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**Fermentation phenomena when different species of micro-organisms are in close association.**

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During the years 1904 and 1905 I became interested in the study of baker's yeast and in fermentation caused by yeast. I made the following observations, mentioned at meetings of the Ceylon Branch of the British Medical Association: :

(1) Baker's yeast both in the East and in London is not a pure culture of a single species of yeast. It is generally composed of two or more species of yeast plus one or two or several species of bacteria. The bacteria isolated from these samples of yeast do not produce gas fermentation in sugars; they produce simple acidity (lactic acid fermentation).

(2) Comparing the fermentation reaction of baker's yeast used *in toto* as obtained from the bakers, sowing particles of the sample in the various sugars, with the fermentation reactions of the isolated pure cultures of the different species of saccharomyces and bacteria composing the sample, I found that the yeast *in toto* ferments a much larger number of carbohydrates than any one of the saccharomyces and bacteria composing the sample. In this country I may say that all the samples of yeast I have examined contain one or more gas forming bacilli.

(3) By mixing artificially all the organisms isolated from the sample of yeast in certain cases the same fermentation reactions are obtained as with the yeast *in toto*. There are apparent exceptions as shown by the following example:

From Orphington, a place in the country near London, I obtained in 1919 a sample of baker's yeast which gas fermented a very large number of carbon compounds, including starch. I in-

vestigated the sample mycologically by plating on maltose and glucose agar, etc., and I isolated and grew in pure cultures what I believed to be all the organisms, saccharomyces and bacteria found in that sample. By mixing all the pure cultures I obtained the same fermentation reactions as with the sample of yeast *in toto* except that the mixture did not touch starch. The experiment was repeated many times with the same result, until finally I had recourse to the following procedure: The surface of the glucose agar plates on which a minute particle of the yeast cake had been plated out were washed with a little peptone water and the washing inoculated into starch. Fermentation took place, the inference being that probably some very minute colonies on the verge of invisibility had escaped my notice when investigating the plates and picking out all the colonies present; the thought came to me also that there might be an ultramicroscopic germ present.

(4) By mixing artificially a non-maltose fermenting saccharomyces (isolated from a sample of baker's yeast) with a bacillus, isolated from the same sample, producing acidity in maltose and several other sugars but never gas in any substance, I obtained fermentation of maltose with production of gas.

(5) By mixing artificially two species of saccharomyces, neither of which gas-fermented saccharose, gas-fermentation of this sugar was obtained.

In recent years I have studied the same and similar phenomena, using mostly pathogenic bacteria: bacilli and cocci, also a pathogenic yeast. In the present paper I will limit myself to give the results of experiments, a number of them already published, with the following organisms:

(a) *Bacilli*: *B. typhosus*, *B. dysenteriae Flexner*, *B. morgani*, *B. proteus*, strain P., *B. coli communior*, *B. kandiensis*, *B. vermiculoides*.

(b) *Cocci*: *Staphylococcus aureus*, two strains of *Streptococcus pyogenes*.

(c) *Yeast*: *Cryptococcus graciloides*.

#### B. TYPHOSUS + B. MORGANI

*Action on Maltose.* *B. typhosus* alone produces acidity, never gas. *B. morgani* alone has no action whatever on maltose; it produces neither acidity nor gas. If a tube of maltose peptone water is inoculated, however, with *B. typhosus* and immediately after with *B. morgani* and placed in the incubator at 35° C. or

37° C. for 48 hours, not only will acidity be produced, but also a large amount of gas. The same result is obtained if the two bacilli are previously mixed in plain peptone water and the peptone water mixture inoculated in tubes of maltose. The same result is also obtained if *B. morgani* is added to *B. typhosus* 24 hours after inoculating with *B. typhosus*; if the interval is more than 24 hours then the production of gas is not so constant.

