Use of CO₂ for More Rapid Growth of the *Nocardia* Species

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Growth of the *Nocardia* species is facilitated by use of 10% CO₂ for initial isolation from clinical material or from laboratory stock cultures.

Nocardia species have been described as aerobic in their oxygen requirements (2-9). Morphological and biochemical differences vary greatly within a species (2-9). With the exception of an *N. caviae* isolate from a baboon (*Papio cynocephalus*) as previously reported (1), limited information is available concerning oxygen requirements. This report discusses the more rapid growth of one *N. caviae* isolate and one each of *N. caviae*, *N. brasiliensis*, and *N. asteroides* stock cultures under an atmosphere of 10% CO₂ as compared with cultivation under strict aerobic and anaerobic conditions.

It may be seen in Table 1 that *N. caviae*, isolated from the baboon (1), grew more rapidly under 10% CO₂ than under aerobic conditions. To compare the baboon isolate with other well-documented *Nocardia* species, stock cultures were obtained from the Bacteriology Service, Brooke Army Medical Center, Ft. Sam Houston, Tex. These were incubated initially at 37 C on 5% whole-blood agar plates under three conditions: 10% CO₂, aerobically, and anaerobically (Table 2). To reduce possible test errors, the original stock cultures were examined in 10 separate experiments.

The following tests were performed in addition to the biochemical studies (1) to demonstrate whether the *N. caviae* baboon isolates grown aerobically or under 10% CO₂ were identical. Individual isolates grown under each atmospheric condition (aerobic and under 10%

CO₂) were subsequently grown under both conditions. Transfers from these colonies were then used for additional tests.

Growth of the *N. caviae* baboon isolate was apparent on whole-blood agar or Sabouraud dextrose agar slants within 24 h under 10% CO₂, whereas under aerobic conditions it required 21 days. Neither the original *N. caviae* baboon isolate nor the stock *Nocardia* sp. cultures grew under anaerobic conditions. Table 1 indicates the growth of the *N. caviae* baboon isolate through four passages under aerobic and 10% CO₂ conditions. As indicated, the isolates grew both aerobically and under 10% CO₂ in approximately the same length of time after four passages (2 days versus 1 day). However, aerobic conditions initially slowed the growth of the 10% CO₂ isolate.

TABLE 2. Growth of *N. caviae*, *N. brasiliensis*, and *N. asteroides* stock cultures under aerobic, anaerobic, and 10% CO₂ atmospheres

Organism	Growth (h)		
	Aerobic conditions	Anaerobic conditions	10% CO ₂
<i>N. caviae</i>	72	No growth	24
<i>N. brasiliensis</i>	96	No growth	24
<i>N. asteroides</i>	72	No growth	24

* On second passage, all three species grew out in 24 h.

TABLE 1. Growth of original *N. caviae* baboon isolates through four passages under aerobic, anaerobic, and 10% CO₂ atmospheric conditions

Isolate or passage	Growth (days)				
	Aerobic conditions		Anaerobic conditions	10% CO ₂	
	A ^a	B ^b		A	B
Test isolate	21	21	No growth	2	1
Passage 1	17	19	No growth	1	1
Passage 2	14	14	No growth	1	1
Passage 3	9	8	No growth	1	1
Passage 4	2	2	No growth	1	1

^a Aerobic isolate, originally recovered in 21 days.

^b 10% CO₂ isolate, originally recovered in 24 h.

Table 2 indicates the observed growth of stock *Nocardia* cultures under aerobic and 10% CO₂ atmospheric conditions. Initially, 10% CO₂ enhanced the growth of the organisms. There were no variations in the repeated tests. Once the stock cultures of *Nocardia* species grew well, there was no difference in growth at either 10% CO₂ or aerobic atmosphere.

These preliminary studies suggest that growth of the *Nocardia* species is promoted under 10% CO₂. To facilitate more rapid growth of these organisms upon initial isolation from clinical specimens and for revitalization of stock cultures, we suggest the use of 5% whole-blood agar plates under an atmosphere of 10% CO₂.

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LITERATURE CITED

1. Boncyk, L. H., B. McCullough, D. D. Grotts, and S. S. Kalter. 1975. Localized nocardiosis due to *Nocardia caviae* in a baboon (*Papio cynocephalus*). Lab. Anim. Sci. 25:88-91.
2. Emmons, C. W., C. H. Binford, and J. P. Utz. 1971. Medical mycology, 2nd ed., p. 94-107, 389-418. Lea and Febiger, Philadelphia.
3. Georg, L. K. 1974. *Nocardia* species as opportunists and current methods for their identification, p. 177-201. In J. E. Prier and H. Friedman (ed.), *Opportunistic pathogens*. University Park Press, Baltimore, London, Tokyo.
4. Gordon, R. E., and J. M. Mihm. 1962. Identification of *Nocardia caviae* (Erikson). Ann. N. Y. Acad. Sci. 98:628-636.
5. Kinch, D. A. 1968. A rapidly fatal infection caused by *Nocardia caviae* in a dog. J. Pathol. Bacteriol. 95:540-546.
6. Kurup, P. V., H. S. Randhawa, and N. P. Gupta. 1970. Nocardiosis: a review. Mycopathol. Mycol. Appl. 40:193-219.
7. Kurup, P. V., H. S. Randhawa, and R. S. Sandhu. 1968. A survey of *Nocardia asteroides*, *N. caviae* and *N. brasiliensis* occurring in soils of India. Sabouraudia 6:260-266.
8. Mostafa, I. E. M., L. Cerny, and J. Cerna. 1972. Canine nocardiosis due to *Nocardia caviae*. Rev. Elev. Med. Vet. Pays Trop. 21:181-189.
9. Smith, H. A., T. C. Jones, and R. D. Hunt. 1972. Veterinary pathology, 4th ed., p. 621. Lea and Febiger, Philadelphia.