THE IDENTIFICATION OF JENSENIA CANICRURIA
BISSET AND MOORE AS A MATING TYPE OF
NOCARDIA ERYTHROPOLIS (GRAY AND
THORNTON) WAKSMAN AND HENRICI

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ABSTRACT. Using determinations of physiological reactions, nocardioophage sensitivities and recombinant production, subcultures of the type strains of Jensenia canicruria Bisset and Moore were found to be identical to subcultures of the type strains of Nocardia erythropolis (Gray and Thornton) Waksman and Henrici. It was suggested that under the rules of nomenclature, J. canicruria should be reduced to synonymy with N. erythropolis. The inclusion of N. erythropolis in the synonymy of Mycobacterium rhodochrous (Overbeck) Gordon and Mihm was rejected by the authors owing to differences between strains included in the synonymy of M. rhodochrous and the strains of N. erythropolis examined.

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Jensenia canicruria Bisset and Moore was first legitimately described as a soil diphtheroid (8). Owing to the great morphological similarity of J. canicruria to members of the genus Nocardia (1, 4, 5, 25), it was suggested that Jensenia was a later synonym of Nocardia (5) and that J. canicruria should be included as a species in the genus Nocardia (5, 6, 20, 21, 22). While agreeing that the genus Jensenia did not appear to be an appropriate designation for the organism described by Bisset and Moore (8), Gordon and Mihm (17)

considered J. canicruria to be a later synonym for Mycobacterium rhodochrous (Overbeck) Gordon and Mihm (15, 16) and included J. canicruria among strains belonging to the species M. rhodochrous. More recently, using numerical analysis, Skyring and Quadling (27) found J. canicruria to cluster among arthrobacters, corynebacteria and brevibacteria. In contrast, Davis and Newton (13) found J. canicruria to be readily separable from corynebacteria, arthrobacters and brevibacteria but J. canicruria fell within the Mycobacterium-Nocardia group. The possible reality of the speculated central location of organisms such as J. canicruria in evolutionary schema (5, 6, 19, 20, 21, 22, 24, 26) perhaps has led to the difficulty of adequately placing this organism or closely related organisms into a taxon acceptable to all investigators. If the speculation is indeed true, the apparent conflict concerning appropriate taxonomy and nomenclature of J. canicruria can be readily understood.

In the past several years recombinational phenomena involving conjugation between organisms originally described as J. canicruria and an organism described as M. erythropolis Gray and Thornton (18) but now considered to be a member of the genus Nocardia, N. erythropolis (Gray and Thornton) Waksman and Henrici (28) have been fully substantiated (2, 3, 9, 10). Thus these strains or species are now among one of the very few bacterial groups definitively shown to display conjugational heredity. As a consequence of this importance, a more definitive nomenclature for J. canicruria should be established.

The present investigation clearly establishes J. canicruria as one of two, or more, mating types of N. erythropolis. Consequently J. canicruria must be considered as a later synonym of N. erythropolis. The present report does not, however, try to suggest the ultimate taxonomic destination of N. erythropolis-like organisms. The answer to this question must remain for the future when precise investigations of the evolution and taxonomy of these organisms have been completed.

MATERIALS AND METHODS

Strains. The origins of the cultures used in these investigations are listed in Table 1. Subcultures of the type cultures of N. erythropolis held by the American Type

Culture Collection (ATCC) and the National Collection of Industrial Bacteria (NCIB), Scotland, are included. N. erythropolis NCIB 9158 is considered to be a subculture of a culture originating in Gray's laboratory and described by Gray and Thornton (18) as M. erythropolis. N. erythropolis ATCC 4277 was derived from NCIB 9158. A subculture of the type culture of J. canicruria, ATCC 11048, held by ATCC is also included although J. canicruria JA-SD 3 has been considered in our laboratory (4, 5) to be an authentic subculture of the type culture, having originated indirectly, from the laboratory of J. canicruria's original describers (8). N. erythropolis JA-SD 2 and J. canicruria JA-SD 3 have been thoroughly described in the past on the basis of their cellular morphology (1, 4, 5, 25) and mating behavior (2, 3, 9, 19). It would appear that the cultures studied here must be considered as authentic representatives of the taxa N. erythropolis (Gray and Thornton) Waksman and Henrici and J. canicruria Bisset and Moore.

