. aeruginosa was much more uniform between active strains as well as between the two trials than in the case of arginine decarboxylase.

For all species producing glutamic acid decarboxylase, the variation between the initial and final pH was quite small and uniform. However, an upward trend in pH was present for those possessing the decarboxylase activity. Since the pH for these species not producing the enzyme was within the same range as for those possessing activity, it was probable that these seven species were not capable of adapting to produce an enzyme for glutamic acid.

The results obtained for the histidine decarboxylase activity are shown in Table 13. The number of species possessing an enzyme active on histidine is greater than for either argi-

Table 12

The ability of some Pseudomonas species to produce L-glutamic acid decarboxylase. (L-glutamic acid solution adjusted to pH 4.0)

Organisms		Trial 1			Trial 2			Average CO_2 liberated
		pH of reaction		CO_2 liberated	pH of reaction		CO_2 liberated	
		Initial	Final	(µl./hr.)	Initial	Final	(µl./hr.)	
Pseudomonas	fragi	4.27	4.50	51	4.08	4.22	38	44
	fluorescens	4.32	4.38	0	4.14	4.18	0	0
	putrefaciens	4.34	4.45	12	4.12	4.26	22	17
	aeruginosa	4.20	4.54	42	4.32	4.68	44	43
	graveolens	4.38	4.70	0	4.30	4.53	0	0
	mucidolens	4.16	4.12	0	4.30	4.34	0	0
	cyanogenes	4.35	4.33	0	4.34	4.32	0	0
	synxantha	4.28	4.38	0	4.32	4.26	0	0
	ichthyosmia	4.33	4.50	0	4.28	4.18	0	0
	viscosa	4.36	4.37	0	4.24	4.42	0	0

Table 13

The ability of some Pseudomonas species to produce L-histidine decarboxylase. (L-histidine solution adjusted to pH 3.0)

Organisms		Trial 1			Trial 2			Average CO_2 liberated
		pH of reaction		CO_2 liberated (µl./hr.)	pH of reaction		CO_2 liberated (µl./hr.)	
		Initial	Final		Initial	Final		
Pseudomonas	fragi	3.73	3.88	67	3.74	3.98	58	63
	fluorescens	3.76	3.81	0	3.82	3.90	0	0
	putrefaciens	3.78	3.76	0	3.60	3.67	0	0
	aeruginosa	4.38	4.47	44	4.02	4.34	60	52
	graveolens	3.72	3.80	6	3.70	3.74	4	5
	mucidolens	3.80	4.30	8	3.72	3.92	6	7
	cyanogenes	3.70	3.82	0	3.88	3.94	0	0
	synxantha	3.65	3.72	13	3.74	3.80	17	15
	ichthyosmia	3.76	3.74	0	3.88	3.92	0	0
	viscosa	3.52	3.62	36	3.70	3.81	35	35

nine or glutamic acid. Ps. fragi, Ps. aeruginosa and Ps. viscosa all show strong production of decarboxylase, while Ps. graveolens, Ps. mucidolens and Ps. synxantha produce smaller amounts of the enzyme. The other four species did not produce measurable amounts of carbon dioxide; therefore, these species are considered not capable of histidine decarboxylase production, at least under the conditions employed.

The results of Epps (1945) suggested that a codecarboxylase was not necessary for L(-)histidine decarboxylase produced by Cl. welchii (perfringens) Type A. If this suggestion were true for the Pseudomonas, then this may be a partial explanation for the large number of species possessing histidine activity.

The pH change is rather uniform for all species with the exception of Ps. aeruginosa, whether the cells possess decarboxylase activity or not.

