Anaerobic Gram-Negative Bacteria in Brewing—A Review

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ABSTRACT

Until recently, gram-negative bacteria have not been considered to be common spoilers of finished (packaged) beer. In this article, however, two gram-negative, obligately anaerobic, beer spoilers of the genera *Pectinatus* and *Megasphaera* are reviewed. Improved detection methods and recent advances in brewing technology leading to lowered O_2 levels in finished beer have led to an increased occurrence of *Pectinatus* and *Megasphaera* bacteria. Their discovery, isolation, nutritional requirements, and end product spoilage patterns have now been reported. The impact of these microbes on the brewing industry is discussed.

Key words: *Pectinatus, Megasphaera*, Anaerobes, Spoilage bacteria, Beer

Very few bacteria are capable of growing in beer. This is due to the selective nature of this fermentation end product. The low pH, alcohol and hop contents, limited energy source(s), and the lack of oxygen all restrict the number of bacteria capable of growth. The organisms that have previously been classified as beer spoilers and that produce end products affecting the flavor and aroma of beer are found in the following genera: *Lactobacillus, Pediococcus, Acetobacter, Acetomonas*, and *Zymomonas*. Wort spoiling coliforms and two coliform bacteria which are occasional beer spoilers, *Enterobacter* and *Obesumbacterium* (*Hafnia*), have often created microbiological problems (13).

Since 1976, two gram-negative, strictly anaerobic bacteria that cause turbidity and off-flavors in beer have been reported. *Pectinatus cerevisiiphilus* (*cerevisiophilus*; 6, 24) was described by Lee et al (16) in 1978 and was also identified by investigators in Germany (2), Scandinavia (5), and Japan (24) very shortly thereafter. *Megasphaera cerevisiae* was initially isolated by Weiss et al (26) in 1976, and although it is apparently an infrequent brewery contaminant, it is also of interest to brewers.

Pectinatus cerevisiiphilus

Isolation and Differentiation

Pectinatus cerevisiiphilus was initially isolated during routine identification of brewery microorganisms. This gram-negative rod was originally thought to be a *Zymomonas* species because of its gram reaction and H_2S production under anaerobic conditions. However, it had characteristics that allowed it to be differentiated quite easily from *Zymomonas* spp. It was found to be a strict anaerobe and was not able to grow on solid media under CO_2 incubation, although it occasionally grew in Gas Pak jars. It fermented several sugars, but no ethanol was detected. Moreover, it was motile but was observed to have a peculiar comblike pattern of flagellar attachment unlike the pattern of polar attachment in *Zymomonas*. It was found not to produce significant amounts of acetaldehyde, a characteristic end product of *Zymomonas*.

Conventional membrane filtration techniques are not practical for the isolation of *Pectinatus* (9). The usual method for isolating *Pectinatus* in solid media requires the use of the Lee tube containing lactate lead acetate (LL) agar (LL agar contains 0.5% yeast extract, 0.3% beef extract, 1.7% sodium lactate [60% syrup], 0.1% ascorbic acid, 0.01% $Na_2S_2O_3 \cdot 5H_2O$, 0.02% lead acetate, 0.0002% methylene blue, 0.2% phenethyl alcohol, and 1.5% agar [17]). The Lee tube (patented; available from Simplee, Inc., Denver, CO 80202) is a double-walled screw-capped tube capable of containing a thin cylinder of media under anaerobic conditions (21). The strict anaerobic requirement of *Pectinatus* normally demands the use of a strongly reduced media containing ascorb acid and sodium thiosulfate. Some workers, however, ha reported that it can be detected in 1–3 weeks by adding deMa Rogosa, Sharpe (MRS) broth (DIFCO) to suspect beer (7). He sterilization of the medium in the Lee tube and subseque incubation in a Gas Pak jar ensures the required anaerob environment. Phenethyl alcohol is included as a component of tl medium because of its selective bacteriostasis of aerobic ar facultative anaerobic gram-negative organisms (3,18,19). Thus, i inclusion into the medium selects for gram-positive organisms a well as the desired obligately anaerobic gram-negative bacteri The use of sodium lactate as the sole carbon source precludes th growth of *Zymomonas* species. Lead acetate is used to differentia the lactic acid-producing bacterial colonies (gram-positive) fro those of the H_2S-producing *Pectinatus* species by coloring th latter colonies black. Inocula from actively growing *Pectinatu* cultures produce visible colonies on LL agar after 2–3 days a 32°C; another 2–3 days are needed to develop the black coloratio (18).

