ENDOGENOUS METABOLISM OF NOCARDIA CORALLINA

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John Gray Robertson

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ABSTRACT

The endogenous metabolism of the soil microorganism N. corallina has been studied with special reference to physiological and structural changes in starvation conditions.

When N. corallina was grown on the surface of nutrient agar growth was characterised by the development of branched hyphae 8-12 μ. long, while in liquid medium bacilli approximately 4 μ. long were produced. Clumping of cells in liquid medium was reduced by growing the organism in cleated flasks on a rotary shaker.

For studies of endogenous metabolism and survival, suspensions of N. corallina were prepared from cultures harvested at full growth and resuspended in phosphate buffer containing magnesium ions. Analyses of total and viable cell counts were affected by clustering of cells and detergents were used to reduce the size of clusters. The cell viability, was estimated using formulae, from the cluster viability and cluster size distribution which were determined using the slide culture technique. The viability of starved cells fell from 99% to 90% over a period of 7 days and subsequently to 50% after a further period of 13 days. A rise in total cell count of 13% was recorded over a 5 day period of starvation. During the first 48 hr. of starvation the bacterial dry wt. fell by 30-40%, and at the same time the initial Q_{O_2} of approximately 10 fell to a value of approximately one. The initial fall in dry wt. was due largely to a decrease in the level of cell polysaccharide from 25% to 5-10% of the cell dry wt. Following this drop in polysaccharide, ammonia was released at a relatively constant rate and at the same time there was a fall in the level of cell protein.

There was a fall in the levels of intracellular free nitrogenous compounds at the onset of starvation but no corresponding release of these substances into the supernatant occurred. Ribonucleic acid appeared to be broken down during starvation. The contribution of the individual cell fractions to the total fall in dry wt. on prolonged starvation were; polysaccharide, 40%; protein, 25%; RNA, 6% and total fatty acids, 5%. The decrease in viability of starved organisms could not be directly correlated with the utilization of any of these cellular components.

Hydrolysis of the total unbound lipid which constituted 15% of the cell dry wt. yielded trehalose, mannose, inositol and glycerol as the water soluble components. Triglycerides were isolated from the total lipids by silicic acid column and thin-layer chromatography. Evidence from thin-layer chromatograms indicated that triglycerides were not major constituents of the total lipids. Incubation of N. corallina with U-^{14}C-palmitate resulted in a large proportion of the radioactivity being incorporated into the triglycerides.

Total fatty acids constituted approximately 12% of the cell dry wt. and contained 3 fractions: (a) C_{10}-C_{20} fatty acids, (b) nocardic acids and (c) a minor unidentified fraction which was more polar than the nocardic acids. Trimethylsilyl derivatives of the methyl nocardates were separated by gas chromatography on the basis of molecular wt. Mass spectrometry of methyl nocardates and TMS derivatives, indicated that the structures of the nocardic acids could vary in 3 ways: in carbon number from C_{36}-C_{48}, in degree of saturation (saturated, mono-unsaturated and diunsaturated acids occurred), and in their isomeric configurations.

Studies on N. corallina using both light and electron microscopy showed clearly the pleomorphic nature of the organism. Parts of the cell surface were covered with fibrous material which appeared to be distinct from the cell wall. Cell division occurred by the formation of septa which were generally associated with extensive cytoplasmic membrane systems. Polyphosphate granules, ribosomes and either polyribosomes or glycogen granules appeared in the cytoplasm during growth. Use of the freeze-etch technique illustrated the granular nature of the cytoplasm, cell wall and membrane surfaces. Starvation of the cells appeared to be associated with (a) a thickening of the cell wall, (b) an increase of the amount of fibrous material per cell, (c) an increase in the size of the polyphosphate granules and (d) the disappearance of large cytoplasmic granules.

Possible implications of the present findings have been considered in relation to previous investigations with this organism and to studies of the endogenous metabolism and survival characteristics of other microbial species.

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