The results obtained for the lysine decarboxylase activity of the various Pseudomonas species are presented in Table 14. The lysine decarboxylase activity level is much more variable than for those enzymes reported above. Ps. aeruginosa produces a much higher activity on lysine than the other species; also the activity is considerably higher than reported for the other amino acid decarboxylases. Ps. viscosa is the only other species showing a high lysine decarboxylase activity, while Ps. fragi, Ps. graveolens, Ps. mucidolens and Ps. cyanogenes all

Table 14

The ability of some Pseudomonas species to produce L-lysine decarboxylase. (L-lysine solution adjusted to pH 3.0)

Organisms	Trial 1			Trial 2			Average CO_2 liberated
	pH of reaction		CO_2 liberated	pH of reaction		CO_2 liberated	
	Initial	Final	(µl./hr.)	Initial	Final	(µl./hr.)	
Pseudomonas fragi	3.32	3.41	12	3.34	3.38	7	9
fluorescens	3.36	3.42	0	3.30	3.40	0	0
putrefaciens	3.28	3.42	0	3.31	3.48	2	0
aeruginosa	3.66	3.85	98	3.64	3.80	82	90
graveolens	3.40	3.46	13	3.32	3.40	11	12
mucidolens	3.34	3.42	14	3.30	3.47	14	14
cyanogenes	3.45	3.75	28	3.50	3.82	30	29
synxantha	3.40	3.42	0	3.40	3.44	0	0
ichthyosmia	3.44	3.44	0	3.42	3.40	0	0
viscosa	3.48	3.70	61	3.44	3.84	68	64

possess a weak lysine decarboxylase activity. The four other species failed to show the presence of the enzyme.

The reaction for the Pseudomonas species active on lysine was within a range of pH 3.4 to 3.7. In lysine decarboxylase activity, the pH of the reaction mixture apparently does not follow the same pattern as found with the pH of the growth medium; lower pH during growth is associated with the higher enzyme activity. For those organisms showing strong lysine decarboxylase activity, the relatively high final pH is not due altogether to the higher reaction pH level but also possibly to the high enzyme activity liberating the amine and causing a shift upward in the final pH.

Gale and Epps (1944) reported that Ps. aeruginosa was one of the better sources of lysine codecarboxylase. The very high lysine decarboxylase activity shown by Ps. aeruginosa and Ps. viscosa in these trials possibly is attributable to the presence of a good supply of the coenzyme. From all reports, the lysine codecarboxylase is needed for the enzyme to be active. Possibly under the conditions of these trials, these two species of Pseudomonas produced an adequate quantity of the coenzyme which resulted in a higher lysine decarboxylase activity than in the case of the other species.

The results obtained for ornithine decarboxylase activity are shown in Table 15. Of the six Pseudomonas species showing ornithine decarboxylase activity, only two species were found

Table 15

The ability of some Pseudomonas species to produce L-ornithine decarboxylase. (L-ornithine solution adjusted to pH 3.2)

Organisms		Trial 1			Trial 2			Average CO_2 liberated
		pH of reaction		CO_2 liberated	pH of reaction		CO_2 liberated	
		Initial	Final	(µl./hr.)	Initial	Final	(µl./hr.)	
Pseudomonas	fragi	3.60	3.55	0	3.60	3.62	0	0
	fluorescens	3.70	3.82	11	3.66	3.80	8	9
	putrefaciens	3.64	3.71	0	3.68	3.79	0	0
	aeruginosa	4.24	4.30	37	4.30	4.47	36	36
	graveolens	3.62	3.65	14	3.70	3.92	16	15
	mucidolens	3.62	3.62	14	3.66	3.84	11	12
	cyanogenes	3.60	3.63	0	3.54	3.60	0	0
	synxantha	3.52	3.56	0	3.56	3.62	0	0
	ichthyosmia	3.64	3.70	8	3.58	3.67	5	6
	viscosa	3.50	3.60	32	3.72	3.89	38	34

to possess fairly strong enzyme activity. Ps. aeruginosa possessed the highest activity on ornithine, yet the amount was only approximately 40 per cent as high as the activity on lysine produced by the same organism. Four species failed to exhibit ornithine decarboxylase activity. The variation in the activity of the different species is smaller than that exhibited for the other enzymes reported, which indicates that either the species are not strong producers of ornithine decarboxylase or that other factors, such as the supply of coenzyme, are limiting their enzyme activity.

