JOURNAL OF BACTERIOLOGY, June 1978, p. 913-919
0021-9193/78/0134-0913$02.00/0

Vol. 134, No. 3
Printed in U.S.A.

Rich Culture Medium for the Radiochemical Labeling of Proteins and Nucleic Acids

MAX P. OESCHGER

Department of Microbiology, Georgetown University, Washington, D.C. 20007

Received for publication 19 December 1977

Yeast extract was treated with tyrosine decarboxylase and used to prepare a rich, complex medium virtually free of tyrosine. The medium supported maximal growth rates for *Escherichia coli* prototrophs, as well as for defined and undefined auxotrophs. It has made possible the efficient radiochemical labeling of cells growing optimally in complex medium and the characterization of mutants with undefined requirements. Similarly prepared media may be useful for the study of fastidious organisms and organisms for which no defined medium has been described.

Oeschger and Berlyn found that many of the temperature-sensitive mutants of *Escherichia coli* which they isolated after mutagenesis carry uncharacterized nutritional requirements (13). This observation is not surprising, since Adelberg and others (1, 6, 7, 9, 11) observed that potent mutagens, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, have the potential to produce multiple mutations. These additional lesions hinder the genetic and biochemical characterization of the mutation of interest in the isolates, especially when a defined medium is required. This problem has been circumvented, with respect to the radiochemical labeling of cell proteins, by the preparation of a complex medium containing yeast extract deficient in one amino acid, tyrosine.

The tyrosine was removed from yeast extract by incubation with the enzyme tyrosine decarboxylase. This treatment selectively removed at least 98% of the tyrosine. Supplementation of a defined medium with the treated extract supported optimal growth of most prototrophic and auxotrophic strains of *E. coli* tested and enabled the efficient radiochemical labeling of their proteins. This medium can also be used for the efficient labeling of nucleic acids.

MATERIALS AND METHODS

Organisms. JC1552, a K-12 derivative isolated by A. J. Clark (2) and obtained from K. B. Low, was used for most of the experiments reported in this paper. The strain is a multiple auxotroph and requires leucine, tryptophan, histidine, arginine, and methionine for growth. Many of the experiments were also carried out with MX386 (14) and MX554, a P1 prophage-cured isolate of MX386 which also requires methionine, and MX399, a strain closely related to MX554 but derived from MX396 (14).

Media. The base medium for all liquid cultures consisted of medium E (15), supplemented to 0.2% glucose and 0.6 mM tryptophan, and 1 mM each leucine, isoleucine, valine, histidine, proline, threonine, arginine, aspartate, glycine, glutamate, serine, glutamine, asparagine, lysine, methionine, and alanine. Where indicated, this medium was further supplemented with either yeast extract or a tyrosine decarboxylase-treated preparation of yeast extract. Plate cultures were carried out on G agar, which was composed of 1% tryptone, 0.1% yeast extract, 0.25% NaCl, and 1.5% agar.

Preparation and treatment of yeast extract. A 20% (wt/vol) solution of yeast extract was prepared in deionized water by adjusting the pH to 5.5 with HCl. The particulate matter, often too fine to be visible to the naked eye, was removed by centrifugation (40,000 × *g* for 20 min), and the supernatant was collected and filter sterilized (0.45-μm nitrocellulose filter). Attempts to remove the particulate matter by filtration with paper designed to collect very fine precipitates (such as Whatman no. 50) were only partially successful. Tyrosine decarboxylase suspended in the same yeast extract solution was added directly to the filter-sterilized solution of yeast extract (25 U/500 ml) and incubated at 37°C without further sterilization. A significant amount of the tyrosine decarboxylase did not dissolve in the yeast extract solution. This material was better left in suspension because its removal (by filtration) dramatically reduced the rate at which the tyrosine was degraded. The reaction was terminated, after the particulate matter contributed by the tyrosine decarboxylase was removed (0.45-μm filtration), by heating at 121°C for 20 min. The treated yeast extract was stored at either −20°C (long-term storage) or 4°C (short-term storage).

Monitoring the removal of tyrosine from yeast extract solutions. Since tyrosine decarboxylase specifically releases the carboxyl carbon from the molecule (4), we followed the breakdown of tyrosine with [*carboxyl*-^{14}C]tyrosine added to portions of the reaction mixture. The initial rate of decarboxylation was assayed by a method adapted from that of Buhler (5). The portions were incubated in closed tubes, the reaction was terminated at the desired times by the addition of H_2SO_4, and the released CO_2 was trapped

in a piece of filter paper impregnated with Protosol. The amount of $^{14}CO_2$ released was then quantitated by counting the papers in scintillation fluid.