In addition, a strain originally designated as M. agreste (18) M. agreste 365, but now usually considered as a strain of N. corallina (28) was included in these studies. N. rubra JA-SD 1 and N. corallina N 305 were also included since they, like N. erythropolis and J. canicruria, had been included in the synonymy of M. rhodochrous by other workers (15, 16).

Tests and culture conditions. Physiological tests like those applied by other investigators to similar strains (15, 16) were used in the present studies. All strains were routinely maintained on nutrient agar slants (Difco Laboratories, Detroit, Michigan) and stored at refrigerator temperatures. Utilization of sugars and polyhydric alcohols was determined on a brom-thymol blue containing medium (23) supplemented with the carbohydrates being tested. In these tests positive utilization was considered as the production of a yellow color in the colony. Acetate, citrate, oxalate and succinate was substituted for glucose in a minimal defined medium (2) to determine the ability of the tested organisms to use these substances as sole carbon and energy sources. The utilization of glucose as the sole carbon-energy source was also determined. Nitrate reduction was ascertained by cultivating the organisms in nitrate containing medium (Difco) and subsequently testing cultures for the presence of nitrate with sulfanilic acid,

TABLE 1. *Sources of strains.*

Authors' Designation and Strain #	Prior Designation and Origin
<i>Nocardia canicruria</i> JA-SD3	<i>Jensenia canicruria</i> , from N. M. McClung, from F. A. Clark from K. A. Bisset
<i>Nocardia canicruria</i> JA-SD7	<i>Nocardia corallina</i> N305, from S. G. Bradley from J. D. Stout
<i>Jensenia canicruria</i> ATCC 11048	<i>Jensenia canicruria</i> 11048, American Type Culture Collection
<i>Nocardia erythropolis</i> JA-SD 2	<i>Nocardia erythropolis</i> 3407, from N. M. McClung from S. A. Waksman
<i>Nocardia erythropolis</i> NCIB 9158	<i>Nocardia erythropolis</i> 9158, from National Collection of Industrial Bacteria
<i>Nocardia erythropolis</i> ATCC 4277	<i>Nocardia erythropolis</i> 4277, from American Type Culture Collection

TABLE 1. Continued

<i>Nocardia rubra</i> JA-SD 1	<i>Nocardia rubra</i> , from N. M. McClung from Centraalbureau voor Schimmelcultures as <i>Proactinomyces ruber</i>
<i>Nocardia corallina</i> 365	<i>Mycobacterium rhodochrous</i> 365, from R. E. Gordon who received it as <i>Mycobacterium</i> <i>agreste</i> 365

TABLE 2. *Reactions of strains to physiological tests and nocardioophage sensitivities*

Test	Authors' strain number							
	JA-SD 3	JA-SD 7	ATCC 11048	JA-SD 2	NCIB 9158	ATCC 4277	JA-SD 1	365
Reaction								
Urease production ^{a/}	+	+	+	+	+	+	-	-
Nitrites from nitrates ^{b/}	-	-	-	-	-	-	+	+
Utilization as carbon ^{c/} and energy source								
Glucose	+	+	+	+	+	+	+	+
Acetate	+	+	+	+	+	+	+	+
Succinate	+	+	+	+	+	+	-	-
Citrate	+	+	+	+	+	+	-	-
Oxalate	-	-	-	-	-	-	-	-
Gelatin hydrolysis ^{d/}	-	-	-	-	-	-	-	-
Starch hydrolysis ^{e/}	-	-	-	-	-	-	-	-
Casein hydrolysis ^{f/}	-	-	-	-	-	-	-	-

TABLE 2. continued

Yellow color on BTB
medium containing g/alpha-D-methyl-
glucoside

alpha-D-methyl- glucoside	-	-	-	-	-	-	-	-
arabinose	-	-	-	-	-	-	-	-
dulcitol	-	-	-	-	-	-	-	-
erythritol	-	-	-	-	-	-	-	-
galactose	-	-	-	-	-	-	+	-
glucose	+	+	+	+	+	+	+	+
inositol	+	+	+	+	+	+	-	-
lactose	-	-	-	-	-	-	-	-
mannose	+	+	+	+	+	+	-	+
mannitol	+	+	+	+	+	+	-	+
raffinose	-	-	-	-	-	-	-	-
sorbitol	+	+	+	+	+	+	-	+
trehalose	+	+	+	+	+	+	+	-
xylose	-	-	-	-	-	-	-	-

