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ENDOGENOUS METABOLISM OF NOCARDIA CORALLINA

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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  $\mu$ . long, while in liquid medium bacilli approximately 4  $\mu$ . 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-<sup>14</sup>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<sub>10</sub>-C<sub>20</sub> 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<sub>36</sub>-C<sub>48</sub>, 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.

## CONTENTS

	SECTION I	page
Chapter 1	INTRODUCTION	1
	Endogenous Metabolism of Microorganisms with Special Reference to <u>Nocardia corallina</u>	1
	The Genus <u>Nocardia</u>	4
	The Species <u>N. corallina</u>	6
	The Chemical Composition of <u>Nocardia</u> Species	9
	Endogenous Metabolism and Bacterial Survival	11
	(a) The effect of the environment on the chemical composition of microorganisms	12
	(b) Studies relating endogenous metabolism to survival capacity	15
	(c) Factors affecting the survival of microorganisms	17
	(d) The functional importance of endogenous metabolism	19
Chapter 2	THE AIM OF THE PRESENT INVESTIGATION	21
	SECTION II	
	EXPERIMENTAL	
Chapter 1	METHODS	23
	<u>Bacteriological Procedures</u>	23
	Organism	23
	Growth in Liquid Medium	23
	Special Growth Flasks	24

	page
Chapter 1      Preparation and Incubation of Cell Suspensions	25
Total Cell Counts	26
Viable Counts	26
Formulae for Estimating Cell Viability from	
Cluster Viability	28
Photography of Cells on Agar Slides	29
<u>Analytical Methods</u>	29
Spectrophotometric Equipment	29
Dry Weight	29
Respiratory Quotients	31
Oxygen Partial Pressure Estimations	31
Total Unbound Lipids	32
Triglycerides	32
Ester determination	32
Glycerol determination	32
Alkaline hydrolysis	33
Total Fatty Acids	33
Alkaline hydrolysis of total cells	33
Acid hydrolysis of total cells	33
Estimation by weight	33
Estimation by chromate oxidation	34
Total Nitrogen	34
Protein	34
Cellular protein	34
Protein in solution	35
Amino Acids	36
Estimation	36
Extraction of intracellular amino acids	36



	page
Chapter 1      Ammonia	37
Total Carbohydrates	37
Total hexose	37
Total reducing sugar	37
Ribose	38
Deoxyribose	39
RNA	39
<u>Preparative Methods</u>	40
Freeze Dried Cells	40
Organic Solvents	40
Potassium Palmitate Solutions	41
Methylation of Fatty Acids	41
Silylation of Hydroxy Esters	41
<u>Chromatography</u>	42
Paper Chromatography of Polyols and Sugars	42
Preparation of samples	42
Analysis of sugars in perchloric acid solutions	
obtained during RNA estimations	42
Solvent systems and detection of components	42
Silicic Acid Column Chromatography	43
Total unbound lipids	43
Total fatty acids	43
Thin-Layer Chromatography	44
Preparation of thin-layer plates	44
Application of samples	45
Solvent systems	45
Identification and isolation of components	45
Autoradiography of thin-layer plates	46

	page
Chapter 1      Gas Chromatography	46
Gas chromatography of methyl esters of fatty	
acids derived from triglycerides	46
Silyloxy derivatives of methyl nocardates	46
Pyrolysis of methyl nocardates	47
Chapter 2      GROWTH CHARACTERISTICS OF <u>N. CORALLINA</u>	48
Development of a Defined Medium	48
Aeration Efficiency of Culture Flasks	48
Oxygen Demand in Liquid Cultures	49
Clumping of Cultures	51
Culture Pigmentation	51
Variations in the Size of Individual Cells	52
Clusters in Suspensions of <u>N. corallina</u> , Their	
Effect on Total and Viable Cell Counts	53
The Effect of Detergents on Cluster Size	53
Growth of <u>N. corallina</u> on Agar Slides	55
<u>Summary</u>	55
Chapter 3      TRIGLYCERIDES IN <u>N. CORALLINA</u>	56
Extraction and Fractionation of Total Lipids	56
Thin-Layer Chromatography of Lipids of <u>N. corallina</u>	57
Identification of Triglycerides in the Lipids of	
<u>N. corallina</u>	58
The Incorporation of U- <sup>14</sup> C Palmitic Acid into the	
Triglyceride Fraction	60
<u>Summary</u>	62

