THE UTILIZATION OF CARBON COMPOUNDS BY NOCARDIA SPECIES[1]

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Organisms belonging to the genus *Nocardia* are distinguished primarily on the basis of colonial morphology, pigment production, and microscopic structure and secondarily by physiological behavior such as liquefication of gelatin, nitrate reduction, hydrolysis of starch, and litmus milk reactions. The actinomycetes are in general fairly inert biochemically, hence species characterization by physiological reactions has not been very rewarding. Detailed studies of single cells of *Nocardia* species as they grew into young colonies enabled the author (McClung, 1949) to separate these organisms into three morphological groups on the basis of their developmental behavior. In general, this development could be correlated with colonial characteristics, especially with the texture of colonies. The present studies were undertaken in order to determine whether or not there are fundamental differences in carbon nutrition which could be related to the morphological groupings, and to determine what carbon compounds serve *Nocardia* species as sources of energy.

The utilization of carbon compounds by actinomycetes has been studied by several authors, including Beijerinck (1900), Münter (1913), Krainsky (1914), Waksman (1919), Lieske (1921), and Gray and Thornton (1928). Taylor and Decker (1947) were able to separate actinomycetes pathogenic for potatoes from nonpathogenic strains on the basis of carbon compound utilization coupled with other reactions. Pridham and Gottlieb (1948) showed that commercially important species of *Streptomyces* could be distinguished on the basis of carbon compounds accepted. No studies of this nature using *Nocardia* species have come to the attention of this author although Pridham and Gottlieb (1948) included a few *Nocardia* and *Micromonospora* species in their studies but reported that results were inconclusive, with one exception (*Nocardia gardneri*), due to the poor growth of these organisms on the medium that they used.

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MATERIALS AND METHODS

In table 1 the organisms used in this study are listed with their sources and morphological groups as determined by the methods previously described (McClung, 1949).

Stock cultures were maintained on glycerol nutrient agar. The methods recommended in *Manual of methods for pure culture study of bacteria* (Committee Bacteriological Technique, 1946) were followed throughout these experiments where possible. The basal medium used for testing carbon compound utilization had the following composition: $NaNO_3$, 2.0 g; K_2HPO_4 (anhydrous), 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $Fe_2(SO_4)_3$, 10.0 mg; $MnCl_2 \cdot 4H_2O$, 8.0 mg; $ZnSO_4 \cdot H_2O$, 2.0 mg; distilled water to make 1,000 ml.[2] Preliminary experiments were run in which 20 mg of biotin and 100 mg of thiamin were added to the basal medium. No strain tested was deficient for these vitamins, but growth was stimulated by their incorporation. Controls containing the vitamins but no carbon source also showed a marked growth, thus rendering comparison with cultures containing carbon sources difficult. For this reason vitamins were not added routinely to the basal medium. The basal medium was made up and adjusted to pH 7.0–7.2, 4.5 ml amounts pipetted into 15 by 125 mm tubes and autoclaved.

The final concentration of carbohydrates, alcohols, DL-inositol, rhamnose, and salicin was 1 per cent, phenol, *o*-cresol, and *m*-cresol 0.1 per cent, and salts of organic acids 0.15 per cent. Because of poor solubility, dulcitol, starch, dextrin, and inulin were added to the basal medium before autoclaving. All others were sterilized by

[2] All water used in these experiments was distilled, then passed through a Barnstad Demineralizer so that the final concentration of ions was less than 0.2 ppm.