When the two bacilli, *B. typhosus* and *B. morgani*, live naturally in close association the same phenomenon takes place as shown by the following observation, which I related in one of my previous papers. The stool of a suspected typhoid case was examined bacteriologically in London, using McConkey's medium plates. An exceptionally large number of white colonies developed; fifteen were picked out and further investigated by replating, etc. Twelve of them consisted of two organisms—*B. typhosus* and *B. morgani*; three of *B. morgani* alone. The mixed cultures derived from the colonies containing only *B. morgani* had no action whatever on that sugar. Mixed cultures obtained artificially by inoculating agar tubes with both *B. typhosus* and *B. morgani* behave in the same manner—namely, when a loopful of the mixed growth is inoculated into a maltose peptone water tube, acid and gas are produced.

*Action on Mannitol.* As is well known *B. typhosus* produces acidity only in mannitol, never gas; *B. morgani* has no action—no acidity or gas is produced. The mixture *B. typhosus* + *B. morgani* produces acidity and gas.

*Action on Sorbite.* *B. typhosus* alone produces acidity, never gas, in sorbite; *B. morgani* alone has no action whatever on sorbite; it produces neither acidity nor gas. The mixture *B. typhosus* + *B. morgani* produces acidity and gas.

TABLE I.

	Lactose	Saccharose	Glucose	Levulose	Maltose	Galactose	Mannitol	Sorbitol
<i>B. typhosus</i>	0	0	A	A	A	A	A	A
<i>B. morgani</i>	0	0	AG	AG	0	AG	0	0
<i>B. typhosus</i> + <i>B. morgani</i>	0	0	AG	AG	AG	AG	AG	AG

O = Negative, neither acid nor gas. A = Acid. G = Gas.

*Action on other Carbon Compounds apart from the above.* The reactions are seen at a glance in Table I, in which the fermentative characters are given of *B. typhosus* alone, *B. morgani* alone, and the mixture of *B. typhosus* + *B. morgani*.

From the table it is seen that certain sugars and other carbon compounds, which when acted upon by *B. typhosus* alone undergo a simple acid fermentation, and when acted upon by *B. morgani* alone do not undergo any fermentation, neither acid nor gas being produced, are capable of undergoing fermentation with production of gas when they are acted upon by the mixture of the two bacilli. In the carbon compounds in which *B. typhosus* alone produces no acidity and *B. morgani* alone neither acidity nor gas their mixture produces neither acidity nor gas.

#### B. TYPHOSUS + B. PROTEUS

The strain of *B. proteus* used (P), as already stated, has no action on maltose, mannitol, or sorbite; it produces neither acidity nor gas in these substances.

*Action on Maltose.* *B. typhosus* alone produces acidity only, never gas; *B. proteus* (P) alone produces neither acidity nor gas; their mixture produces acidity and gas.

*Action on Mannitol.* *B. typhosus* produces acidity, never gas, in this alcohol. *B. proteus* (P) has no action on it; neither acidity nor gas is produced. Their mixture, however, produces acidity and gas.

TABLE II.

	Lactose	Saccharose	Glucose	Levulose	Maltose	Galactose	Mannitol	Sorbite
<i>B. typhosus</i>	0	0	A	A	A	A	A	A
<i>B. proteus</i> (P)	0	0	AG	AG	0	AG	0	0
<i>B. typhosus</i> + <i>B. proteus</i> (P)	0	0	AG	AG	AG	AG	AG	AG

0 = Negative, neither acid nor gas. A = Acid. G = Gas.

*Action on Sorbite.* *B. typhosus* produces acidity only; *B. proteus* neither acidity nor gas; their mixture causes production of acidity and gas.

#### B. DYSENTERIAE FLEXNER + B. MORGANI

*Action on Mannitol.* *B. dysenteriae Flexner* produces acidity only, never gas; *B. morgani* has no action on it; neither acidity

nor gas is produced. The mixture *B. dysenteriae* + *B. morgani* produces acidity and gas.

*Action on Maltose.* *B. dysenteriae Flexner* produces only acidity, never gas; *B. morgani* produces neither acidity nor gas; their mixture produces acidity and gas.

**B. DYSENTERIAE FLEXNER + B. PROTEUS**

*Action on Mannitol.* *B. dysenteriae Flexner* produces acidity, never gas; *B. proteus* (P) produces neither acidity nor gas; their mixture produces acidity and gas.

