THE EFFECT OF STERILIZATION OF MEDIA UPON THEIR GROWTH PROMOTING PROPERTIES TOWARD BACTERIA

ELLIS I. FULMER, ARTHUR L. WILLIAMS AND C. H. WERKMAN

Contribution from the Laboratories of Chemistry and Bacteriology, Iowa State College

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Data presented by Fulmer and Huesselmann (1927) showed that sterilization of yeast growth media under pressure produces a yeast growth stimulant. The production of the stimulant was always associated with caramelization of the medium. The standard medium contained per 100 cc. 0.188 gram ammonium chloride, 0.180 gram dipotassium phosphate and 10 grams of sucrose. Sterilization under pressure of the sucrose and ammonium salt (preparation A) and of sucrose and dipotassium phosphate (preparation B) likewise led to the formation of the stimulant. For each preparation there was an optimum concentration of the caramelized medium for growth, the exceeding of which depressed the growth. The preparations differed in stability toward hydrochloric acid; the ammonium chloride preparation was not decolorized by addition of the acid, i.e., is "acid fast" while the phosphate caramel was practically decolorized by such a treatment. The various preparations were not significantly different in degree of stimulation. The stimulation was shown to be due to changes produced in the sugar by the reagents and was not a pH effect.

Data presented recently by Lewis (1930) showed that "media containing glucose, maltose, lactose, galactose or levulose with various nitrogenous compounds when sterilized at 122°C. for fifteen minutes" formed substances which inhibited the growth of *Phytomonas malvaceara*. He attributes the inhibitory effect to "conversion of the nitrogen compound into a form which is not suitable for assimilation by some species of bacteria." He

did record experiments with a medium not containing nitrogen. In the treated medium containing sodium ammonim phosphate, the growth of the following organisms was not inhibited, *Serratia marcescens, Salmonella enteritidis, Escherichia coli, Aerobacter aerogenes, Pseudomonas fluorescens* and *Ps. aeroginosa,* while the growth of *Phytomonas melvaceara* was inhibited. With peptone as a source of nitrogen, the treated medium inhibited the growth of *Staphylococcus albus, Staph. aureus, Sarcina lutea, Bacillus mycoides,* and *B. anthracis,* while the non-inhibited species included *E. coli, B. subtilis, A. aerogenes, Serratia marcescens,* and *Ps. fluorescens.*

During the course of work in these laboratories on the fermentation changes produced by *Aerobacter pectinovorum* it was noted that growth was always better in the medium sterilized for fifteen minutes at 15 pounds pressure than in the medium sterilized by filtration. The medium contained per 100 cc., 0.6 gram ammonium chloride, 0.2 gram dipotassium phosphate and 5 grams of glucose. These results were analogous to those reviewed above for yeast and indicated the production of a bacterial growth stimulant during the process of sterilization.

In order to determine the relative degree of stimulation brought about by caramelization, the flasks of media in each experiment were adjusted to the same pH and inoculated with one drop of an actively growing culture. The flasks were watched closely for the first appearance of growth and the time recorded.

To four of eight flasks containing 5 per cent glucose solution, ammonium chloride and dibasic potassium phosphate were added in the right amounts. The medium was then sterilized at 15 pounds pressure for fifteen minutes and the salts added to the other four flasks of medium. The pH was adjusted to 6.8 in all the flasks and they were inoculated. Within twenty-four hours the four flasks containing the caramelized medium showed growth, while it was seventy-two hours before growth was apparent in the four which were not caramelized. This would indicate that either the caramel or some substance which is formed simultaneously with the former acts as a stimulant toward the organism used.

To determine if this substance might act as a stimulant under different conditions, two other similar series were arranged, one at a pH of 7.05 and the other at 6.6. Within twenty-four hours after inoculation all media which contained some caramel, showed growth. The uncaramelized media showed growth after seventy-two hours; apparently, stimulation may occur at any pH suitable for the growth of the organism.

In order to determine whether or not an increase in the concentration of caramel would cause an increase in stimulation, the medium containing phosphate and ammonium chloride was sterilized under 15 pounds pressure for five hours which caused an

TABLE 1

FLASK NUMBER	PER CENT OF CARAMELIZED SOLUTION	PER CENT OF UNCARAMELIZED SOLUTION
1	0	100
2	0	100
3	0	100
4	10	90
5	20	80
6	30	70
7	40	60
8	50	50
9	60	40
10	70	30
11	80	20
12	90	10
13	100	0

intense but clear coloring of the medium. This corresponds to the standard caramel solution prepared by Fulmer and Huesselmann, and portions of it were added to uncaramelized medium to form the concentrations shown in table 1.

The flasks were then sterilized in live stream at atmospheric pressure for ten minutes. The pH was adjusted to 6.8 and the flasks were inoculated. Flasks number 1, 2, and 3 were used as checks. Twelve hours later there was growth in all flasks which contained caramel. It was very evident that the media with the higher per cent of caramel contained the most growth. In fact the medium consisting of 100 per cent caramel solution

appeared to have the greatest stimulating effect. The medium which contained no caramel required about fifty hours for appearance of growth.

A commercial caramel of unknown origin and composition was tried. A slight stimulation was observed but not comparable to that obtained above.

Another caramel was prepared as follows: 100 grams of glucose were heated to a temperature of 200°C. and held at this temperature for twenty minutes, cooled and dissolved in water to make 250 cc. of solution. Varying quantities of this solution were added to an uncaramelized medium. This caused a slight stimulation but not comparable to that obtained by sterilization of the medium.

TABLE 2

ORGANISM	TIME FOR GROWTH TO BE APPARENT	
	Caramelized medium	Uncaramelized medium
	hours	*hours*
Aerobacter faeni	12	36
Esch. freundii	15	36
Actinomyces, sp?	22	
Aerobacter aerogenes	12	24
Serratia marcescens	15	15*
Esch. coli	15	15*
B. subtilis	36	36*

* The growth was greater in the caramelized medium.

An attempt was made to determine whether or not the stimulant produced during sterilization could be removed by decolorizing the medium with charcoal. A commercial charcoal, Norite A, was boiled and washed several times with distilled water to remove any soluble substances which might be present. A little of this charcoal was added to each of four flasks, two of which contained caramelized media and two, uncaramelized. After standing for three to four hours the charcoal was filtered off and the medium sterilized in live steam at atmospheric pressure for ten minutes. The pH was adjusted to 6.8 and the flasks were inoculated. Within twelve hours growth was very evident in

all four flasks. The experiment was repeated with the same results. It was then noticed that traces of charcoal had passed through the filter and were present in the medium. The experiment was again repeated and in this case the medium was filtered a second time through a Berkfeld type of filter. Within fifteen hours there was a heavy growth in the medium which had contained the caramel. No growth was evident in the other flasks at the end of ninety-two hours. This proves without doubt that the stimulation is not due to the caramelization of the medium but to some substance which is produced at the same time as is the caramel. The stimulation in this case cannot be attributed to the effect of the charcoal, since each flask had precisely the same treatment.

It seemed desirable to try the effect of a caramelized medium upon other organisms capable of growth on ammonium salts as a source of nitrogen. The results are given in table 2.

The last four organisms in table 2 are four of the five organisms which Lewis was able to grow on the caramelized medium. The data above show that these organisms are actually stimulated. It may be concluded, then, that the sterilization of media under pressure may lead to the production of growth stimulants for yeast and for the bacteria studied. Decolorization of the caramelized medium with charcoal (Norite A) does not remove the bacterial growth stimulant.

REFERENCES

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