TABLE 2. continued

Lysis by nocardiphage^{h/}

MNP1	+	+	+	+	+	+	-	-
MNP7	+	+	+	+	+	+	-	-
φC	+	+	+	-	-	-	-	-
φEC	+	+	+	+	+	+	-	-

a/ Negative reactions recorded after 21 days incubation. Positive reaction observed within 3 d.

b/ Reactions recorded after 5 d incubation.

c/ Reactions recorded after 14 d incubation

d/ Reactions recorded after 21 d incubation.

e/ Reactions recorded after 5 d incubation.

f/ Reactions recorded after 14 d incubation.

g/ Reactions recorded after 3 d incubation.

h/ Reactions recorded after 1 d incubation.

alpha-naphthylamine reagents. The ability to liquefy gelatin was determined in stab inoculated nutrient gelatin cultures (Difco). Tubes containing gelatin cultures were chilled in ice water to detect the presence of liquefied gelatin after appropriate incubation intervals. Urease production by the strains was determined by observing the presence or absence of indicator color changes in cultures on urea broth (Difco) or urea solid medium (Difco). The ability to hydrolyze starch was ascertained by growing the strains on nutrient agar supplemented with 0.2% soluble starch; after appropriate incubation the plates were flooded with 3% I-KI solution to detect the presence or absence of the starch-iodine complex surrounding the growth. Casein hydrolysis was determined by observing a hydrolyzed area surrounding colonies in cultures grown on nutrient agar containing 1% casein. All cultures for the above tests were incubated at 30C for appropriate intervals.

Sensitivity of the strains to infection by nocardiphage was determined using a previously described method (11). The nocardiphages used were: MNP1 and MNP7 (6) and ϕ EC and ϕ C (11). MNP1, MNP7, and ϕ EC were propagated on N. erythropolis JA-SD 2; ϕ C was propagated on J. canicruria JA-SD 3.

Fertility of mutants of the tested strains were determined using methods previously described (2). Strains were considered to be fertile if, upon mating, prototrophic recombinants, at least 10-fold greater than background mutations of individual controls, were recovered on glucose containing mineral salts medium. All mating attempts were carried out a minimum of 5 times.

RESULTS

The results of the physiological tests and nocardiphage sensitivities are shown in Table 2. All cultures designated as N. erythropolis or J. canicruria produced identical reactions in these tests with the exception of the test of sensitivity to nocardiphage ϕ C. All examined cultures labeled N. erythropolis were resistant to the action of this nocardiphage, while all J. canicruria cultures were sensitive. Of the other cultures, that labeled N. corallina N305 was found to be identical in all respects to those labeled J. canicruria. The cultures labeled N. rubra and

M. agreste were found to differ in several respects from those strains above and from each other.

The results of the attempted matings of the examined cultures are shown in Table 3. All mating attempts between direct line derivatives of cultures designated as N. erythropolis and derivatives of cultures designated as J. canicruria or N. corallina N305 were fertile. Matings of N. erythropolis by N. erythropolis and J. canicruria by J. canicruria, considered in more detail elsewhere (2, 10), were infertile. Matings of N. rubra derivatives with either N. erythropolis or J. canicruria derivatives were infertile in all mating attempts. Matings were not carried out with the strain designated as M. agreste.

