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IDENTIFICATION OF THE NEWLY DESCRIBED *MYCOBACTERIUM PORIFERAE* FROM TUBERCULOUS LESIONS OF SNAKEHEAD FISH (*CHANNA STRIATUS*)

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Abstract—A mycobacterium isolated form a cultured snakehead with nodular lesions was identified on the basis of high performance liquid chromatography (HPLC) profile of cell wall mycolic acids, and confirmed by conventional tests, as *Mycobacterium poriferae*, a species previously isolated only from a marine sponge. The profiles of *M. poriferae*, *Mycobacterium aurum* and *Mycobacterium parafortuitum* are here reported for the first time.

Key words: *Mycobacterium poriferae*, HPLC, fish tuberculosis, bacterial identification, *Channa striatus*, aquaculture.

Résumé—Une mycobactérie isolée chez *Channa striatus* qui présentait des lésions nodulaires a été identifiée sur la base du profil des acides mycoliques de la paroi cellulaire à travers (HPLC) et confirmée par les tests conventionnels comme étant *Mycobacterium poriferae*, une espèce précédemment isolée chez une éponge marine uniquement. Les profils de *M. poriferae*, de *Mycobacterium aurum* et de *Mycobacterium parafortuitum* sont ici produits pour la première fois.

Mots-clefs: *Mycobacterium poriferae*, HPLC, tuberculose de poisson, identification bactérien, *Channa striatus*, aquaculture.

INTRODUCTION

Mycobacterium poriferae was first described by Padgitt and Moshier [1] who isolated this new species from the marine sponge *Halichondria bowerbanski*; to our knowledge no other isolation of this organism has been reported. The characterization of a strain of *M. poriferae* isolated from a cultured freshwater fish, the snakehead (*Channa striatus*, family *Channidae*), is here reported.

Granulomatous lesions, mainly due to *Mycobacterium marinum*, are not unusual in fish. In such animals infections due to *Mycobacterium fortuitum* [2], *Mycobacterium chelonae*

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[3], *Mycobacterium scrofulaceum* [4], *Mycobacterium simiae* [4], *Mycobacterium gordonae* [5] and to other unidentified nontuberculous mycobacteria have, sporadically, also been reported [5]. The identification procedures that allowed us to recognize, for the first time, that the species *M. poriferae* may be the cause of fish tuberculosis are described.

MATERIALS AND METHODS

The strain (S7) was isolated at the Aquatic Animal Health Research Institute of Bangkok (Thailand), from a snakehead presenting nodular lesions at the viscera, a typical feature of fish tuberculosis.

For its identification, both conventional tests [6, 7] and high performance liquid chromatography (HPLC) of cell wall mycolic acids were investigated. Conventional procedures included a wide panel of cultural and biochemical tests (Table 1).

The HPLC analysis was performed according to a technique developed at the Center for Disease Control (U.S.A.) [8]. Briefly, a 10 μl loopful of colonies grown at 30°C for 1 week on Middlebrook 7H11 medium was saponified with methanolic potassium hydroxide (25% KOH in 50% methanol) in an autoclave for 1 h at 121°C, and, after the acidification

Table 1. Results of standard biochemical, cultural and inhibition tests on our isolate of *M. poriferae* compared with those of the type strain

	Our strain	Type strain	
	S7	A	B
Niacin	–		–
Nitrate reduction	–	–	
Thermostable catalase	+	+	
Pyrazinamidase	+	+	
β-glucosidase	–		+
Tween 80 hydrolysis (10 days)	+	+	
Tellurite reduction	+		+
Arylsulfatase (3 days)	–	–	
Urease	+	+	
Catalase (over 45 mm of foam)	+		+
Photochromogenicity	–	–	
Scotochromogenicity[a]	+	+	
Growth at 25°C	+	+	
Growth at 37°C	+	+	
Growth at 45°C	–	–	
MacConkey	–		–
Tolerance to			
p-Nitrobenzoate (50 μg/ml)	–		–
NaCl (5%)	+	+	
Thiophene-2-carboxylic hydrazide (5 μg/ml)	+	+	
Thiacetazone (10 μg/ml)	+		+
Hydroxylamine (500 μg/ml)	–	–	
Isoniazid (1 μg/ml)	+		+
Oleate (250 μg/ml)	+		+
Ethambutol (1 μg/ml)	–		+
p-Aminosalicylate (1 μg/ml)	+		+
Toluidine blue (300 μg/ml)	–		–
Growth rate	Rapid	Rapid	
Colonial morphology	Smooth	Smooth	

(A) Padgitt and Moshier [1].
(B) Tests performed by us.
[a]Pinkish orange pigmentation.

with 18.5% HCl, mycolic acids were extracted with chloroform and derivatized to their UV-absorbing *p*-bromophenacyl esters [9]. After further acidification and chloroform extraction, a high molecular weight internal standard (Ribi, ImmunoChem, U.S.A.) was added and 5 μl of the sample were injected into the reverse phase C-18 ultrasphere-XL cartridge column of a HPLC System Gold model (Beckman, U.S.A.), equipped with a 166 model detector set at 260 nm. The elution conditions changed linearly, from 98% methanol:2% dichloromethane to an 80:20 mixture in 1 min and to a 35:65 one over the next 9 min (flow rate = 2.5 ml/min).

The HPLC profile was visually compared with the ones of our mycolic acids library, which includes, besides the profiles of a wide collection of clinical isolates, those of almost all type strains listed in Bergey's manual of systematic bacteriology [10].

RESULTS

On the basis of conventional tests results (Table 1) the strain appeared to be a rapidly growing scotochromogenic mycobacterium, able to grow between 25 and 37°C but not at higher temperatures, which did not match any of the species considered by clinical microbiology manuals [6, 7].