TABLE 1

Test organisms used in carbon compound utilization experiments

STOCK NO.	NAME	SOURCE	MORPHOLOGICAL GROUP
1	*Proactinomyces ruber* (Casabó) Bald.	Centraalbureau voor Schimmelcultures, Baarn	2
2	*Nocardia* sp.	Soil	2
3	*P. pseudomadurae* Bald.	CBS	3
4	*P. agnosus*	CBS	1
6	*P. polychromogenes* (Vallée) Jensen	CBS	1
7	*P. polychromogenes*	Waksman 3409A	2
8	*N. erythropolis* (Gray and Thornton) Waksman	Waksman 3407	1
9	*P. restrictus* Turfitt	CBS	1
10	*P. asteroides* (Eppinger) Bald. var. *crateriformis* Bald.	CBS	2
12	*Nocardia* sp.	Oil Sands	2
14	*Nocardia* sp.	K. L. Jones	1
15	*Nocardia* sp.	Soil	1
16	*Nocardia* sp.	Soil	1
25	*Nocardia* sp.	Soil	3
29	*Nocardia* sp.	Soil	3
32	*Nocardia* sp.	Soil	3
33	*Nocardia* sp.	Soil	2
36	*Nocardia* sp.	Soil	3
40	*Nocardia* sp.	Soil	3
42	*Nocardia* sp.	K. L. Jones	2
44	*Nocardia* sp.	Soil	3
48	*Streptomyces* sp.	Soil	
52	*Nocardia* sp.	O. A. Plunkett	3
53	*Streptomyces* sp.	Air	
54	*Nocardia* sp.	E. Munch-Petersen	3
57	*Jensenia canicruria* Bisset and Moore*	F. A. Clark	1
72	*N. asteroides* (Eppinger) Blanchard	Drake University	3
73	*N. asteroides* " "	NRRL No. B-970	3
74	*N. rubra*	NRRL No. B-685	2
75	*N. globerula* (Gray) Bergey *et al.*	NRRL No. B-1306	2
76	*N. opaca* (den Dooren de Jong) Bergey *et al.*	American Type Culture Collection 4276	2
77	*N. rangoonensis* (Erikson) Bergey *et al.*	ATCC 6860	3
78	*N. corallina* (Bergey *et al.*) Bergey *et al.*	ATCC 4273	2
79	*N. convoluta* (Gray and Thornton) Bergey *et al.*	ATCC 4275	2
80	*N. cuniculi* Snijders	ATCC 6864	3
81	*N. blackwellii* (Erikson) Bergey *et al.*	ATCC 6846	3
82	*N. leishmanii* Chalmers and Christopherson	ATCC 6855	3
83	*N. sylvodorifera* A. Cast	ATCC 7372	3
84	*N. asteroides* (Eppinger) Blanchard	ATCC 3308	3
87	*N. polychromogenes* (Vallée) Waksman	CBS	2
88	*N. caprae* (Silberschmidt) Waksman	CBS	3
89	*N. asteroides* (Eppinger) Blanchard	CBS	2
90	*N. aquosus* Turfitt	CBS	1
91	*N. caviae* (Snijders) Waksman	CBS	2
92	*N. asteroides* (Eppinger) Blanchard	C. W. Emmons NIH 9903	3
93	*N. asteroides* " "	C. W. Emmons NIH 9935	3

* This organism included for comparative purposes.

filtration through Seitz EK filter pads and added aseptically to the basal medium. All carbon compounds used were of the purest grade available from Pfanstiehl, Eastman Kodak Co., Fisher Scientific Co., or General Biochemical Co., and in most cases the same lot of a particular compound was used throughout the experiment. The paraffin (mp 56 to 58, Fisher Scientific Co.) was tested by adding 50 mg to each tube of basal medium before autoclaving. After the carbon sources were added, the medium was incubated to determine sterility.

Inoculum was prepared from two week old glycerol nutrient agar slant cultures. A small amount of the organism was placed in one ml of sterile distilled water in a test tube. The

TABLE 2

Growth of Nocardia species on basal medium plus various carbon compounds

CARBON SOURCES	GROUP 1									GROUP 2													
	4	6	8	9	14	15	16	57	90	1	2	7	10	12	33	42	74	75	76	78	79	87	89	91
D-Arabinose	*	+							t								+		+	+		+	+	t
L-Arabinose	+		+		+		+	t	t				t			+				+	t		+	+
D-Ribose	+	+	+	+	+	+	+	+	+	+	+	+	t	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	t	+	+	+	+	+	+	+		+	+	+	+	t	+		+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Levulose	+	+	+	t	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Sorbose	+					t		+	t														t	t
Cellobiose	+			+			+		t			+			+		+					+	t	
Lactose							+		t												+		t	+
Maltose	+	+	+	t	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melibiose	+		t	+					+		+										+		+	t
Sucrose	+	+	+	t	+	+	+	+	t	+	+	+		+	+	+	+	t	+	+	+	+		+
Trehalose	+	+	+	t	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melezitose	+			+			+	+	t		+			+	+	+	+		+	+	+	t		
Raffinose	+			+					t								+				+	t	+	
Rhamnose						+	+		t	+	+	+			+	+					+			t
Dulcitol							+				+				+		+				t	t		
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+		+	+	+	+	t	+	+		+	+	+	+	+	+	+	+	+	+	+	+
D-Sorbitol	+	+	+	+	+	+	+	+	+	+	+	t		+	+	+	+	+	+	+	+	+	+	+
Salicin		+	+		+	+	+				+	+	t	+	+		+	+				+	+	+
DL-Inositol	+		+	+	+	+	+	+	+		+	t	t	+	+	+	+			t	+	+	+	+
Dextrin	+	+	+	+	+	+	+	+	t	t	+	+	+	+	+	+	+	+	t	t	t	+	+	+
Inulin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	t	+	+	+	+
Starch	+	+	+	+	+	+	+	+	t	+	+	+	+	+	+	+	+	t	+	t	t	+		
Na-acetate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Na-citrate		+				+		+			+			+	+		+	+	+		t	+		
Na-formate	+			+																				
Na-oxalate											+									+		+		
Na-propionate	+	+	+		+		+		+	+	+	+		+	+	+	+		+	+	+	+	+	+
Na-salicylate											+													
Na-succinate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				+	+	+
Na-tartrate											+				+						+	+		
m-Cresol																t								t
o-Cresol						+	+				+						+					t		t
Phenol										+				t						+				
Paraffin	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* Blanks indicate no growth or utilization; + growth and utilization; t slight growth, probable utilization.

