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Antibiotic Resistances of Yogurt Starter Cultures *Streptococcus thermophilus* and *Lactobacillus bulgaricus*

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Twenty-nine strains of *Lactobacillus bulgaricus* and 15 strains of *Streptococcus thermophilus* were tested for resistance to 35 antimicrobial agents by using commercially available sensitivity disks. Approximately 35% of the isolates had uncharacteristic resistance patterns.

Many antibiotics are used in the dairy industry for mastitis therapy, and this results in their being excreted in milk. From the 1950s to the mid-1970s, there were reports concerning the presence of antibiotics in milk (7, 11, 16). There were also attempts to grow antibiotic-resistant cultures of lactobacilli for the production of yogurt (15).

In most countries, legislation restricts the sale of milk containing antibiotics, as recommended by the World Health Organization (FAO/WHO), (1, 11, 12). The FAO/WHO and the Codex Alimentarius (12) state, "yogurt is a coagulated milk product obtained by lactic acid fermentation through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Its microorganisms in the final product must be viable and abundant." French legislation also requires that the product contain at least 10^7 viable bacteria per g (10).

Although it has been stated that there is no reasonable justification for using antibiotic-resistant strains in the manufacture of yogurt (12), there are few publications concerning the antibiotic resistance of these strains. The most comprehensive studies are limited in either number of cultures or the types of antibiotics tested, and no information is given concerning the origin of the cultures used (8, 13, 15).

Yogurt contains very high concentrations of viable microorganisms, and therefore, because of the concern in recent years about the unnecessary use of antibiotic-resistant strains, it was believed pertinent to survey the resistances of cultures selected for yogurt manufacture.

MATERIALS AND METHODS

Organisms. Strains of *L. bulgaricus* and *S. thermophilus* were isolated or obtained during a screening program between 1973 and 1975 for those suitable for use in the manufacture of yogurt so that a regular sequence of starter cultures could be maintained. All strains were identified according to the descriptions of Rogosa (14) and Deibel and Seeley (2) and were chosen for characteristics most suitable for the manufacture of yogurt: rapid growth, acid production, and good flavor enhancement.

The *L. bulgaricus* strains used in this study were obtained as follows: YL-1, from the Laboratory of Applied Industrial Biology, St.-Ouer, France; YL-2, from yogurt bought in Lausanne, Switzerland; YL-4, from Christian Hansen's Laboratories, Copenhagen, Denmark; YL-5, from yogurt bought in Sofia, Bulgaria; YL-6/YL-12, from Wieby Laboratories, Nieboll, Germany; YL-13, an old culture, origin unknown; YL-14 and YL-15, from yogurt bought in Prague, Czechoslovakia; YL-17, YL-18, and YL-20, from Universita Cattolica del Sacro Cuore, Piacenza, Italy; LB-1 and LB-2, from the National Institute for Agronomical Research, Jouy-en-Josas, France; LB-3, from fresh milk, Canton du Valais, Switzerland; LB-4, from Chambourcy cheese factory, Marseille, France; LB-5, from Nestlé Industrial Laboratory, Orbe, Switzerland; LB-6, from Unigate Technical Centre, Bradford-on-Avon, England; LB-7/LB-12, from scum in the preparation of Emmenthal-type cheese, cheese factory, Nogent-en-Bassigny, France.

The *S. thermophilus* strains used in this study were obtained as follows: YS-1, from the Laboratory of Applied Industrial Biology, St.-Ouer, France; YS-2 and YS-3, from yogurt bought in Lausanne, Switzerland; YS-4, from Christian Hansen's Laboratories, Copenhagen, Denmark; YS-5, from yogurt bought in Sofia, Bulgaria; YS-6/YS-12, from Wiesby Laboratories, Nieboll, Germany; YS-14 and YS-15, from yogurt bought in Prague, Czechoslovakia; YS-16, and old culture, U.S.A., origin unknown.

Sensitivity test procedure. Cultures of *L. bulgaricus* were grown in lactobacilli MRS broth (Difco Laboratories, Detroit, Mich.). Cultures of *S. thermophilus* were grown in HJ medium (5) containing tryptone (3%, wt/vol), yeast extract (1%, wt/vol), beef extract (0.2% wt/vol), lactose (0.5% wt/vol), and KH_2PO_4 (0.5% wt/vol) in distilled water, with final pH adjusted to 6.5. The medium was sterilized at 121°C for 15 min. Cultures were plated in their respective solid media to which agar was added (1.5, wt/vol) before sterilization. Actively growing bacterial cultures (0.1 ml) were mixed with sterile agar medium (10 ml, 45°C) as the plate was poured. Dispens-o-Discs (Difco, Detroit, Mich.) were used; we also prepared disks of 0.5 and 1.0 μg of ampicillin, 0.5 and 1.0 U of penicillin G, and 1.0 and 5.0 μg of tetracycline (Sigma Chemical Co., St. Louis, Mo.). The disks were placed on the agar surface, and the plates were incubated for 16 h. Resistance was defined no zone of growth inhibition around the disk. All incubations were at 40°C.

