sugarjournal.com | September 2026

# Sugar Journal

YOUR **BEST RESOURCE** FOR THE **GLOBAL SUGAR** AND **ETHANOL INDUSTRIES**

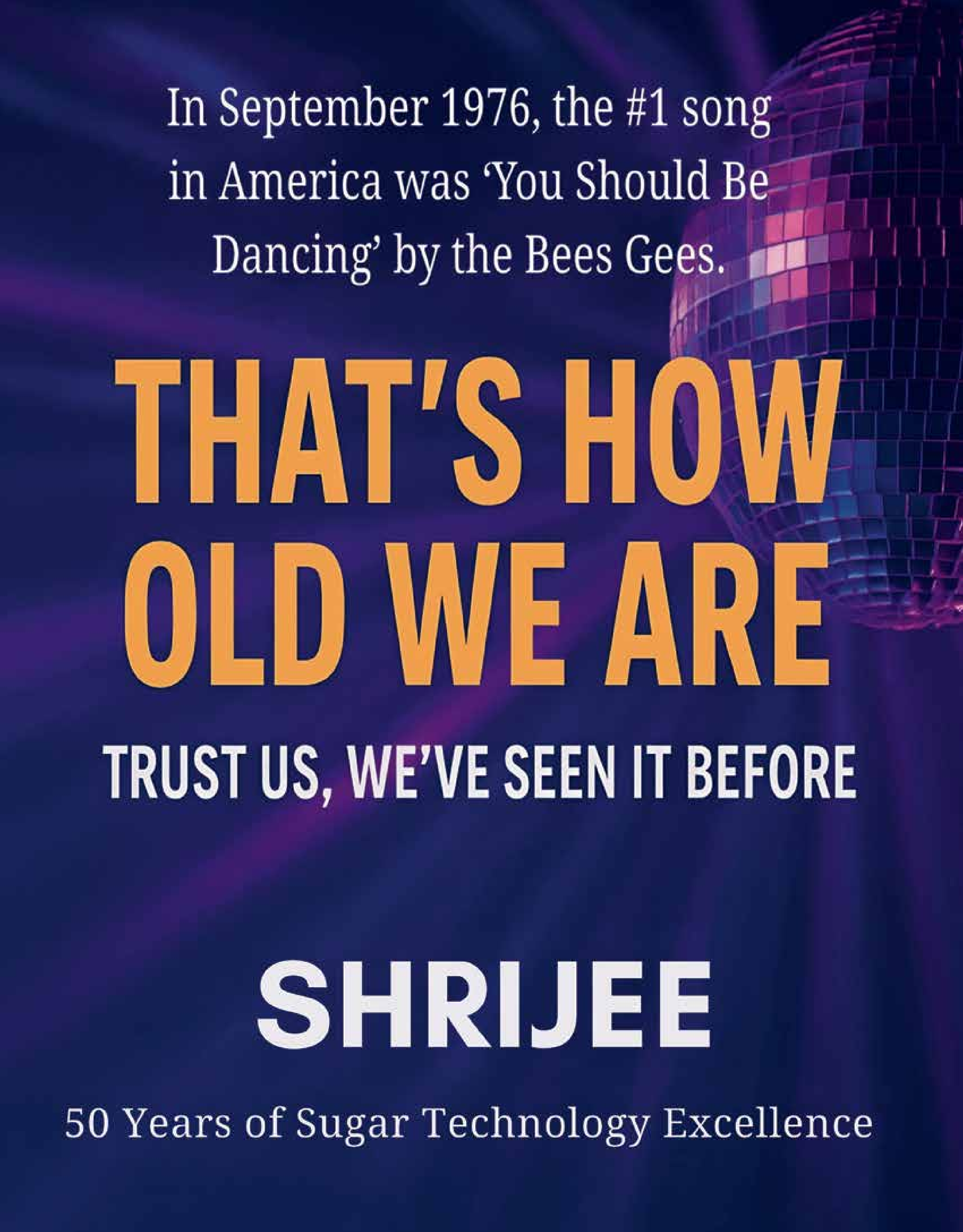
# LATIN AMERICAN SUGAR INDUSTRY

*also*

## MICROBIAL ISOLATES SUGAR BEET FACTORY JUICES

**AGRICULTURE | PROCESSING | REFINING | ENERGY | BY-PRODUCTS**

*Translations in Spanish and Portuguese*


In September 1976, the #1 song in America was 'You Should Be Dancing' by the Bees Gees.
THAT'S HOW OLD WE ARE
TRUST US, WE'VE SEEN IT BEFORE
SHRIJEE
50 Years of Sugar Technology Excellence


# RAISING CANE

## THE ALL-NEW CH SERIES SUGAR CANE HARVESTERS

We started in the fields, threading our way through the stalks. We talked – and listened – to sugar cane growers like you. We spent hours in the cab. And when we were through, we came up with an all-new CH Harvester. From a large, spacious cab with plenty of comfort and visibility, to more automation technology to increase productivity and decrease stress, to the sleek new styling – the new CH Series is our most advanced and capable harvester yet.

