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Microbiological Spoilage of Mayonnaise and Salad Dressings[1]

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Saccharomyces bailii was isolated from two-thirds of the spoiled mayonnaise and salad dressing samples examined. Most of the rest were spoiled by *Lactobacillus fructivorans*. However, one sample contained large numbers of both *S. bailii* and *L. plantarum*. Two of the spoiled samples also contained small numbers of bacilli. *Bacillus subtilis*, *B. pumilis*, *B. polymyxa*, *B. megaterium*, and *B. licheniformis* were found in one sample and *B. subtilis* and *B. pumilis* in another. Small numbers of *B. subtilis* and *B. licheniformis* were also present in one unspoiled sample. Several media were evaluated for the isolation of *L. fructivorans*. *S. bailii* and *L. fructivorans* vigorously fermented glucose. The concentration of glucose in the spoiled samples ranged from 0 to 38.5 g/kg and from 1.3 to 17.8 g/kg for the unspoiled samples.

Spoilage in mayonnaise and salad dressings results from a variety of causes including separation of the emulsion, oxidation and hydrolysis of the oils by chemical or biological action, and growth of microorganisms that produce gas or off-flavors (9).

Microbiological spoilage of these products is generally caused by yeasts and bacteria. Williams and Mrak (30) reported gassy spoilage of a starch-based salad dressing to be caused by a yeast similar to *Zygosaccharomyces globiformis*. Fabian and Wethington (7) found samples of salad dressing and French dressing to be spoiled by an unidentified species of *Zygosaccharomyces*. Similarly, Appleman et al. (3) observed large numbers of an unidentified species of *Saccharomyces* in spoiled mayonnaise but also found *Bacillus subtilis* to be abundant. Pederson (19) reported *B. vulgatus* to be responsible for spoilage in a Thousand Island dressing. The work of Charlton et al. (5) appears to be the first report of salad dressing spoiled by lactobacilli. The species involved was considered new and described as *Lactobacillus fructivorans*.

The question of survival of pathogenic bacteria in mayonnaise and salad dressings has previously been investigated, and the studies indicate that the products themselves generally represent no health hazard because of survival or multiplication of pathogenic bacteria (4, 8, 16, 24, 27).

Our work was undertaken because the microbiological spoilage of mayonnaise and salad dressing still occurs frequently and the microorganisms responsible for spoilage have generally not been well studied. Considerations are also given to methods of detecting the spoilage microorganisms and reasons for their growth in these products.

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MATERIALS AND METHODS

Source of samples. Samples of spoiled mayonnaise and salad dressings were received from the Mayonnaise and Salad Dressings Institute and represented various brands manufactured throughout the United States. Unspoiled samples were obtained from local markets and also represented various brands.

Plating procedures. An 11-g sample was placed in 99 ml of 0.1% sterile peptone water and mixed in a Waring Blendor for 10 min at low speed. Subsequent higher dilutions with peptone water were made from this mixture. Preliminary experiments with diluents showed that 0.1% peptone water, as recommended by Straka and Stokes (22) for other food products, gave two to five times more colonies than distilled water and was comparable to solutions of glucose (15 and 30%), glycerol (5, 10, and 25%), and sodium chloride (5 and 10%).

Plate count agar (PCA; Difco) with 100 μg of cycloheximide per ml for inhibiting fungi was used to determine the number of aerobic bacteria. Anaerobic and microaerophilic bacteria were detected by placing a loopful of undiluted sample into a tube of thioglycollate broth (BBL). After growth was observed, the broth was streaked onto plates of anaerobic agar (BBL) which then were incubated in a nitrogen atmosphere. The cultures from these plates were maintained on APT agar (BBL). Later, samples were

plated with Lactobacillus Selective (LBS) agar (BBL) instead of inoculating into thioglycollate broth.

The samples were examined for yeasts and molds by plating on malt-glucose-agar (7) and on YXT agar (0.4% yeast extract, 0.4% glucose, 1.0% malt extract, and 1.5% agar, in distilled water). Tetracycline HCl (30 μg/ml) was added to both media to inhibit bacteria. The number of yeasts recovered on YXT agar and malt-glucose-agar were comparable, and only counts from the YXT agar are reported.

All plates were incubated at 28 C. Yeast counts were made at 5 days, and bacteria on PCA plates were counted at 3 days, but those on LBS agar were counted at 10–14 days.