TABLE 2—*Concluded*

CARBON SOURCES	GROUP 3																				STREPTOMYCES SP.	
	3	25	29	32	36	40	44	52	54	72	73	77	80	81	82	83	84	88	92	93	48	53
D-Arabinose	+	*	+	+		+	+													+	+	+
L-Arabinose	+	+	+	+	+	+	+	+	+	+	+	+		+	+	t	t		+	+	+	
D-Ribose		+	+	+			+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+		+	+			+	+	+	+	t	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	t	+	+			+	+	+		+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Levulose		+	+				+	t	+	+	+		+	+	t	+	+	+	+	+	+	
D-Mannose	+	+	+	+	+	+	+	t	+	+	+	t	+	+	+	+	+	t	+	+	+	+
L-Sorbose	+			+							+									t		
Cellobiose				+	+	+	+				+				+					t	+	+
Lactose	+			+	+		+								+	+			t	t	+	
Maltose	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		+	+	+	+
Melibiose	+	+	+	+	+		+		+	+		+					t		+	t	+	t
Sucrose	+		+	+	+	+	+		+	+	+		+	+	+	+	+	+	+	+	+	t
Trehalose		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
Melezitose	+	+	+	+	+	+	t	t					+	+	+		+	t		t		
Raffinose	+	+	+	+	+	+	+			+	+				+				+	+	+	t
Rhamnose	+		+	+		+	+				t		+	+		+	+		+	t	+	
Dulcitol			+	+		+	+			t						t			+		+	
Glycerol	+	+	+	+	+	+	+	+	+		+	t	+	+	+	+	+	+			+	+
D-Mannitol	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Sorbitol	+	+	+	+	+	+	t	+	+	+	+		+	+	+	+	+	+		+	+	+
Salicin		+	t	+	+	+	+		+	+	+				+			+	+		+	+
DL-Inositol		+	+	+	+	+	+	+	+	t	+			+	+	+	t		+	+	+	
Dextrin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inulin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	t	+	+	+	+	+
Starch	+	+	+	+	+	+	+	+	+	+	+	+	t	t	+	+	+	+	+		+	+
Na-acetate	+	+	+		+		+	+	+	+	+	+	+	+	+	t	+	+	+	+	+	+
Na-citrate				+	+		+	+							+	t	t				+	t
Na-formate								+				+									+	
Na-oxalate		+		+			+			+	+							+	t		+	
Na-propionate		+		+			+		+	+	+		+	t	+	+	+	+	+	+		
Na-salicylate																						
Na-succinate		+	+	+	+		+	+	+	+	+	t		t	+	t	+	+	+	+	+	+
Na-tartrate	+		+				+			+			t			t		+	+		+	
m-Cresol					t																	
o-Cresol																				t		
Phenol																						
Paraffin	+		+	+				+	+	+	+	t	+	+		+	+		+	+		

* Blanks indicate no growth or utilization; + growth and utilization; t slight growth, probable utilization.

inoculum was finely divided by rubbing against the sides of the tube with a glass rod and then washed through three 10 ml changes of sterile distilled water. The water was decanted after centrifuging the organism to the bottom each time. Finally 10 ml of water were added, and this suspension served as the inoculum. The test medium was inoculated by adding one drop of the suspension to each tube by means of a sterile pipette. All tests were made in triplicate, and controls containing no carbon source were inoculated at the same time. The viability and purity of the inocula were tested each time by inoculating a drop onto glycerol nutrient agar slants. Incubation was at room temperature (about 25 C) for two weeks. Results were read

by comparing the test cultures with controls containing no carbon source. No attempt was made to differentiate between good and poor use of a particular compound, except if only a trace of growth occurred, it was recorded as "t". In most cases this is thought to be slight utilization but would be of no value in species differentiation. Doubtful cases were repeated, and all refusals of ten carbon sources (L-arabinose, D-ribose, D-levulose, D-xylose, maltose, sucrose, salicin, Na-acetate, Na-succinate, and Na-propionate) which seemed to be most promising for species separation were repeated. Finally all tests were repeated from two to six times over a period of two years.