Pectinatus can also be grown and maintained in media such a MRS broth or Nakagawa broth (24) if the medium is cultured wit a minimum headspace and if a heavy inoculum is used. *Pectinatu* has been grown on thioglycollate agar pour plates in Gas Pak jar at 30°C. The resulting colonies are circular, entire, convex t pulvinate, white, glistening, and opaque (15). Laboratory culture develop a higher tolerance to oxygen (9).

When Lee and his co-workers described this new beer-spoilin anaerobe and proposed its inclusion in the genus *Pectinatus*, ther was disagreement from Kirchner and his co-workers, who believe that the new microbe should belong in the genus *Bacteroides* unde the species name *serpens* (14). Kirchner based his conclusion on th similarity of the fermentation end products, the mole percen guanine plus cytosine (mol% G+C) of the DNA, and the motilit (peritrichous flagella). Newer evidence, however, has supporte the decision made by Lee and his co-workers to form a new genu for the newly isolated bacterium (17). The major difference between the genus *Pectinatus* and *Bacteroides* are their pathwa differences for the production of propionic acid as well a immunological cross reactivity and flagellar arrangement.

Mechanism of Propionate Synthesis

In order to synthesize propionate, *Pectinatus* reduces fumarate to succinate followed by a decarboxylation of succinate to propionate (11). *Bacteroides*, a possibly related microorganism, produces propionate by a direct reductive mechanism (acrylate pathway). The key enzyme in the former pathway (Fig. 1) is succinate oxidoreductase. The enzyme succinate oxidoreductase can be competitively inhibited by malonate, leading to decreases in propionic acid production and a corresponding increase in acetic acid production. This specific reduction of propionic acid by malonate inhibition differentiates between the two pathways.

Immunological Cross-Reactions

Polyvalent rabbit antisera against a number of *Pectinatus* species and other brewery contaminants have been raised by injecting rabbits with formaldehyde-killed cell suspensions. The resulting antisera were then tested for cross reactivity by immunofluorescent staining as seen in Table I (11). Serum raised against *Pectinatus* ATCC 33332 reacted strongly with other *Pectinatus* species but not against other microorganisms, including those of the *Bacteroides* genus. Haikara reported three distinct strains of *Pectinatus* from the 11 isolates that she tested (6). Precipitin patterns (immunoelectrophoretic reaction of acid

extracts from *Pectinatus* versus rabbit antisera raised against each isolate) were used to group the isolates with similar proteins on their cell surfaces (Table II). Completion of the above tests enabled Haikara and co-workers to conclude that *Pectinatus* belonged in its own genus (11,12).

Morphological and Biochemical Characteristics of *Pectinatus cerevisiiphilus*

"Bergey's Manual" (15) describes the genus *Pectinatus* as follows:

> Slightly curved rods, 0.7–0.8 μm in diameter and 2.0–32 μm or more in length, with rounded ends; they occur singly, in pairs, and only rarely in short chains. Shorter, younger cells do not show a helical shape, but the elongated older cells tend to form helices. After cultures have passed the stationary and declining phases, round cell forms are often observed. Gram-negative. Young cells move very actively, giving the appearance of an "X" shape as they swim. Older and longer cells have a snakelike motion as they move. Flagella emanate from only one side of a cell. Flagella are not limited to the center portion of the concave side of the cell as they are in the genus *Selenomonas*. The number of flagella per cell depends upon the cell size and its condition, but generally ranges from 1 to 23 or more. The organisms are obligately anaerobic, nonsporeforming mesophiles. They grow between 15 and 40° C, with optimum growth at ≈32° C. Originally isolated from spoiled, packaged beer. The mol% G+C of the DNA is 39.8 (T_m).