Identification of microorganisms. The yeast isolates were identified by using the culture techniques of Wickerham (29) and the classification system proposed by van der Walt (26). The lactobacilli were identified by using criteria given by *Bergey's Manual*, 7th ed., Rogosa and Sharpe (20), Charlton et al. (5), and Vaughn et al. (23). The medium used for carbon assimilation studies of the lactobacilli was that given for gas detection by Gibson and Abd-el-Malek (10). Growth in the presence of bile salts was determined by the method of Wheater (28), and the Voges-Proskauer tests were made with paper test-strips (PathoTec, General Diagnostics Division, Warner-Chilcott Laboratories, Morris Plains, N.J.). Bacilli were identified on the basis of the scheme in *Bergey's Manual*, 7th ed.

Determination of glucose. Glucose was extracted from the mayonnaise and salad dressings by the following procedure. A 5-g sample was mixed with 5 ml of distilled water and added to 30 ml of cold 95% ethanol. The mixture was centrifuged at 10,000 $\times$ *g* for 20 min at 5 C. The clear supernatant was reduced to 5 to 8 ml in vacuo at 55 C, the *p*H was set at 7.0 with 1 N NaOH, and the volume was brought up to 10 ml with distilled water. Recovery of glucose by this method was estimated to be 75 $\pm$ 5% based on addition of known amounts of glucose to selected samples.

Glucose was detected chromatographically by using descending paper chromatography (Whatman no. 1 paper) with a butanol-pyridine-water (6:4:3, v/v) solvent system. The spots were located by first spraying the chromatograms with $AgNO_3$ in acetone and then spraying with NaOH in ethanol. Quantitative determinations of glucose were made as outlined in the glucose oxidase-peroxidase method of Hill and Kessler (12), but the buffer was changed to 0.8 M

TABLE 1. *Numbers of microorganisms present in spoiled and unspoiled mayonnaise and salad dressings*

Samples	Type of dressing	Sample *p*H	Bacteria present on			Yeasts (no./g)
			Thioglycolate[a]	LBS[b] (no./g)	PCA (no./g)	
Spoiled						
1	Blue cheese	4.1	−		205,000	107,000
2	Mayonnaise-like[c]	4.1	+		400	0
3	Mayonnaise-like	3.9	−		180	44,500
4	Mayonnaise-like	3.9	−		0	39,500
5	Mayonnaise-like	3.8	−		0	10,900
6	Mayonnaise-like	3.8	−		0	8,250
7	Mayonnaise-like	3.6	−		0	14,550
8	Mayonnaise-like	3.9	−		0	165,500
9	Mayonnaise-like	3.7	−		0	32
10	Mayonnaise-like	3.7	−		0	100
11	Mayonnaise-like	3.7	−		0	36
12	Mayonnaise-like	3.7	−		0	5
13	Mayonnaise	4.0			0	11,750
14	Mayonnaise	4.0			0	17,350
15	Mayonnaise	3.8		8,100	0	0
16	Mayonnaise	3.7		9,000,000	0	0
17	Mayonnaise	3.8		12,600,000	0	0
Unspoiled						
1–4	Mayonnaise	4.0–4.2	−		0	0
5–6	Salad[d]	3.7	−		0	0
7–8	Italian	3.4–3.8	−		0	0
9	Thousand Island	3.7	−		0	0
10	Blue cheese	4.6	−		70	0

[a] Indicated by − no growth; + growth.
[b] LBS, Lactobacillus Selective; PCA, plate count agar.
[c] Not identified as to whether mayonnaise or salad dressing.
[d] Mayonnaise-like dressing which contains a cooked or partly cooked starch or flour paste.

tris(hydroxymethyl)aminomethane and the dye to *o*-tolidine dihydrochloride.

RESULTS

Microbiological analyses of 17 spoiled samples and 10 unspoiled samples are given in Table 1. Eleven of the spoiled samples contained yeasts, four had bacteria, but two had both bacteria and yeasts. Only one of the unspoiled samples contained microorganisms, a small number of bacilli. Molds were not detected in any of the samples. Thioglycollate broth was used to detect lactobacilli throughout most of the study, but it was then found that LBS agar allowed good growth and a plate count could also be made.

Although a variety of samples from different parts of the country were spoiled by yeasts, *Saccharomyces bailii* Lindner was the only species isolated. All of the isolates formed abundant ascospores on malt extract and yeast-malt-agars (29), and there were usually four spores in each conjugated ascus (Fig. 1).

These isolates of *S. bailii* gave a vigorous gaseous fermentation of D-glucose but no fermentation of D-galactose, maltose, lactose, or raffinose. Sucrose was fermented latently by isolates from nine of the 13 samples containing yeasts. The gaseous fermentation of sucrose began 12 to 56 days after inoculation of the fermentation medium and was quite vigorous. All isolates assimilated D-glucose, D-galactose, ethanol, glycerol, ribitol, D-mannitol, D-glucitol, and acetate (weak), and some isolates also assimilated L-sorbose, trehalose, and calcium 2-keto-D-gluconate (weak); the other carbon sources used in standard assimilation tests (29) were not utilized. There was no liquefaction of gelatin.