The extent of the reaction was monitored by taking 0.5-ml portions from a volume of the same reaction mixture containing [^{14}C]tyrosine as described above and transferring them directly to dry, empty scintillation vials. The samples were acidified by the addition of 25 μl of concentrated H_2SO_4 and incubated at room temperature for at least 1 h to allow for the release of $^{14}CO_2$. A 10-ml amount of Biofluor was added and the samples were counted.

Cultivation of cells. Liquid cultures, started from cells freshly grown at 25°C on G agar plates, were grown at 37°C with vigorous aeration. The initial density of the cultures never exceeded 10^8 cells per ml, and the cells were usually diluted at least one time and allowed to regrow before any experimental work began.

Incorporation of radiochemicals. Log-phase cultures were used for the isotope incorporation experiments. The incorporations were terminated by mixing samples with 10 volumes of ice-cold 5% trichloroacetic acid. The samples were held at 4°C for a minimum of 10 min, and the precipitates were collected on GFA (Whatman) filters and washed with 5% trichloroacetic acid and ethanol (twice with each). The disks were dried and counted.

Analytical procedures. Unless otherwise noted, all samples were counted on GFA filters in 5 ml of scintillation fluid {5.0 g of 2,5-diphenyloxazole (PPO) and 0.1 g of 1,4-bis[2]-(5-phenyloxazolyl)benzene (POPOP) per liter of toluene} with an Intertechnique scintillation spectrometer. The cell densities were monitored at 600 nm with a Coleman Junior II spectrophotometer, and cell numbers were calculated from a standard curve relating absorbance to colony-forming units.

Chemicals. Yeast extract (lot no. 575449 and 590523) and tryptone were purchased from Difco Laboratories. Tyrosine decarboxylase (lot no. 45A151) was purchased from Worthington Biochemicals. L-Amino acids of the highest purity were purchased from Sigma Chemical Co. [*U*-^{14}C]tyrosine (specific activity, ca. 500 mCi/mmol) was a product of Amersham/ Searle, and [*carboxyl*-^{14}C]tyrosine (specific activity, 54.6 mCi/mmol) and [^{3}H]uridine (specific activity, 36.7 Ci/mmol) were products of New England Nuclear Corp. New England Nuclear also supplied the POPOP, PPO, Protosol, and Biofluor used in this work. All other chemicals were of reagent grade.

RESULTS

Preparation of digested yeast extract. Yeast extract solutions (20%) were prepared for decarboxylation as described above. The progress of the decarboxylation was monitored by addition of [*carboxyl*-^{14}C]tyrosine to portions of the yeast extract-tyrosine decarboxylase suspension. The initial rate of decarboxylation was determined by capturing and quantitating the $^{14}CO_2$ released. Projections from these results suggested that, under the conditions used, all of the tyrosine would be removed within 24 h. Direct measurement showed that only 2% of the label remained in the yeast extract at this time. Further incubation did not reduce the level of residual label, nor did the addition of more enzyme, more nonradioactive tyrosine, or more undigested yeast extract. I then confirmed, by observing the rapid decarboxylation of additional [*carboxyl*-^{14}C]tyrosine introduced into a portion of the reaction mixture, that the tyrosine decarboxylase retained activity. These results indicate that the residual radioactivity might not have been tyrosine and that the removal of tyrosine was at least 98% complete. The treatments were routinely carried out for 24 to 36 h to ensure that the reaction had gone to completion.

The reaction was terminated by heating at 121°C. Incubation of a portion of the heat-treated medium with [*carboxyl*-^{14}C]tyrosine resulted in the release of less than 0.05% of the radioactivity in 16 h, indicating that little or no tyrosine decarboxylase activity remained after this treatment.

The treated medium was stored at either −20 or 4°C and its pH was adjusted just before use. Once the pH was raised into the range of 6.8 to 7.2, the 20% yeast extract solutions slowly accumulated precipitate.

Chemical characterization. Amino acid analyses of yeast extract before and after treatment confirmed that the majority of the tyrosine had been removed (Table 1). More importantly, the incubation of yeast extract with tyrosine decarboxylase had little or no effect on the levels of the other amino acids (Table 1).

