DOI 10.15199/64.2026.1.2

Non-specific microbiological contamination of unpasteurized beer

Niespecyficzne zanieczyszczenie mikrobiologiczne niepasteryzowanego piwa

Sylwia Andrzejczak-Grządko
(ORCID: 0000-0001-9646-6144),
Olga Konkol
Uniwersity of Zielona Góra, Institute of Biological Science, Department of Biotechnology, Szafrana 1, 65-516 Zielona Góra, Poland, s.andrzejczak-grzadko@wnb.uz.zgora.pl

Paweł Kąkol
Haust Brewery, Pl. Pocztowy 9, 65-305 Zielona Góra, Poland

Keywords
unpasteurized beer, microbial contamination, Paenibacillus

ABSTRACT
Unpasteurized beer is susceptible to microbial contamination due to the nature of the beverage and the way it is produced. *Lactobacillus spp.* is the most commonly reported contaminant in beer, and many bacterial species are unable to survive in such an environment. However, it turns out that some non-specific bacteria are able to survive and multiply in beer. Our study analyzed several unpasteurized beers from a local brewery. In addition to standard microbial contaminants, an unusual microorganism – *Paenibacillus glucanolyticus* – was found in the analyzed beer. Analyses confirmed that *P. glucanolyticus* is not only able to survive in beer, but also, by metabolizing the beer's components, it can lead to pronounced organoleptic changes. This species is known to spoil food products, but so far, it has not been identified as one that can adversely affect stored beer.

Słowa kluczowe
niepasteryzowane piwo, zanieczyszczenie mikrobiologiczne, Paenibacillus

ABSTRAKT
Niepasteryzowane piwo jest podatne na zanieczyszczenie mikrobiologiczne ze względu na charakter napoju i sposób jego produkcji. *Lactobacillus spp.* jest najczęściej zgłaszanym zanieczyszczeniem w piwie, a wiele gatunków bakterii nie jest w stanie przeżyć w takim środowisku. Okazuje się jednak, że niektóre niespecyficzne bakterie są w stanie przeżyć i rozmnażać się w piwie. W naszym badaniu przeanalizowaliśmy kilka niepasteryzowanych piw z lokalnego browaru. Oprócz standardowych zanieczyszczeń mikrobiologicznych, w analizowanym piwie znaleziono nietypowy mikroorganizm – *Paenibacillus glucanolyticus*. Analizy potwierdziły, że *P. glucanolyticus* nie tylko jest w stanie przetrwać w piwie, ale także, metabolizując składniki piwa, może prowadzić do wyraźnych zmian organoleptycznych. Wiadomo, że ten gatunek może psuć produkty spożywcze, ale jak dotąd nie zidentyfikowano go jako gatunku mogącego niekorzystnie wpływać na przechowywane piwo.

INTRODUCTION

Beer is a low-alcohol beverage produced by the metabolic changes of yeast cells in hopped malt wort or hopped wort obtained from malt and unmalted raw materials (Boulton and Quain 2006). For centuries, it has been valued for its durability and biological stability, which results from the fact that beer is an unfavorable environment for the development of many microorganisms. This is mainly due to the presence of ethanol (0.5-10%) and high concentration of carbon dioxide (about 0.5%) produced by yeast fermentation, but also iso-α-acids from hops, low pH (3.8-4.7) and low oxygen levels (Bamforth 2023). Despite the exceptionally unfavorable conditions, some microorganisms manage to survive in beer. The development of undesirable microorganisms in beer can result in numerous product defects, such as changes in appearance (e.g., discoloration of beer, viscosity, turbidity), flavor and aroma defects, disturbances in the fermentation process, loss of colloidal stability or excessive fermentation (Esmaeili et al. 2015). The risk of infection increases in the case of craft beers, and contamination is caused by errors resulting from technical or process defects in the brewery, as well as overall hygiene of the personnel and the facility (Baiano 2020).

In the brewing industry, microorganisms responsible for beer spoilage are defined as those that were not intentionally introduced and can survive and multiply in wort, fermenting wort, filtered beer or packaged beer (Eßlinger, 2009). Gram-negative bacteria have a hydrophobic cell membrane that makes them resistant to bitter compounds present in beer. However, only a few Gram-negative bacteria are responsible for beer spoilage (Paradh and Hill 2016). The most dangerous microorganisms responsible for beer spoilage, especially in unpasteurized beer, are anaerobic ones from the genus *Pectinatus*. Their presence in the product is marked by an unpleasant odor resulting from the production of hydrogen sulfide, methyl mercaptan and a range of fatty acids. *Pectinatus* bacteria also excretes organic acids such as acetic, lactic, propionic and succinic acids (Paradh 2015). Acetic acid bacteria, on the other hand, metabolize ethanol to acetic acid, giving beer a vinegary taste (Bouchez and De Vuyst 2022). Lactic acid bacteria (LAB), including those from the *Lactobacillus* and *Pediococcus* genus, constitute most of the Gram-positive bacteria that manage to grow and multiply in beer. Their presence mainly causes beer souring (Riedl et al. 2019).

