STUDIES ON BACTERIAL INTERACTION: I. THE EFFECT OF TRYPSIN ON THE CELLULASE ACTIVITY OF *CELLULOMONAS FLAVIGENA*[1]

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Since cellulose is the most abundant of all carbohydrates and since only a relatively small amount of that produced annually is utilized by man, one of the most attractive possibilities in the realm of microbiology is the conversion of cellulose to more valuable compounds by biological means. Ever since the early definitive studies of Omelianski (1902), efforts have been directed toward the realization of this possibility. Many bacteria have been shown to possess enzymes capable of digesting cellulose, but purification of cultures has typically resulted in a diminution of cellulose activity. Several explanations have been advanced to account for this reduction in activity, many being based on the idea of beneficiation of the medium through the production by the "contaminants" of vitamins (Cowles and Rettger, 1931) or other required substances (Burroughs *et al.*, 1950). It is the purpose of the present paper to suggest that increased cellulase activity so often noted in mixed bacterial cultures may result from the direct action of enzymes on the cellulase synthesizing organisms.

MATERIALS AND METHODS

The organism used in these studies, *Cellulomonas flavigena*, was obtained from Dr. E. T. Reese of the Quartermaster General Laboratories, in Philadelphia, Pa. This species was isolated and its cellulase activity demonstrated by members of the staff of that laboratory (Reese, 1948, Siu, 1950). The stock culture was propagated in triplicate on sucrose agar containing peptone and yeast extract. These cultures were stored at 10° C after growth was apparent, transfers being made at 60 day intervals.

The cells used in the investigation were cultured on sucrose or cellulose agar. Good growth was obtained on the former in 3 days; growth on cellulose agar was less rapid, being apparent only after 20 days. In either case, the cells were suspended in buffered distilled water (pH 7.0) and were washed 3 times. After 24 hr. incubation at 10° C, the suspension was so diluted that a 1: 20 dilution transmitted 60 percent of the incident light in an Evelyn photoelectric colorimeter when transmission through distilled water was taken as 100 percent.

Trypsin was used in this preliminary study because it was readily available and because it has a fairly broad action spectrum. Trypsinized cells were obtained by incubating washed cells in a 1: 20,000 solution of trypsin at pH 7.0. Following 30 min. incubation in a 37° C water bath, the trypsinized cells were washed 3 times to remove the residual trypsin prior to use. Cells not treated with trypsin were subjected to the same incubation and washing before use.

Ground cells were obtained by triturating washed, stored cells in a mortar. Alumina was used to enhance abrasion. When the resultant slurry was trypsinized, boiled trypsin was added to the control preparation, it being impractical to attempt to remove the residual trypsin by washing.

The activity of the various preparations was measured by manometric techniques described by Umbreit *et al.* (1949). The substrates used were carbo-

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hydrates having glucose and/or its isomers as the basic unit. All were used in M/50 concentration calculated as glucose equivalents.

EXPERIMENTAL RESULTS

C. flavigena, cultured on sucrose agar, was tested for its capacity to oxidize glucose, sucrose, cellobiose, cellulose, starch and inulin, both before and after trypsinization. The oxidation of starch and inulin followed the pattern obtained with cellulose; the balance of the substrates followed the oxidative pattern established for glucose. Since the oxidation of these substrates thus fell into two groups, the data obtained with glucose and cellulose are reported as representative. Figure 1 summarizes the data obtained in this part of the investigation.

From the curves presented in figure 1, it is obvious that cells of *C. flavigena* which had been cultured on sucrose agar were able to oxidize glucose but unable to oxidize cellulose prior to trypsin treatment. After exposure to trypsin the cells oxidized glucose more rapidly. Oxygen uptake by trypsinized cells in the presence