# Sugar Journal

# Table of Contents September 2026

## Features

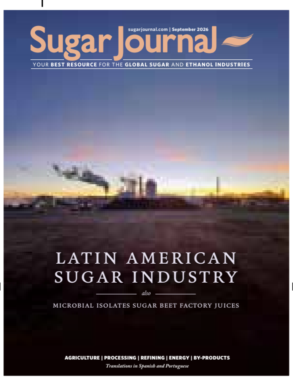


## Departments

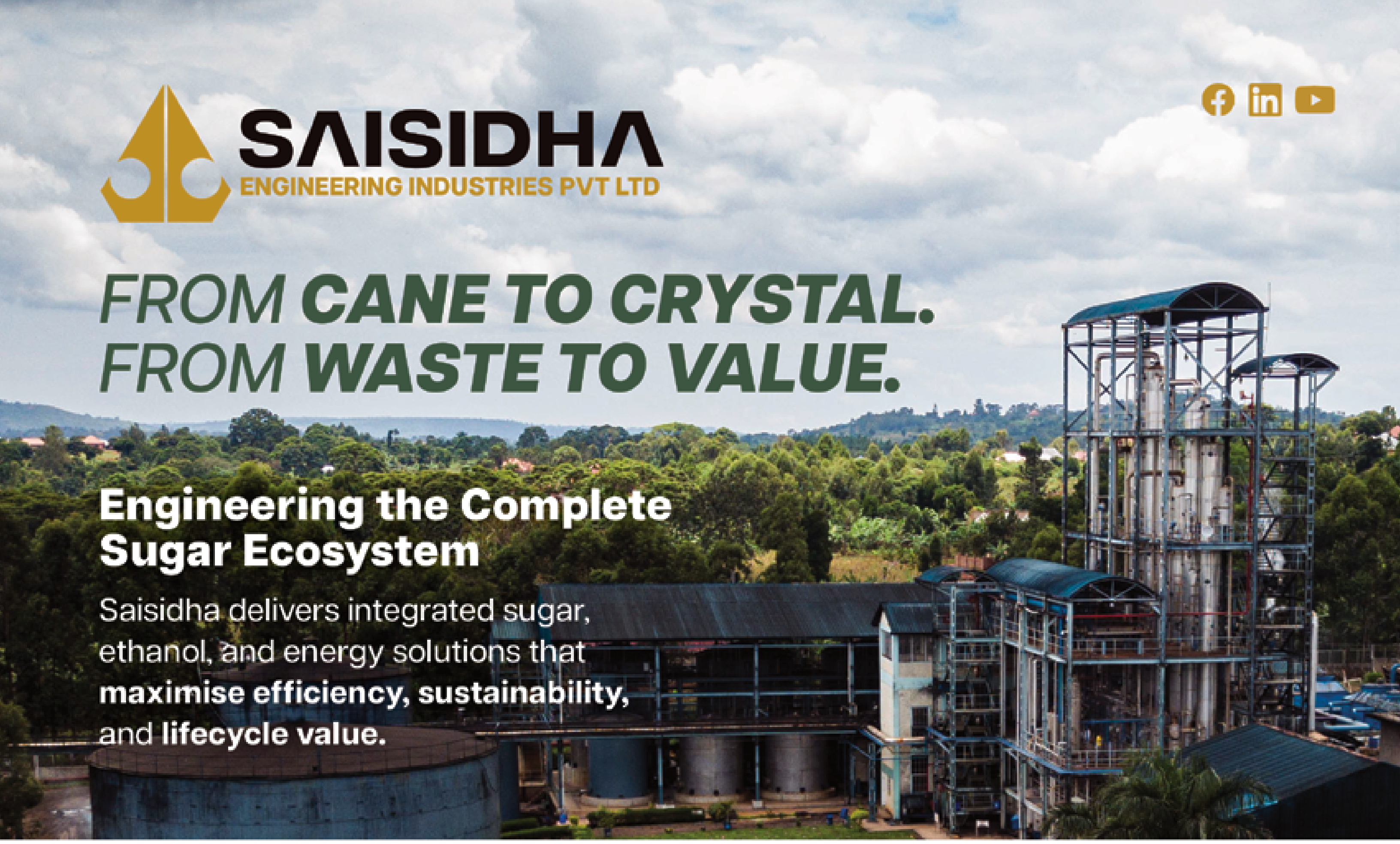


## Columns






Cover
Lovell Factory,
Western Sugar Cooperative.




**Sugar &** Refining | **Distillery** & Ethanol | **Energy &** Co-generation | **Boilers &** Process Equipment

## Global Presence

**Africa:** Uganda | Tanzania | Kenya | Nigeria | South Africa | Zambia | Ethiopia

**Asia:** Indonesia | Vietnam | Sri Lanka

**Americas & Others:** Mexico | Guyana | Costa Rica | Belize | Fiji

- **Project across 22+ Countries**
- **Strong footprint in Africa & Emerging Markets**
- **Trusted by public & private sector groups**

Scan to know more about **SAISIDHA**

info@saisidha.com
sanjaybhosale@saisidha.com

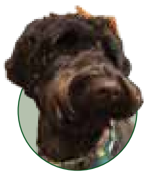
WWW.SAISIDHA.COM

Whatsapp +91 - 9822 022 259

# Publisher's Corner

La industria azucarera ha tenido la suerte de beneficiarse de personas cuyas historias son tan notables como sus logros profesionales. Tanto Alfonso Fanjul, Jr. y Alejandro Morales proceden de familias con raíces de Cuba que abandonaron su tierra natal tras la toma de poder Castrista, dejando atrás las vidas que conocían para comenzar nuevos retos en los Estados Unidos. Empezando con poco o nada, se enfrentaron al enorme desafío de construir nuevas vidas y carreras, pero gracias al trabajo duro, la perseverancia y la determinación, ambos lograron un gran éxito en sus respectivas áreas de la industria azucarera. Sus historias son especialmente significativas porque representan logros muy significativos. A lo largo de la historia, varias personas han llegado a Estados Unidos desde Cuba, Vietnam y varios países, donde la libertad, la oportunidad y la posibilidad de construir un futuro mejor podían parecer inalcanzables. Sus travesías nos recuerdan no solo el valor que se necesita para dejarlo todo atrás y empezar de nuevo, sino también las oportunidades y libertades que tenemos la suerte de contar en Estados Unidos.

The sugar industry has been fortunate to benefit from individuals whose stories are as remarkable as their professional accomplishments. Alfonso Fanjul, Jr. and Alejandro Morales both came from families with roots in Cuba who left their homeland following Castro's takeover, leaving behind the lives they had known to begin again in the United States. Starting over with little, they faced the enormous challenge of building new lives and careers, yet through hard work, perseverance, and determination, both went on to achieve great success in their respective areas of the sugar industry. Their stories are especially meaningful because they represent something much larger. Throughout our history, people have come to America from Cuba, Vietnam, and countless other countries where freedom, opportunity, and the chance to build a better future may have seemed unattainable. Their journeys remind us not only of the courage it takes to leave everything behind and start again, but also of the opportunities and freedoms we are fortunate to have in the US.