The sixth set of trials involves the ability of the Pseudomonas species to produce tyrosine decarboxylase, the results being presented in Table 16. Tyrosine decarboxylase production, as far as the ten species of Pseudomonas studied are concerned, is limited to Ps. viscosa. The amount of tyrosine activity produced, when compared to the other decarboxylases, would have to be classified as moderate or weak. Gale (1943), working with E. coli, showed that where the species possessed the ability to produce several amino acid decarboxylases, the tyrosine activity produced was considerably smaller than for most of the other decarboxylases. In examining the values for the five decarboxylases produced by Ps. viscosa, the tyrosine activity is smaller than any of the other enzymes produced.

A summary of the amino acid decarboxylases produced by the

Table 16

The ability of some Pseudomonas species to produce L-tyrosine decarboxylase. (L-tyrosine solution adjusted to pH 3.5)

Organisms	Trial 1			Trial 2			Average CO_2 liberated
	pH of reaction		CO_2 liberated	pH of reaction		CO_2 liberated	
	Initial	Final	(µl./hr.)	Initial	Final	(µl./hr.)	
Pseudomonas fragi	4.04	4.28	0	4.22	4.21	0	0
fluorescens	4.26	4.59	0	4.22	4.43	0	0
putrefaciens	4.12	4.17	0	4.20	4.22	0	0
aeruginosa	4.92	5.65	0	4.97	5.41	0	0
graveolens	4.08	4.24	0	4.10	4.22	0	0
mucidolens	4.10	4.16	0	4.14	4.25	0	0
cyanogenes	4.06	4.20	0	4.08	4.21	0	0
synxantha	4.12	4.38	0	4.08	4.24	0	0
ichthyosmia	4.24	4.60	0	4.18	4.34	0	0
viscosa	4.14	4.63	28	4.22	4.52	20	24

different species of Pseudomonas as presented in Tables 11 through 16 is shown in Table 17. It will be noted that all species were capable of production of at least one decarboxylase while none of the ten species produced enzymes for all six amino acids tested. The number of Pseudomonas species which produced the specific decarboxylases varied from one for tyrosine to six for histidine, lysine and ornithine. The data indicate also that no absolute correlations exist between the species and the decarboxylases produced; e.g., there were no cases where production of a decarboxylase specific for one substrate was always associated with action on a particular one or definite group of substrates.

Effect of pyridoxine on decarboxylase activity

After the study of the extent of decarboxylase production by the selected Pseudomonas species, it seemed desirable to study the effect of pyridoxine upon the activity of amino acid decarboxylases. Bellamy, Umbreit and Gunsalus (1945) stated that organisms capable of growing without pyridoxine were able to synthesize a codecarboxylase. Later Gunsalus, Umbreit, Bellamy and Foust (1945) described the active codecarboxylase as a phosphorylated pyridoxal compound. Thus, trials with selected Pseudomonas species were made to determine if addition of pyridoxine to the growth medium or to the reaction mixture would increase the decarboxylase activity.

Table 17

The production of amino acid decarboxylases by some Pseudomonas species. (Tabulations based on results from Tables 11-16)

Organisms	Amino acids					
	Arginine	Glutamic acid	Histidine	Lysine	Ornithine	Tyrosine
Pseudomonas fragi	+	+	+	+	-	-
fluorescens	-	-	-	-	+	-
putrefaciens	-	+	-	-	-	-
aeruginosa	+	+	+	+	+	-
graveolens	-	-	+	+	+	-
mucidolens	+	-	+	+	+	-
cyanogenes	-	-	-	+	-	-
synxantha	-	-	+	-	-	-
ichthyosmia	+	-	-	-	+	-
viscosa	+	-	+	+	+	+

The basal medium of casamino acids-glucose-phosphate was prepared and sterilized as described in "Methods". To 100 ml. of the sterilized basal medium was added 1 ml. of a sterilized solution containing 500γ of pyridoxine. Fifty ml. portions of the basal medium fortified with pyridoxine were inoculated with Ps. aeruginosa, Ps. viscosa, Ps. putrefaciens and Ps. fluorescens and incubated for 24 hours at 37° C. for the first and 21° C. for the latter three. In all cases bottles of the basal medium were inoculated with each of the four organisms for use as controls. After incubation, the cells were harvested and cell suspensions prepared as in "Methods". For measuring the amount of decarboxylase activity, 0.5 ml. quantities of the various cell preparations were added to each of four Warburg flasks. All flasks contained, in addition to the cell suspensions, 2.0 ml. of phosphate buffer of pH 6.0. Two of these four flasks also contained 50γ of pyridoxine and 3γ of adenosine triphosphate. To the side arm of all flasks was added 0.5 ml. of the specific amino acid for measurement of decarboxylase activity.