*Action on Maltose.* *B. dysenteriae Flexner* produces only acidity, never gas; *B. proteus* (P) produces neither acidity nor gas; their mixture produces acidity and gas.

TABLE III.

	Lactose	Saccharose	Glucose	Levulose	Maltose	Galactose	Mannitol	Sorbite
<i>B. dysenteriae Flexner</i>	0	0	A	A	A	A	A	0
<i>B. morgani</i>	0	0	AG	AG	0	AG	0	0
<i>B. proteus</i> (P)	0	G	AG	AG	0	AG	0	0
<i>B. dysenteriae Flexner</i> + <i>B. morgani</i>	0	0	AG	AG	AG	AG	AG	0
<i>B. dysenteriae Flexner</i> + <i>B. proteus</i> (P)	0	0	AG	AG	AG	AG	AG	0

O = Negative, neither acid nor gas. A = Acid. G = Gas.

**B. TYPHOSUS + B. MORGANI + B. PROTEUS**

*Action on Maltose, Mannitol and Sorbite.* *B. typhosus* alone produces acidity, no gas. *B. morgani* alone produces neither acidity nor gas. *B. proteus* (P) alone produces neither acidity nor gas. The mixture *B. morgani* + *B. proteus* produces neither acidity nor gas. The mixture of the three germs produces acidity and gas.

**B. COLI COMMUNIOR + B. KANDIENSIS (VAR. KANDIENSOIDES)**

*Action on Adonite* *B. coli communior* produces neither acidity nor gas. *B. kandiensis*, acidity only, never gas. The mixture *B. coli communior* + *B. kandiensis* will produce acidity and gas, although slowly. The tubes must be kept at 37° C. for at least a week.

**STAPHYLOCOCCUS AUREUS + B. MORGANI**

*Action on Maltose.* *Staphylococcus aureus* alone (Ross Institute Strain) produces acidity, no gas in maltose; *B. morgani*

alone produces neither acidity nor gas. The mixture *Staphylococcus aureus* + *B. morgani* produces acidity and gas.

STAPHYLOCOCCUS AUREUS + B. PROTEUS

*Action on Maltose.* *Staphylococcus aureus* alone (Ross Institute Strain) produces acidity, no gas in maltose; *B. morgani* alone produces neither acidity nor gas. The mixture *Staphylococcus aureus* + *B. proteus* produces acidity and gas.

STREPTOCOCCUS PYOGENES + B. MORGANI

*Action on Maltose.* *Streptococcus pyogenes* (Strain Public Health Laboratory, London,) alone produces acidity, no gas; *B. morgani* produces neither acidity nor gas. The mixture *Streptococcus pyogenes* + *B. morgani* produces acidity and gas, but it may take eight or ten days at 37° C. before the gas appears.

STREPTOCOCCUS PYOGENES + B. PROTEUS

*Action in Maltose.* *Streptococcus pyogenes* (Strain Public Health Laboratory, London,) alone produces acidity, no gas; *B. proteus* produces neither acidity nor gas. The mixture *Streptococcus pyogenes* + *B. proteus* produces acidity and gas, but it may take eight or ten days at 37° C. before the gas appears.