The final pH was taken of all cultures.

RESULTS

The results of these studies are presented in table 2 where the organisms are listed according to the three morphological groups referred to previously.

All organisms tested used glucose, mannose, dextrin, and inulin. None used *m*-cresol; Na-salicylate was used by only one strain; and phenol, by two. All other carbon sources were used by five or more of the strains tested. Compounds which would be most useful for differentiation of species (i.e., those which were used by about half of the organisms and refused by about half) were melibiose, raffinose, rhamnose, and Na-citrate. The other compounds were either used by most or refused by most, so would be useful in differentiation of particular species but of limited value in separating large groups.

The pH of the medium changed during incubation from 0 to 1.5, always toward the alkaline side, but varied considerably with both the carbon source and organism. Different trials would also produce variations in the final pH. Since the changes in pH could not always be related to growth, final pH was of little value in determining whether or not a particular carbon compound was used. Carbon sources used by more than half of the organisms tested include: L-arabinose, D-ribose, D-xylose, D-galactose, D-levulose, maltose, sucrose, trehalose, melezitose, glycerol, mannitol, sorbitol, salicin, DL-inositol, starch, Na-acetate, Na-propionate, Na-succinate, and paraffin. Those refused by one-half or more of organisms tested were D-arabinose, cellobiose, lactose, melibiose, L-sorbose, raffinose, rhamnose, dulcitol, Na-citrate, Na-

TABLE 3

Carbon compounds utilized by six different isolates of Nocardia asteroides

CARBON SOURCES	NOCARDIA ASTEROIDES ISOLATES 72	73	84	89†	92	93
D-Arabinose	*					+
L-Arabinose	+	+	t	+	+	+
D-Ribose	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+
D-Galactose	+	+		+	+	+
D-Glucose	+	+	+	+	+	+
D-Levulose	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+
L-Sorbose		+		t		t
Cellobiose		+		t		t
Lactose				t	t	t
Maltose	+	+	+	+	+	+
Melibiose	+		t	+	+	t
Sucrose	+	+	+		+	+
Trehalose	+	+	+	+	+	+
Melezitose			+			t
Raffinose	+	+		+	+	+
Rhamnose		t	+		+	t
Dulcitol	t				+	
Glycerol		+	+	+	t	
D-Mannitol	+	+	+	+	+	+
D-Sorbitol	+	+	+	+		+
Salicin	+	+		+	+	
DL-Inositol	t	+	t	+	+	+
Dextrin	+	+	+	+	+	+
Inulin	+	+	t	+	+	+
Starch	+	+	+		+	
Na-acetate	+	+	+	+	+	+
Na-citrate			t			
Na-formate						
Na-oxalate	+	+			t	
Na-propionate	+	+	+	+	+	+
Na-salicylate						
Na-succinate	+	+	+	+	+	+
Na-tartrate	+				+	
m-Cresol						
o-Cresol						t
Phenol						
Paraffin	+	+	+	+	+	+

* Blanks indicate no growth or utilization; + growth and utilization; t slight growth, probable utilization.

†This organism belongs to morphological group 2 although most isolates of *Nocardia asteroides* are morphologically group 3.

formate, Na-oxalate, Na-salicylate, Na-tartrate, *o*-cresol, and phenol.

DISCUSSION

No relationship was found to exist between carbon compound utilization and the morphological groups. Since no two organisms used exactly the same carbon sources, the possibility of using carbon compound utilization as a means of species differentiation is strongly suggested. However, table 3 shows that the carbon compounds used by six strains of *Nocardia asteroides* are not the same. This indicates that different isolates of the same organisms differ in their ability to use carbon compounds. However, identification of *Nocardia* species would be helped if information concerning their behavior on judiciously selected carbon compounds was included in descriptions of new species.

These studies reveal that carbon compounds having an α-glucoside linkage (maltose, starch, dextrin, trehalose) are used more often by *Nocardia* species than those having the β-glucoside linkage (cellobiose, lactose). This suggests that α-glucosidase (maltase) is more prevalent among these organisms than β-glucosidase (emulsin).

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SUMMARY

Forty-three isolates of *Nocardia* species, two strains of *Streptomyces* species and *Jensenia canicruria* were tested for their ability to utilize thirty-nine carbon compounds in chemically defined media. Glucose, mannose, dextrin, and inulin were used by all organisms tested. No organism used *m*-cresol. No two isolates used exactly the same series of carbon compounds. This suggests that carbon compound utilization would be useful in identification and differentiation of *Nocardia* species.

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