Biological characterization. Medium E, supplemented with dextrose and amino acids as described in Materials and Methods, was used as the base medium for all of the experiments. The addition of yeast extract to this base medium markedly enhanced the rate of growth of *E. coli* cells, reducing the mean generation times by 25 to 30% (Table 2). The treatment of the yeast extract had no effect on its ability to stimulate the growth of the bacterial strains tested (Table 2). Full stimulation of growth was obtained with 0.2% yeast extract supplementation, and concentrations up to 0.8% did not affect this rate (Table 3). A 25-fold dilution of exponentially growing cells with base medium supplemented with 0.8% treated yeast extract produced no lag or alteration in growth rate (data not shown). These results indicate that treatment of the yeast extract with tyrosine decarboxylase did not remove any growth-stimulating component(s) or generate any toxic product(s).

I next compared the incorporation of radiolabeled tyrosine into cells growing in the base medium and in the base medium supplemented

to 0.4% treated or untreated yeast extract. The medium supplemented with the treated yeast extract was superior in tyrosine incorporation to both the medium supplemented with the untreated yeast extract and the base medium without supplementation (Fig. 1). Hence, supplementation of the defined medium with treated yeast extract not only enhanced the rate of cell growth, but, for reasons that are not clear, stimulated to an even greater extent the rate of [^{14}C]tyrosine utilization (cf. Table 2 and Fig. 1). Incorporation began immediately after the addition of tyrosine, and the rate remained constant until at least 50% of the tyrosine was incorporated (Fig. 1). The initial rate of tyrosine incorporation by cells which had been cultured in the medium was proportional to cell density (Fig. 2). In contrast, just after dilution with fresh treated medium, the incorporation was not proportional to cell density (Fig. 3), whereas a similar dilution with treated medium which had previously been used for cell culture gave proportional results (data not shown). I conclude from these results that the treated yeast extract contained either residual tyrosine or an inhibitor of tyrosine uptake which was removed by growing cells.

TABLE 1. *Amino acid composition of untreated and tyrosine decarboxylase-treated yeast extract*

Amino acid	Relative concn[a] (nmol/ml)	
	Untreated[b]	Treated[b]
Ala	185.04	182.95
Arg	911.66	803.33
Asp	49.06	48.73
Glu	96.34	94.59
Gly	44.95	44.55
His	0.47	0.49
Ile	40.90	39.73
Leu	68.64	66.61
Lys	47.28	45.76
Met	16.01	15.64
Phe	33.05	29.45
Pro	61.73	80.00
Ser	67.26	66.37
Thr	36.31	36.27
Trp	2.61	2.70
Tyr	6.25	—[c]
Val	2.70	2.58

[a] Values were obtained with 1:2,000 dilutions of 20% yeast extract solutions. The determinations were made with a Beckman model 121-M amino acid analyzer fitted with an Autolab printing integrator.

[b] Before analysis, samples of the 20% yeast extract solutions were precipitated with equal volumes of 10% trichloroacetic acid. After the solutions stood at 4°C for 20 min, any precipitate which may have formed was removed by centrifugation (none was detected). The trichloroacetic acid was removed by three water-saturated diethyl-ether extractions, and the residual ether was removed by evaporation. Trichloroacetic acid-precipitated samples were diluted an additional 1,000-fold before analysis.

[c] —, Not detectable.

Figure 4 shows the effects of the level of yeast extract supplementation on the rate of incorporation of added radiochemically labeled tyrosine. The culture growing in medium supplemented with 0.8% treated yeast extract showed a reduced rate of uptake compared with that of the companion cultures, but no change in the extent of incorporation (Fig. 4a). After an additional cell doubling, this effect disappeared (Fig. 4b). These results are consistent with the treated yeast extract containing an inhibitor of tyrosine uptake which was removed by cell growth. In addition, the medium must contain some residual tyrosine because tyrosine auxotrophs of *E. coli* formed microcolonies on plates supplemented with treated yeast extract. An estimate of the maximal amount of residual tyrosine in the treated yeast extract can be made from the data in Fig. 3 by assuming that the reduction in the rate of incorporation was due solely to residual tyrosine and not to an inhibitor of tyrosine uptake. A plot of the dilution of the specific activity of the [^{14}C]tyrosine (calculated by dividing the maximum relative incorporation per cell per minute by the observed relative incorporation per cell per minute) versus the amount of treated yeast extract added gave a straight line. The slope of the line gives the level of tyrosine in the treated medium and indicated that, at

