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Selective Medium for Distinguishing Micrococci from Staphylococci in the Clinical Laboratory

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A nitrofuran-containing medium, FTO agar, supported the growth of *Micrococcus* and prevented the growth of *Staphylococcus*. Its potential as a differential medium is considered worthy of clinical trial.

A medium selective for corynebacteria in skin samplings was described in 1969 by Smith (12, 13). Known as FTO agar, it contains a nitrofuran [Furoxone: 1-*N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidene; Norwich Pharmacal, Norwich, N.Y.]. Our use of the medium for axilla ecology studies soon revealed that certain aerobic cocci flourished on FTO, exhibiting more resistance to the selective agent than even the corynebacteria (J. C. Curry and G. E. Weise, Abstr. Annu. Meet. Am. Soc. Microbiol. 1972, E57, p. 10). Since FTO was designed to inhibit staphylococci, it was hypothesized that the resistant skin cocci were micrococci; colony morphology (generally large, circular, yellow, and dull) and Gram stain results (gram-positive cocci in tetrads and pairs) supported this. On axilla contact plates of FTO agar, the colonies that developed first (1 or 2 days) appeared indifferent to the selective agent and were easily distinguished from the slow-growing (4 days) corynebacteria (small, beige-to-pink colonies). To provide us with some assurance that the cocci colony counts on FTO were micrococci, known strains of *Micrococcus* and *Staphylococcus* were tested for their capacity to grow on FTO. The results of these preliminary studies indicated that FTO resistance was common to all *Micrococcus* species, with sensitivity common to all *Staphylococcus* species (J. C. Curry and G. E. Borovian, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, E39, p.7).

Considered of potential value to clinical microbiologists, these studies have since been expanded to include 74 strains of *Micrococcus* species and 119 strains of *Staphylococcus* species. Seventy-two strains representing seven *Micrococcus* species from skin (66 to 72% GC) were obtained from Wesley E. Kloos of North Carolina State University. Characterized as *Micrococcus* species by an extensive array of traditional biochemical methods and cell wall and deoxyribonucleic acid base analyses (1, 2, 4, 8, 9), these included: ten strains each of *M. kristinae*, *M. sedentarius*, *M. roseus*, *M. lylae*, *M. nishinomyaensis*, *M. varians*, and *M. luteus* and two strains of *Micrococcus* sp. Upon receipt, all cultures were transferred to fresh slants of brain heart infusion agar (BHIA), incubated for 2 days at 32°C, and stored refrigerated. Two strains from the collection of Lever Brothers, *Micrococcus* S and *M. varians* MS102, brought the total number of 74.

Also obtained from W. E. Kloos and thoroughly identified (6, 7, 10, 11) were 100 strains of 10 *Staphylococcus* species from skin (30 to 37% GC): 10 strains each of *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. cohnii*, *S. xylosus*, *S. saprophyticus*, *S. capitis*, *S. hominis*, *S. simulans*, and *S. warneri*. Also included were eleven strains of Baird-Parker's "*Micrococcus*" subgroups: two strains each of subgroups 1 and 6; three strains each of subgroups 2 and 3; and one strain of subgroup 5. Upon receipt, BHIA slants were prepared as above. Eight strains from the collection of Lever Brothers (*S. aureus* ATCC 6538, *S. epidermidis* ATCC 17917, *S. warneri* ATCC 155, *Staphylococcus* sp. SS-1 and ATCC 8425, and three antibiotic-resistant hospital isolates of *Staphylococcus*) brought the total number to 119.

FTO agar is conveniently prepared in 700-ml amounts in 1-liter Erlenmeyer flasks. Tryptic soy agar (Difco) or Trypticase soy agar (BBL), fortified with 0.1% yeast extract and 0.5% Tween 80 (sorbitan monooleate), is the basal medium of Smith that fosters the growth of corynebacteria. It is also an excellent medium for *Micrococcus-Staphylococcus* testing, although fortification is probably unnecessary. After autoclaving and cooling to 48°C, 0.1% of a 0.5% acetone stock of Oil Red O and 10% of a 0.05% acetone stock of Furoxone were added. To prevent flocculation, the latter was added slowly from a 100-ml graduate into swirling

agar. Flasks were then left open or loosely covered in the water bath, to allow acetone volatilization prior to pouring and hardening plates on a level surface.

Broth subcultures (BHI) were prepared from all stock slants and subsequently streaked directly onto FTO agar by sterile swab. Control streak plates were also prepared using plate count agar. After 2 or 3 days of incubation, the amount of growth on FTO, as compared to the controls, served as a qualitative measure of FTO resistance. The results were dramatic, with positive growth of all *Micrococcus* strains (74) and negative growth of all *Staphylococcus* strains (119).

Additionally, 18 strains of *Micrococcus* species were subjected to both qualitative (streak plate) and quantitative FTO agar plate tests. The latter were easily prepared by pipetting 1 ml from appropriate decimal dilutions onto the agar surface of leveled plates, which were then left undisturbed for absorption of liquid (a few hours or, if more convenient, overnight). FTO is a very dry medium and no predrying of the agar surface or inversion of finished plates during incubation is necessary. Control counts were obtained by standard pour plate method using BHIA. All plates were incubated for 4 days at 35°C, prior to counting colonies and conversion to count/milliliter of broth cultures. Table 1 shows that most of the strains tested (13 of 18) were relatively unaffected by Furoxone, as evidenced by comparable BHIA and FTO counts. Furthermore, FTO plate counts can be utilized to quantitate at least those most common to skin, *M. luteus* and *M. varians* (8), as well as the less common species, *M. roseus* and *M. kristinae*. Some strains of *M. nishinomyaensis*, *M. lylae*, and *M. sedentarius* would be missed by this method. Thus, even though we recognize that FTO agar, like all selective agars, has some limitations, it can be useful for skin ecology studies for disclosing micrococcal populations within the staphylococci-dominated axillary flora. Important to diagnostic studies, however, is the finding that even those few that were inhibited when diluted for plate counts were at least able to initiate growth on FTO streaks, using undiluted broth cultures.