The four species to be studied were selected on the basis that two were capable of forming five decarboxylases and the other two produced only one enzyme of this type. One of the organisms producing five enzymes was the only one capable of tyrosine decarboxylation.

Bellamy and Gunsalus (1944) reported that the pyridoxine

requirement for decarboxylase production is several-fold the concentration for maximum growth. The addition of 500γ of pyridoxine per 100 ml. of medium was considered to be in excess of the requirement for growth, since the cell populations on the casamino acids-glucose-phosphate medium were as large as those on the medium fortified with pyridoxine. In all previous trials, the growth medium had not contained any added growth factors; however, many could have been present in the technical grade of the casamino acids used. Also to be considered is the ability of the Pseudomonas species to synthesize adequate amounts of the various growth factors, including the ones required for the production of decarboxylase and codecarboxylase.

The results obtained with the addition of pyridoxine to the basal medium and to the reaction flasks are presented in Table 18. In examining data on the five enzymes produced by Ps. viscosa, when grown in the presence of pyridoxine, histidine and tyrosine decarboxylases are increased slightly. The increase for histidine activity based on one trial indicates that the pyridoxine may be partially responsible by causing a more rapid production of the enzyme. The amount of difference between tyrosine activity on the fortified and control growth media should be questioned as being due to the added pyridoxine. According to Bellamy and Gunsalus (1944), increased tyrosine decarboxylase activity is due to a greater concentration

Table 18

The effect of pyridoxine in the growth medium and in the reaction flasks upon the decarboxylase activity of the Pseudomonas species. (Average of two flasks in the same trial)

Organisms	CO_2 liberated (µl./hr.)											
	Arginine		Glutamic acid		Histidine		Lysine		Ornithine		Tyrosine	
	B_6[a] added	control	B_6[a] added	control	B_6[a] added	control	B_6[a] added	control	B_6[a] added	control	B_6[a] added	control
Ps. viscosa	46	55	0	0	21	23	46	48	30	23	19	19
Ps. viscosa + B_6[b]	52	50	0	0	34	31	53	47	30	32	26	22
Ps. aeruginosa	35	36	25	27	41	36	75	72	33	36	0	0
Ps. aeruginosa + B_6[b]	36	33	40	41	43	40	76	84	38	35	0	0
Ps. putrefaciens	0	0	23	22	0	0	0	0	0	0	0	0
Ps. putrefaciens + B_6[b]	0	0	19	21	0	0	0	0	0	0	0	0
Ps. fluorescens	0	0	0	0	0	0	0	0	10	13	0	0
Ps. fluorescens + B_6[b]	0	0	0	0	0	0	0	0	11	11	0	0

[a] 50 γ pyridoxine and 3 γ of A. T. P. added to the Warburg flasks.

[b] 500 γ pyridoxine added per 100 ml. media.

of pyridoxine than is needed for optimum functional growth. The results on lysine, arginine and ornithine decarboxylases were very inconsistent, since some controls show more activity than the corresponding fortified media, indicating that pyridoxine may not be the limiting factor for optimum activity by these three enzymes.

Based on limited trials with Ps. aeruginosa, the growth medium fortified with pyridoxine gave increased activity of glutamic acid decarboxylase. The other four decarboxylases produced by this organism failed to show an increased activity. An interesting point is that the histidine activity of this species failed to show the difference that was observed between the fortified and control growth media with Ps. viscosa.

Ps. putrefaciens apparently is not affected by the addition of pyridoxine. As shown previously, glutamic acid is the only amino acid for which a decarboxylase is produced by this organism and in these limited trials the amount of activity did not increase with the addition of the pyridoxine to the growth medium.