TABLE 2. *Effect of yeast extract supplementation of the defined base medium[a] on the mean generation times of three E. coli strains*

Yeast extract added (%)	Mean generation time (min)[b] for:					
	JC1552		MX554		MX399	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
None	40.0 ± 0.7	40.0 ± 0.7	48.0 ± 1.6	48.0 ± 1.6	50.0 ± 4.0	50.0 ± 4.0
0.4	31.7 ± 1.8	28.7 ± 0.4	32.5 ± 0.4	33.5 ± 0.4	36.7 ± 0.8	36.0 ± 1.4
0.8	26.0 ± 0.8	27.0 ± 2.0	32.0 ± 0.8	32.0 ± 0.0	35.0 ± 0.8	36.0 ± 0.8

[a] The defined base medium was as described in the text.

[b] Average values from three or four independent determinations of log-phase cultures growing at 37°C.

TABLE 3. *Effect of the amount of treated yeast extract supplementation of the defined base medium[a] on the mean generation time of strain JC1552*

Amt of treated yeast extract (%)	Mean generation time (min)[b]
0	40.0 ± 0.7
0.1	34.0 ± 2.5[c]
0.2	28.0 ± 1.6
0.3	28.0 ± 1.4
0.4	29.0 ± 0.8
0.6	29.0 ± 1.2
0.8	27.5 ± 0.4

[a] The defined base medium was as described in the text.

[b] Average values from three independent determinations of log-phase cultures growing at 37°C.

[c] This generation time was maintained throughout log-phase growth.

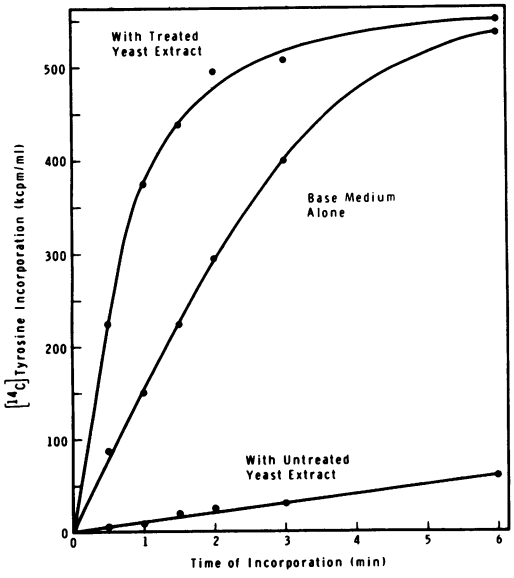

FIG. 1. *Incorporation of [^{14}C]tyrosine by strain JC1552. JC1552 was grown at 37°C in base medium alone and in base medium supplemented to 0.4% with either treated or untreated yeast extract. When the cultures reached a density of 4 × 10^8 cells per ml, 10 μl of tyrosine (50 μCi/ml) was added per 0.8-ml cell culture. At the times indicated, 0.1-ml portions of the culture were withdrawn and added to ice-cold 5% trichloroacetic acid, and the precipitates were collected, washed, and counted as described in the text.*

most, 4.3 nmol of tyrosine remained per ml of 0.4% treated yeast extract. Growth to 4 × 10^8 cells per ml from an inoculum of 1 × 10^8 cells per ml (hence a net increase of 3 × 10^8 cells per ml) was sufficient to remove any residual tyrosine and inhibitors of tyrosine uptake from 0.4% yeast extract solutions (Fig. 2 and 4).

When more radioactive tyrosine was added, more time was required for 50% incorporation (Fig. 5). The line connecting the points in Fig. 5 did not extrapolate to zero, but to a value indicating the presence of an amount equivalent to 1.5 nmol of nonradioactive tyrosine per ml. This value should approximate the size of the intracellular tyrosine pool, because the cells had been grown to a level where all extracellular tyrosine should have been exhausted. In terms of carrying out efficient pulse-labeling experiments, the level of tyrosine in the cell did not pose a problem. When as little as 0.6 nmol of radioactive tyrosine was added per ml of culture, it was rapidly and efficiently incorporated, with 50% utilization taking place in approximately 30 s (Fig. 5).