RESULTS AND DISCUSSION

All cultures were sensitive to the following antibiotics: 0.5 μg of ampicillin, 10 μg of aureomycin, 5 U of bacitracin, 50 μg of carbenicillin, 30 μg of cephaloridin, 30 μg of cephalothin, 5 μg of chloromycetin, 2 μg of clindamicin, 1 μg of dicloxacillin, 30 μg of doxycycline, 2 μg of erythromycin, 3 μg of Mandelamine, 5 μg of methicillin, 15 μg of oleandomycin, 0.5 U of penicillin G, 5 μg of rifampin, 10 μg of terramycin, and 1 μg of tetracycline. It was of interest that none of the strains was resistant to penicillin, tetracycline, or erythromycin, since resistance has been previously reported (4, 15).

The results of antibiotic resistance studies are summarized in Tables 1 and 2. Many of our strains exhibited both a marked increase in resistance and different patterns of resistance compared with the results reported by Reinbold and Reddy (13) and Marth and Ellickson (8).

Most *S. thermophilus* and *L. bulgaricus* strains were resistant to both neomycin (5 μg) and polymyxin B (300 U), both of which have been used in mastitis therapy. Apart from these two antibiotics, our results for *S. thermophilus* (Table 1) show, compared with the study of Reinbold and Reddy (13), a marked increase in the numbers of strains resistant to those antibiotics as well as with previously unreported resistances to dihydrostreptomycin, furadantin, novobiocin, cloxacillin, oleandomycin, and oxacillin. All of these antibiotics are used in mastitis control. It is of interest that most strains of *S. thermophilus* exhibited resistance to streptomycin, whereas previously strains had been reported sensitive to 0.1 μg/ml (8). Although our study did not contain all of the antibiotics previously reported to be used in mastitis control (13), all of our strains of *S. thermophilus* were resistant to at least two (in three instances, five) of these antibiotics; the strains of Reinbold and Reddy (13), were resistant to only one, poly-

TABLE 1. *Antibiotic resistance[a] patterns for strains of S. thermophilus*

Antibiotic	Concn	Bacterial strains														
		YS-1	YS-2	YS-3	YS-4	YS-5	YS-6	YS-7	YS-8	YS-9	YS-10	YS-11	YS-12	YS-14	YS-15	YS-16
Cloxacillin[b]	1 μg					R										
Colimycin	10 μg	R	R	R	R	R	R	R	R	R	R	R[c]	R	R	R	R
Dihydrostreptomycin[b]	10 μg	R	R	R	R	R	R	R	R	R	R		R	R		R
Doxycycline	10 μg															
Furadantin[b]	50 μg		R	R		R	R									
	300 μg															
Gentamycin	10 μg	R	R	R	R	R	R			R	R	R	R	R		R
Kanamycin	5 μg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	30 μg	R	R		R	R	R	R	R	R	R		R	R		R
Lincomycin[b]	2 μg															
Mandelamine	2 μg															
Mycostatin	100 U	R	R	R		R	R	R		R	R	R	R	R	R	R
Nalidixic acid	30 μg	R	R	R	R	R	R	R	R	R	R	R[c]	R	R	R	R
Neomycin[b]	5 μg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	30 μg	R	R	R	R	R	R	R	R	R	R	R	R	R		R
Novobiocin[b]	10 μg	R						R								
	30 μg	R														
Oleandomycin	5 μg		R													
Oxacillin[b]	1 μg							R								
Polymyxin B[b]	50 U	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Sulfamethoxazole-	300 U	R	R	R		R	R	R		R	R		R	R	R	R
trimethoprim	25 μg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Streptomycin	2 μg	R	R	R	R	R	R	R	R	R	R		R	R	R	R
	10 μg	R	R	R	R	R	R	R		R	R		R	R		R
Sulfadiazine	300 μg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Sulfamethoxypyridazine	300 μg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

[a] Resistance is denoted by R; unless so indicated, strains were sensitive.
[b] Antibiotics reported by Reinbold and Reddy (13) to be used in mastitis therapy.
[c] Indicates tests where stimulation of growth occurred around the Dispens-o-Disc.

