Microbial Isolates From North American Sugar Beet Factory Juices and Biofilms

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ABSTRACT

During sugar beet processing, microbes from infected roots, storage piles, and soils carry over throughout sugar extraction, creating operational challenges and resulting in sucrose losses. In this study, diffusion tower juice and biofilm samples were obtained from 18 North American sugar beet factories for microbial sampling to characterize issues relating to raw sugar manufacturing, such as increased sample viscosity from bacterial exopolysaccharide (EPS) production. A broad sampling strategy was applied to obtain as many different microbial isolates as possible for experimental characterization. Most microbes isolated were bacteria with a few yeast identified as well. In total, 379 isolates, belonging to 22 genera, were obtained from 33 diffuser tower juice samples, and 233 isolates, belonging to 26 genera, were obtained from 21 biofilm samples. A subset of 54 isolates representing some of the most common genera (*Leuconostoc*, *Peribacillus*, *Bacillus*, *Pantoea*, *Rahnella*, *Acinetobacter*, and *Weissella*) were grown in high sucrose-containing medium. Among these, 10 strains were identified as capable of significantly increasing viscosity in the flask cultures in which medium exhibited a more gel-like consistency, rather than free-flowing. This increased viscosity effect was likely due to EPS production.

Additional Key Words: Sugar beet, sugarbeet, *Beta vulgaris*, processing, sucrose, biofilm, exopolysaccharides (EPS), viscosity, *Leuconostoc*, *Weissella, Bacillus, Peribacillus, Pantoea, Rahnella, Acinetobacter*

INTRODUCTION

Approximately 5.2 million short tons of beet sugar were produced in the U.S. in the fiscal year 2021-22, accounting for 56.3% of total domestic sugar production (Abadam, 2023). During growth and storage of sugar beets, microbial infection of plant roots can cause devastating crop loss, sucrose degradation and carryover of microbes and associated soil into the factory processing streams that can cause sucrose losses and operational challenges (Majumdar et al., 2022; Solomon, 2009; Strausbaugh, 2016; Strausbaugh et al., 2011). Various microbial contaminants have been reported to reduce processing efficiency during sugar beet extraction, necessitating control measures such as the addition of antimicrobial agents, maintenance of high temperatures in the diffusion tower of 70-73°C, and heating of press water to 90°C (Arvanitis et al., 2004; Asadi, 2007; Holland et al., 1990; McGinnis, 1982; Šereš et al., 2017). Culture-dependent microbiological studies to isolate and identify microbes present and characterize their behaviors are central to ongoing efforts to reduce microbial contamination and improve factory sanitation, but these approaches also have limitations such as potential culture bias and low throughput (Abdel-Rahman et al., 2023; Robles-Gancedo et al., 2009). Additionally, culture-independent methods such as amplicon-based sequencing provide a more comprehensive profile of microbes present including those that may be unculturable in the laboratory (Bill, et al., 2024). Commonly reported microbes include lactic acid bacteria such as *Leuconostoc* and *Lactobacillus*, thermophilic species of *Bacillus* and *Clostridium*, and yeasts (Pollach et al., 2002; Robles-Gancedo et al., 2009; Tallgren et al., 1999). Additionally, microbes infecting the beet roots in the field or storage piles such as *Candida*, *Fusarium*, and *Penicillium,* which are associated with increased invert sugars and raffinose, may also carry over into the extraction process (Bill, et al., 2024 under review; Kusstatscher et al., 2021; Kusstatscher et al., 2019). In addition to consumption of sucrose, microbial metabolism results in various byproducts that can interfere with factory operations. These biproducts include ethanol, organic acids that lower the pH of raw juice (lactic acid, acetic acid, butyric acid), gases that affect heat exchange (H_2S, H_2, CO_2), and polysaccharides that block filtration (primarily dextran) (Kohout et al., 2020; Pollach et al., 2002; Tallgren et al., 1999; Wojtczak et al., 2013). Microbial contaminants may also form biofilms on factory surfaces that necessitate cleaning and increase operating costs (Abdel-Rahman et al., 2023; Galié et al., 2018)).

Recent advances in high-throughput sequencing have greatly advanced the present understanding of the sugar beet-associated microbiome in growing fields and storage piles (Mendes et al., 2011; Wolfgang et al., 2023), and will increase our understanding of the factory beet juice microbiome (Bill, et al., 2024; Zhang et al., 2022). Further research may elucidate the microbes which have the most detrimental effect on sugar extraction processes and environmental factors that lead to the greatest levels of such microbes. In the present study, microbial isolations were carried out using diffusion tower juice and biofilm samples collected at 18 sugar beet factories in North America. While various microbiological studies have previously been performed at beet sugar factories, microbes were often classified in broad categories such as "Aerobic mesophiles" or "Anaerobic thermophiles" (Kohout et al., 2020; Robles-Gancedo et al., 2009),which can contain overlapping groups of bacteria and do not provide a complete picture of microbial diversity. Another understud-

ied aspect this study attempted to address was the sampling of biofilms, which are commonly found on exposed factory surfaces (Abdel-Rahman et al., 2023; Galié et al., 2018). Biofilms are laborious to sanitize and may also be sources of microbial contamination. The microbial isolates collected in this study lay the groundwork for future studies on how various microbes contribute to factory losses, and the efficacy of various biocides to reduce their impact in factories. A portion of isolates also underwent preliminary assessment for their ability to produce polysaccharides in flask culture that increase culture viscosity that may be predictive of impacts during sugar beet processing.

