cocci or short rods and comparatively few long rods. More than half of these strains are non spore-formers. Most of them were found to be Gram positive.

5. The ammonification capacity is very feeble except in the arable soils.
6. More or less nitrification occur in all soils examined.
7. The denitrification occurs in the arable soils but none in others.
8. The fixation of nitrogen by *Azotobacter* was observed only in soil 2#.
9. The fermentation of cellulose was found in the arable soils only.
10. The most striking fact is that so many organisms which are able to on the nitrogen free medium, were found in the virgin soils.

ON THE PRODUCTION OF ACETYLMETHYLCARBINOL AND 2,3- BUTYLENEGLYCOL BY MICROBES I.

MOST MICROBES SEEM TO HAVE AN ABILITY
OF ACETOIN-PRODUCTION.

By

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The production of Acetylmethylcarbinol (acetoin) and its reduction product 2,3-butyleneglycol by various microbes (about 20 species of bacteria, 10 species of yeast, only one fungus, *Mucor mucedo*) has often been reported by many investigators.

As to the mode of the production of acetoin and 2,3-butyleneglycol, Neuberg stated that, by the aid of carboligase, acetaldehyde, which is formed in a nascent state as an intermediate product in the fermentation, undergoes an acyloin condensation with aldehyde which is added to the fermented liquid, and gives rise to acetoin, and the acetoin thus formed is reduced into 2,3-butyleneglycol. On the other hand, Kluyver explained this mechanism by the catalytic hydrogen-transformation theory (Wasserstoffübertragungstheorie). Kostytschew suggested that the carboligatic condensation is to be due to a specific reaction-ability of pyruvic acid. Although the latter two investigators denied the existence of carboligase, which was suggested by Neuberg as a specific enzyme being capable of condensing aldehydes, they did not absolutely

deny the enzymic formation of acetoin.

The writer also does not deny the enzymic formation. But the writer does suggest that acetoin may also be formed by the simple chemical action of nascent acetaldehyde, and that all organisms which produce acetaldehyde as an intermediate product of fermentation may be able to produce acetoin or 2,3-butyleneglycol.

The production of acetoin called the attention of many investigators as an important reaction since it is suggested as the first stage of the carbon-chain-synthesis in the organism. If it be so, it seems to be very natural to suppose that all organisms may have the ability of producing acetoin or 2,3-butyleneglycol, because all organisms have the ability of fat-synthesis.

It will not be useless, therefore, to determine whether all microbes produce these compounds or not, for the purpose of the investigation of the mechanism and the physiological significans of acetoin-production. From this point of view the writer examined the production of these compounds by Kluyver's method, and confirmed the fact that all the microbes tested produced more or less of both acetoin and 2,3-butyleneglycol or at least one of them. The results obtained are shown in the following table.

Experimental.

As medium 80c.c. of koji extract (10° Balling) was used for yeast and fungus. For bacterium 50c.c. of neutralized koji extract (10° Balling) containing 1g. of calcium carbonate was used. 2 platinum ears of microbes were inoculated to each.

TABLE.

Microbes	Acetoin	2,3-Butylene-glycol	Temp.	Duration of culture
B. <i>acidi-lactici</i> 131	+++	+	35°	7 days
" <i>aquatis sulcantus</i>	++++	++	"	"
" <i>aureus</i>	++	++	"	"
" <i>butyricus</i>	++	+++	"	"
" <i>centrosporus</i>	±	++	"	"
" <i>cereus</i>	+++	++	"	"
" <i>coli</i> C	++++	not tested	"	"
" <i>coli</i>	+	—	"	"
" <i>cominicus</i>	++++	+++++	"	"
" <i>erythrogenes</i>	—	±	"	"
" <i>enteritidis</i> Gaertner	+	±	"	"
" <i>fluorescens liquefaciens</i>	±	+	"	"
" <i>Granulobacter saccharobutyricum</i>	+	±	"	"
" <i>lactis innocuum</i>	±	—	"	"
" <i>Loeffler typhi</i>	±	—	"	"
" <i>mycoides roseus</i>	+++	+	"	"

