their significance in relation to flavor and odor changes in irradiated meat may be evaluated.

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AMINO ACIDS IN FERMENTATION

Utilization of C^{14} Leucine and C^{14} Glycine by *Saccharomyces cerevisiae*

J. W. SPANYER, Jr., and ALAN T. THOMAS

Brown-Forman Distillers' Corp., Louisville 1, Ky.

The utilization of leucine and glycine by *Saccharomyces cerevisiae* was investigated using leucine-2-C^{14} and glycine-2-C^{14} in microfermentations. The amino acids were separated by monodimensional paper chromatography. The distribution of the tagged carbon was traced by radioautographs. The carbon skeleton of leucine, unlike that of glycine, is not utilized to any appreciable extent in the synthesis of other amino acids.

RECENT WORK (*4*) verifies that *Saccharomyces cerevisiae* deaminates and decarboxylates α-amino acids to form alcohols as indicated by Ehrlich and Neubauer and Formherz.

Thorne (*5,6*) has shown that differences exist in the utilization of amino acids, not only when tested individually, but when tested in the presence of other amino acids. He concluded that amino acids were integrated intact into yeast protein.

The authors (*4*) found that a relatively constant amount of leucine was unaccounted for in a leucine balance under their standardized conditions. While Gilvarg and Bloch (*2*) have shown that the carbon skeletons of some amino acids come from acetate carbon and some from glucose, during yeast metabolism, it is not improbable that the carbon skeleton of one amino acid is utilized in the synthesis of another amino acid.

For the investigation of the utilization of radioactively tagged amino acids by yeast, leucine and glycine were chosen, as they represent a well utilized and a poorly utilized amino acid (*6*).

Table I. Media for Test Tube Fermentations

Component	Medium 1, G.	Medium 2, G.
Dextrose	126	126
Monopotassium phosphate	0.055	0.055
Potassium chloride	0.0425	0.0425
Calcium chloride	0.0125	0.0125
Magnesium sulfate	0.0125	0.0125
Ferric chloride	0.0002	0.0002
Manganese sulfate	0.0002	0.0002
Yeast extract[a]	50 ml.	.....
Yeast culture	30 ml.	30 ml.
Demineralized water, make vol. to	1 liter	1 liter

[a] 80 grams of Difco yeast extract per liter of demineralized water.

Experimental

Materials and Equipment. Chromatographic chambers, one 28 × 28 × 28 inch and one 14 × 28 × 28 inch cabinet.

Chromatographic paper, Whatman No. 1, $18^1/_4 \times 22^1/_2$ inches.

Solvent systems (3). Phenol and water in the ratio of 4 to 1 and with 20 mg. of 8-quinolinol per 500 ml. of solvent. A beaker containing 0.3% ammonia was placed in the cabinet when this solvent was used.

2-Butanol and 3% ammonia in the ratio of 3 to 1.

2-Butanol, water, and formic acid in the ratio of 120 to 40 to 1.

Densitometer, Macbeth Ansco Model 12.

Film, Kodak, medical x-ray film, No-Screen, 11 × 14 inches.

Xerograph, Xerox, Model D, Haloid Co., Rochester, N. Y.

Leucine-2-C^{14}, Tracerlab, Inc., Boston, Mass.

Glycine-2-C^{14}, Tracerlab, Inc., Boston, Mass.

Fermentation. A series of fermentations was conducted in test tubes using 5 ml. of fermentation medium. The composition of the media used is given in Table I. The dextrose and mineral salts were dissolved, combined, and sterilized. The yeast extract was sterilized separately. The mineral salts were in the same ratio as in the previous work with 10- and 1-liter fermentations (*4*), but the concentration was cut in half. Tests indicated that yeast growth was not impaired at this lower mineral level. This reduction was merely a precaution to minimize the possibility of salt effects at high hydrolyzate concentrations in subsequent chromatographic analyses.

Sterilized stock solutions of leucine and of glycine were prepared. Aliquots of either the leucine or glycine solutions were pipetted into sterilized test tubes to give the desired amino acid levels, as shown in Table II. A standard solution of leucine-2-C^{14} was prepared, having an activity of 17.7×10^3 disintegrations per second per milliliter. The activity of a comparable glycine-2-C^{14} solution was 1.48×10^5 disintegrations per second per milliliter. Two milliliters of radioactive leucine were added to each tube in the leucine fermentation runs, while radioactive glycine was substituted in the glycine tests.