MATERIALS AND METHODS

Sample collection. In total, 18 sugar beet factories (Table 1) contributed samples to the study during the 2022-2023 campaign. Factory staff collected diffusion tower juice from the bottom of the tower and biofilm samples from multiple factory locations and added these to cryotubes containing sterile glycerol solution to yield a final concentration of 20% glycerol (Cabrera et al., 2020; Amberg, 2005). Many factories collected multiple samples during the processing campaign from as early as December 2022 and as late as August 2023 depending on location with the intent to capture as much microbial diversity as possible in the samples given that many microorganisms are "unculturable" (Vartoukian et al., 2010). One-third of biofilm collection sites were noted by factory operators (Table 2).

The samples were then frozen at −20 °C and shipped overnight on ice packs to the USDA-ARS laboratory in Fargo, ND, where they were held at −80 °C until being shipped overnight on dry ice to the USDA-ARS laboratory in New Orleans, LA. Upon arrival, the sample cryovials were stored at −80 °C.

Preparation of beet sugar growth media. Factory beet juice was used to prepare agar plates to aid in recovery of a greater diversity of microbes present in factory samples. Diffusion tower juice was collected from American Crystal Sugar Company, Moorhead Factory, MN and stored at −80 °C and shipped overnight on dry ice to the USDA-ARS laboratory in New Orleans, LA and stored at −20 °C. Raw juice was thawed at 4 °C and centrifuged at 10,000 × g for 20 minutes to remove solid debris. To the supernatant, 3 g/L yeast extract, 6 g/L peptone, and 20 g/L agar were added before autoclaving as similarly performed previously (Bruni et al., 2022).

Microbial isolation & identification. Small amounts (<50 µL) of the frozen factory samples were serially diluted in sterile water and spread onto beet agar plates, which were incubated aerobically at 28 °C until colonies appeared. Approximately 12 colonies were picked for isolation and identification from each factory sample that was cultured. Whenever possible we tried to pick as many different colony morphologies as possible. However, if most of the colonies looked the same on the plate, colonies were then randomly picked. Due to the tendency for large mucoid colonies to form on the beet juice agar, de Man, Rogosa, and Sharpe (MRS) (DeMan et al., 2003), and nutrient agar (Research Products International, Mt. Prospect, IL USA) plates were used for re-streaking to obtain single colony axenic cultures. All culturing was done aerobically at 28 °C. After 3 rounds of streaking, an isolated colony was picked and suspended in 50 µL of autoclaved ultrapure water to use as a PCR template. The 16S rRNA gene was PCR-amplified with primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Heuer et al., 1997), using Extaq Hot Start DNA polymerase (Takara, San Jose, CA USA)). PCR products were purified with Clean and Concentrator-5 kits (Zymo Research, Irvine, CA USA) and Sanger sequenced at Eurofins Genomics, LLC (Louisville, KY USA). The chromatogram files were imported into Geneious Prime Version 2023.0.1 (Dotmatics, Boston, MA USA), which was used to trim the primer-binding regions, assemble the forward and reverse reads, and BLASTn query the consensus sequences against the NCBI 16S RefSeq database to identify the nearest related organism. In a few cases, fungal isolates were identified and thus the ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) primers were used for PCR and sequencing (Smith & Peay, 2014; Walters et al., 2016), and the consensus sequences were BLASTn queried against the NCBI ITS RefSeq database. The isolates were identified by their closest BLAST hit in the NCBI RefSeq 16S and ITS databases and had an average of 99.48% sequence identity to their best hits. Additionally, the colony morphology of bacterial isolates on beet juice agar plates was recorded as either mucoid (slimy or gummy), rough (dull and irregular), or smooth (circular and generally translucent) (Ayers et al., 1979; Breakwell et al., 2017).

Rarefaction analysis. Rarefaction analysis is a method to examine the effect of sample size on species richness (Raup, 1975; Sanders, 1968).The analysis presented in this study was performed in Microsoft Excel using a custom function to randomly sample a numbered list of isolates or samples without replacement. The list of isolates and samples were divided into increasing intervals of 51 (612 total isolates, divided by 12) and 5 (55 total samples, divided by 11), respectively. At each interval, 10 random samplings were performed. For the purposes of the rarefaction analysis, isolates with different species as the best 16S rRNA gene or ITS1 region BLAST hit were considered unique taxa, though we acknowledge that these methods are no longer considered sufficient to properly classify isolates at the species level. Nevertheless, we argue that having enough sequence variation to change the top BLAST hit is enough for estimations of microbial diversity, similar to how amplicon-based sequencing studies now use amplicon sequence variants (ASVs) in similar analyses (Callahan et al., 2017) .