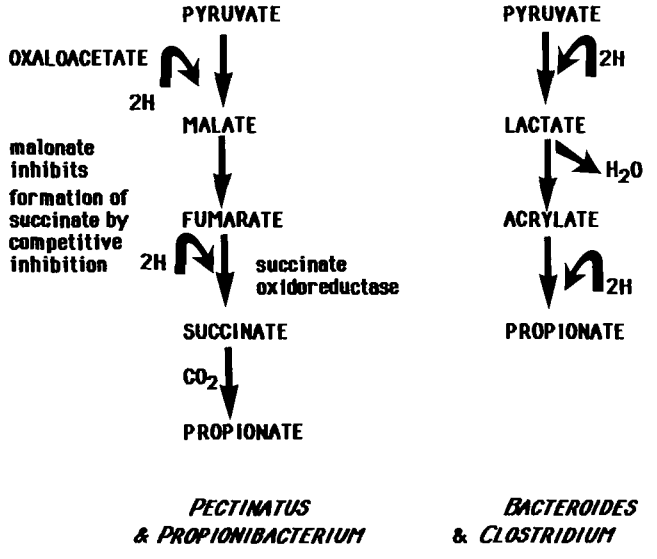

Fig. 1. Two pathways for the production of propionic acid (10).

TABLE I
Cross Reactivity of Serum Raised Against *Pectinatus* ATCC 33332[a]

Organism	Antiserum Dilution 1:30	1:100	1:500
Pectinatus ATCC 33332	++++	++++	++++
Pectinatus ATCC 29359	+++	+	–
Pectinatus DSM 20465	+++	+	–
Pectinatus VTT-E-80121	+++	+	–
Bacteroides fragilis VTT-E-81133	–	–	–
Bacteroides sp. VTT-E-81134	–	–	–
Bacteroides subtilis VTT-E-68013	–	–	–

[a] From Haikara et al 1981 (11).

Freeze fracture electron microscope studies by Haikara (11) show ultrastructures unique to *Pectinatus*. They appear to possess a typically gram-negative outer membrane, but the cytoplasmic membrane contains invaginations, mesosomes, and a thick peptidoglycan layer—all of which are characteristic of gram-positive bacteria (11). *Pectinatus* is thought to be an intermediate form between gram-negative and gram-positive bacteria.

A number of researchers have detailed the utilization of sugars by *Pectinatus* species (2,8,12,15,23). These results are summarized in Table II. Of particular note in this table is the wide range of sugars that *Pectinatus* spp. are able to utilize as carbon sources. Listed contradictions in results may result from variations in testing methods. Also of interest are the results of Back et al (2) and Schisler et al (22), who demonstrated the ability of *Pectinatus* to use lactic acid and pyruvic acid. Haikara also indicated that *Pectinatus* groups I and III were urease negative whereas group II was urease positive (11).

Alcohol tolerance tests indicate that *Pectinatus* is capable of growing in media containing up to 4.5%, w/v, ethanol (12,23). *Pectinatus* also has a pH optimum greater than 4.5, although it will tolerate a pH of 3.7. These organisms are therefore well suited for growth in beer.

The metabolic end products produced by *Pectinatus* spp. include propionic acid, carbon dioxide, acetic acid, succinic acid, and acetoin. It is the only brewing bacterium that produces propionic acid (22). The relative amounts of the listed end products are, however, dependent upon the substrate utilized by the organism (Table III). In addition to these organic acids, *Pectinatus* produces significant quantities of methyl mercaptan, dimethyl sulfide, and hydrogen sulfide. Up to 80, 13, and 290 μg/L, respectively, can be found in contaminated beer (11). All three of these levels are at or above taste thresholds (11).