Another feature of the medium supplemented with treated yeast extract was that it permitted the radiochemical labeling of RNA with uridine. Although increasing levels of yeast extract supplementation decreased the rate of uridine incorporation, the rates remained sufficiently high for most experimental procedures (Fig. 6).

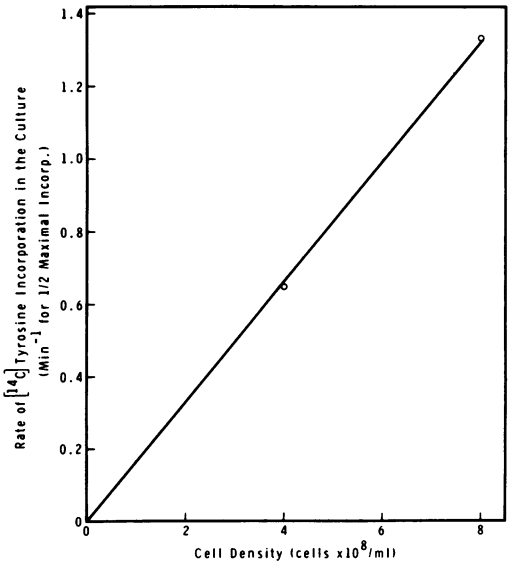

FIG. 2. *Rate of [^{14}C]tyrosine incorporation as a function of cell density. Strain JC1552 was grown at 37°C in base medium supplemented to 0.4% treated yeast extract from initial inocula of 10^8 cells per ml. Ten microliters of [^{14}C]tyrosine (50 μCi/ml) was added to parallel 0.8-ml cultures when they reached densities of 4 × 10^8 and 8 × 10^8 cells per ml. Portions (0.1 ml each) were removed at the times indicated in Fig. 1 and were treated with 5% trichloroacetic acid; the precipitates were collected, washed, and counted as described in the text. Each rate of incorporation was determined by taking the reciprocal of the time required for half-maximal incorporation.*

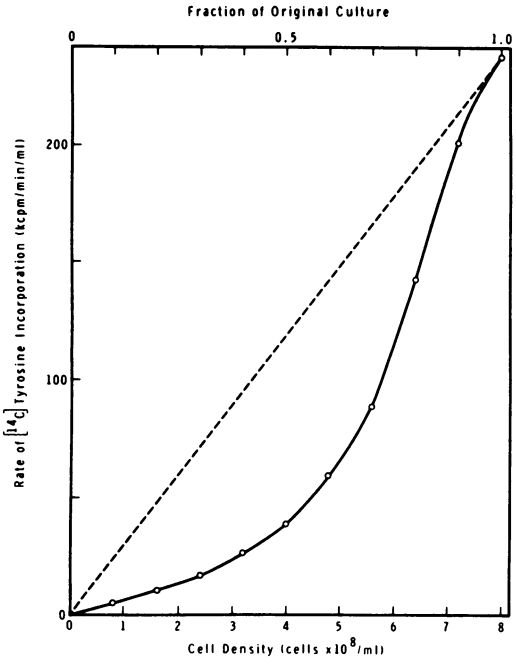

FIG. 3. *Effect of cell density and medium on [^{14}C]tyrosine incorporation. Strain JC1552 was grown at 37°C in base medium supplemented to 0.4% treated yeast extract to a density of 8 × 10^8 cells per ml. Portions of the culture were diluted, as indicated on top of the figure, into fresh, prewarmed 0.4% treated medium. Cell densities after dilution are indicated at the bottom of the figure. After an equilibration time of 3 min, 4 μl of [^{14}C]tyrosine (50 μCi/ml) was added to each of the cultures (0.5 ml). Incorporation was terminated after 1 min by the addition of 5% trichloroacetic acid. The precipitates were collected, washed, and counted as described in the text. (-----) Curve expected if the medium were free of tyrosine or an inhibitor of tyrosine uptake.*