Measurement of microbial culture viscosity. Bacterial isolates were grown overnight at 28 °C at 250 RPM as precultures in 5 mL of either MRS broth or tryptone sucrose yeast (TSY) broth containing 50 g/L sucrose (adapted from tryptone glucose yeast extract (TGY) medium) (Haynes et al., 1955)). *Leuconostoc* and *Weissella* isolates were grown in MRS as precultures since these strains sometimes became too viscous to pipette in the presence of sucrose. All other precultures were grown in TSY. The precultures were then used to inoculate 50 mL TSY medium containing 120 g/L sucrose in 250 mL culture flasks to OD_{600} of 0.05. The flask cultures were shaken at 250 RPM for 24 hours at 28 °C. OD_{600} was read with a spectrophotometer and culture viscosity was measured using a Brookfield model DV-II+ viscometer equipped with a UL spindle and small sample adapter. Culture samples were first visually classified among

one of three groups: watery, intermediate, and viscous. Watery samples had no significant visual increase in viscosity, while viscous samples had consistencies resembling gels (with intermediate class falling in between). Viscous samples required dilution prior to viscometry analysis, whereby 100 mL of deionized water was added to the 50 mL cultures and mixed thoroughly by shaking the flask; watery and intermediate class samples were analyzed as-is. Based on their initial qualitative classification, samples were measured with at least two viscometer speeds (20 and 50 RPM for watery; 1 and 2 RPM for intermediate; 5, 10 and 20 RPM for diluted viscous samples). Multiple speeds were used to assess for shear thinning phenomena, which is characteristic of polysaccharide solutions (Evageliou, 2020; Xu et al., 2009; Yang et al., 2019). An observation of shear thinning would provide evidence in support of the presence of polysaccharides within the culture broth, showing that the bacteria studied are responsible for EPS formation. All viscosity measurements (for individual 50 mL cultures) were done in duplicate.

RESULTS

In total, 18 sugar beet factories contributed 55 cryostock vial samples to this study (Table 3). This number includes 33 diffusion tower juice samples, 21 biofilm samples, and 1 unknown sample, which was only identified by factory but not sample type. The factories are geographically distributed across the sugar beet producing regions of North America, representing 9 U.S. states and 1 Canadian province. The samples were collected as early as December 2022 and as late as August 2023. This design was intended to maximize the microbial diversity in the samples to ensure as many representative organisms as possible could be collected. Relating to this, factories that begin their processing campaign earlier in the year were asked to wait to collect samples until at least December, when microbial contamination typically increases in most factories with the exception of the factory in Brawley, CA, which processes on an alternative schedule (English, 2020; Strausbaugh, 2018).

The main goal of this work was to obtain representative isolates of the most abundant microbial contaminants of beet sugar juice and biofilms, rather than a comprehensive profiling of the microbiome recently reported by others (Bill, et al., 2024). As such, greater emphasis was placed on sample count rather than the depth of sampling, i.e. the number of isolates per sample. Based on this logic, roughly 12 colonies were picked from each sample, eventually resulting in 612 isolates. The isolates were identified by sequencing of their 16S or ITS1 rRNA genes and found to belong to 37 genera, including many previously reported genera such as *Leuconostoc*, *Bacillus*, and *Rahnella* (Figure 1). There were 499 Gram-positive bacteria compared to 103 Gram-negative bacteria. *Leuconostoc* was the most abundant genus by far, represented by 365 isolates. Only 10 fungal isolates were obtained. Proportional to the number of viable samples, 379 isolates were obtained from 33 diffusion tower juice samples, 223 isolates were obtained from 21 biofilm samples, and 10 isolates were obtained from the 1 unlabeled sample. There were 14 genera common to both juice- and biofilm-derived isolates while 9 were unique to juice samples and 13 were unique to biofilm samples. Additionally, 2 genera were unique to the "unknown" sample. Rarefaction analysis was applied to examine the recovery rate of novel taxa as a function of sampling (Figure 2). The data did not appear to have reached an asymptote, suggesting that additional taxa would continue to be recovered as more samples are collected and more isolates are picked per sample.

A set of 54 bacterial isolates, which represent 7 of the 8 most abundant genera, were assessed for their ability to increase the viscosity of the growth medium when grown as flask cultures. The viscosity of flask cultures were both visually classified and measured using a Brookfield model DV-II+ viscometer (see methods). Roughly equal numbers of juice- (n=25) and biofilm- (n=29) derived isolates were characterized overall, but certain genera were more abundant in either juice or biofilm samples, such as *Rahnella* and *Acinetobacter*. In total, 37 flask cultures were classified as watery (Table 4), 7 flask cultures were classified as intermediate (Table 5), and 10 flask cultures were classified as viscous (Table 6). In total, the juice and biofilm isolate flask culture samples had similar absolute numbers of watery and intermediate class samples (Figure 3; juice, watery: n=18; juice, intermediate: n=4; biofilm, watery: n=19; biofilm, intermediate: n=3). However, the biofilm-derived isolates had roughly double the number of viscous culture samples compared to juice (juice, viscous: n=3; biofilm, viscous: n=7). Although the sample size in this case is relatively small, these results seem consistent with previous observations that that EPS from biofilm isolates results in higher viscosities than EPS from juice (planktonic) isolates (Yang et al., 2019). Finally, there seemed to be a weak correlation (Cramers V, 0.316) between increased viscosity and earlier observations of mucoid colony morphology on beet juice agar during the microbial isolations (Cramer, 1946). To verify this, the isolates were streaked again onto beet juice agar plates. Indeed, mucoid morphology appeared to be more common in strains producing viscous (8 of 10) and intermediate viscosity (7 of 7) flask cultures when compared to the watery cultures (17 of 37). The highest viscosity was observed in flask cultures of *Leuconostoc* isolate 48-3 followed by *Weissella* isolate 15-1 and *Pantoea* isolate 19-7. Interestingly, isolates obtained from both juice and biofilm samples produced viscous cultures. Furthermore, a mild shear thinning effect was observed in most of the viscous culture samples as the RPMs were increased. Shear thinning is a typical effect reported for polysaccharide solutions (Evageliou, 2020; Xu et al., 2009).