Takahashi (24) compared the amounts of organic acid found in normal and *Pectinatus*-contaminated beers. The results are shown in Table IV. The synthesis by *Pectinatus* of large amounts of propionic acid has been utilized by investigators to identify the organism in contaminated beer using gas chromatography (8,12,22). Seidal and co-workers (23) tested a series of isolates from

TABLE II
Utilization of Various Carbon Sources by *Pectinatus* Isolates[a]

Peptone Yeast Extract + Substrate	Isolate ATCC 33332	ATCC 29359	DSM 20466	DSM 20465
Arabinose	+[b]	+	+	+
Cellobiose	+	+	–	+
Erythritol	+	+	+	+
Fructose	+	+	+	+
Galactose	+	+	+	+
Glucose	+	+	+	+
Glycerol	+	+	+	+
Glycogen	–	–	–	–
Lactose	–	–	+	–
Maltose	+	–(+)	–	+
Mannitol	+	–(+)	+	+
Mannose	+	+	+	+
Raffinose	–	–	–	–
Rhamnose	+	+	+	+
Ribose	+	+	+	+
Sorbitol	+	+(–)	+	+
Sorbose	+	+(–)	–	–
Starch	–	–	–	–
Sucrose	–	–	–	–
Trehalose	–	–	–	–
Xylose	–	+	+	–
Immunological grouping	I	II	II	III

[a] Adapted from references 12, 16, and 17.
[b] Turbid culture with pH < 5.5.

Pectinatus- and *Megasphaera*-contaminated beers for their ability to grow in a variety of different beers. A summary of their results is found in Table V.

Control Methods in the Brewery

Pectinatus cerevisiiphilus is difficult to detect in the brewery. Haikara (10) indicated that the most common source of contamination was bottling and capping equipment in summer. A major concern was the slow rate of the microbe's reproduction in media—a great deal of harm might be done to the product before detection. Lee and co-workers reported (17) that *Pectinatus* is rather easy to kill with heat and that 1 min at 58°C is adequate to control the organism. This is less than one pasteurization unit, where 1 PU is defined as 1 min at 60°C. *Lactobacillus* contaminants can be much more resistant (25). Ordinary sanitizing agents such as chlorine and iodophors are also very effective against *Pectinatus* (17). Seidal and co-workers, however, warned that newer conservation measures involving the reuse of sanitizers may lead to the use of depleted chemicals, which may allow the subsequent survival of *Pectinatus* spp. and *Megasphaera* spp. (24).

More recent work by Haikara (6) involving the serological characterization of *Pectinatus* isolates indicates that the *Pectinatus* bacterium may be capable of being sequestered in the brewery to be released at a later time. She was able to show that serologically identical *Pectinatus* strains were isolated from a brewery with intervals of four years between the isolations. This work also indicated that three separate strains of *Pectinatus cerevisiiphilus* may be implicated as beer spoilers.

MEGASPHAERA SPECIES

Initially isolated in 1976 by Weiss and co-workers (26), the genus *Megasphaera* was first cultured from several bottles of diet pilsner. The subsequent cultivation and characterization of this organism required patience as it became apparent that this organism was the most strict anaerobe known to exist in the brewing operation (23). *Megasphaera* produces product defects in beer that have been variously described as rotten, putrid, penetrating, stinking, and foul tasting. Recent work by Engelmann and Weiss (4) and by Haikara (8) has been instrumental in the characterization of this beer-spoiling organism and in the naming of the species *Megasphaera cerevisiae* (4).

Isolation of *Megasphaera*

The initial isolation described by Seidal and co-workers (23) of *Megasphaera* spp. used a beer agar with 1% glucose and 1% peptone; a micro assay culture agar (Difco 0319-01, MAC Agar), Schaedler broth (Oxoid CM 497), MRS agar (Merck no. 10660), or a Standard I agar from Merck (no. 7881). Currently, a peptone-yeast extract medium (PY-medium) is used by Engelmann and Weiss (4) for substrate-utilization tests. Its composition (in g/L) is as follows: peptone, 5; tryptone, 5; yeast extract, 10; Na_2HPO_4, 2; cysteine-HCl, 0.5. Tween 80 is added at 1 ml/L. Various substrates such as glucose, fructose, lactate, or pyruvate have been added at 5 g/L. The medium is autoclaved, cooled under nitrogen, and normally poised at pH 7.0. On agar, *Megasphaera* forms small whitish, smooth, and shiny colonies after strict anaerobic incubation; in fluid media, a homogeneous turbidity forms with a loose sediment (26).