DISCUSSION

It has been proposed by several workers (5, 6, 20, 21, 22) that J. canicruria is a Nocardia. Accordingly, J. canicruria should then be renamed N. canicruria. In fact this has been suggested by some workers (5) but the designation, N. canicruria, has not become firmly established in the literature (13, 17, 27). The present studies preclude such nomenclature. In all, excepting one, tests applied in the present investigation, N. erythropolis cultures and organisms designated J. canicruria behaved identically. Furthermore, matings of direct line derivatives of N. erythropolis with J. canicruria derivatives in all instances proved to be fertile as shown in present studies and in other reports from our laboratories (2, 3, 9, 10). On such bases it would be unwise to exclude J. canicruria and N. erythropolis from a single taxon. Since direct subcultures of the type cultures of both of these designated taxa are in all ways identical in reaction to the tests applied here and capable of bacterial sexual phenomena, these cultures designated J. canicruria can only be considered as mating type variants of N. erythropolis. Thus J. canicruria Bisset and Moore must be considered as a later synonym for N. erythropolis (Gray and Thornton) Waksman and Henrici. The culture herein designated N. corallina N305 must also be identified as a mating type variant of N. erythropolis. In all likelihood the culture N. corallina N305, in its past, was either misidentified as belonging to the taxon N. corallina or was mistakenly labeled.

TABLE 3. *Strain fertility in mating attempts*

Authors' Strain Number	Mated with Authors' strain number				
	JA-SD 3	JA-SD 7	JA-SD 2	ATCC 4277	JA-SD 1
JA-SD 3	<u>a/</u>	-	+	+	-
JA-SD 7	-	-	+	+	-
JA-SD 2	+	+	-	-	-
ATCC 4277	+	+	-	-	-
JA-SD 1	-	-	-	-	-

a/ A negative sign indicates that recombinants were not recovered in repeated mating attempts. A positive sign indicates that recombinants were recovered in mating attempts.

It is quite clear from the present studies that J. canicruria is a later synonym of N. erythropolis, and according to nomenclatural rules (14) should be included in the synonymy of N. erythropolis. However, should N. erythropolis be relegated to the synonymy of M. rhodochrous as suggested by Gordon and Mihm (15, 16)? In our opinion, M. rhodochrous is composed of organisms too diverse in character to be included as members of a single species. Similar conclusions were reached by several workers. Schneidau and Shaffer (26) investigated several strains previously examined by Gordon and Mihm (15, 16) and found the dissimilarity of the strains' biochemical characteristics too great to allow their inclusion in one species, M. rhodochrous. Anderson and Bradley (6) deemed it necessary to place two strains labeled M. rhodochrous in divergent phylogenetic groups based on actinophage susceptibilities. Similar diverse phylogeny is indicated in the results of Kwapinski (24). In his studies, closely related but separable, antigenically determined phylogenetic groups typified by strains of J. canicruria, M. rhodochrous and N. rubra were recognized. Thus three organisms previously considered as strains of M. rhodochrous (15, 16) were conflictingly found to typify separable antigenic groups (24). In investigations of infra-red absorption patterns of Nocardia and related organisms (7), other workers concluded that the species N. rubra, N. corallina and N. asteroides belonged to one distinct group (subdivision of the group into distinct species was not ruled out) while N. erythropolis, N. polychromogenes and other species comprised another separable group. Excepting N. asteroides, the species studied by infra-red absorption are similar or identical to species examined by Gordon and Mihm (15, 16, 17) who previously had placed these species in the synonymy of the single species, M. rhodochrous. Castelnuevo et al. (12) included four strains designated M. rhodochrous in their studies of Nocardia and Mycobacterium. They identified one strain labeled M. rhodochrous as N. rubra; another was identified as N. corallina while the identity of the two remaining strains was uncertain.

From the results of the present investigation and of others cited, it is evident that the application of the name M. rhodochrous to a single, presently well defined species is uncertain. According to nomenclatural rules (14) an essential point in nomenclature is to avoid the use of names which

may cause error or ambiguity. On this basis the strains examined here cannot be legitimately considered in the synonymy of M. rhodochrous (Overbeck) Gordon and Mihm.

The hypothesis that a continuum of species exists in the genus Nocardia has been advanced, in varying degrees of formality, by many investigators employing different approaches to determine degree of species relationship (5, 6, 7, 19, 20, 21, 22, 24, 26). Since Nocardia may well be a continuum of species, we recognize that the limits of species variation in the genus may have to be artificially and arbitrarily defined. It is only through the means of a thorough study of the genus and its species in all aspects thought to be taxonomically significant will a meaningful classification of nocardial species be realized. Consequently while this report has attempted to clarify the nomenclature of only few enigmatic nocardial strains, we recognize that a fully satisfactory treatment of these or any other nocardial strains awaits future study and agreement.

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