DISCUSSION

The negative impact of microbial contaminants on sugar crop processing has long been recognized (Solomon, 2009), and the sugar production industry continues to seek improvements in methods to detect and reduce microbial load (Abdel-Rahman et al., 2023; Bill, et al., 2024; Holland et al., 1990; Kusstatscher et al., 2019; Robles-Gancedo et al., 2009). The isolation work in this study provided hundreds of relevant isolates that are central to performing future crucial experiments testing the efficacy of antimicrobial agents and characterizing bacterial exopolysaccharides. Towards this goal, a broad sampling of diffusion tower juice and biofilms from sugar beet factories across North America was undertaken to obtain a representative collection of microbial contaminants. The broad design of the sampling scheme has resulted in a remarkably diverse collection of isolates. This diversity likely reflects the wide geographic and temporal range of sampling. Additionally, this study revealed significant microbial diversity present in biofilms throughout the sugar beet

factories, which do not seem to have been systematically studied previously. Biofilms are specialized microbial communities encased in extracellular matrix comprised of exopolysaccharides, proteins, and extracellular DNA that usually exhibit unique rheological and structural characteristics that typically have increased resistance to antimicrobial measures (Galié et al., 2018; Jeon et al., 2023). These biofilms are of interest as potential sources of re-contamination, and a more systematic study would be required to understand the factors affecting biofilm formation and composition in beet sugar factories.

Although the rarefaction curves suggest that additional sampling would produce more isolates belonging to novel taxa, the goal of this work was to obtain representative and relevant isolates for experimental characterization. This appears to have been generally achieved, as the isolates obtained in this study include diverse genera and are taxonomically similar to those previously identified in both culture-dependent and culture-independent studies (Bill, et al., 2024; Pollach et al., 2002; Robles-Gancedo et al., 2009; Tallgren et al., 1999; Zhang et al., 2022). Admittedly, the culturing conditions used in this study did likely lead to the omission of some previously reported genera such as the strictly anaerobic *Clostridium* and *Thermoanaerobacter* (Wiegel, 1981), *Lactobacillus* that can be microaerophilic or anaerobic requiring addition of reducing agent and anaerobic culture conditions (De Angelis, 2016), and thermophiles like *Thermoanaerobacterium* and *Thermoanaerobacter* (Bill, et al., 2024; Kohout et al., 2020; Lee et al., 1993). It is also possible that fewer fungi were isolated if some of these microorganisms were less able to tolerate elevated temperatures during processing and collection from the diffusion tower (Robles-Gancedo et al., 2009). Such strains could be obtained through more targeted sampling or isolation strategies from the remaining frozen factory samples or other culture collections.

A major impact of microbial contamination is the production of viscous polysaccharides (Hector et al., 2016), which interfere with filtration and other processing steps during raw sugar extraction (Ernst et al., 2024; Evageliou, 2020; Soliman, 2007; Borji et al., 2019). A polysaccharide of special concern has been dextran, a polymer composed primarily of α-1,6 linked glucose subunits (Díaz-Montes, 2021; Ernst et al., 2024; Passerini et al., 2015; Purama et al., 2009). Indeed, many of the strains that caused significant viscosity in flask cultures were lactic acid bacteria such as *Leuconostoc* and *Weissella*, whose genomes tend to encode for dextran production (Qi et al., 2023; Yu et al., 2022). In contrast, it would be interesting to identify the composition of the viscous polysaccharides produced by *Pantoea* spp. isolates 7-5, 19-4, and 19-7, as this genus is not known to produce dextran. It is also worth noting differences in apparent polysaccharide production between isolates identified as the same genus through 16S rRNA sequence similarity. This shows further studies are needed to associate particular microbes with increased viscosity, which is crucial to mitigating viscosity problems during processing. While microbes are likely killed during juice heating, microbial-derived exopolysaccharides (EPS) such as dextran are likely to persist and cause operational challenges downstream of the initial microbial degradation of sucrose. Furthermore, some studies suggest that microbes producing higher viscosity EPS may be more likely to adhere to surfaces and form biofilms (Yang et al., 2019) . In summary, the microbial isolates collected in this study are valuable for future studies aimed at identifying microbial susceptibility to antimicrobials as well as characterizing exopolysaccharide production and providing potential solutions to the operational challenges such as increased viscosity and biofilm formation resulting from microbial contamination.

