UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

15 MAY 1991

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: SACB Review of Supplemental Information Submitted by Mycogen Corporation on Criteria for Identification of Pseudomonas fluorescens Colonies.

TO: Phil Hutton (PM-18)
Insecticide-Rodenticide Branch
Registration Division (H7505C)

FROM: Roy D. Sjoblad, Ph.D., Microbiologist
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THROUGH: Reto Engler, Ph.D., Chief
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Mycogen Corporation has submitted information on a proposal to determine whether any viable cells after fermentation and subsequent fixation procedures may be Pseudomonas spp. A "bean sheet" was not submitted with the data package.

SACB Conclusion: The proposed method - which relies on colony characteristics, and pigment production on semi-selective agar; and subsequently solely on the Gram-stain reaction, is totally inadequate for evaluating a viable bacterium as a Ps. fluorescens.

SACB Review of data:

Pseudomonas Isolation Agar (PIA) is used to select for viable microorganisms when material is plated out on PIA after the kill fixation incubation period.

If colonies on the plate are neutral color, and are round with the entire edge slightly raised in the center, then they will be tested further. If colonies do not fit these characteristics they will not be examined further. Blue-green or blue pigmented colonies will not be further tested because it will be assumed that these colonies are Pseudomonas aeruginosa.

Colonies fitting the above morphological description and which are not blue-green, will be subjected to a Gram-stain. Gram-positive bacteria will not be tested further. Gram-negative rods will be tested further by growth on L-agar plates [redacted]

[redacted]

SACB Discussion: The approach of relying solely on colony morphology and enhancement of pigment formation to conclude whether a bacterium growing on a semi-selective medium is Pseudomonas fluorescens is wholly inadequate. Subsequent sole reliance on the Gram-stain reaction to decide which isolates would need further testing also is totally unacceptable.

It is fundamental to bacterial taxonomy that individual bacteria must be examined microscopically and be described morphologically for shape, flagella presence and arrangement, in addition to the Gram-stain reaction. While colony appearance and pigment production are useful in the first stages of classification, they are by no means sufficiently stable or consistent markers for bacterium identification.

After the morphological stage of examination, it might be concluded that the isolate is not Pseudomonas. However, any Gram-negative polarly flagellated rod at this stage can be considered as a presumptive Pseudomonas sp. Even a non-motile Gram-negative rod may be a Pseudomonas sp. Such isolates should be subjected to the appropriate - and at least, key - nutritional/biochemical analyses to determine if it is Ps. fluorescens. Pigment production and analysis are needed for correct placement of Pseudomonas. When one is comparing an isolated bacterium with a known bacterium, the known bacterium probably should also be assayed at the same time using the same methods. Absolute confirmation of the isolate as a viable form of the engineered bacterium requires an appropriate probe for the inserted genetic material. Mycogen Corp. has not specified what is meant by

[redacted]

SACB is concerned that the proposed scheme for identification of isolates as Pseudomonas fluorescens does not reflect even the most basic application of appropriate microbial taxonomy; especially since the Agency has made it clear that the organism is to be killed.