Vivian
Office Mascot

Romney K. Richard
Editor


# Sugar Journal

**VOLUME 89 | NUMBER 4**



3803 Cleveland Ave.
New Orleans, LA 70119 USA
504.482.3914
www.SugarJournal.com



## Editorial Staff

**Editor**
Romney Kriedt-Richard
romney@sugarjournal.com

**Executive Editor**
Charley Richard, Ph.D.
charley@sugarjournal.com

**Contributing Editors, Latin America**
Juliusz Lewinski, Ph.D.
Guilherme Rossi Machado Jr.
Raul O. Castillo, Ph.D.

**Contributing Editor, Africa**
Peter Lyne

**Contributing Editor, Australia**
Robert Quirk

**Contributing Editor, United States & Canada**
Steve Clarke, Ph.D.

## Business Staff

**Publisher**
Romney Kriedt-Richard
romney@sugarjournal.com

**Production Manager**
Britt Benoit
art@sugarjournal.com

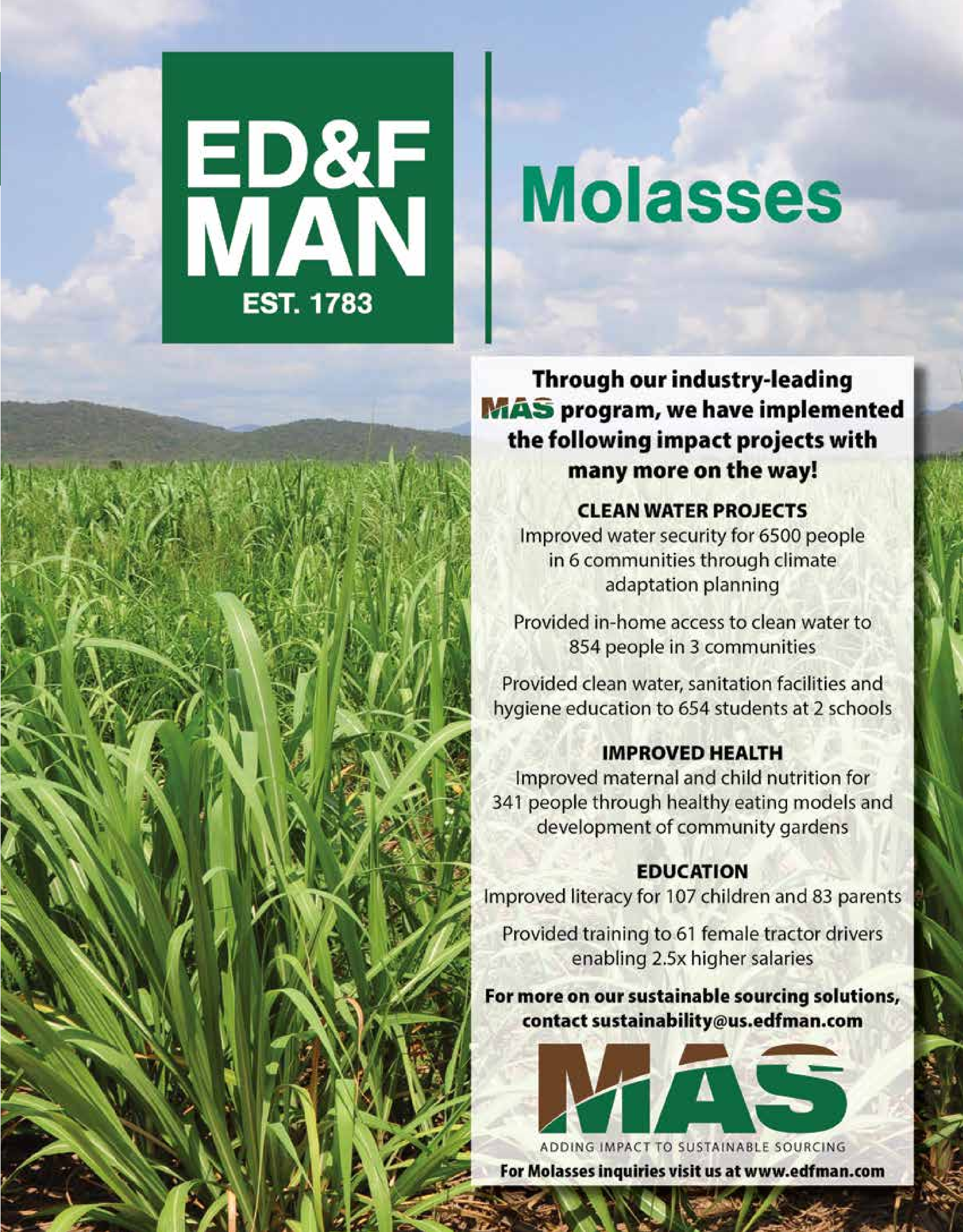
**Circulation Manager/ Accounting**
Debbie Helmstetter
debbie@sugarjournal.com