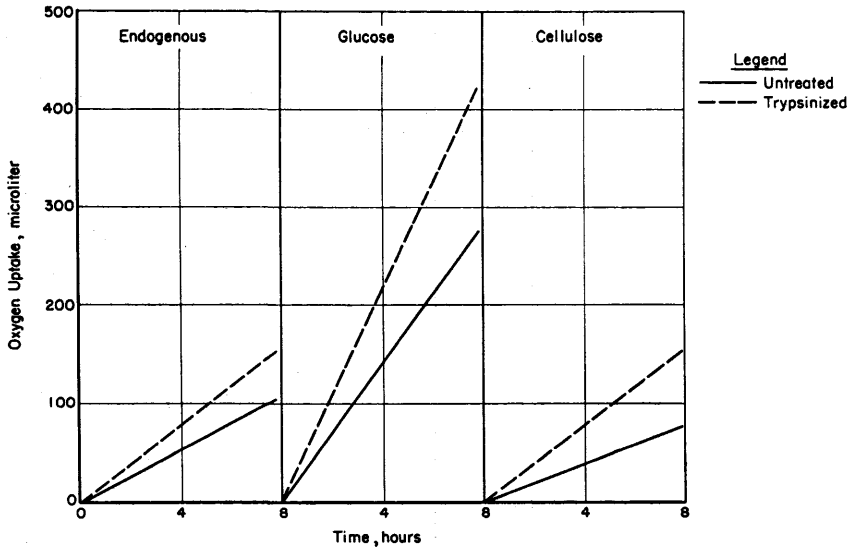

FIGURE 1. Oxygen uptake by *C. flavigena* (cultured on sucrose) before and after trypsinization.

of cellulose was greater than oxygen uptake by untreated cells; however, endogenous utilization of oxygen was also increased in trypsinized cells, and so the increased oxygen uptake in the presence of cellulose was not significant.

The data obtained when "cellulose-grown" cells were tested for their capacity to oxidize the test substrates are presented in figure 2.

From the data shown in figure 2, it is apparent that "cellulose-grown" cells oxidized glucose and to a lesser extent cellulose. Trypsinization of these cells increased the rate of oxidation of glucose but not that of cellulose. The endogenous oxygen utilization by these cells was not affected by treatment with trypsin.

It was hoped that treatment of *C. flavigena* with trypsin would bring about an increase in the rate of cellulose utilization, since it has been claimed that cellulase might be found on, or in the cell membrane (Rahn and Leet, 1949). Since trypsin neither unmasked cellulase on the "sucrose-grown" cells nor removed cellulase from "cellulose-grown" cells, it was supposed that cellulase was protected by virtue of its position or by some other as yet unelucidated mechanism. This supposition was tested by subjecting "sucrose-grown" cells to the action of trypsin after they had been ground in a mortar. The preparations were tested for their ability

to oxidize the substrates previously mentioned. The data obtained are presented in figure 3.

It is evident that the endogenous rate of oxygen utilization was high with both of these preparations. The shape of the curves obtained indicates that growth was

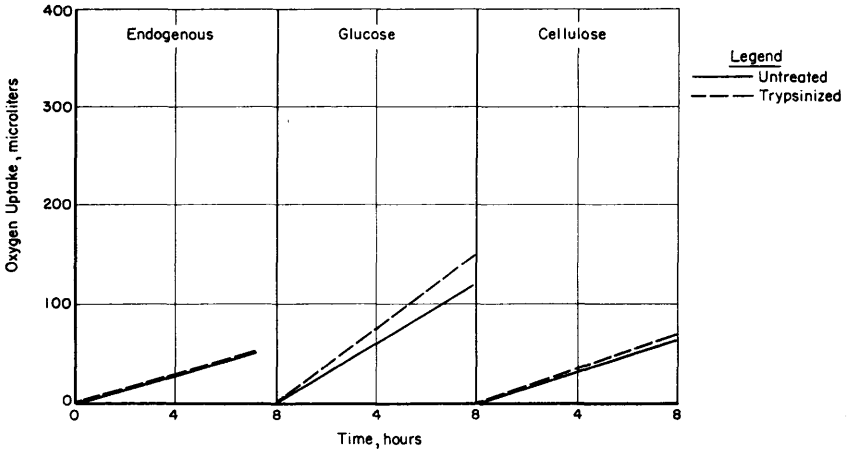

FIGURE 2. Oxygen uptake by *C. flavigena* (cultured on cellulose) before and after trypsinization.