Two of the spoiled samples contained small numbers of bacilli in addition to the other micro-

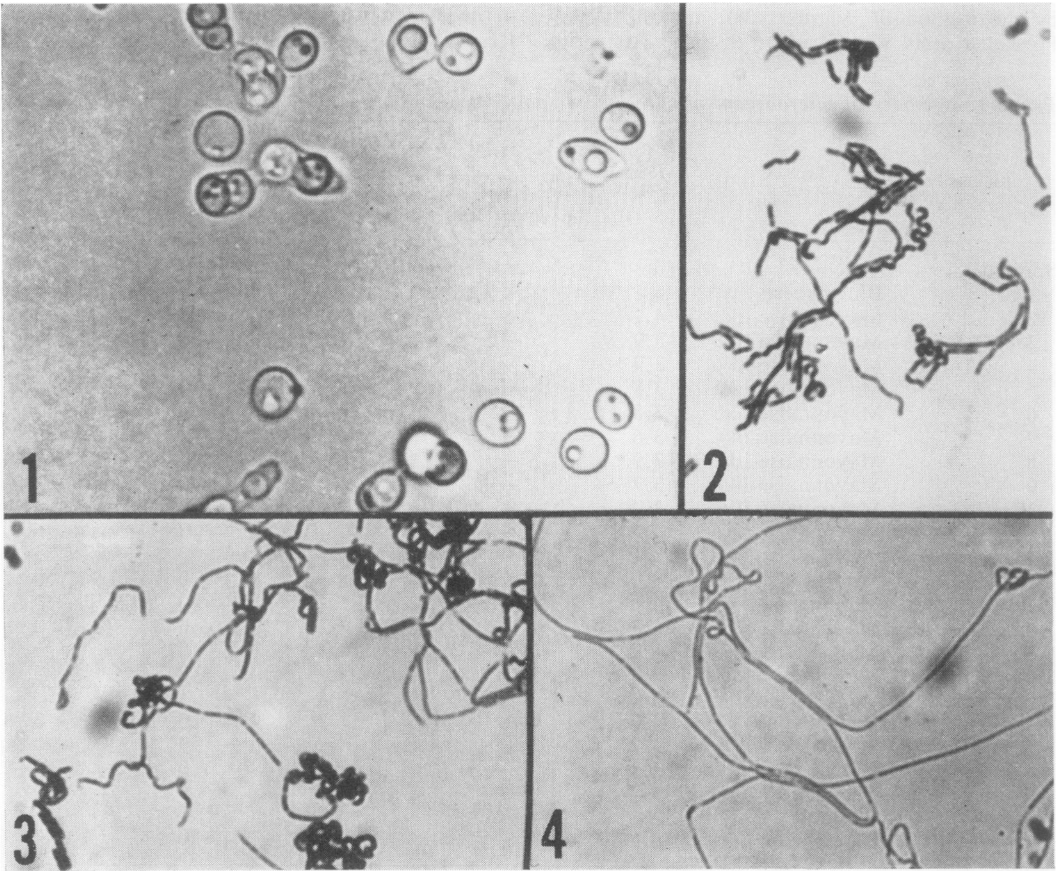

FIG. 1-4. (*1*) *Saccharomyces bailii. Vegetative cells and conjugated asci with ascospores.* × *1,000.* (2-4) *Lactobacillus fructivorans. Various degrees of filament formation by three different strains. All show coiling of the filaments.* × *1,250.*

organisms present. *B. subtilis*, *B. pumilis*, *B. polymyxa*, *B. megaterium*, and *B. licheniformis* were found in sample 2, and *B. subtilis* and *B. pumilis* in sample 3. *B. subtilis* and *B. licheniformis* were present in unspoiled sample 10.

Apparently lactobacilli were responsible for spoilage in the rest of the samples. *L. plantarum* was isolated (on PCA) in high numbers from spoiled sample 1, but *S. bailii* was also abundant. This was the only sample with high numbers of both bacteria and yeasts.

L. fructivorans was isolated from spoiled samples 2, 15, 16, and 17. The number per gram in sample 2 was not determined since isolation was in the thioglycollate broth. The other three samples were plated with LBS agar, and the numbers ranged from 8,100 to 12,600,000 per g. The amount of filament production differed considerably among the isolates, but all had the curious property of producing curves or coils in the filaments of cells (Fig. 2–4).