DATA AVAILABILITY

The 16S rRNA and ITS1 sequences for the isolates have been deposited in the National Center for Biotechnology Information GenBank database and can be found by the accession numbers PQ691418-PQ692019 and PQ687024-PQ687033**.**

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Table 1. Participating factories and their locations and parent companies.

Factory	Location	Company
1	Croswell, MI	Michigan Sugar
2	Billings, MT	Western Sugar
3	Fort Morgan, CO	Western Sugar
4	Moorhead, MN	American Crystal Sugar
5	Sebewaing, MI	Michigan Sugar
6	Mini-Cassia Paul, ID	Amalgamated Sugar
7	East Grand Forks, MN	American Crystal Sugar
8	Wapheton, ND	Minn-Dak Farmer's Cooperative
9	Twin Falls, ID	Amalgamated Sugar
10	Caro, MI	Michigan Sugar
11	Hillsboro, ND	American Crystal Sugar
12	Nampa, ID	Amalgamated Sugar
13	Sydney, MT	Sydney Sugar
14	Lovell, WY	Western Sugar
15	Bay City, MI	Michigan Sugar
16	Scottbluff, NE	Western Sugar
17	Taber, Alberta, Canada	Lantic Sugar
18	Brawley, CA	Spreckels Sugar

Table 2. Collection sites of biofilm samples were reported for one-third of sugar beet factory biofilm samples (SN).

SN	Site Collected	Date Received
3	Pre-limer cell 2	1-5-2023
4	Pre-limer cell 3 and 4	2-6-2023
11	Diffuser	1-27-23
12	Thick juice pump	
14	Diffusion/Cossette mixer	2-2-2023
20	Inside and outside beet room	2-3-2023
22	Whites, chips and tails	2-6-23
23	3rd floor 2nd Carb filters	1-06-2023
27	Pre-limer cell 4	3-22-2023
28	Thin juice pump	1-11-2023
35	Raffinerie Tirlemontoise (RT) and Slope (SL) Diffusor	8-16-23
36	Raffinerie Tirlemontoise (RT) and Slope (SL) Diffusor	6-28-23

Table 3. Summary of sugar beet factory samples and resulting numbers of isolates identified, with distribution of genera based on full-length 16S rDNA or ITS1 region rDNA Sanger sequencing.

	Viable vials	Isolates	Genera
All samples	55	612	37
Juice samples	33	379	22
Biofilm samples	21	223	26
Unknown[a]	1	10	6

[a] *Only the factory name was legible.*

Table 4: Measured viscosities of culture broths from biofilm- and diffuser juice-derived isolates from sugar beet factories in North America classified as "watery" viscosity (1-20 cP at 20 RPM)

Genus	Isolate	Source	Colony morphology	Meas. Visc. (cPs) at 20 RPM	Meas. Visc. (cPs) at 50 RPM
Acinetobacter	60-12	Biofilm	Smooth	1.71 ± 0.04	1.72 ± 0.02
Acinetobacter	21-5	Biofilm	Smooth	1.35 ± 0.00	1.40 ± 0.01
Acinetobacter	21-8	Biofilm Smooth	1.22 ± 0.02	1.20 ± 0.00	
Acinetobacter	38-11	Biofilm	Smooth	1.58 ± 0.06	1.59 ± 0.03
Acinetobacter	46-12	Biofilm	Smooth	1.55 ± 0.06	1.58 ± 0.03
Bacillus	16-7	Biofilm	Smooth	2.00 ± 0.02	1.88 ± 0.01
Bacillus	36-6	Biofilm	Mucoid	1.94 ± 0.02	1.81 ± 0.01
Bacillus	37-11	Juice	Smooth	14.5 ±0.35	--a
Bacillus	16-1	Biofilm	Smooth	1.70 ± 0.02	1.70 ± 0.00
Bacillus	45-1	Juice	Mucoid	1.71 ± 0.04	1.74 ± 0.06
Bacillus	45-9	Juice	Mucoid	20.3 ± 0.0	-- a
Bacillus	18-5	Juice	Mucoid	1.71 ± 0.04	1.68 ± 0.01
Bacillus	68-1	Juice	Rough	1.64 ± 0.02	1.61 ± 0.01
Bacillus	49-2	Biofilm	Mucoid	2.09 ± 0.02	2.08 ± 0.03
Bacillus	18-1	Juice	Mucoid	2.27 ± 0.02	2.24 ± 0.04
Bacillus	49-1	Biofilm	Mucoid	2.00 ± 0.02	1.94 ± 0.02
Leuconostoc	14-9	Biofilm	Mucoid	3.65 ± 0.03	3.72 ± 0.01
Pantoea	15-4	Juice	Mucoid	15.7 ± 0.3	-- a
Pantoea	3-4	Juice	Mucoid	14.9 ± 0.0	-- a
Pantoea	9-1	Juice	Smooth	1.61 ± 0.02	1.60 ± 0.02
Pantoea	19-1	Biofilm	Mucoid	5.66 ± 0.11	5.03 ± 0.04
Pantoea	25-2	Biofilm	Smooth	2.03 ± 0.02	2.04 ± 0.03
Pantoea	24-11	Juice	Mucoid	5.58 ± 0.00	5.17 ± 0.01
Peri bacillus	51-4	Biofilm	Mucoid	2.00 ± 0.07	2.06 ± 0.08
Peri bacillus	18-2	Juice	Smooth	3.59 ± 0.02	3.38 ± 0.Ql
Peribacillus	22-11	Juice	Smooth	1.94 ± 0.02	1.88 ± 0.01
Peribacillus	4-1	Juice	Smooth	2.25 ± 0.08	2.13 ± 0.03
Peribacillus	4-7	Juice	Smooth	3.33 ± 0.13	3.05 ± 0.04
Peribacillus	36-1	Biofilm	Smooth	3.24 ± 0.04	3.18±0.01
Peribacillus	36-9	Biofilm	Smooth	5.03 ± 0.02	4.70 ± 0.Ql
Peri bacillus	51-1	Biofilm	Smooth	2.50 ± 0.01	2.50 ± 0.02
Peribacillus	71-1	Biofilm	Smooth	2.60 ± 0.02	2.49 ± 0.Ql
Rahnella	24-4	Juice	Mucoid	3.17 ± 0.06	3.15 ± 0.02
Rahnella	26-4	Juice	Smooth	2.94 ± 0.04	2.94 ± 0.01
Rahnella	35-2	Juice	Mucoid	3.59 ± 0.06	3.55 ± 0.07
Rahnella	39-3	Juice	Mucoid	6.27±0.13	5.91 ± 0.06
Weissella	31-12	Biofilm	Mucoid	4.97 ± 0.06	4.85 ± 0.08