The solutions in the test tubes were evaporated to dryness on a water bath. The dextrose–mineral salt solution, yeast extract, demineralized water, and yeast were pipetted into the test tubes as listed in Table II, in the proportions as indicated in Table I, to give a final volume of 5 ml.

Each tube was carefully shaken by hand four times a day during the fermentation. After 72 hours of fermentation, the entire contents of each microfermentor was centrifuged to remove the yeast. The yeast solids were washed twice with sterile demineralized water. The centrifugate and washings were combined and evaporated to the original volume.

Ten-liter pilot fermentations with yeast extract and added leucine indicated a 94 to 97% utilization of the available sugar. Liter fermentations with glycine, without yeast extract, showed very little cell growth and only 10% utilization of the sugar. Sugars were not run on the microfermentors but comparison of the weight of yeast grown per milliliter indicated a nutrient utilization of 80 to 90% with added leucine and yeast extract. Removal of the yeast extract reduced the fermentation efficiency of the leucine-rich fermenters by about half.

The microfermentations of glycine without yeast extract compared very favorably with the results of the 1-liter fermentations. The weight of yeast indicated a fermentation efficiency of 10 to 14%. The results of glycine with yeast extract, which provided an available amino acid pool, were erratic.

Glycine fermentations were tested for methanol and formic acid by the spot test procedures of Feigl (*1*). The limit of identification for methanol was 3.5 γ at a dilution limit of 1 to 20,000. The limit of identification for formic acid, at a dilution limit of 1 to 20,000 was 1.4 γ. The results on both tests were negative.

Chromatographs. Both the yeast and centrifugate were acid hydrolyzed. The protein hydrolyzates were chromatographed as described in an earlier paper (*3*), except that the chromatograms were not developed with ninhydrin. The chromatographic sheets were 18 × 22½ inches while the x-ray film used in subsequent radioautography was 11 × 14 inches. This permitted the running of extra chromatograms on each sheet. The additional applications were spotted on either side of the ones to be radioautographed. These extra chromatograms were developed with ninhydrin and provided an indication of the quality and degree of development of the invisible portion. Various concentrations of the radioactive standard were applied in a series of spots on each sheet to serve as a quantitative guide in the determination of the radioactivity present in the chromatogram.

Radioautography. The trimmed chromatographs were carefully placed on 11 × 14 inch medical No-Screen x-ray films and were inserted in light-proof cardboard folders. The folders were loaded in a photographic darkroom with the only light source being a 10-watt bulb, shielded by a Wratten Series 6B filter. The light source must be a minimum of 4 feet from the point of film exposure. The film was exposed to the undeveloped radioactive chromatograms for 60 days before film development.

Table II. Amino Acid Level in Microfermentations

Fermentation[a] Number	Yeast Extract Added	DL-Leucine, Mg./Ml.	DL-Glycine, Mg./Ml.
5	Yes	0	...
6	Yes	0.3	...
7	Yes	0.6	...
8	Yes	1.2	...
9	No	0	...
10	No	0.3	...
11	No	0.6	...
12	No	1.2	...
17	Yes	...	0
18	Yes	...	0.3
19	Yes	...	0.6
20	Yes	...	1.2
21	No	...	0
22	No	...	0.3
23	No	...	0.6
24	No	...	1.2

[a] Prefix *MF* used in Figures 1 and 2 to indicate microfermentors. Each fermentation was run in quadruplicate so the letters *A* through *D* were added as a suffix to identify each individual fermentation.