Ps. fluorescens likewise failed to respond to pyridoxine, either as the growth factor or as a component of the reaction mixture, thus indicating that pyridoxine was not the limiting factor in ornithine decarboxylase production.

Based on the results presented, there was no agreement on whether the addition of pyridoxine to the growth medium gave

increased activity for the same decarboxylase produced by the different species of Pseudomonas. In no instance were the four species of Pseudomonas tested stimulated by the presence of pyridoxine in the growth medium to form amino acid decarboxylases other than those reported in Table 17.

The addition of pyridoxine and A T P to the reaction flasks, for functioning as codecarboxylase, did not bring about any certain increase in the decarboxylase activity for the Pseudomonas species studied. Gunsalus, Bellamy and Umbreit (1944) reported that pyridoxine and A T P were capable of functioning as the coenzyme for decarboxylase produced by S. faecalis when coenzyme concentration was the limiting factor. Since addition of these two factors to the reaction flasks did not yield increases in enzyme activity, it seems probable that adequate amounts of the coenzyme or other needed factors are present within the cell.

DISCUSSION

Ten species of Pseudomonas commonly found in milk and milk products were used to determine their ability to decarboxylate the amino acids arginine, glutamic acid, histidine, lysine, ornithine and tyrosine, using the Warburg technique to measure the carbon dioxide liberated by the cells. The use of amino acids of the L form was preferred because the presence of the D form might have produced misleading results. The species tested and found to contain one or more decarboxylases were Ps. fragi, Ps. fluorescens, Ps. putrefaciens, Ps. aeruginosa, Ps. graveolens, Ps. mucidolens, Ps. cyanogenes, Ps. synxantha, Ps. ichthyosmia and Ps. viscosa. The fact that these ten species were capable of decarboxylase production is, however, in direct conflict with the findings of Gale (1946), who stated that no organisms belonging to the genus Pseudomonas had been found to produce decarboxylases.

The inclusion of the specific substrate in the growth media, as shown in Table 1, resulted in the production of measurable amounts of the specific decarboxylase. The best results were obtained, however, by using the more complete media. Thus it appears that the specific substrate is responsible for the formation of the adaptive enzyme and, in addition, the decarboxylase is produced in greater amounts on a rich medium than in a merely adequate medium. These results confirm those of Gale (1946), who reported that when organisms are grown in a

medium free of amino acids, the amino acid decarboxylases are not produced. The addition of the amino acid substrate to the growth medium results in the production of the corresponding decarboxylase, provided the enzyme potential is present in the organism.

In testing the ten species of Pseudomonas, two major temperature ranges are involved. Experimental trials incorporating a representative species for 21 and 37° C. temperature ranges were carried out using Ps. fragi and Ps. aeruginosa, respectively. The growth temperature seems to play a very important role in the production of the amino acid decarboxylase by both species tested. Ps. fragi produces the largest amount of enzyme activity at 18° C., which is lower than the recognized optimum growth temperature and Ps. aeruginosa produces the most activity at its optimum growth temperature of 37° C. Eggerth (1939) also noted that decarboxylase production by some organisms was greater when growth took place at temperatures lower than the optimum for growth.

The age of the culture was shown in Table 4 to be an important factor in the amount of decarboxylase formed by Ps. fragi. The higher activity occurring at 24 hours with the slightly lower cell population than at 30 hours suggests that the formation of the enzyme apparently begins somewhere in the logarithmic phase and increases in activity as growth proceeds, with the greatest activity occurring near the end of

the logarithmic growth phase. Gale reported (1940a) that decarboxylase activity did not occur in young cells of E. coli, but strains capable of enzyme formation produced the enzyme late in the growth period. Lagerborg and Clapper (1952) reported that for S. faecalis activity could be demonstrated between 16 and 24 hours, with the optimum being with the 24 hour incubation period.