Higher viscosity samples could not be measured at 50 RPM due to exceeding the upper limit for instrument torque required to determine viscosity.

Table 5: Measured viscosities of culture broths classified as "intermediate" viscosity (30-230cP at 1 RPM) from biofilm- and diffuser juice-derived isolates from sugar beet factories in North America

Genus	Isolate	Source	Colony morphology	Meas. Visc. (cPs) at 1 RPM	Meas. Visc. (cPs) at 2 RPM
Leuconostoc	5-1	Biofilm	Mucoid	57.3±1.3	48.3 ± 1.3
Leuconostoc	25-7	Biofilm	Mucoid	47.7 ± 2.1	45.8 ± 0.6
Leuconostoc	2-6	Biofilm	Mucoid	37.2 ± 4.2	36.6 ± 4.2
Leuconostoc	52-11	Juice	Mucoid	222.9 ± 3.8	210.9 ± 3.4
Leuconostoc	47-1	Juice	Mucoid	128.1 ± 7.2	123.0 ± 7.6
Leuconostoc	62-9	Juice	Mucoid	190.2 ± 3.4	175.1±1.5
Pantoea	7-5	Juice	Mucoid	51.0 ± 0.8	48.5 ± 0.6

Table 6: Measured viscosities of culture broths from biofilm- and diffuser juice-derived isolates classified as "viscous" that required dilution to enable measurement (diluted with 100 mL water added to 50 mL cultures) of viscosity (2-57cP at 5 RPM).

Genus	Isolate	Source	Colony morphology	Meas. Visc. (cPs) at 5 RPM	Meas. Visc. (cPs) at 10 RPM	Meas. Visc. (cPs) at 20 RPM
Leuconostoc	2-3	Biofilm	Mucoid	2.94 ± 0.08	2.94 ± 0.00	2.88 ± 0.04
Leuconostoc	48-3	Juice	Mucoid	56.9 ± 0.5	52.0 ± 0.1	-- a
Pantoea	19-4	Biofilm	Mucoid	21.7 ± 1.1	18.6 ± 0.8	15.6 ± 0.6
Pantoea	19-7	Biofilm	Mucoid	33.9 ± 0.5	28.2 ± 0.4	22.5 ± 0.2
Peribacillus	13-5	Juice	Smooth	14.4 ± 0.6	12.7 ± 0.4	11.1±0.4
Weissella	56-6	Biofilm	Mucoid	2.58 ± 0.08	2.55 ± 0.0	2.46 ± 0.04
Weissella	15-1	Juice	Mucoid	36.9 ± 4.4	29.8 ± 3.8	24.4 ± 2.6
Weissella	31-2	Biofilm	Smooth	3.90 ± 0.08	3.75 ± 0.04	3.56 ± 0.02
Weissella	53-1	Biofilm	Mucoid	5.28 ± 0.00	5.19 ± 0.04	5.01 ± 0.04
Weissella	53-10	Biofilm	Mucoid	5.34 ± 0.25	5.04 ±0.08	4.79 ± 0.11

[a]*Higher viscosity samples could not be measured at 20 RPM due to exceeding the upper limit for instrument torque required to determine viscosity*