To provide FTO selectivity information, minimum inhibitory concentration (MIC) values for the agent itself against a variety of microorganisms were determined by the gradient plate method (3). Table 2 shows the dramatic contrast in resistance between the staphylococci (less than 10 μg/ml) and the micrococci (more than 1,000 μg/ml). A perusal of other MIC values shows that FTO as prepared herein (50 μg of Furoxone per ml) would normally support (in addition to *Micrococcus*) most corynebacteria, *Candida albicans*, *Brevibacterium ammoniagenes*, *Lactobacillus casei*, and some yeasts.

TABLE 1. *Growth of micrococcus species on FTO agar*

Strain no.[a]	Species	Qualitative (streak plate)[b]		Quantitative (plate count/ml)[c]	
		FTO	PCA	FTO (surface)	BHIA (pour)
WK132	*Micrococcus* sp.	+++	+++	4,500,000	4,600,000
ATCC 416	*M. roseus*	++	++	70,000	60,000
ATCC 516	*M. roseus*	++++	++++	7,000,000	9,000,000
ATCC 272	*M. luteus*	++++	++++	15,000,000	15,000,000
CCM852	*M. luteus*	++++	++++	12,000,000	18,000,000
S	*Micrococcus* sp.	++++	++++	26,000,000	25,000,000
ATCC 9341	*M. varians*	++++	++++	52,000,000	60,000,000
MS102	*M. varians*	++++	++++	14,000,000	14,000,000
CCM884	*M. varians*	++++	++++	49,000,000	53,000,000
ATCC 27573	*M. sedentarius*	++	+++	NC[d]	3,000,000
PM297	*M. nishinomyaensis*	++++	++++	4,300,000	5,600,000
LK335	*M. nishinomyaensis*	++++	++++	19,000,000	23,000,000
CCM2140	*M. nishinomyaensis*	+	+++	<10,000	9,100,000
JL205	*M. nishinomyaensis*	+	+++	<10,000	13,000,000
WK312	*M. lylae*	++++	++++	22,000,000	22,000,000
ATCC 27568	*M. lylae*	+	++++	20,000	31,000,000
MK322	*M. kristinae*	++++	++++	44,000,000	60,000,000
GH270	*M. kristinae*	++++	++++	40,000,000	50,000,000

[a] Courtesy of W. E. Kloos, North Carolina State University, with one exception (*Micrococcus* sp. S, from the collection of Lever Brothers).

[b] +, Light; ++, moderate; +++, heavy; ++++, luxuriant. PCA, Plate count agar.

[c] Expressed as colony-forming units.

[d] NC, Not countable. Positive growth, but decimal dilutions did not follow.

TABLE 2. *Gradient plate MIC values for Furoxone*

Organism (source)	MIC of Furoxone (μg/ml)
Staphylococcus sp. (saliva)	0.3
Staphylococcus warneri ATCC 155	1.4
Streptococcus sp. (tongue)	1.9
Escherichia coli ATCC 10536	2.0
Staphylococcus epidermidis ATCC 17917	2.1
Staphylococcus aureus ATCC 6538	2.3
Streptococcus sp. (saliva)	2.8
Yeast (axilla)	3.1
Actinomyces sp. (tongue)	3.5
Streptococcus salivarius ATCC 9756	5.0
Staphylococcus sp. (saliva)	6.1
Corynebacterium sp. (axilla)	10
Streptococcus sp. (saliva)	25
Streptococcus faecalis ATCC 10541	25
Pseudomonas aeruginosa ATCC 9721	44
Yeast (axilla)	(>50)
Corynebacterium sp. (axilla)	64
Corynebacterium sp. (axilla)	67
Micrococcus sp. (axilla)	100
Nocardia sp. (saliva)	200
Lactobacillus casei ATCC 4646	(>500)
Corynebacterium sp. (saliva)	(>500)
Corynebacterium sp. (saliva)	(>500)
Brevibacterium ammoniagenes ATCC 6871	(>1,000)
Candida albicans ATCC 10231	(>1,000)
Corynebacterium sp. (axilla)	(>1,000)
Micrococcus roseus ATCC 416	(>1,000)
Micrococcus roseus ATCC 516	(>1,000)
Micrococcus luteus ATCC 272	(>1,000)
Micrococcus varians ATCC 9341	(>1,000)
Micrococcus luteus S (axilla)	(>1,000)
Micrococcus sp. (axilla)	(>1,000)

Note that the streptococci, although less sensitive than staphylococci, would also be inhibited on 50 μg of FTO per ml. In general, MIC information is helpful for interpretation of mixed population results, with colony characteristics usually sufficient to differentiate major types present. MIC values can also be used as guides for adjusting the selectivity of the medium if so desired. At 50 μg of Furoxone per ml, for instance, some corynebacteria but no micrococci would be inhibited.

The primary finding, that FTO permits the growth of *Micrococcus* and prevents the growth of *Staphylococcus*, has led to the conclusion that a simple streak plate can serve as a first step towards identifying an unknown coccus. Since the medically important organisms, the staphylococci, provide negative results, positive controls with a known *Micrococcus* species are recommended. It is further suggested that this Furoxone-sensitivity characteristic of the staphylococci might also be applied to routine sensitivity testing by adding a Furoxone disk (50 μg/ml) to the battery normally used.

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