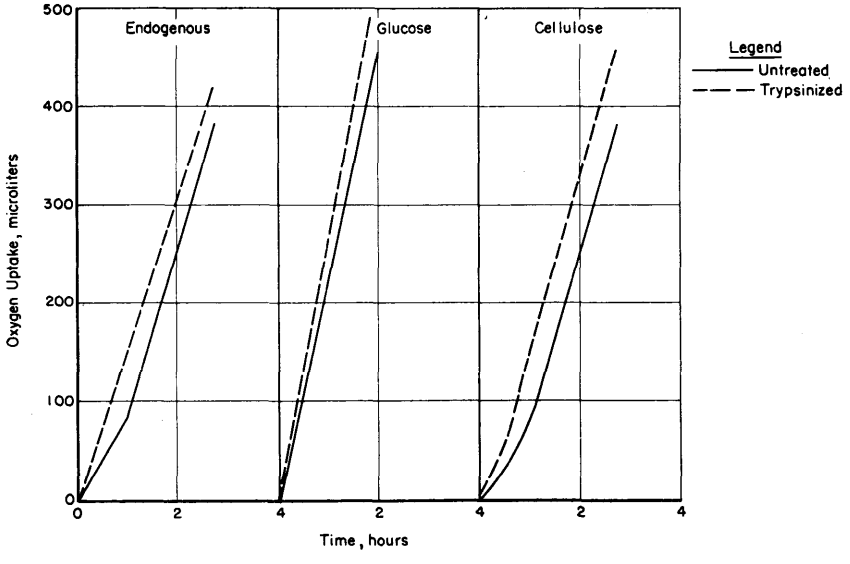

FIGURE 3. Oxygen uptake by *C. flavigena* (cultured on sucrose) before and after trypsinization.

probably taking place in the Warburg flasks. The most interesting observation is that the slurry from "sucrose-grown" cells was capable of oxidizing cellulose after being ground and subjected to the action of trypsin. Similar results were obtained when "cellulose-grown" cells were treated in like manner.

DISCUSSION

In attempting to relate the data obtained to events as they may occur in mixtures of organisms, it is necessary to make a few assumptions. Although many mixtures of microorganisms are characterized by a fairly constant rate of

reproduction, increases in numbers are often transitory. It should be noted that although we are accustomed to speaking of the "protein-sparing action of carbohydrates", this phenomenon may not be too common. In complex mixtures of organisms and substrates, of which sewage is an example, both carbohydrates and proteins are digested readily. It is therefore reasonable to assume that proteolytic enzymes are liberated into the same medium in which those capable of hydrolyzing carbohydrates are liberated. Peltier and Schroeder (1949) have shown this to be the case with *Bacillus subtilis* and have concluded that production of amylase without concurrent release of proteolytic enzymes by strains of this species would be a remarkable event.

In the light of available information, it is possible to postulate that in mixed bacterial cultures at least some synergistic reactions are brought about through the direct action of enzymes on bacterial cells. Although there is no reason to believe that trypsin is produced by bacteria, there are similar proteolytic enzymes that are known to be synthesized by these organisms.

Enzymes similar to trypsin might be considered to have a dual action; altering the permeability of living cells, and releasing enzymes from disrupted cells. The enzymes released from cellular debris would increase the concentration of diffusable substances which could be utilized more rapidly by viable cells exposed to the action of proteolytic enzymes. The data presented indicate that trypsin probably increased the permeability of viable cells as well as liberating cellulase from cellular debris.

In natural mixtures of microorganisms, these events might lead to a cycle in which proteolytic enzymes would increase certain activities of viable cells thus enhancing reproduction. As the cells multiplied, other factors might intervene and reduce the numbers of living cells. The dead and dying cells would then be subject to digestion during which process enzymes would be released. These enzymes in turn would act, increasing the concentration of diffusable substances and completing the cycle. The result of such a course of events would be increased activity of all cells with a net increase in utilization of a particular substance.

While the above discussion is oversimplified and admittedly speculative, it does suggest a somewhat different approach for further study of microbial interactions.

SUMMARY

Cellulomonas flavigena is capable of oxidizing cellulose when the cells are cultured on cellulose-containing medium, but not when the cells are cultured on sucrose-containing medium. Treatment of both preparations with trypsin increases the rate of glucose oxidation but has no effect on the rate of cellulose oxidation. When either preparation is ground prior to trypsin treatment, its capacity to oxidize cellulose is enhanced. An attempt is made to relate the data obtained to events as they might occur in mixed culture.

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