Methods for detecting microbiological beer contamination can be divided into qualitative and quantitative. The former allows for the detection and identification of specific strains and their metabolites, which leads to the confirmation or exclusion of their presence in the product. Quantitative microorganism determination indicates the scale of contamination and allows determining whether it is at an acceptable level according to the applicable standards. These methods can be traditional or use faster and modern techniques (Hill, 2015; Siegrist et al. 2015; Tompkins et al. 2018).

The aim of this study was to evaluate the microbiological quality of unpasteurized and unfiltered craft beers and to identify potential beer-spoiling microorganisms using selected microbiological methods.

MATERIALS AND METHODS

Beer

The study analyzed unpasteurized and unfiltered top-fermented beers obtained from a local craft brewery. The composition and key parameters of the tested beverages are presented in Table 1.

Tab. 1. Composition of beer for 1000 liters of wort.

Name (beer number)	American Wheat (1)	(W) Life (2)	American Saison (3)	Ox Bile (4)
Yeast	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
Alcohol	4,7%	6,6%	7,0%	8,5%
Malt	Pilsner 50 kg, Light Wheat 50 kg	Pale Ale 90 kg, Rye 25 kg, RED 5 kg, Chocolate 5 kg, Roasted 5 kg	Pilsner 100 kg, Munich 25 kg, Wheat 5 kg	Pilsner 125 kg, Pale Ale 40 kg, Wheat 25 kg
Hops (iso-alpha-acids)	Citra (13%) 1,3 kg, Columbus (13%) 0,1 kg, Azacca (12,5%) 1,3 kg	Columbus (14%) 0,3 kg, Simcoe (12,8%) 1,0 kg	Columbus (13,9%) 0,2 kg, Mosaic (12%) 1,0kg	Centennial (8,7%) 1,5 kg, Chinook (11%) 3,2 kg
Final extract	3,3 P	2,4 P	2,0 P	4,9 P
Other components	-	Zinc sulfate 0,7 g	Dried lemon peel 0,1 kg, Coriander 0,2 kg	Sugar 10 kg, Zinc sulfate 0,65 g
Aging time	approx. one month	7-8 weeks	over half a year	3-4 month

The beers were brewed and matured according to the producer's recipe and bottled according to the producer's established scheme, i.e., poured into dark bottles disinfected with peracetic acid. The bottled product was left at room temperature until analysis. For the tests, 6 bottles of each type of beer were used, which were opened on the day of the test. The beer (W)Life was analyzed twice. Two different batches of the beverage, produced and bottled independently, were used for the analyses.

Media

In the study, several enriched and selective-differential media were used, allowing for the isolation of a wide range of microorganisms. These included: MRS agar (de Man, Rogosa, and Sharpe), DRBC agar (Dichloran Rose Bengal Chloramphenicol), BHI agar (Brain Heart Infusion), MacConkey agar, and Mannitol-Salt agar (GrasoBiotech, Poland).

Evaluation of Microbiological Contaminants in Beer

Beer samples were analyzed as bottled products. The first inoculation was performed on the day of bottling, and subsequent analyses were carried out every two weeks. For each medium, 100 µl of beer was inoculated onto five replicate plates. All plates were incubated at 28°C.

Resulting cultures were evaluated macroscopically based on colony morphology. When necessary, isolates were re-streaked onto appropriate media to obtain single colonies. Bacterial and yeast isolates were examined microscopically using both live preparations and stained smears (crystal violet for bacteria, methylene blue for yeast).

In addition, beer samples were assessed organoleptically, including gushing observations, turbidity, and odor changes. The organoleptic assessment was carried out by the individuals performing the microbiological tests. They were not experts, and the evaluation was based on their subjective impressions in comparison with the original characteristics of the beer.

Identification of microorganisms

Isolated microorganisms were identified using the MALDI-TOF method (Invac, Poland).