For S. faecalis, Cl. welchii (perfringens) and E. coli, incubation periods of 10 to 16 hours have been reported by Gale (1946) as being optimum for formation of the decarboxylases. There is some question as to why Ps. fragi was slower in decarboxylase formation since very little growth occurred as late as 18 hours. Gale (1941) gives a partial explanation for this slow decarboxylase formation by reporting that when growth takes place in the presence of a carbohydrate, the appearance of the enzyme within the cell is delayed. He did not indicate if the delay was due to need for acidity development before enzyme formation could begin or if the higher acidity produced may slow down cell growth and thus delay enzyme formation.

The Clark and Lubs phosphate buffer proved to be more stimulatory in the growth medium for the development of the decarboxylase activity than the Clark and Lubs phthalate or McIlvaine citrate-phosphate buffer. The phosphate buffer apparently plays a dual role, acting as a source for phosphate

for proper cell function and exerting a buffering effect during the growth of the cells. Apparently the buffers used were not markedly toxic, since all were favorable to the formation of decarboxylases by one or more organisms.

The pH of the reaction becomes all important because the cells may possess the amino acid decarboxylase in adequate quantities, yet little or no activity of the enzyme on the amino acid will be measured unless the reaction range is correct. The enzyme does not react with the substrate unless an acid condition prevails. The reaction pH ranges for activity between the decarboxylases and the amino acids were found to be as follows: arginine, 3.2 to 5.0; glutamic acid, 3.6 to 4.6; histidine, 2.5 to 5.5; lysine, 2.7 to 5.5; ornithine, 3.6 to 5.6; and tyrosine, 4.0 to 5.0. These reaction ranges for enzymes from Ps. fragi, Ps. aeruginosa and Ps. viscosa are in close agreement with those reported by Gale (1940a, 1940b) for E. coli and S. faecalis. As the pH of the reaction moves from the optimum range and approaches 6.0, the amount of activity decreases and in every case activity is almost completely lacking at pH 6.0.

Those species which produce enzymes active upon more than one amino acid may show extremely strong activity for one amino acid and a very weak reaction on all of the others. Another variation in activity occurs between the species which produce a decarboxylase active for the same amino acid. For example,

Ps. aeruginosa is an extremely strong producer of lysine decarboxylase, while the other five species producing lysine decarboxylase were moderate to weak in activity. Working with several strains of Bact. (Escherichia) coli, Gale (1940a) reported that in order to obtain linear decarboxylation, it was necessary to vary the amount of cells used in each manometer according to the amount of activity present. The enzyme-producing ability of the cells (where the potential is present) is extremely variable for all Pseudomonas species studied, indicating that the variation in occurrence as well as degree of activity apparently is characteristic of the organism and not the enzyme.

In studying any enzyme reaction, the question always arises regarding the coenzyme and its effect on the rate of activity. The decarboxylases are no exception, as many reports are available as to the source, composition and function of the codecarboxylases. Gale and Epps (1944) reported that a coenzyme was necessary for the action of decarboxylases on lysine, tyrosine, arginine and ornithine. Previous studies indicate that apparently no coenzyme is required for glutamic acid and histidine decarboxylases to be activated toward their substrates.

Several trials using pyridoxine in the casamino acids-glucose-phosphate medium indicate that this compound may have a small effect on increasing the enzyme activity for some amino

acids. Of the two organisms which produce five enzymes, Ps. viscosa and Ps. aeruginosa each showed some response to the added pyridoxine for only one decarboxylase. The two other organisms, Ps. putrefaciens and Ps. fluorescens, failed to show any increase in the decarboxylase activity due to the presence of pyridoxine in the growth medium.

Bellamy and Gunsalus (1944), working with the tyrosine decarboxylase of S. faecalis, reported the production of eight to ten times more enzyme when the cells were grown in a pyridoxine-fortified medium. The data in Table 18 show that no such response was obtained with any of the Pseudomonas species studied; in all cases the increase was less than one-fold. These questionable small increases in decarboxylase activity by some of the species also indicate that either these Pseudomonas species do not require the coenzyme or that the cells are capable of producing all of their metabolic requirements. Jensen (1951), growing Ps. aeruginosa in a medium containing milk as the amino acid source, found that large quantities of the various B-vitamins, including pyridoxine, were synthesized and stored within the cell.