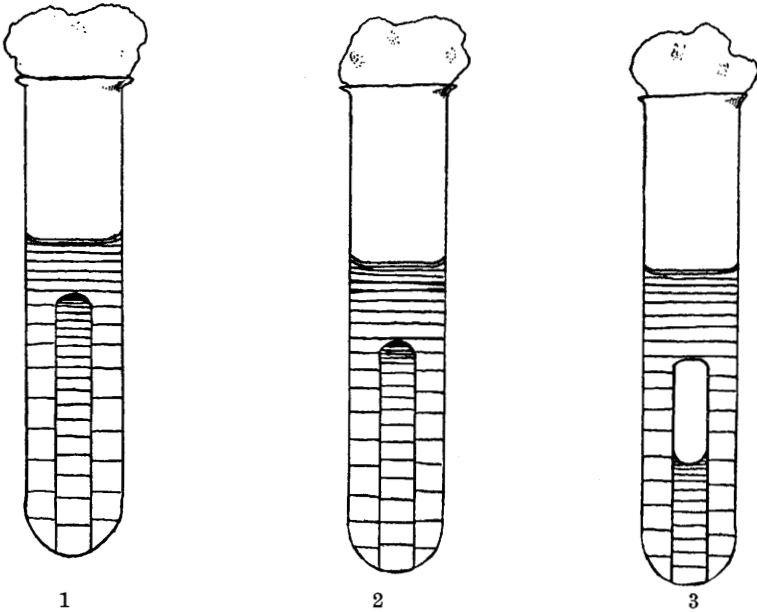
BACILLUS VERMICULOIDES + CRYPTOCOCCUS GRACILOIDES

These two organisms live apparently in symbiosis and it took me more than a year to separate them. The symbiotic mixture produces, though very slowly, gas in glucose, levulose and maltose. The cryptococcus alone does not produce either acid or gas; the bacillus alone produces acidity only.

TABLE IV.

	Lactose	Glucose	Levulose	Maltose	Galactose	Saccharose	Mannite	Dulcite	Salicin	Inosite	Dextrin	Inulin	Sorbite	Adonite	Arabinose	Raffinose	Insodulcite	Erythrite	Glycerine	Gelatine
Cryptococcus graciloides Castellani	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacillus vermiculoides Castellani	A	A	A	A	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cryptococcus graciloides + B. vermiculoides	A	AG	AG	AG	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

A = acid. G = Gas. O = absence of gas or coagulation. S = slight.



1. Mannitol peptone water tube inoculated with *B. dysenteriae Flexner*; gas absent. 2. Mannitol peptone water tube inoculated with *B. morgani*; gas absent. 3. Mannitol peptone water tube inoculated with *B. dysenteriae Flexner* + *B. morgani*; gas present.

#### RESUME AND CONCLUSIONS

“Baker’s yeast” as obtainable in Ceylon and in England is not a pure culture of yeast, but is generally composed of two or more species of *Saccharomyces* and one or two or several species of bacteria living in close association. The whole yeast as a rule gas-fermented more carbohydrates than any of the isolated germs did, either *Saccharomyces* or bacteria. A bacillus, which produced only acidity, not gas, in maltose, would produce gas when *Saccharomyces* was added to it, was inactive on maltose and was isolated from the same sample.

In recent years I have used for my experiments mostly pathogenic bacteria and also a pathogenic yeast, *Cryptococcus graciloides*.

Simple acid fermentation of certain carbon compounds caused by micro-organisms which never produce gas may in a number of instances be transformed into fermentation with the production of gas by adding a micro-organism which, alone, is inert, viz., produces neither acidity nor gas on those particular carbon

compounds. For example: *B. dysenteriae Flexner* produces acidity, never gas, in maltose and mannitol; *B. morgani* produces neither acidity nor gas in those two carbohydrates; the mixture *B. dysenteriae Flexner* + *B. morgani* produces, however, acidity and gas. For the phenomenon to take place several conditions are necessary; the most important one seems to be that the added bacillus, though inert on those particular carbon compounds, must be capable of producing fermentation with gas in glucose.

## 3025

**Electrocardiographic studies of the action of propylene and some other anesthetic gases.**

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Earlier experimental observations<sup>1</sup> and certain clinical experiences indicated the advisability of making an electrocardiographic study of the behavior of the heart under the influence of propylene, the results of which are here reported.

In four dogs and three cats the administration of propylene in concentrations from 25 per cent (less than sufficient to induce or maintain anesthesia) up to 40-50 per cent caused ectopic\* ventricular beats, which promptly ceased when the gas was discontinued or the concentration lowered. With higher concentrations the ectopic beats usually became more and more numerous, often so much so that an ectopic ventricular tachycardia resulted. Under these conditions also discontinuance of the gas or a sufficient lessening of its concentration resulted in a return to the normal mechanism. In control observations with ether, chloroform, nitrous oxide, ethylene, and acetylene in varying concentrations ectopics were not observed.

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<sup>1</sup> Halsey, J. T., Reynolds, C., and Prout, W. A., *J. Pharm. and Exp. Ther.*, 1925, xxvi, 479.

\* Originating at various points in each ventricle.