The only profile of our mycolic acid HPLC library which showed a very close similarity with that of our isolate (Fig. 1) was that of the type strain of *M. poriferae* (ATCC 35087). The differentiation of the HPLC pattern of *M. poriferae* was easily achieved from those of *Mycobacterium aurum* and *Mycobacterium parafortuitum* (Fig. 1), the species most closely related, on the basis of conventional tests [1], to this organism.

A very good agreement was present between conventional test results of our isolate and those reported in the sp. nov. description of *M. poriferae* [1]. Several features, previously not investigated on *M. poriferae*, were studied in parallel on our isolate and the reference strain, and they also gave compatible results. Only ethambutol tolerance was in disagreement.

The agreement of mycolic acid profile with that of the type strain, and the results of cultural and biochemical tests strongly suggest that the strain belongs to the recently described species *M. poriferae*.

DISCUSSION

Fish tuberculosis is of interest to man, both from the economic point of view and because of the well documented possibility of transmission of cutaneous infections in humans.

The economic impact is surely relevant in the present case, as the snakehead is a popular edible fish, cultured mainly in Thailand. Even if nothing is known about the clinical significance of *M. poriferae*, its isolation from a fish with typical nodular lesions supports the hypothesis of its pathogenic activity in such animals.

The lack of reports on infections caused by *M. poriferae* in man does not rule out its possible role in human pathology, as this could be due to lack of awareness of this new organism and the difficulty of identification. Thus, the occupational risk for aquaculture operators cannot, at present, be ignored.

Although the snakehead is a fresh-water fish, while the type strain was isolated from a marine sponge [1], the aquatic environment may represent an important link between

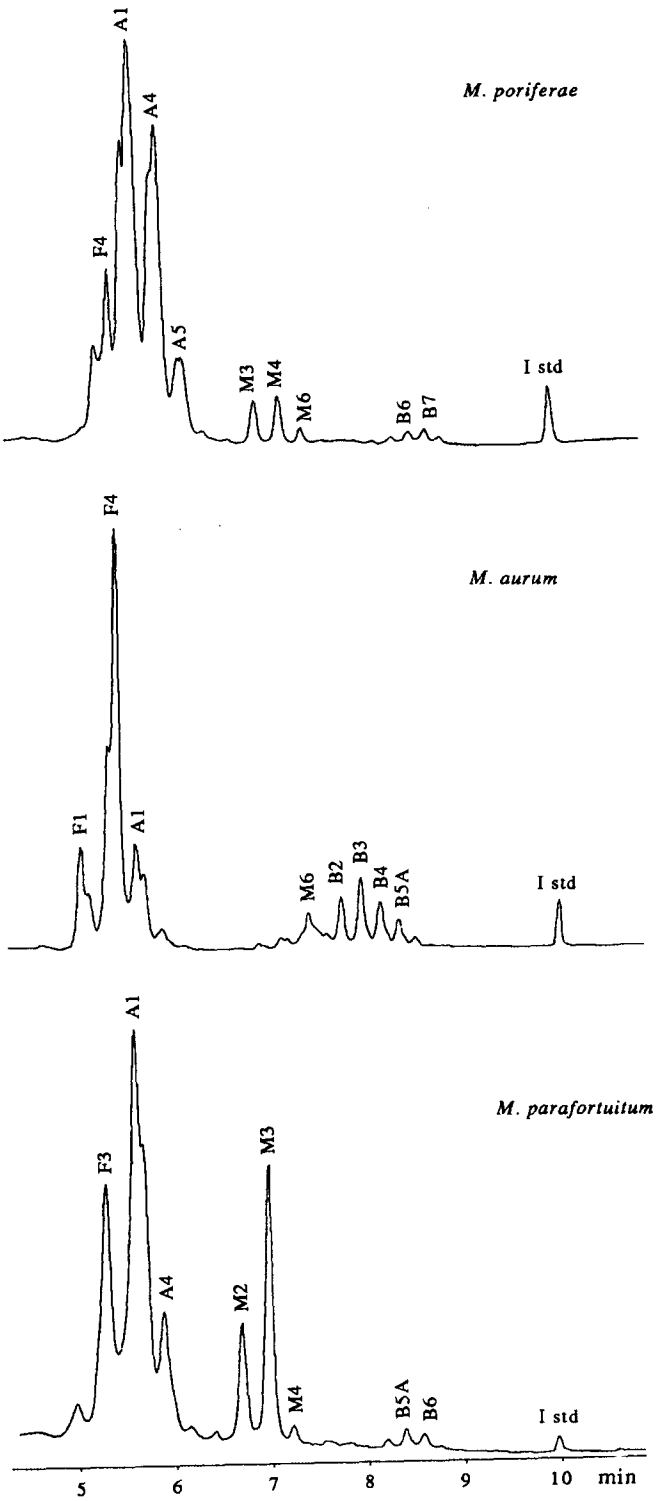

Fig. 1. Representative patterns of bromophenacyl esters of mycolic acids determined by HPLC from *M. poriferae* (ATCC 35087), *M. aurum* (ATCC 23366) and *M. parafortuitum* (ATCC 19686). Peaks identification according to Glickman *et al.* [15].

the only two reported isolations of *M. poriferae*. The trash marine fish with which snakeheads are fed, may, in fact, constitute a possible vehicle between the two habitats.

The ability of HPLC analysis of cell wall mycolic acids to support the identification of the frequently encountered mycobacteria is well documented [8, 11–13] and it has also been confirmed for some less common species [14]. The present case, in which HPLC has been of paramount importance in the speciation of an extremely rare isolate like *M. poriferae*, not achievable with conventional tests alone, suggests that this technique is not yet fully exploited.

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