DISCUSSION

Choice of amino acid. Tyrosine was chosen for this work for a number of reasons. First, tyrosine is a biochemical end product and not normally an intermediate in any microbial metabolic pathway; thus the label in tyrosine is transferred specifically into protein. Second, tyrosine is rapidly taken up and efficiently utilized by prototrophic strains of *E. coli* (J. Majors and M. P. Oeschger, unpublished data). This allows the use of prototrophic strains of *E. coli* in protein labeling experiments without requiring washings, cell transfers, or other special manipulations. Third, tyrosine can be removed from complex mixtures by a gentle, specific, and simple enzymatic procedure employing tyrosine decarboxylase. This enzyme is active under mild conditions (pH 5.5) and has no special requirements (such as a metal, which would remain to adulterate the treated medium). The enzyme does require pyridoxal phosphate (10), but, when prepared from cells grown in the presence of pyridoxine derivatives, the enzyme is fully active without any special additions (3, 4). One of the products of its reaction, CO_2, is volatile, and so under acidic conditions the reaction is driven toward completion. The other product, tyramine, does not appear to interfere with the uptake or utilization of exogenous tyrosine. The tyrosine decarboxylase preparation itself does not contribute significantly to the composition of the medium since only minute amounts of the enzyme are required. Finally, tyrosine is commercially available as a radiochemical with high specific activity (both ^{3}H and ^{14}C), which makes it an effective amino acid for protein labeling. Since tyrosine contains nine carbon atoms, a higher ^{14}C specific activity can be obtained with uniformly labeled tyrosine than with most of the other amino acids. This fact helps to compensate for the lower-than-average molar abundance of tyrosine in most proteins (12).

Composition of the complex medium. Initially, I attempted to pattern the treated medium after R broth (8), a medium routinely used in my laboratory. Preliminary experiments with the two complex components of the medium, yeast extract and tryptone (Difco), showed incomplete decarboxylation of the tyrosine in the tryptone. Oeschger and Berlyn (unpublished data) found that they could enzymatically remove the tyrosine from Casamino Acids (Difco) and therefore substituted Casamino Acids for tryptone. Treated yeast extract-Casamino Acids medium (13) was used for some time until a batch of Casamino Acids (Difco lot no. 601802) was obtained which apparently contained an inhibitor of tyrosine decarboxylase. A mixture of amino acids, as listed in Materials and Methods, was then substituted for the Casamino Acids. Yeast extract and amino acids at the levels used do not possess strong buffering potential, so the medium presented here was based on a well-buffered, chemically defined medium, medium E. The defined medium, when supplemented with glucose or glycerol, satisfies the carbon, nitrogen, and ionic requirements of the cells and so permits latitude in the level of yeast extract supplementation. Other defined media may be substituted.

The problems associated with the removal of tyrosine from tryptone and Casamino Acids have never been encountered with yeast extract. (The numbers of two recently used lots are listed in Materials and Methods.) The base medium supplemented with treated yeast extract has been used for the successful culture of hundreds

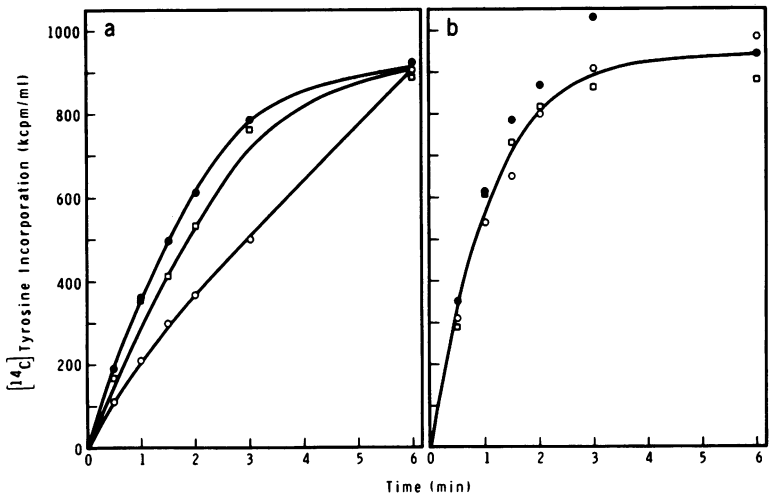

FIG. 4. *Effects of cell density and concentration of treated yeast extract on [^{14}C]tyrosine incorporation. Strain JC1552 was grown at 37°C in the base medium supplemented to 0.1%, 0.4%, or 0.8% treated yeast extract from inocula of 10^8 cells per ml. Ten microliters of [^{14}C]tyrosine (50 μCi/ml) was added to each of the cultures (0.8 ml) and, at the times indicated, 0.1-ml portions were removed to 5% trichloroacetic acid. The precipitates were collected, washed, and counted as described in the text. Cultures were at a density of (a) 4×10^8 or (b) 8×10^8 cells per ml. Symbols: (●) 0.1%, (□) 0.4%, and (○) 0.8% yeast extract supplementation.*