DNA isolation

The Genomic Mini kit (A&A Biotechnology, Poland) was used to isolate genomic DNA from the *Paenibacillus glucanolyticus* isolate, and the isolation was carried out according to the manufacturer's instructions.

Detectionof the *horA* gene in the *Paenibacillus glucanolyticus* strain

The PCR reaction was performed using primers specific for the *horA* gene according to Bergsveinson (2017). The PCR reaction used a ready-made, standard PCR mix (A&A Biotechnology, Poland), containing *Taq* DNA polymerase (0.1 U/µl), $MgCl_2$ (4 mM), and dNTPs (0.5 mM of each dNTP). The reaction composition was set according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Four types of unpasteurized and unfiltered craft beers were analyzed for organoleptic changes and microbiological contamination over a 10-week period. Despite being bottled on the same day, the beers differed in composition, appearance, and fermentation parameters, which influenced their stability.

After 10 weeks, all beers exhibited strong gushing and visible sediment formation. Significant aroma deviations were also noted: American Wheat developed a sweet, honey-like smell, (W)Life an unpleasant pungent odor, and American Saison a fruity, strongly alcoholic aroma. Only Ox Bile retained a profile close to the original product.

Saccharomyces cerevisiae was detected in all beers throughout the study. Initial yeast counts varied considerably between samples – from several hundred cells/ml in American Wheat to over 10^6/ml in American Saison. Increasing turbidity correlated with rising yeast numbers, which exceeded 10^6/ml in all beers by the final sampling point.

Microbiological analysis confirmed contamination in three beers: American Wheat, (W)Life, and American Saison. In all three, *Lactobacillus brevis* was isolated, appearing approximately four weeks after bottling and persisting until the end of the study in American Saison. The presence of LAB suggests secondary contamination occurring after wort boiling, likely during yeast pitching or bottling. Single colonies of *Bacillus cereus, Bacillus licheniformis,* and *Staphylococcus delphini* were also isolated. These bacteria are not typical

beer spoilers but may originate from raw materials or insufficient hygiene during production.

The (W)Life beer showed additional contamination with *Paenibacillus glucanolyticus* and an unidentified spiral-shaped microorganism. The spiral bacteria reached concentrations above 10^6/ml at weeks 4 and 6 but were not detected later, suggesting limited survival under beer conditions. Their identification requires molecular methods, as MALDI-TOF analysis did not yield a match.

In contrast, *P. glucanolyticus* did not appear in early inoculations, indicating initially low cell numbers, but its later detection demonstrates that this species is capable of surviving and multiplying in beer. Determining its actual abundance was difficult due to the characteristic spreading growth of this bacterium on microbiological media, which prevents reliable colony counting.

Members of the genus *Paenibacillus* are widespread in nature, particularly in soil and plant-associated environments, and include species with pathogenic, spoilage-related, and industrially relevant properties (Shida et al. 1997, Durak et al. 2006, Grady et al. 2006). Given that *P. glucanolyticus* is capable of metabolizing various polysaccharides such as lignin, cellulose, xylan, curdlan and pustulan (Alexander and Priest 1989, Mathews 2016), its enzymatic activity may contribute to its ability to persist in beer and could potentially explain the organoleptic changes observed in the contaminated samples. Although it has been associated with dairy spoilage and, in rare cases, human infection (Ferrand et al. 2013), it has not previously been reported as a contaminant of beer. Its detection in two independent batches suggests that this species may represent a previously unrecognized source of contamination in brewing.

Although some *Paenibacillus* strains may carry the hop-resistance gene *horA* (Haakensen and Ziola, 2008), PCR analysis did not confirm its presence in the tested isolate. This does not exclude the possibility of gene variants or sequence divergence preventing detection.

The differences in contamination patterns between beers likely result from variations in ingredients, malt types, and production conditions. The repeated detection of *P. glucanolyticus* in two independent batches of (W)Life strongly suggests that the contamination originated from raw materials rather than accidental environmental exposure.

Overall, the results clearly demonstrate that unpasteurized and unfiltered craft beers are highly susceptible to microbiological contamination, and that the composition of raw materials, production conditions and beer style play a decisive role in shaping their microbiological stability. The findings obtained in this study highlight several key aspects that are essential for understanding the sources and consequences of contamination in craft brewing. These observations form the basis for the conclusions presented below.