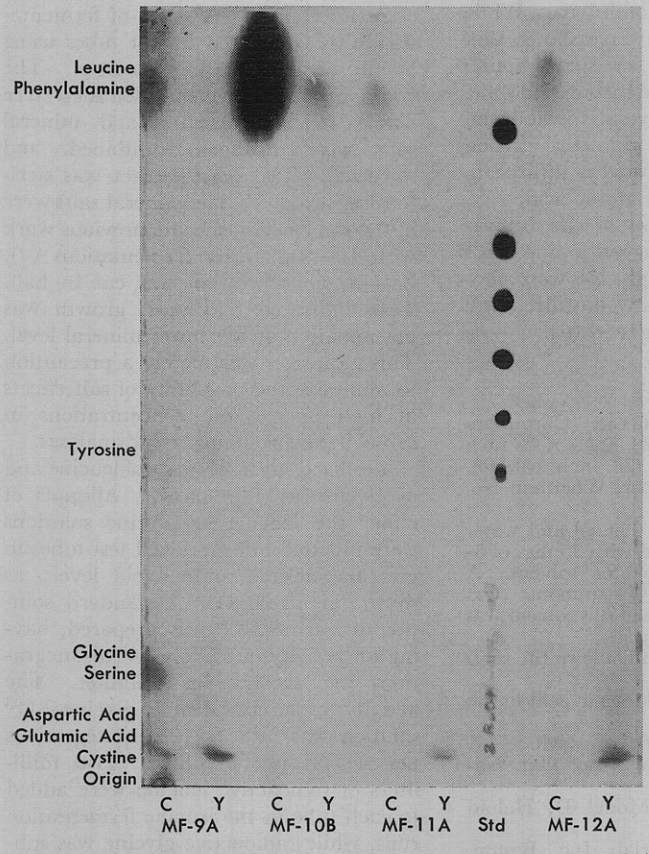

Figure 1. Typical radioautograph with leucine-2-C^{14}

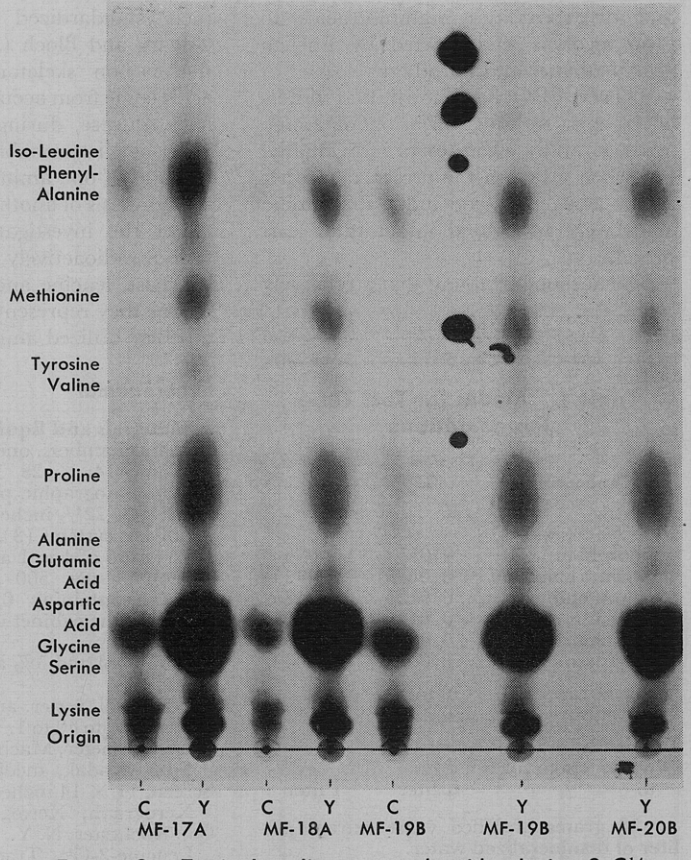

Figure 2. Typical radioautograph with glycine-2-C^{14}

Table III. Distribution of Leucine in Radioautographs

Fermenter	Yeast Extract Added								No Yeast Extract							
	5		6		7		8		9		10		11		12	
Sample	C[a]	Y[b]	C	Y	C	Y	C	Y	C	Y	C	Y	C	Y	C	Y
	Amino Acid Spot[c]															
Glycine									T[d]	..	..	..	..	..	..	..
Leucine	2	2	2	1	..	1	3	T	12	T	14	T	11	T	12	..
Tyrosine	1	..	1	..	..	..	..	..	..	1	..	..	..	..	..	1
Alanine-tyrosine									..	1	..	..	..	T	..	T
Arginine-histidine									..	T	..	..	..	T	..	T
Aspartic acid–glycine									2	T	..	..	..	T	..	T
Glycine-serine									2	..	..	..	..	..	..	..
Histidine-threonine	1	..	1	..	T	..	1	..			..	..	..	..	..	..
Leucine-phenylalanine	2	2	2	T	..	1	3	T	9	..	11	..	7	..	6	..
Aspartic acid–glutamic acid–cystine	T	..	T	..	..	..	T	..	T	2	T		..	1	..	1

[a] C indicates centrifugate.
[b] Y indicates yeast.
[c] Rating of spot is given as nearest multiple of background density of 0.15.
[d] T indicates presence of a trace.