In dairy products these ten species of Pseudomonas could be stimulated to produce decarboxylases which would be active on amino acids, whether the amino acids resulted from action of the organism itself or from proteolysis by other microorganisms.

The formation of the amine by decarboxylase activity may function as a protective mechanism against accumulations of hydrogen ions, as suggested by Hanke and Koessler (1924a). Since carbon dioxide is used by many bacteria, the amino acid decarboxylases may produce carbon dioxide for use by such organisms. Further, a reaction may result between the amine and other metabolic products, such as an alcohol; the resulting ester could be responsible for the aroma that characterizes many of the species studied. The flavor and aroma which are attributed to Pseudomonas when present in fluid milk, butter and cottage cheese may be due to the above mechanism.

SUMMARY AND CONCLUSIONS

Ten selected species of Pseudomonas, isolated from dairy products, were used for testing amino acid decarboxylase activity. The activity of the enzyme was determined by measuring with the Warburg respirometer the carbon dioxide liberated in the reaction between the decarboxylases and the substrates arginine, glutamic acid, histidine, lysine, ornithine and tyrosine. All of the amino acids used were of the L form.

The buffer selected for all determinations, whether in the growth medium or the Warburg reaction flasks, was Clark and Lubs phosphate. This phosphate buffer appeared to be much more beneficial in the growth medium than the Clark and Lubs phthalate or McIlvaine citrate-phosphate buffer. The phosphate buffer apparently did not exert as strong a buffering effect, thus allowing the pH to drop to lower levels during the growth of the cultures, thus the low pH is more conducive to higher decarboxylase production.

When the production of the enzyme was measured on various media, no activity occurred unless the specific amino acid substrate was added to or present in the growth medium. From these trials it is believed that the decarboxylases are adaptive enzymes produced only when the specific substratum is present in free form in the growth medium. However, many species lack the potential to produce certain of the decarboxylases, even under

otherwise very favorable conditions.

When incubation was at 18° C., a 24 hour culture of Ps. fragi gave higher decarboxylase values than at 15, 21 or 24° C. Incubation of Ps. aeruginosa cultures for 24 hours at 37° C. gave higher activity than at 25, 30 or 45° C. Incubation at 18° C. was adopted in all subsequent trials for all species having the lower optimum growth temperature.

Time trials at the optimum temperatures for production of the decarboxylase by various species showed that incubation for 24 hours at 18 or 37° C. (depending on the species tested) was conducive to the formation of more enzyme activity than 18, 30, 36 or 42 hours. Apparently the amino acid decarboxylases are not produced in appreciable quantities early in the growth cycle, but,as the cell populations for 24 and 30 hours indicate, the activity reaches maximum at about the time the active cell growth ceases.

The pH at which reaction of the various decarboxylases upon the six amino acids occurs ranges from pH 2.5 to 5.6, with the majority being optimally active for carbon dioxide liberation within the range of 3.25 to 4.25. As the reaction levels approached pH 6.0, the amount of enzyme activity decreased markedly. In no case were there measurable amounts of carbon dioxide liberated when the reaction level exceeded pH 6.0.

The distribution of the decarboxylases within the ten

species of Pseudomonas is extremely variable. Not only was the number of decarboxylases produced by each species unpredictable, but also the amount of activity for the different decarboxylases was variable within each species. Decarboxylase activity of some species was not strong for any of the amino acids employed; other species produced a very strongly active enzyme system for one amino acid and a very weakly active enzyme for another.

Ps. fluorescens, Ps. putrefaciens, Ps. cyanogenes and Ps. synxantha each produced only one decarboxylase; Ps. ichthyosmia produced two decarboxylases; Ps. graveolens produced three decarboxylases; Ps. fragi and Ps. mucidolens each produced four decarboxylases while Ps. aeruginosa and Ps. viscosa each produced five of the six decarbosylases for which tests were made.

The addition of pyridoxine, which had been reported as being required for the coenzyme effect, to the casamino acids-glucose-phosphate medium caused a very small increase in the production of some decarboxylases by Ps. viscosa and Ps. aeruginosa. In all cases where enchancement occurred, the increase in enzyme activity was less than one-fold.

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