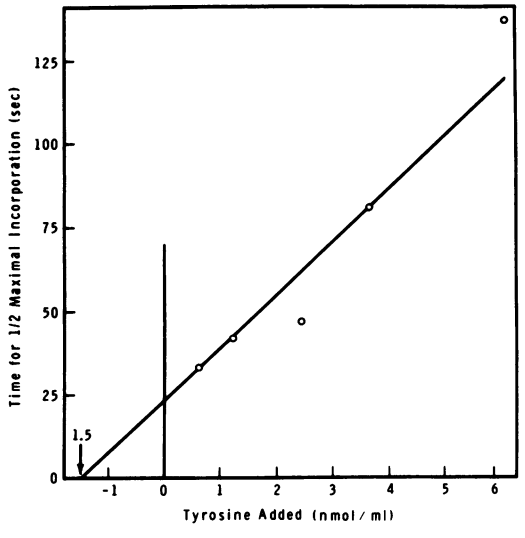

FIG. 5. *Effect of the amount of tyrosine added on the rate of tyrosine incorporation. Strain JC1552 was grown in base medium supplemented to 0.4% with treated yeast extract. When the culture density reached 8×10^8 cells per ml, 5, 10, 20, 30, or 50 μl of [^{14}C]tyrosine (50 μCi/ml) was added to each of the five parallel 0.8-ml cultures, and 0.1-ml portions were removed at the times indicatd in Fig. 1 to 5% trichloroacetic acid. The precipitates were collected, washed, and counted as described in the text. Each time for one-half of maximal incorporation was determined from plots of incorporation versus time.*

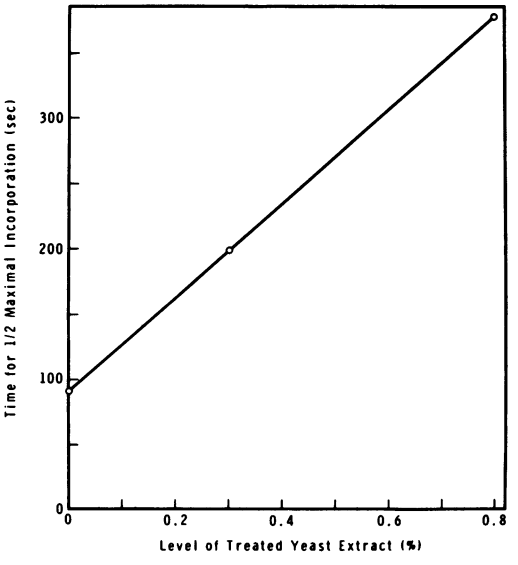

FIG. 6. *Effect of the level of treated yeast extract on the rate of [^{3}H]uridine incorporation. Strain MX399 was grown in base medium alone and base medium supplemented to 0.3 and 0.8% with treated yeast extract. When the cultures reached a density of 6×10^8 cells per ml, 10 μl of [^{3}H]uridine (1 mCi/ml) was added to each of the three 1.0-ml cultures. At various times 0.1-ml portions were withdrawn to 5% trichloroacetic acid. The resulting precipitates were collected, washed, and counted as described in the text.*

of nitrosoguanidine-induced mutant strains of *E. coli* (G. T. Wiprud and M. P. Oeschger, unpublished data). Many of these isolates cannot be cultured in defined medium with either amino acid or Casamino Acids supplementation alone.

Properties of the treated yeast extract. Although chemical analysis of the treated yeast extract suggested that all of the tyrosine had been removed, biological characterization indicated that a small amount remained (up to 4.3 nmol/ml of medium containing 0.4% treated yeast extract). The residual tyrosine and any components which might inhibit the uptake of tyrosine were removed by growing cells in the medium. An increase of less than 3×10^8 cells per ml apparently removed these components from media containing up to 0.4% treated yeast extract. Higher levels of yeast extract required additional cell growth before optimal incorporation was obtained (Fig. 4).