Morphological and Biochemical Characterization

The type species of *Megasphaera* listed in "Bergey's Manual" (15) is that of *M. elsdenii*, a rumen microbe, which has recently been shown to be distinct from the beer spoiling organism, *M. cerevisiae*, by DNA/DNA hybridization studies (4). However, the beer isolates closely resemble the phenotypic characteristics of the genus *Megasphaera*. A description of the new species, *Megasphaera cerevisiae*, has been developed as follows:

> Slightly elongated cocci, 1.3 to 1.6 μm in diameter, occurring singly, in pairs and occasionally in short chains. Gram-negative. Nonsporulating. Nonmotile. Strictly anaerobic. Colonies on PYL (lactate) or PYF (fructose) agar are whitish, smooth and flat, 2–3 mm in diameter. Growth of *Megasphaera* species isolated from beer occurs between 15 and 37°C (but not at 40°C) with an optimum at 28°C. Catalase and benzidine test negative. H_2S is formed. Nitrate is not reduced to nitrite, indole is not produced. Growth in prereduced PY broth is poor, but abundant growth is obtained in PYL and PYF media. Trace to minor amounts of acetic acid, propionic acid, iso and *n*-butyric acid, iso and *n*-valeric acid, caproic acid and gas is produced in PY broth,

TABLE III
Conversion of Substrate to Organic Acids by *Pectinatus*[a]

Substrate	Organic Acid (meq acid produced/L)			
	Acetic	Propionic	Lactic	Succinic
Fructose	74.2	35.5	···	···
Glucose	89.2	52.3	4.8	45.3
Lactate	186.7	63.0	···	0.5
Maltose	103.3	2.3	···	6.8

[a] From Lee et al 1978 (16).

TABLE IV
Organic Acids in Normal and *Pectinatus*-Contaminated Beers[a]

Organic Acid	Normal Beer (mg/L)	Contaminated Beer (mg/L)
Gluconic acid	29[b]	14
Pyroglutamic acid	115	96
Lactic acid	58	42
Acetic acid	90	221
Pyruvic acid	50	···
Malic acid	65	···
Propionic acid	22	933
Citric acid	104	···
Succinic acid	38	241

[a] From Takahashi 1983 (24).
[b] Final cell concentration approximated to be only 10^5/ml.

TABLE V
Growth of *Pectinatus* and *Megasphaera* In Different Beers

Variable/ Bacteria	Beer Variety					
	Diet Pilsner	Old Beer	Light Full Beer	Yeastless Wheat Beer	Export	Light Doppelbock
°Plato	8.4	11.8	11.6	12.7	12.7	18.4
Alcohol, % w/v	3.5	3.5	3.9	4.0	4.3	6.5
Bittering units	23.6	27.3	27.8	13.4	30.4	32.8
pH	4.75	4.00	4.49	4.39	4.42	4.53
Pectinatus						
cerevisiiphilus	+2[b]	+>14	+4	+7	+7	−14
MKP 1	+4	+>14	+4	+4	+7	−28
MKK 1	+5	−28	+4	+4	+7	−28
Wa/1b	+3	−28	+4	+4	+5	−14
Megasphaera						
sp. III 2e	+14	−14	+4	+4	+14	−28
sp. V_1	+14	−28	+4	+10	+14	−28
elsdenii	−28	−14	−28	−28	−28	−28

[a] From Seidal et al 1979 (23).
[b] + = Growth, − = no growth; the numbers following the ± indicator give the number of days after the inoculation where the strongest multiplication occurred or, in the negative case, when the test was interrupted. The various *Pectinatus* and *Megasphaera* isolates used were given test numbers as their exact species were not determined.