LIST OF FIGURES

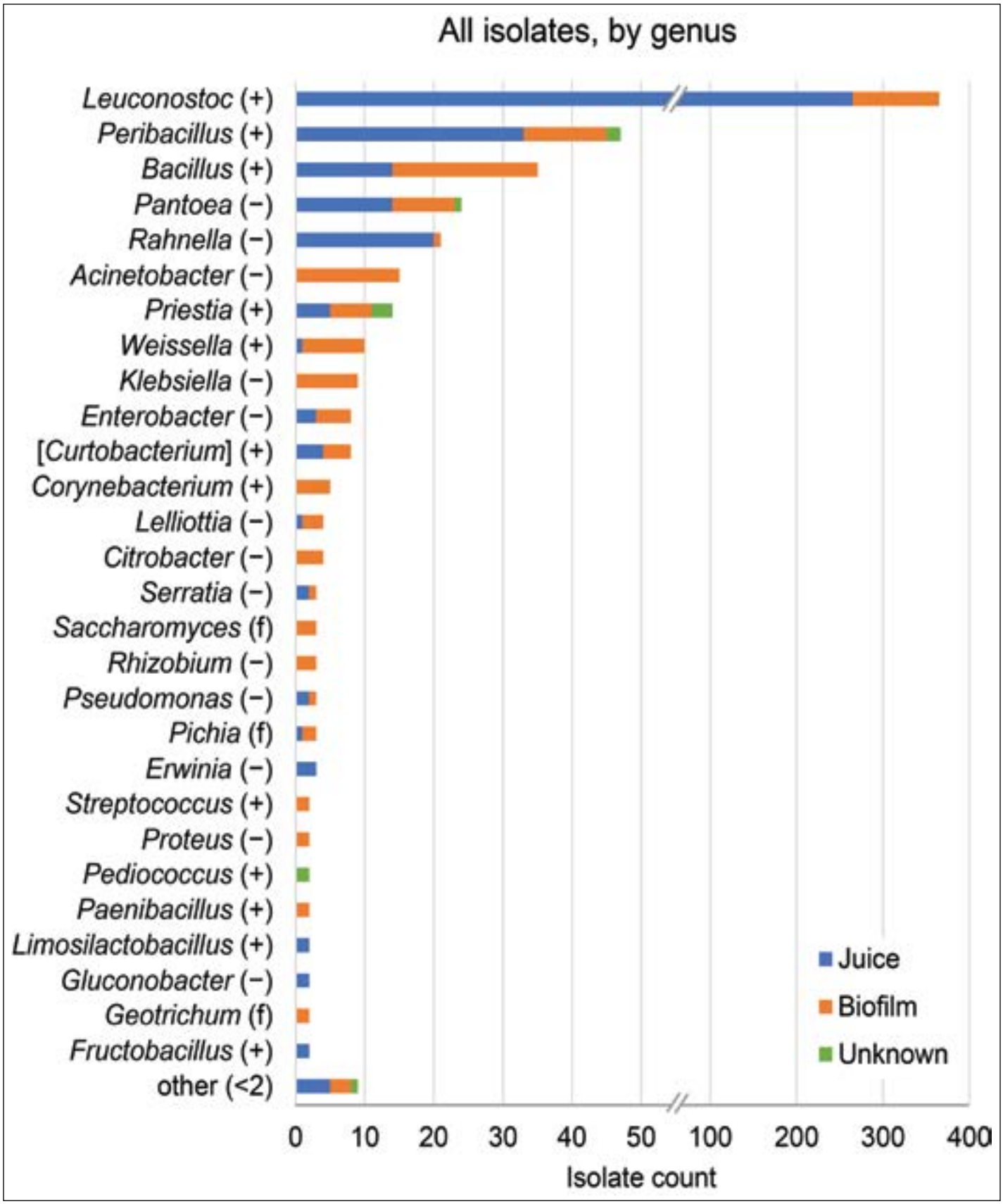

Figure 1. Microbial isolates tallied by genus from sugar beet factories in North America. Gram-positive bacteria are indicated by (+), Gram-negative bacteria are indicated by (−), and fungi are indicated by (f). The 9 genera that had only 1 isolate each were grouped into the category "other". Note: The NCBI taxonomy database entry indicates that the type strain [*Curtobacterium*] *plantarum* ATCC 49174 should be transferred into the genus *Pantoea*.