Table IV. Distribution of Glycine in Radioautographs

Fermenter	Yeast Extract Added								No Yeast Extract						
	17		18		19		20		21		22		23		24
Sample	C[a]	Y[b]	C	Y	C	Y	C	Y	C	Y	C	Y	C	Y	Y
	Amino Acid Spot[c]														
Alanine	..	..	..	..	..	..	..	..	T[d]	2	..	..	T	..	T
Aspartic acid	..	..	..	..	..	..	..	..	2	..	3	T	3	..	..
Glutamic acid	..	..	..	..	..	..	..	..	..	2	T	2	..	2	1
Glycine	..	?[e]	..	?	..	?	..	?	..	2	1	4	..	5	3
Isoleucine	..	1	..	2	..	1	..	T	..	2	..	2	T	2	T
Leucine	..	..	..	..	..	..	..	..	..	2	..	2	T	2	1
Lysine	4	13	3	11	3	10	..	11	2	2	1	2	3	1	2
Methionine	..	2	..	2	..	2	..	2	..	T	..	T	..	T	T
Proline	..	3	..	2	..	2	..	2	1	1	2	2	1	2	1
Tyrosine	..	2	..	2	..	2	..	2	..	1		T	T	1	..
Alanine–glutamic acid	..	T	..	T	..	2	..	T	T	2	1	2	1	2	T
Alanine-tyrosine	..	..	..	..	..	..	..	..	..	T	T	2	T	2	T
Arginine-histidine	..	..	..	..	..	..	..	..	T	2	T	2	T	3	2
Cystine-serine	..	?	..	?	..	?	..	?	..	2	..	5	..	5	3
Glycine-serine	3	26	3	25	4	25	..	21	T	7	2	8	2	9	4
Histidine-threonine	3	?	2	?	4	?	..	?	..	2	T	2	T	2	T
Isoleucine-phenylalanine	2	3	T	2	2	2	..	2	..	T	..	2	..	2	1
Leucine-phenylalanine	..	..	..	..	..	..	..	..	..	2	T	2	T	3	T
Methionine-valine	..	2	..	2	..	2	..	T	T	2	T	2	T	2	1
Tyrosine-valine	..	2	..	1	..	T	..	T	..	2		2	..	2	..
Aspartic acid–cystine–glutamic acid	3	9	2	8	3	7	..	3	4	2	5	3	5	5	2
Aspartic acid–glycine-serine	4	23	3	23	5	24	..	19	2	7	3	12	2	8	5

[a] C indicates centrifugate.
[b] Y indicates yeast.
[c] Rating of spot is given as nearest multiple of background density of 0.15.
[d] T indicates presence of a trace.
[e] ? indicates presence of a spot that could not be read—e.g., too dark or blending with an adjacent spot.

The film densities were read on a Macbeth Ansco densitometer.

Xerographs of typical radioautographs are given in Figures 1 and 2. The distribution of amino acids in the radioautographs is as indicated in Tables III and IV.

Discussion

The radioautographs can be interpreted only qualitatively, but the spot densities of the various amino acids furnish a rough comparison of the amount of amino acid utilized. Table III shows that leucine was taken up, intact, by yeast in the presence of yeast extract, but not in its absence. There was very little activity in any of the amino acid spots except leucine. The carbon skeleton of leucine is not utilized to any appreciable extent in the synthesis of other amino acids; but, in the presence of a mixture of amino acids, it is presumably taken up in the formation of proteins and other nitrogenous compounds.

The carbon skeleton of glycine is utilized to some extent by yeast in the synthesis of amino acids (Table IV). The absence of methanol or formic acid in the fermented media indicated that deamination or decarboxylation of the glycine was improbable. These results, plus the wide distribution of radioactivity in the yeast protein, suggest that glycine is utilized as a carbon-carbon unit in amino acid formation. Fermentation with glycine, in the absence of yeast extract, is incomplete and retarded, yet the radioautographs indicated that appreciable amounts were incorporated into the yeast protein.

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