TABLE VI
End Products Produced by *Megasphaera* from Various Substrates[a]

Strain	Substrate	End Products[b] Acetic Acid	Propionic Acid	Butyric Acid	Valeric Acid	Caproic Acid	H_2	CO_2
M. cerevisiae	glucose[c]	...	...	...	...	...	...	...
20462	fructose	0.10	0.07	0.27	0.17	0.24	0.38	0.65
	lactate	0.15	0.11	0.17	0.42	0.03	0.04	0.22
M. elsdenii	glucose	0.32	0.02	0.41	0.03	0.09	1.51	0.71
20460	fructose	0.34	0.00	0.38	0.00	0.04	1.23	0.77
	lactate	0.26	0.24	0.14	0.16	0.01	0.40	0.34
Megasphaera sp.	glucose[c]	...	...	...	...	...	...	...
20461	fructose	0.10	0.02	0.23	0.09	0.26	0.39	0.63
	lactate	0.19	0.15	0.11	0.37	0.00	0.04	0.19

[a] From Engelmann and Weiss 1985.
[b] Amount in mmol produced per mmol of substrate utilized by resting cells.
[c] Substrate not metabolized.

TABLE VII
Biochemical Characteristics of *Megasphaera* Isolates[a]

Characteristics	*Megasphaera* Beer Isolates	*Megasphaera elsdenii*
Growth		
@ 15°C	+	−
@ 40°C	−	+
Nitrate reduction	−	−
H_2S production	+	+
Indole production	−	−
Utilization of:		
Lactate	+	+
Pyruvate	+	+
Succinate	−	−
Glucose	−	+
Fructose	+	+
Arabinose	Variable	−
Maltose	−	+

[a] From Engelmann and Weiss 1985.

with iso-valeric acid being the predominant product. End products of fructose and lactate fermentation by resting cells are shown in Table VI. Pyruvate is also metabolized yielding mainly butyric acid. Succinate is not attacked. Fructose is fermented by all strains, arabinose by some of the strains. Unheated glucose, ribose, xylose, mannose, maltose, cellobiose, trehalose, and glycerol are not fermented. Peptidoglycan of the cell wall is of the m-Dpm-direct type. In addition, the peptide subunit contains one putrescine residue covalently bound to the alpha-carboxyl group of the glutamic acid. The mol% G+C of the DNA is 42.4–44.8 T_m (4).

The biochemical characteristics of the beer isolates of *Megasphaera* that are useful in differentiating the organisms from *Megasphaera elsdenii* as well as other beer-spoiling organisms are shown in Table VII.

Megasphaera species will grow in peptone-yeast extract (PY) media and in wort, as well as in sugar-free media containing protein hydrolysis products (12). However, growth in sugar-free media is poor. Back and co-workers (2) stated that *Megasphaera* species prefer lactate and pyruvate as a carbon source and grow less well when required to use the typical fermentation sugars, glucose and maltose. Fructose is, however, well utilized by the organism (1). In the presence of a low-level, lactic acid bacterial infection, utilization of the lactic acid by *Megasphaera* can result with biochemical end products including butyric acid, butanol, and valeric acid (26) being even more foul than those produced by the lactic acid bacteria (15). *Megasphaera* is a more likely contaminant in recent years because of technological advances in bottling that have resulted in lower air contents in bottled products.

Megasphaera tolerates hops to 32–35 bittering units (EBC) and alcohol to 5.5%, w/v. It also appears to have a pH minimum of 4.3. End products of metabolism of *Megasphaera* spp. include a variety of short-chain fatty acids such as acetic, propionic, butyric, valeric, and caproic acids (Table VI). The relative proportions of these end products is dependent upon both the isolate of *Megasphaera* and the substrate being utilized. When grown on PY medium, *Megasphaera* spp. also produced minor amounts of the straight-chain fatty acids; the predominant end product found was iso-valeric acid. This is thought to be a result of amino acid degradation (4).

Utilization of these end products as indicators for detection of the presence of *Megasphaera* spp. has been suggested by Haikara (8). The enrichment of suspect beer with lactate or heat-treated glucose accelerates the rate of growth of the *Megasphaera* species leading to a decrease in the time required before visual or gas chromatography detection is possible.

DNA/DNA hybridization studies by Engelmann and Weiss (4) on 12 different brewery isolates of *Megasphaera* along with *M. elsdenii* indicated that all 12 of the brewery isolates belong to one genospecies with no detectable specific genomic relationship occurring to *M. elsdenii*. However, the phenotypic characteristics of the beer isolates cannot be attributed to either *Acidominococcus* or *Veillonella*, so Engelmann and Weiss have proposed a single new species, *M. cerevisiae*, to accomodate the organism.