1. Three of the four analyzed craft beers contained microorganisms capable of surviving and multiplying in the beer environment, leading to pronounced organoleptic changes and a deterioration of product quality.
2. *Lactobacillus brevis* was the most frequently isolated spoilage bacterium, indicating secondary contamination during fermentation or bottling, which is typical for unpasteurized and unfiltered beers.
3. *Paenibacillus glucanolyticus* was detected in the (W)Life beer, a species not previously described as a beer contaminant. Its presence in two independent batches suggests that the source of contamination was the raw materials rather than accidental environmental exposure.
4. The presence of unidentified spiral-shaped bacteria, reaching high cell numbers during the middle stage of the study, indicates the possible occurrence of microorganisms in beer that are difficult to identify using routine methods.
5. The absence of the *horA* gene in the *P. glucanolyticus* isolate does not exclude its presence; however, it suggests that the ability of this strain to survive in beer may rely on mechanisms other than classical hop-compound resistance.

FUNDING

Funding: This work was supported by the Marshal's Office of the Lubuskie Province as part of the "Small grants for universities from the Lubuskie Province" program.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data przyjęcia: 28.10.2025

Data recenzji: 19.11.2025

REFERENCE

[1] Alexander B., Priest F.G. (1989). *Bacillus glucanolyticus*, a new species that degrades a variety of beta-glucans. International Journal of Systematic Bacteriology, 39,112-115. https://doi:10.1099/00207713-39-2-112

[2] Baiano A. Craft beer: An overview. (2021). Comprehensive Reviews in Food Science and Food Safety, 20, 1829-1856. https://doi:10.1111/1541-4337.12693

[3] Bamforth CW. (2023). Beer: Tap Into the Art and Science of Brewing (4th edn). New York: Oxford University Press.

[4] Bouchez A, De Vuyst L. (2022) Acetic Acid Bacteria in Sour Beer Production: Friend or Foe? *Front Microbiol* 13:957167. https://doi: 10.3389/fmicb.2022.957167

[5] Boulton C, Quain D. (2006)Brewing Yeast and Fermentation. New Jersey: John Wiley & Sons

[6] Durak MZ, Fromm HI, Huck JR, Zadoks RN, Boor KJ. (2006) Development of molecular typing methods for *Bacillus* spp. and *Paenibacillus* spp. isolated from fluid milk products. *J Food Sci* 71:M50–M56. https://doi: 10.1111/j.1365-2621.2006.tb08907.x

[7] Esmaeili S, Mogharrabi M, Safi F, Sohrabvandi S, Mortazavian AM, Bagheripoor-Fallah N. The common spoilage microorganisms of beer: occurrence, defects, and determination- a review. *Carpathian J Food Sci Technol* (2015) 7:68–73.

[8] Eßlinger HM. (2009) Handbook of Brewing: Processes, Technology, Markets. Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA.

[9] Ferrand J, Hadou T, Selton-Suty C, Goehringer F, Sadoul N, Alauzet C, Lozniewski A. (2013) Cardiac device-related endocarditis caused by *Paenibacillus glucanolyticus*. *J Clin Microbiol* 51:3439-42. https://doi: 10.1128/JCM.00864-13.

[10] Grady EN, MacDonald J, Liu L, Richman A, Yuan ZC. (2016) Current knowledge and perspectives of *Paenibacillus*: a review. *Microb Cell Fact* 15:203. doi: 10.1186/s12934-016-0603-7

[11] Haakensen M, Ziola B. (2008) Identification of novel horA-harbouring bacteria capable of spoiling beer. *Can J Microbiol* 54:321-5. https://doi: 10.1139/w08-007

[12] Hill AE. (2015) Traditional methods of detection and identification of brewery spoilage organisms. In: Hill A, editor. Brewing Microbiology. Woodhead Publishing, Cambridge

[13] Mathews SL, Grunden AM, Pawlak J. (2016) Degradation of Lignocellulose and Lignin by *Paenibacillus glucanolyticus*. *Intern Biodeter Biodegr* https://doi: 10.1016/j.ibiod.2016.02.012

[14] Paradh AD. (2015) Gram-negative spoilage bacteria in brewing. In: Hill AE, editor. Brewing Microbiology. Managing Microbes, Ensuring Quality and Valorising Waste. Elsevier

[15] Paradh AD, Hill AE. Review: Gram Negative Bacteria in Brewing. *Advanc Microbiol* (2016) 6:195-209. https://doi: 10.4236/aim.2016.63020

[16] Riedl R, Dünzer N, Michel M, Jacob F, Hutzler M. (2019) Beer enemy number one: genetic diversity, physiology and biofilm formation of Lactobacillus brevis. *The Institute of Brewing & Distilling* 125:250-260. https://doi: 10.1002/jib.553