These isolates of *L. fructivorans* and the type culture NRRL B-1841 produced gas from D-glucose, D-fructose, sucrose (weak), maltose (weak), and malate (weak) but did not utilize D-galactose, L-arabinose, D-xylose, cellobiose, melezitose, acetate, or soluble starch as judged by the failure to produce gas or change the *p*H of the culture medium. The isolates gave a negative Voges-Proskauer reaction and did not grow in the presence of bile salts.

Sucrose is commonly used as a sweetener in mayonnaise and salad dressings (31), but the presence of other fermentable carbohydrates was suggested since *L. fructivorans* ferments sucrose only weakly and not all isolates of *S. bailii* are able to ferment this sugar. The use of corn syrup, invert sugar, and other such sugar sources is permitted in food dressings (6), and they are frequently less costly and more convenient to handle than sucrose.

Paper chromatography showed that all samples, except spoiled sample 2, contained glucose. Most of the samples also contained other reducing sugars with concentrations about as great as that of glucose. The concentration of glucose in the spoiled samples, excluding sample 2, ranged from 3.0 to 38.5 g/kg and from 1.3 to 17.8 g/kg for the unspoiled samples.

Representative strains of *L. fructivorans*, NRRL B-3796 through B-4003, and of *S. bailii*, NRRL Y-7253 through Y-7262, have been retained in the ARS Culture Collection.

DISCUSSION

PCA has been recommended for the detection of bacteria causing spoilage in mayonnaise and salad dressings (2), and, although the bacilli and *L. plantarum* were detected on this medium, *L. fructivorans* was not. Thus, it becomes apparent that LBS agar or some similar medium must also be used if lactobacilli, such as *L. fructivorans*, are to be detected in these products.

L. fructivorans was first isolated from spoiled salad dressing by Charlton et al. (5) and described as a new species. Later, Vaughn et al. (23) studied this species and regarded it as valid, but more recently it has been considered a synonym of *L. brevis* (*Index Bergeyana*, 1966). Data presented here indicate that it may indeed be a distinct species. *Bergey's Manual*, 7th ed. lists *L. brevis* as forming acid from D-galactose, D-xylose, and L-arabinose. Neither the strain originally described as *L. fructivorans* nor the present strains form acid from these three sugars. In addition, *L. fructivorans* was described as forming curved filaments, a characteristic shared by our strains but not one typical of *L. brevis*.

The role of *L. plantarum* in the spoilage of the blue cheese dressing is difficult to assess since a large number of yeasts also were present. Similarly, the occurrence of small numbers of bacilli in two of the spoiled samples and one unspoiled sample may not be significant. However, Iszard (13) reported *B. petasites* to cause gaseous fermentation in a salad dressing; Appleman et al. (3) reported large numbers of *B. subtilis* and an unidentified yeast to be present in spoiled mayonnaise, and Pederson (19) attributed spoilage of a Thousand Island dressing to *B. vulgatus*.

The sources of the spoilage microorganisms have been generally attributed to contaminated ingredients (1, 11, 17, 32) and to unsanitary manufacturing equipment and surroundings (3, 7, 30). Zuccaro et al. (33) studied the ability of salad dressing spoilage microorganisms to survive in oil-water mixtures at different pasteurization temperatures. Iszard (14, 15) suggested controlling spoilage through addition of lactic acid to the dressings; Shapiro and Holden (21) proposed various antibiotic and chemical dips to decrease the microflora of a packaged salad mix.

One facet brought out in our study, and apparently not previously considered as a factor influencing spoilage, has been the sugar concentration in the products. Traditionally, sucrose has been used as a sweetener and, although it is only slowly fermented by *L. fructivorans*, many isolates of *S. bailii* produce a latent but vigorous fermentation of this sugar. Because of this delay, the products may not show gaseous fermentation until quite sometime after they have left the manufacturing plant. Delayed fermentation of sucrose also has been reported for many other species of *Saccharomyces* (18).

The practice of substituting syrups and other glucose-containing sweeteners for sucrose seems to further complicate the problem of spoilage since both *L. fructivorans* and *S. bailii* readily ferment glucose. One would also expect other spoilage microorganisms to make good growth and perhaps form gas in the presence of glucose where they might not if only sucrose were present.

Control of spoilage in mayonnaise and salad dressing thus becomes a problem of manipulating ingredients. The concentration of acetic acid in the products is usually relied upon to prevent the growth of spoilage microorganisms (25), but there is little difference in *p*H between spoiled and unspoiled samples (Table 1). The judicious use of sweeteners might also help decrease the amount of spoilage that occurs.

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