

protein allowed to flocculate with subsequent centrifugation. Standards received the same ingredients as the experimental tubes but the trichloroacetic acid was added directly after the thiamine and incubation was omitted. Controls were run with the fish suspension subjected to 100° for 20 minutes prior to the addition of the thiamine followed by incubation. For assay two 4 ml samples of the resulting supernatant were used. A simplified modification¹² of the Melnick and Field¹³ colorimetric diazonium method was employed to determine residual thiamine. It was found that the zeolite adsorption could be omitted without interference of color formation leading to erroneous analyses providing the absorption of light by the xylene extract was measured in a Klett-Duboscq visual colorimeter.

None of the 4 species of marine teleosts tested showed the presence of thiaminase in a saline suspension of its minced viscera. A similar suspension of whole minced quahogs (*Venus mercenaria*), which has already been shown to contain thiaminase,⁹ destroyed 33% of the added thiamine in 2 hours. No appreciable heat-destruction of thiamine was found

to occur under the conditions of the incubation.

The absence of thiaminase in most marine teleosts is still inexplicable. In the 13 species so far investigated only the herring, *Clupea harengus*, has been demonstrated to contain the enzyme. The conclusion of Deutsch and Hasler⁶ that the whiting does not contain thiaminase has been confirmed. In their experiments with cod and haddock, Deutsch and Hasler⁶ used eviscerated fish. Since the enzyme occurs primarily in the viscera, if at all, this leaves the status of these fish doubtful. Explanation of the occurrence of thiaminase in some fish and not in others may rest on a phylogenetic basis as has been suggested.⁶ In that case extensive investigation of additional marine fish as well as freshwater, estuarine, and marine invertebrates and primitive chordates is requisite.

Summary. In 4 species of marine teleosts no thiaminase was found according to the procedure described. This brings to a total of 12 the marine teleosts in which there appears to be no thiaminase. In only one species, the Atlantic herring, has its presence been demonstrated.

¹² Hoehberg, M., and Melnick, D., *J. Biol. Chem.*, 1944, **150**, 53.

¹³ Melnick, D., and Field, H., Jr., *J. Biol. Chem.*, 1939, **127**, 515.

I wish to thank Captain Jared Vincent for supplying me with the Atlantic whiting used in this investigation.

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Utilization of Certain Rare Sugars by Microorganisms.

CHESTER M. MCCLOSKEY AND J. R. PORTER.*

From the Departments of Chemistry (Organic Division) and Bacteriology, The State University of Iowa, Iowa City.

The use of common sugars and alcohols in bacteriological work to differentiate and characterize species is well known. However, some of the so-called "rare" sugars and their derivatives have been used much less frequently for such purposes.¹ The failure to employ such substances in the past has been due in

part to their high cost, or to the fact that they have been unavailable on the market.

In connection with other studies in the Division of Organic Chemistry at the State University of Iowa, several unusual sugars were prepared in sufficient quantities to permit their use in a few bacteriological studies. To

* We wish to acknowledge and thank Professor George H. Coleman for his help in the preparation of some of the sugars used in this study.

¹ See for example, Koser, S. A., and Saunders, F., *J. Bact.*, 1933, **26**, 475; Sternfeld, L., and Saunders, F., *J. Am. Chem. Soc.*, 1937, **59**, 2653.

TABLE I.
Utilization of Unusual Sugars in 24-72 Hr by Certain Microorganisms.

Organism	Turanose	Tagatose	Neolactose	Primeverose	Vicianose	β - β -Trehalose	$\pm$ (β -D-glucosyl)- D-mannose	2-methyl-glucose	3-methyl-glucose	6-methyl-glucose	Inositol	α -Schardinger Dextrin	β -Schardinger Dextrin
<i>Esch. coli</i>	AG	—	—	—	AG	AG	AG	—	—	—	—	—	—
<i>Esch. coli</i>	—	AG	—	—	—	—	AG	—	—	—	—	—	—
<i>Esch. coli</i> -v- communior	AG	—	—	—	—	—	AG	sl.A	—	—	—	—	—
<i>A. aerogenes</i>	—	A	—	—	—	—	—	—	—	—	—	—	—
<i>A. oxytocolum</i>	AG	AG	—	AG	AG	AG	AG	—	—	—	—	—	—
<i>K. pneumoniae</i> "995"	—	AG	—	—	—	AG	AG	—	—	—	—	—	—
<i>K. pneumoniae</i> "1090"	—	AG	—	—	AG	AG	AG	—	—	—	—	—	—
<i>K. pneumoniae</i>	—	—	—	—	AG	A	AG	—	—	—	—	—	—
<i>Paracolon bacillus</i>	—	—	—	—	—	AG	AG	—	—	—	—	—	—
<i>E. typhosa</i> "86"	—	A	—	—	—	—	—	—	—	—	—	—	—
<i>E. typhosa</i> "H"	—	A	—	—	—	—	—	—	—	—	—	—	—
<i>E. typhosa</i> "O"	—	sl.A	—	—	—	—	—	—	—	—	—	—	—
<i>S. paratyphosa</i>	—	AG	—	—	—	—	—	—	—	—	—	—	—
<i>S. schotmuellerii</i>	—	AG	—	—	—	—	—	—	—	—	—	—	—
<i>S. schotmuellerii</i>	—	AG	—	—	—	—	—	—	—	—	—	—	—
<i>S. enteritidis</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>S. typhimurium</i>	—	AG	—	—	—	—	—	—	—	—	—	—	—
<i>Sh. dysenteriae</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Sh. paratyphenteriae</i> "Flexner"	—	A	—	—	—	—	—	—	—	—	—	—	—
<i>Sh. paratyphenteriae</i> "Hiss Y"	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Sh. paratyphenteriae</i> "Sonne"	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Sh. paratyphenteriae</i> "Strong"	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Bacillus macerans</i>	AG	—	sl.A	AG	AG	AG	AG	—	—	—	—	AG	AG
" polymyxa (3 strains)	AG	—	A	AG	AG	AG	AG	—	—	—	—	AG	AG
<i>Saccharomyces cerevisiae</i>	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Carlsbergensis</i>	sl.A	—	—	—	—	—	—	—	—	—	—	—	—
<i>Bayensis</i>	sl.A	—	—	—	—	—	—	—	—	—	—	—	—
<i>globosus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—