**Advertising Representative**
Jane Robinson
jane@sugarjournal.com



SUGAR JOURNAL (ISSN #0039-4734) is published monthly by Kriedt Enterprises, Ltd.
Editorial & Executive Office at 125 South Cortez Street, New Orleans, Louisiana 70119, U.S.A.
Telephone: (504) 482-3914 · Cell: (504) 628-3533 · Email: Romney@sugarjournal.com · Web site: www.sugarjournal.com
Periodical postage paid at New Orleans, Louisiana 70113, and additional entries.
POSTMASTER send change of address to Sugar Journal, 125 South Cortez Street, New Orleans, Louisiana 70119
Subscription rates, including all countries — 1 year $55.00 U.S., $90.00 foreign.


Copyright 2026 by Kriedt Enterprises Ltd. Printed in U.S.A.


ED&F MAN
EST. 1783
Molasses
Through our industry-leading MAS program, we have implemented the following impact projects with many more on the way!
CLEAN WATER PROJECTS
Improved water security for 6500 people in 6 communities through climate adaptation planning
Provided in-home access to clean water to 854 people in 3 communities
Provided clean water, sanitation facilities and hygiene education to 654 students at 2 schools
IMPROVED HEALTH
Improved maternal and child nutrition for 341 people through healthy eating models and development of community gardens
EDUCATION
Improved literacy for 107 children and 83 parents
Provided training to 61 female tractor drivers enabling 2.5x higher salaries
For more on our sustainable sourcing solutions, contact sustainability@us.edfman.com
MAS
ADDING IMPACT TO SUSTAINABLE SOURCING
For Molasses inquiries visit us at www.edfman.com

# Alfonso Fanjul, Jr. 1937 – 2026

Alfonso "Alfy" Fanjul Jr., a Cuban-born billionaire who escaped Fidel Castro's revolution, rebuilt his family's sugar enterprise in Florida, and played a pivotal role in transforming Florida Crystals into one of the nation's leading agribusiness empires, passed away in August at the age of 89.

As co-chairman and chief executive of Florida Crystals, Fanjul, alongside his three younger brothers, expanded the family business into one of the nation's largest privately held sugar and real estate conglomerates.

Together with his siblings José, Alexander, and Andrés, Fanjul led the accumulation of a fortune estimated in the billions through ventures in sugar production, refining, real estate, renewable energy, and international investments.

Born in Havana in 1937, Fanjul was the son of Alfonso Fanjul Sr. and Lillian Rosa Gomez-Mena, both descendants of Spanish immigrants. Their union merged two prominent Cuban sugar dynasties, which together controlled ten mills and extensive real estate holdings. By 1959, the majority of these assets had been seized by Castro's regime, prompting the family's relocation to Florida.

