Some Physiological Studies of *Fusobacterium nucleatum*

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Summary

Fusobacterium nucleatum has been associated with various forms of periodontal disease in both man and animals. It is among the most frequently detected of the cultivable bacteria from subgingival plaque and appears to be associated with both diseased and clinically healthy sites. Recent studies suggest that there are three sub-species or "biotypes" of *F. nucleatum* and it appears that different biotypes may be related to disease-active and -inactive sites. It may be hypothesized that differences in disease-association between these *F. nucleatum* biotypes may be related to differences in their physiology and metabolism. Accordingly, strains belonging to the biotypes *F. nucleatum subspecies nucleatum* (strain ATCC 25586), *F. nucleatum subspecies polymorphum* (strain AHN 4237) and *F. nucleatum subspecies vincentii* (strain D212B-2), were grown in continuous culture in a chemically-defined medium (CDM) and some aspects of their physiology examined under different growth conditions.

The three test strains were found to share a number of physiological properties. For example, they grew well in various CDM's, with or without added carbohydrate, over a pH range of about 6 to 8; the optima being between 7.0 and 7.8. Growing in a carbohydrate-free CDM, glutamate, serine, histidine and lysine were the key amino acids from which both carbon and energy were obtained and the acidic fermentation end-products were acetate : butyrate : formate (1.5 : 1 : 0.5), irrespective of growth rate. Also, in terms of growth energetics, the yield and maintenance energy requirements of the test strains were similar. Strain AHN 4237 was also able to grow on a peptide fraction prepared from a commercial peptone, obtaining the four key growth-promoting amino acids from small peptides.

When a CDM containing carbohydrate as well as amino acids was used, the individual strains behaved somewhat differently. For example, AHN 4237, grown under a variety of physiological conditions, utilised 32 - 83% of the available glucose, converting it to a mixture of acids, including lactate, but converting little to intracellular polyglucose (IP).

Strain D212 B-2, depending upon pH, utilised some 40-65% of the glucose and converted a significant proportion to IP. Similarly, strain ATCC 25586 utilised 70-96% of the glucose, a small amount of which was converted to IP, particularly at higher pH's. This strain also responded well to both fructose and galactose but, like the other strains, did not utilise sucrose. When grown in a CDM in which glucose was the primary energy source, most of the glucose was consumed and converted principally to lactate and butyrate, with virtually no IP production. Moreover, growth yields showed that glucose was a more efficient energy source than the amino acids, from which only 1 mole of ATP per mole is derived.

It was concluded that the three test strains show similar physiological behaviour - all can metabolize both carbohydrate and amino acids (or small peptides). This property could help to explain the widespread distribution of *F. nucleatum* in the mouth. However, no obvious physiological differences were found that might account for suggested differences in disease association between the test strains.

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