Chapter 4	FATTY ACIDS IN <u>N. CORALLINA</u>	63
	Extraction, Fractionation and Identification of Fatty Acids from <u>N. corallina</u>	64
	Silylation of Methyl Nocardates	67
	Gas Chromatography of the TMS Derivatives of Methyl Nocardates	67
	Mass Spectrometry of the TMS Derivatives of Methyl Nocardates	68
	Pyrolysis of Nocardic Acids	69
	Oxidation of Nocardic Acids	70
	Fractionation of Methyl Nocardates on Silver Nitrate Impregnated Plates	71
	<u>Summary</u>	72
Chapter 5	VARIATIONS IN DRY WEIGHT, TOTAL AND VIABLE COUNTS AND RESPIRATORY ACTIVITY IN STARVED SUSPENSIONS OF <u>N. CORALLINA</u>	73
	Variations in Viable and Total Cell Counts and in Dry Weight	74
	Respiration Rates for Cell Suspensions of <u>N. corallina</u>	76
	Respiration Experiments with Acetate Grown Cells	77
	Respiration Experiments with Cells Incubated with Palmitate	77
	<u>Summary</u>	78

Chapter 6	CHANGES IN THE LEVELS OF LIPID, CARBOHYDRATE AND	
	PROTEIN IN STARVED SUSPENSIONS OF <u>N. CORALLINA</u>	79
	Lipid Content of Cells of <u>N. corallina</u>	79
	Fatty Acid Levels in <u>N. corallina</u>	80
	Total Nitrogen, Ammonia, Protein and Carbohydrate	
	Levels Related to Changes in Dry Weight	81
	Changes in Levels of Total Fatty Acids, Proteins,	
	Carbohydrate, Dry Weight and Cluster Viability	
	During Endogenous Incubation	82
	Changes in Levels of Total Fatty Acids and Correlation	
	of Protein Breakdown with Ammonia Production	83
	Correlation of Breakdown of Cellular Carbohydrate with	
	Production of Ammonia	84
	<u>Summary</u>	85
Chapter 7	CHANGES IN THE LEVEL OF RNA IN STARVED SUSPENSIONS	
	OF <u>N. CORALLINA</u>	87
	Phosphate Buffer Supernatants	87
	0.2N HClO <sub>4</sub> Fraction	88
	0.1N HClO <sub>4</sub> Fraction	90
	Levels of RNA in Cell Suspensions Incubated Under	
	Endogenous Conditions	91
	<u>Summary</u>	92
Chapter 8	STUDIES ON THE ULTRASTRUCTURE OF N. CORALLINA	93
	Methods and Materials for Electron Microscopy	93
	Results and Discussion	95

Chapter 8	General Morphology and Growth Characteristics	
	in Liquid Medium	95
	Cell Coat	97
	Cell Wall	100
	Cytoplasmic Membrane	100
	Intracytoplasmic Membrane Systems	101
	Cytoplasm	102
	Polyphosphate Granules	103
	Nuclear Material	105
	Cell Division	105
	<u>Summary</u>	106

### SECTION III

DISCUSSION	108
Growth Characteristics of <u>N. corallina</u>	108
Lipid Studies	110
Changes in Viability, Total Cell Counts, Dry Weight and Respiratory Activity in Starved Cell Suspensions	112
Changes in Levels of Intracellular Components During Endogenous Metabolism	114
Correlation Between Cell Survival and Endogenous Metabolism	117
REFERENCES	
APPENDIX I. Culture media.	
APPENDIX II. Derivation of formulae for estimating cell viability from cluster viability and cluster size distribution.	