Control of *Megasphaera* in the Brewery

Control of *Megasphaera* spp. in beer and in the brewery can be accomplished through the use of oxidizing sanitizers. Note that while *Megasphaera* is considered to be a beer spoiler, it is capable of growing in wort under strictly anaerobic conditions.

CONCLUSIONS

Pectinatus and *Megasphaera* are beer-spoilage microrganisms of increasing concern to the brewery. The end products of metabolism of these two organisms are extremely distasteful, and their growth in beer even to cell numbers $< 10^6$/ml renders it undrinkable. These two organisms are rare and make up less than 1–2% of the total beer-spoiling flora (1). They are generally found in aging tanks or krausening cellars or on the bottling line; 60% of all such contaminations in Germany occur in this area (1). They were not generally found in bottled beer in Germany because adequate pasteurization destroys both *Pectinatus* and *Megasphaera*, but they have been noted in kegs. This is likely due to a greater statistical probability of growing these few cells when larger numbers are present. Although they are not generally noted

in the brewery past the polishing filter, Seidel et al (23) proposed that they can occur during the filling process.

The explanation for the recent "discovery" of these beer spoilers is of interest. The technical expertise required to isolate obligately anaerobic microorganisms has been available for a number of years. Brewing microbiologists, however, have never considered the need to culture strict anaerobes in a brewery, because the brewery itself was not strictly anaerobic at any point. Recent advances in filling technology have now allowed the oxygen content of the finished beer to be decreased from > 1 mg/L down to 0.4–0.8 mg/L (23). It is interesting to note that *Pectinatus* and *Megasphaera* were first isolated in large modern breweries where these kinds of techniques would be more common.

In addition to these two factors, the recent trend towards lighter (lower alcohol, less hopped) beer may also be selecting a more favorable environment for the establishment and growth of these organisms. Their ability to use alternate carbon sources such as lactate or unused sugars makes them especially dangerous in a mixed infection of the beer, and in fully fermented, low-alcohol beers (i.e., low-calorie, diet beers), which are normally thought to be less prone to infection because of their reduced fermentable sugar content.

The origin of these microorganisms is not clear at present. Both organisms have been isolated from water and sewer pipes in the brewery as well as from lubricating oil (17). With the present day trend to light beers and the lowered O_2 content in these beers, the brewing microbiologist should consider these organisms as a serious potential problem that may become more significant in the future. Because the origin of these microorganisms in the brewery is still under investigation, only general preventative maintenance techniques are useful. The slow growth of these organisms (4–6 days) precludes the use of preventative testing, because by the time the organisms have been identified, the beer has spoiled. This is due to the fact that a relatively small initial number of organisms will result in production of detectable amounts of objectionable end product.

Just because these organisms are generally found in large modern breweries does not mean they do not exist in the smaller brewery. The conditions, however, in the smaller brewery may be less than ideal for the propagation of these organisms. *Pectinatus* and *Megasphaera* are both easily controlled with oxidizing sanitizers, so that plant hygiene performed in a thorough manner should reduce their occurrence in the brewery. Care must be taken to ensure that sanitizers are used at the recommended concentration and that areas that may be excluded from contact with oxygen are adequately flushed with sanitizer.

If *Pectinatus* and *Megasphaera* infections do occur in the brewery, it is possible to detect the metabolic end products 1–2 days before turbidity occurs. This is too late to save the beer, but may prevent shipping of the product (8). Plants that are experiencing persistant problems should consider elimination of fob water sprays as well as extensive treatment of all their equipment with chlorine sanitizers (but perhaps not iodine, references 7, 17) or with strong oxidizing agents to try to control the problem (7).

Pectinatus and *Megasphaera* are organisms that can cause severe taste and odor problems in beer. Because of their strictly anaerobic nature, however, they should be easier to confine to a role of occasional spoilers than the lactic acid bacteria or some coliforms, which are able to survive a wider range of brewery conditions. Both microbes appear to be unable to survive in high-gravity beers (Table V, 23), although survival of these organisms under high-gravity conditions was not tested in a recent study by Magnus et al (20).

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