Nutritional Control of Cell Division in a Species of Erwinia[1]

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While studying the protoplasting of gram negative bacteria it was noted that cells of an *Erwinia* species became 15-30 μ in length after growth on a casitone-proteose peptone-dextrose medium, but grew only to a size of 1 to 2 μ when cultured on heart infusion agar. This large variation in size was reversible within 12-16 hours. Cell size was observed microscopically; however, it was noted that colonies consisting of small cells were smooth whereas, colonies composed of large cells were of the rough type.

Cell suspensions were streaked and single colonies of rough or smooth type cells were isolated to determine if any colonies were composed of both

type cells. These studies revealed that cell size was nutritionally controlled. Mutation was also ruled out because all cells and colonies were of one type, either large and rough or small and smooth, depending on the medium. The possibility also existed that the long cells were, in reality, unbroken

chains of small cells. Examination with the phase, dark-field or electron microscope revealed that the long cells did not possess cross walls or septa and could, therefore, be considered only as single cells. It was, therefore, concluded that some component of the casitone-proteose peptone-dextrose medium was responsible for inhibiting the division mechanism of these bacteria. This concept was further substantiated when growth and division studies revealed that cells grown in the casitone-proteose peptone-dextrose medium divided at a greatly decreased rate after the 10th to 12th hour of incubation although growth (dry weight basis) continued at a rate greater than control cells growing in nutrient broth.

In order to define the nutritive condition causing cell elongation, each constituent of the casitone-proteose peptone-dextrose medium (also including yeast extract and sodium chloride), was individually added to nutrient broth, since this medium allowed only short-cell development. Dextrose was the nutrient responsible for long-cell development. Autoclaving of dextrose was not necessary since filtered dextrose also caused cell elongation to occur.

Two obvious effects of growth in glucose are to lower pH and to change the O/R potential of a medium. Although growth in 1% dextrose resulted in a pH of 4.5 to 4.7 after 16 hours in unbuffered media, cell elongation still occurred in buffered media at a pH range of 6.2 to 6.7. An O/R influence was also discounted since cystine or cysteine at various levels could not substitute for dextrose. Long-cell development occurred on nutrient agar in the absence of dextrose only when the cells were grown at 38 C. (usual incubation was 25 C.). Overall growth was, however, greatly retarded.

Other carbohydrates and sugar alcohols were tested in nutrient agar to determine if the reaction was specifically mediated by dextrose. Compounds tested included starch, sucrose, lactose, mannitol, arabinose, rhamnose, inulin, raffinose, xylose, galactose, potassium gluconate, levulose, and maltose. Long-cell development occurred only in the presence of dextrose, sucrose, mannitol and galactose. There was no correlation between utilization of the compound and cell size; nor was any correlation apparent between chemical structure and ability to promote small- or long-cell development.

Because it appeared that dextrose degradation was yielding a compound that played an important role in the overall reaction, and because the degradation product might be difficult to identify in a complex such as nutrient broth, it appeared desirable to use a chemically-defined medium for this organism. Starr and Mandel (1950) reported a synthetic medium, for 13 strains of *Erwinia caratovora,* consisting of mineral salts, phosphate buffer and glucose. Although their formulation did not allow growth of our organism, supplementation with organic nitrogen in the form of an amino acid allowed growth. Thirteen amino acids were added individually to the Starr-Mandel medium (alanine, aspartic acid, glutamic acid, arginine, isoleucine, methionine, phenylalanine, serine, valine, glycine, histidine, leucine, and proline) and it was observed that all except serine allowed good growth in 40 hours. Aspartic acid allowed best growth in 16 hours. Cell size was small to intermediate (1 to 5 μ) in the presence of each amino acid except DL serine where cell size was small. Thereafter, DL aspartic acid was used as the sole source of organic nitrogen in the medium, although asparagine and glutamine also allowed good growth to occur.

Other compounds were added to the synthetic medium to determine if they would induce long-cell development. These included all naturally-occurring purines and pyrimidines (adenine, guanine, cytosine, uracil and

thymine), B vitamins (pantothenate, riboflavin, B_{12}, choline, folic acid, nicotinic acid, biotin, thiamine, and pyridoxal hydrochloride), and the salts of fatty acids (formate, pyruvate, malate, succinate, fumarate, butyrate and a ketobutyrate). At no time did cell elongation occur to the same extent that it did when cells were grown on nutrient agar plus 1% dextrose, except when pyruvate was present (succinate and biotin allowed some elongation).

In further attempts to induce long-cell formation, each individual component of the synthetic medium was titrated against other components of the medium. Regardless of the level of each component, cell elongation was not enhanced; instead it appeared that the levels normally employed were optimal for the elongation being obtained. Because these studies revealed that the inorganic ammonium salt (NH_4Cl) was not needed in the medium when organic nitrogen (DL aspartic acid) was present, the NH_4Cl was left out of the medium in future studies.

Although inorganic nitrogen was not needed for optimal growth, it is well known that amino acids are formed from inorganic nitrogen and carbon skeletons derived from the metabolism of dextrose. Therefore, the individual amino acids were again studied to determine if a relationship existed between elongation and amino acid metabolism. The results were excellent. Maximum cell length, in nutrient agar plus 1% dextrose, was about 30μ, but three amino acids (DL serine, DL methionine, or DL phenylalanine) caused the formation of cells about 300 μ long in 16 hours with only slight retardation in overall growth (turbidity). Two other amino acids (DL threonine or DL tryptophan) caused the extremely long cell formation to occur in 24 hours. The effect could also be demonstrated in the absence of dextrose although overall growth was greatly retarded. This amino acid effect was found to be negated by DL alanine, NH_4Cl, $(NH_4)_2SO_4$, and to a limited extent by NH_4NO_3 and DL isoleucine. Para-aminobenzoic acid also antagonized the effect, but overall growth (turbidity) was greatly reduced. Long-cell development was also retarded by high levels of pantothenic acid.

D or L forms of serine or methionine were added to the medium to determine if optical configuration of the amino acid was important. Only the D forms allowed long-cell development to occur. The optical configuration of aspartic acid was not important and reversal of long-cell development could be accomplished by adding either D or L alanine to the synthetic medium. Reversal of long-cell development by *B* alanine was not possible.

Various forms of serine or methionine were incorporated into the medium to determine if precursor substances or substitutions within the molecules had any effect on the reaction. Compounds tested included serine ethyl ester, o-methylserine, betaine, glycylserine, phosphoserine, ethanolamine, sarcosine and acetylmethionine. The results were negative, except that phosphoserine did cause some elongation.

Because the division enzymes of cells are involved and because the location of these enzymes is an intriguing problem, we have attempted to determine how the enzymes become localized in the approximate center of a dividing cell, employing photomicrography of continuously growing cultures. Unless migration of these enzymes is postulated, they can localize and function in the center of a cell only if they remain at the division end of a cell and the cell continues to grow from that one end only. Once enough growth has occurred at this metabolically active and growing end and formation of compounds such as D serine are occurring at a site now removed from these enzymes, the enzymes could be competitively reactivated by compounds such as alanine and division could again occur. Pre-

liminary studies have shown that these bacteria grow only from the division end.

LITERATURE CITED

Starr, M. P. and M. Mandel. 1950. The nutrition of phytopathogenic bacteria. IV. Minimal nutritive requirements of the genus Erwinia. J. Bacteriol. 60: 669-672.