TABLE 2. *Antibiotic resistance[a] patterns for strains of L. bulgaricus*

Antibiotic	Concn	Bacterial strains																												
		YL-1	YL-2	YL-4	YL-5	YL-6	YL-7	YL-8	YL-9	YL-10	YL-11	YL-12	YL-13	YL-14	YL-15	YL-17	YL-18	YL-20	LB-1	LB-2	LB-3	LB-4	LB-5	LB-6	LB-7	LB-8	LB-9	LB-10	LB-11	LB-12
Cloxacillin[b]	1 µg																												R	
Colimycin	10 µg	R	R	R	R	R	R		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Dihydrostreptomycin[b]	10 µg																												R	R
Doxycycline	10 µg							R																						
Furadantin[b]	50 µg																									R				
	300 µg																									R				
Gentamicin	10 µg																									R			R	
Kanamycin	5 µg															R					R				R	R	R	R	R	R
	30 µg																				R				R	R	R	R	R	R
Lincomycin[b]	2 µg							R		R			R																	
Mandelamine	2 µg							R																						
Mycostatin	100 U	R	R		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R	R	R
Nalidixic acid	30 µg	R	R	R	R	R	R	R	R	R	R	R	R^c	R	R	R^c	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Neomycin[b]	5 µg	R		R		R	R	R	R	R	R		R^c	R		R^c	R	R		R	R	R	R	R	R	R	R	R	R	R
	30 µg						R				R		R			R^c					R				R	R	R		R	R
Novobiocin[b]	10 µg																									R				
	30 µg																									R				
Oleandomycin	5 µg																													
Oxacillin[b]	1 µg																													
Polymyxin B[b]	50 U	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Sulfamethoxazole-trimethoprim	300 U	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R^c
	25 µg			R		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R^c
Streptomycin	2 µg																												R	R
	10 µg																												R	R
Sulfadiazine	300 µg	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Sulfamethoxypyridazine	300 µg														R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

[a-c] See Table 1.

myxin B (50 U). Our study would therefore appear to show a marked increase in the number of *S. thermophilus* strains resistant to these antibiotics. Our overall results for strains of *S. thermophilus* resemble the results obtained by Reinbold and Reddy (13) for *S. faecalis* more closely than for *S. thermophilus*. Comparison of the results for *S. thermophilus* (Table 1) with those for *L. bulgaricus* (Table 2) shows that one cannot clearly distinguish between the groups by comparing antibiotic resistances. With the exception of strains YS-2, YS-7, and YL-8, both groups were resistant to the same antibiotics.

Our results with *L. bulgaricus* strains (Table 2) also show an increase in the numbers of strains resistant to polymyxin B (300 U), neomycin (30 µg), lincomycin, novobiocin, and doxycycline. All of these antibiotics with the exception of doxycycline are reportedly used in mastitis therapy. To our knowledge, resistances to lincomycin and doxycycline have not been previously reported in *L. bulgaricus*. However, unlike the results with *S. thermophilus*, we found a reduction in the number of strains resistant to certain antibiotics for which resistances were previously reported. For example, no strains were resistant to tetracycline or oleandomycin, and only one was resistant to mandelamine. In consequence, unlike *S. thermophilus*, our strains of *L. bulgaricus* were not resistant to more antibiotics used in mastitis therapy than were strains reported by Reinbold and Reddy (13). However, our strains of *L. bulgaricus* that exhibited resistance to the most antibiotics were isolated from raw milk and thus were more recently isolated from nature.

The reasons for our observed increases in antibiotic resistances are not yet known. There may be differences due to the method of determining resistance, the basal media used, the typing of strains, the accidental presence of antibiotics in milk, mutation, or transmission of resistances. Although no studies have reported attempts to demonstrate genetic exchange mechanisms in yogurt cultures, evidence is accumulating that such mechanisms exist for other bacteria commonly found in dairy products (3, 6, 9). Whatever the reason, our results show that certain strains which would otherwise be used in the manufacture of yogurt may have numerous unnecessary antibiotic resistances. Therefore, for manufacture of yogurt, we consider it prudent to use strains that are resistant to as few antibiotics as possible in order to reduce an unnecessary distribution of antibiotic-resistant strains. For this reason, of the strains used in this study, we do not recommend that the following strains be used for the manufacture of yogurt: *S. thermophilus* strains YS-1, YS-2, YS-3, YS-5, YS-6, and YS-7 and *L. bulgaricus* strains YL-8, YL-10, YL-13, YL-17, LB-3, LB-7, LB-8, LB-9, LB-10, LB-11, and LB-12.

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