B. Megaterium	++	+++	35°	7
" mesentericus fuscus	++++	++++	"	"
" mesentericus vulgatus	++++	++	"	"
" natto H.	++++	++++	"	"
" natto S.	++++	++++	"	"
" neopolitans	++++	++++	"	"
" oxalaticus	++++	++++	"	"
" prodigiosus	++	+	"	"
" pumilus	++++	++++	"	"
" proteus	+	++	"	"
" subtilis 106	++	+	"	"
" stutzeri	+++	+++	"	"
" typhi murium	+++	++	"	"
" vulgaris	++++	++++	"	"
Micrococcus ureae	+	—	"	"
Sarcina albidia	+	—	"	"
Staphylococcus candidus	++++	+	"	"
" candidans	+	+	"	"
ureae	+	—	"	"
Spirillum rubrum	+	—	"	"
Iron bacteria ?	++++	not tested	"	"
Azotobacter Beijerincki	+++	++	"	"
Actinomyces No. 1.	+	—	32°	21
" No. 3.	+	+	32°	21
Saccharomyces apiculatus	++	+	27°	7
" cartilaginosus	—	±	"	"
" ellipsoideus	++	—	"	"
" exiguus	±	—	"	"
" Ludwigii	±	—	"	"
" Pastorianus	++	+	"	"
" saké A. No. 3.	—	—	"	"
" shaoahing No. 1.	+	±	"	"
Beer yeast (Asahi Brewery)	±	—	"	"
Distillery Race 2	++	+	"	"
Carlsberg bottom yeast No. 1.	+	—	"	"
Johanisberg wine yeast	+	—	"	"
Zygosaccharomyces Barkeri	+	—	"	"
" Major	+++	+	"	"
" salsus	++	++	"	"
" soja A.	++	+	"	"
Debaryomyces globosus	++	+	"	"
Schwanniomyces occidentalis	+	+	"	"
Pichia membranaefaciens	—	—	"	"
Willia anomala	+	+	"	"
Schizosaccharomyces octosporus	+	—	"	"
" Santawensis	+	—	"	"
Endomyces fibuliger	—	—	"	"
Mycoderma M. No. 1.	—	—	"	"
Oidium lactis	++	+	"	"

<i>Oidium albicans</i>	+	+	27°	7
<i>Monilia candida</i>	++	+	"	"
<i>Monilia</i> (California Univ.)	++	+	"	"
<i>Torula</i> B.	—	—	"	"
<i>Chalara mycoderma</i>	—	+	"	"
<i>Aspergillus oryzae</i> A.	++	—	35°	7
" <i>mellius</i>	±	—	"	10
" <i>niger</i>	++	—	"	7
<i>Penicillium glaucum</i>	—	±	"	"
" <i>purpurenum</i>	++	+	"	"
<i>Monascus purpureus</i>	+	—	"	"
<i>Rhizopus Deleamar</i>	++	++	"	"
" <i>chiunian</i>	—	±	"	"
<i>Mucor racemosus</i>	+++	—	"	"
" <i>alternans</i>	++	—	"	"
<i>Dematium pullulans</i>	+	+	"	"
<i>Cunninghamiella elegans</i>	—	—	27°	"
<i>Citromyces Pfefferianus</i>	++	++	35°	"
<i>Chlamydomucor javanicus</i>	+	+	"	"
<i>Sachsia</i> sp.	++	+	"	"

Some microbes, which did not show the production of these compounds in the experiments mentioned above, were cultured in 1 litre of Hayduck's medium containing 10 per cent of fructose, at 27° for 14 days, and the detection was carried out as follows: Acetoin was detected after extracting with chloroform, and the glycol was tested in the alcohol-ether extract after condensing it in vacuum.

TABLE

	Acetoin	2,3-Butyleneglycol
<i>Sacch. sake</i> A. No. 3.	+++	+++++
<i>Pichia membranaefaciens</i>	+	++
<i>Endomyces fibuliger</i>	+	++
<i>Mycoderma</i> M. No. 1.	++	++++
<i>Torula</i> B.	+	+++

Cunninghamiella elegans was tested again after cultivating it in 80c.c. of koji extract containing 0.002 per cent methyleneblue, at 27° for 7 days. This time acetoin alone was detected.

It will be seen from the results of these experiments that all microbes seem to have the ability of producing acetoin or 2,3-butyleneglycol in the fermentation of sugar. This fact forms a contribution in support of my hypothesis regarding acetoin-production.