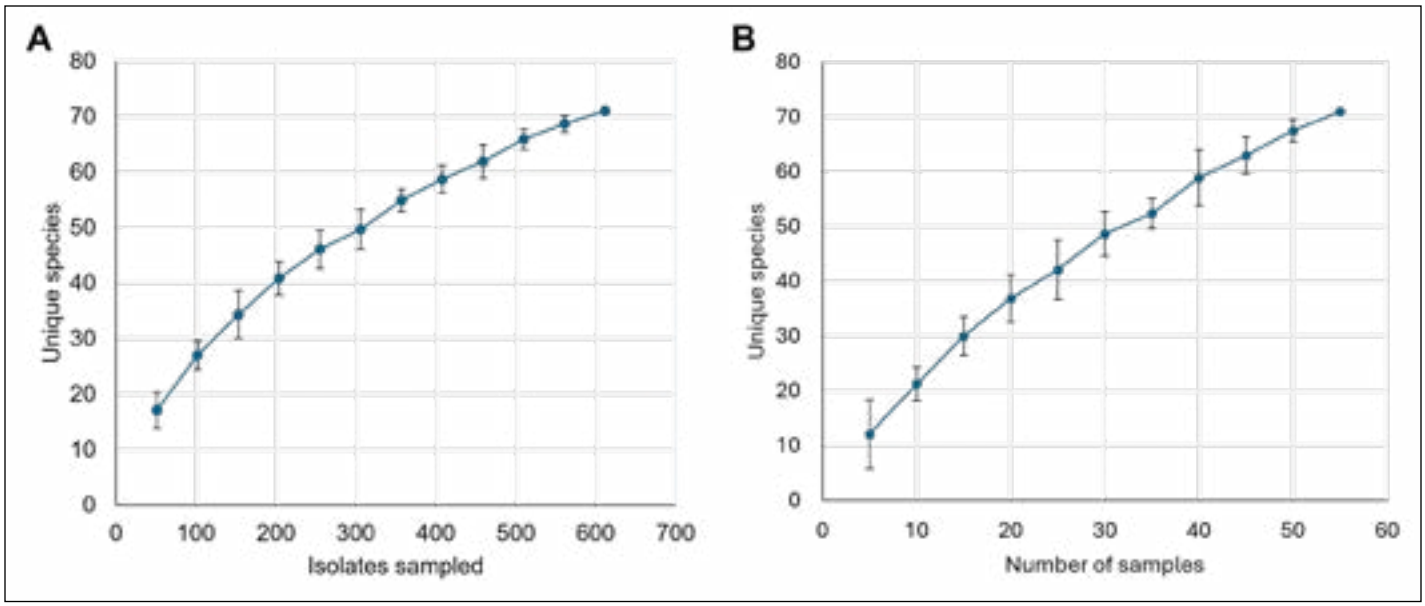

Figure 2. Rarefaction analysis estimating the isolation rate of novel taxa from sugar beet factories in North America. Each point represents the average of 10 random subsamples at intervals of A) isolates and B) factory samples. Error bars denote the standard deviation.

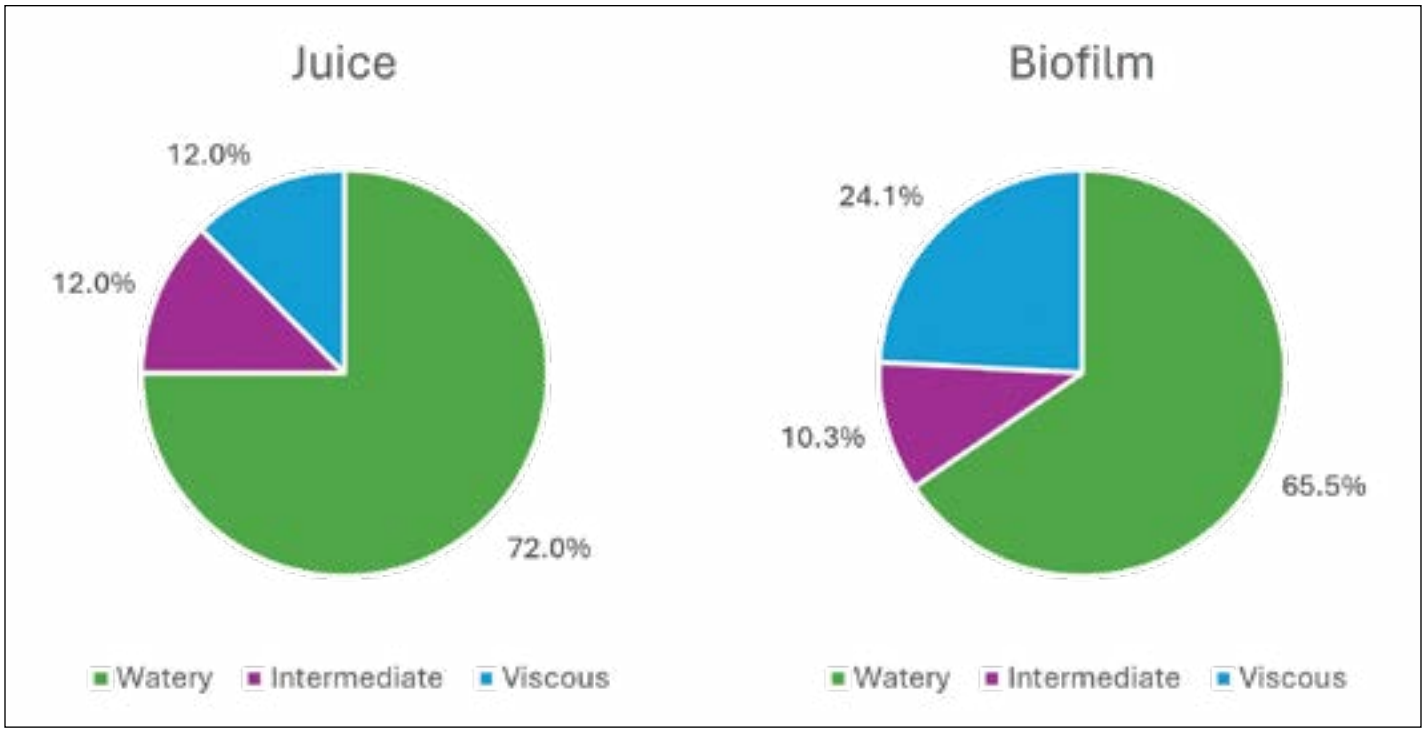

Figure 3. Relative proportion of juice and biofilm samples across different viscosity classifications from sugar beet factories in North America.

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