A—Acid; sl.A.—slight acid; AG—Acid and gas.

our knowledge these compounds have not been employed previously in this respect. Thus the present report is a brief communication dealing with the utilization of certain rare sugars and sugar derivatives by microörganisms.

Methods. Thirteen sugars were available for this study. Vicianose,² primeverose,³ levoglucosan,³ and β,β -trehalose⁴ were all prepared by deacetylation of their acetates. Tagatose,⁵ 2-methyl-D-glucose,⁶ 3-methyl-D-glucose,⁷ 6-methyl-D-glucose,⁸ neolactose,⁹ 4-(β -D-glucosyl)-D-mannose,¹⁰ and turanose¹¹ were synthesized according to technics described in the literature. The α - and β -Schardinger dextrans were kindly furnished by Dr. T. J. Shoch of Corn Products Refining Co., Argo, Illinois. Concentrated solutions of the sugars were prepared separately in distilled water, and sterilized by passing through sintered glass filters. The concentrations were such that upon adding 0.1 ml of the sterile solution to 4.9 ml of previously sterilized meat-infusion broth (pH 7.2) a final concentration 0.2 or 0.5% sugar resulted.

Eighteen bacterial species and 4 yeasts were used in this work. The bacteria were stock strains, and the yeasts were obtained from Dr. L. J. Wickerham of the Northern Regional Laboratory in Peoria, Illinois.

The utilization of the sugars was determined

² McCloskey, C. M., and Coleman, G. H., *J. Am. Chem. Soc.*, 1943, **65**, 1778.

³ McCloskey, C. M., Kirby, R., and Coleman, G. H., *Ind. Eng. Chem.*, 1944, **36**, 1040.

⁴ McCloskey, C. M., Pyle, R. E., and Coleman, G. H., *J. Am. Chem. Soc.*, 1944, **66**, 349.

⁵ Reichstein, T., and Bosshard, W., *Helv. Chim. Acta.*, 1934, **17**, 753.

⁶ Lieser, T., and Leekzyck, E., *Ann.*, 1934, **511**, 137.

⁷ Sundberg, R. L., McCloskey, C. M., Rees, D. E., and Coleman, G. H., *J. Am. Chem. Soc.*, 1945, **67**, 1080.

⁸ Bell, D. J., *J. Am. Chem. Soc.*, 1936, **58**, 859.

⁹ Richtmyer, N. K., and Hudson, C. S., *J. Am. Chem. Soc.*, 1935, **57**, 1716.

¹⁰ Isbell, H. S., *J. Research Nat. Bur. Standards*, 1930, **5**, 1185.

¹¹ Hudson, C. S., and Paesu, E., *J. Am. Chem. Soc.*, 1930, **52**, 2522.

by the conventional method of detecting acid and gas production; bromocresol purple was employed as the indicator of acid formation, and a Durham tube was used to detect gas. The bacteria were incubated at a temperature of 37°C, and the yeasts at 25° to 30°C. All tubes were observed for a period of one week.

Results. The results of this study are briefly summarized in Table I. It will be seen that the methylated sugars, levoglucosan, neolactose (galactose + altrose), and the Schardinger dextrans are not readily utilized by bacteria or yeasts. It is of interest that neolactose is not fermented by any of the coliform bacteria which normally utilize lactose. Primeverose and turanose were utilized by certain *Escherichia-Aerobacter-Klebesella* species or strains, and these sugars may be of some value for differential purposes, if a larger number of organisms were employed. Tagatose appears to have some importance in separating certain coli-aerogenes forms, and for differentiating between *Eberthella* and *Shigella* species. This sugar may also be of importance in characterizing some of the *Salmonella* species. Ordinary trehalose has an α,α configuration and is fermented by some of the coliform bacteria and *Salmonella* species.¹² The β,β -trehalose used in this study differs from the ordinary trehalose in that it is attacked by certain coliform bacilli, but is not utilized by any of the *Salmonella* species tested.

In general, none of the sugars is of any value in separating the species of yeasts employed: only *Saccharomyces Carlesbergensis* and *Sacch. Bayensis* produced slight acid from turanose.

Summary. Thirteen rare sugars, including turanose, tagatose, neolactose, primeverose, vicianose, β,β -trehalose, three methylated glucoses, and the Schardinger dextrans were tested for their utilization by 18 bacterial species and 4 yeasts. Some of these sugars appear to be of value for differentiating certain members of the enteric group of bacteria.

¹² Bergey, D. H., Breed, R. S., Murray, E. G. D., and Hitchens, A. P., *Bergey's Manual of Determinative Bacteriology*, 5th Ed., Williams & Wilkins Co., Baltimore, 1939.