Specific advantages of the medium. The removal of tyrosine from yeast extract as described in this report provides a simple and inexpensive way to prepare a very rich medium for specific radiochemical labeling. This medium will support a broad range of auxotrophic strains, including nutritional mutants which arise as a result of mutagenesis. The medium also supports a maximal growth rate, thus permitting experiments which require extensive cell multiplication to be carried out easily in one day, even at nonoptimal temperatures. The medium also allows radiochemical labelings for the study of the physiology and gene expression of cells growing optimally in a rich, complex medium.

Yeast extract is known to contain limiting amounts of thymine (K. B. Low, personal communication), and media containing yeast extract have been used without special treatment for the labeling of DNA with thymidine (J. E. Cronan, Jr., personal communication). I have shown that RNA can be efficiently labeled with uridine in treated, yeast extract-supplemented medium. These experiments have been successfully carried out with prototrophic strains. Thus, the same complex medium can be used for the labeling of DNA, RNA, and proteins.

The enzymatic depletion of a specific component in a complex medium could be used to prepare media for the labeling of fastidious microorganisms and organisms for which no defined medium has been described. With the appropriate enzymes, an arginine- and lysine-free complex medium could be prepared which would be useful for the radiochemical analyses of peptides after tryptic digestion. It is clear that this medium and others like it may be useful in many areas of experimental work.

ACKNOWLEDGMENTS

I thank J. E. Cronan, Jr., for suggesting the use of enzymes to remove amino acids from complex hydrolysates; Patrice Cuccaro, Gary Theall, and Maureen Beurskens for their help in carrying out some of the experiments; Patrice Cuccaro for her help in drafting the manuscript; Anne Morris Hooke for critically reading the manuscript and Lou Henderson for performing the amino acid analyses.

This work was supported by grant BMS-75-21305 from the National Science Foundation.

LITERATURE CITED

1. **Adelberg, E. A., M. Mandel, and G. C. C. Chen.** 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788–795.
2. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
3. **Bellamy, W. D., and I. C. Gunsalus.** 1943. Growth requirements of *Streptococcus fecalis* for tyrosine decarboxylation. J. Bacteriol. **46**:573.
4. **Bellamy, W. D., and I. C. Gunsalus.** 1944. Tyrosine decarboxylation by streptococci: growth requirements for active cell production. J. Bacteriol. **48**:191–199.
5. **Buhler, D. R.** 1962. A simple scintillation counting technique for assaying $^{14}CO_2$ in Warburg flasks. Anal. Biochem. **4**:413–417.
6. **Calendar, R., B. Lindquist, G. Sironi, and A. J. Clark.** 1970. Characterization of *rep* mutants and their interaction with P2 phage. Virology. **40**:72–83.
7. **Clarke, S. J., B. Low, and W. H. Konigsberg.** 1973. Close linkage of the genes *serC* (for phosphohydroxy pyruvate transaminase) and *serS* (for seryl-transfer ribonucleic acid synthetase) in *Escherichia coli* K-12. J. Bacteriol. **113**:1091–1095.
8. **Duberstein, R., and M. P. Oeschger.** 1973. Growth of bacteriophage H on male and female strains of *Escherichia coli*. J. Virol. **11**:460–463.
9. **Guerola, N., J. L. Ingraham, and E. Cerda-Olmedo.** 1971. Induction of closely linked multiple mutations by nitrosoguanidine. Nature (London) New Biol. **230**:122–125.
10. **Gunsalus, I. C., W. D. Bellamy, and W. W. Umbreit.** 1944. A phosphorylated derivative of pyridoxal as the coenzyme of tyrosine decarboxylase. J. Biol. Chem. **155**:685–686.
11. **Hirota, Y., F. Jacob, A. Ryter, G. Buttin, and H. Nakai.** 1968. On the process of cellular division in *Escherichia coli*. I. Asymmetrical cell division and production of deoxyribonucleic acid-less bacteria. J. Mol. Biol. **35**:175–192.
12. **Lehninger, A. L.** 1970. Biochemistry, p. 93. Worth Publishers Inc., New York.
13. **Oeschger, M. P., and M. K. B. Berlyn.** 1975. Regulation of RNA polymerase synthesis in *Escherichia coli*: a mutant unable to synthesize the enzyme at 43°. Proc. Natl. Acad. Sci. U.S.A. **72**:911–915.
14. **Oeschger, M. P., and S. L. Woods.** 1976. A temperature-sensitive suppressor enabling the manipulation of the level of individual proteins in intact cells. Cell **7**:205–212.
15. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.