Fanjul's life represents one of South Florida's most notable immigrant success stories. Prior to Fidel Castro's revolution, the Fanjul family owned one of Cuba's largest privately held sugar enterprises, encompassing extensive plantations, mills, and related businesses. Following the nationalization of their holdings without compensation, the family sought refuge in the United States, bringing with them only their expertise in the sugar industry. By the 1970s, the brothers had established an even greater fortune in Florida, positioning the family among the most affluent and politically influential in Palm Beach.

Affectionately known as "Alfy," the erudite and distinguished Fanjul, along with his brother José "Pepe," became the public faces of the family's business empire and emerged as prominent figures in Palm Beach society and philanthropy.


SUGAR TECHNOLOGY INTERNATIONAL

ADVANCED SUGAR PROCESSING SOLUTIONS

TOWER CONTINUOUS VACUUM PAN

The STI Tower Continuous Vacuum Pan (TCVP) features a vertical, stacked design that minimizes floor space while enabling high-efficiency and continuous sugar crystallization. The TCVP uses natural circulation with a long massecuite flow path for crystal growth without the use of mechanical circulators, hereby optimizing crystal quality and C.V with stable operation. The modular system allows for capacity expansion, online cleaning with minimal downtime. High thermal efficiency through increased heating surface and the use of low-pressure or recompressed vapor.

SUGAR DRYER COOLER

The STI Sugar Dryer Cooler is designed for raw, white, and refined sugar applications, using a counter-current operation that reduces dryer length and power consumption while maintaining high efficiency. It features process control and automation, optional air preheating, and operates under slight vacuum. The system produces refined sugar with a significantly lower tendency to cake and can process up to 120 tons per hour in a single unit. The STI Dryer cooler technology is adaptable to the upgrading of existing dryers.

SERVICES
- Project Management
- Engineering
- Technical Advisory Audits
- Procurement and Logistics

EQUIPMENT DESIGN & SUPPLY
- Continuous Vertical Crystallizer
- High Circulation Rate CVP
- Juice Clarifier System
- Syrup Clarifier System
- Continuous Massecuite Reheater
- Sugar Conditioning System
- CO2 Remelt Decolorization & Filtration
- Direct Contact Heater & Condenser
- Entrainment & Spin Separator

CONTACT US
1309 West, 15th Street, Suite 160
Plano, Texas 75075, USA
Telephone: +1 214 784-2917
Sales@groupsti.com
www.groupsti.com

*Dear Members of the Fanjul Family and the ASR/FCC Team,*

*On behalf of everyone at Western States Machine Company, we extend our heartfelt condolences on the passing of Alfonso "Alfy" Fanjul, Jr., whose leadership helped shape Florida Crystals, Domino Sugar, and ASR.*

*Alfy was a visionary leader, a respected steward of the sugar industry, and a devoted family patriarch. Through Florida Crystals, Domino Sugar, ASR, and the broader enterprises he helped lead, his resilience, integrity, and commitment to his employees and community left a lasting impact on all who had the privilege of knowing and working with him.*

**"The greatest leader is not necessarily the one who does the greatest things, but the one who inspires others to carry forward a lasting legacy."**

*Our thoughts and prayers are with the Fanjul family and the entire Florida Crystals, Domino Sugar, and ASR teams during this difficult time. May you find comfort in Alfy's extraordinary legacy, the companies he helped build, and the many lives he touched.*

*With deepest sympathy,*
*Western States Machine Company*
*Board of Directors, Management, and Employees*


# The Sweet Success of Latin America's Smaller Sugar Exporters

BY VLADISLAV VOROTNIKOV

In a global sugar market defined by scale, Paraguay and Guatemala are betting on something different. Rather than competing head-on with Brazil, the two exporters are carving out lucrative niches with premium sugars that appeal to buyers seeking quality, traceability and sustainability. And the strategy seems to pay off.

In a market where Brazil's scale is almost impossible to challenge, smaller exporters are being forced to rethink the traditional sugar playbook. With vast production capacity, established logistics networks and the ability to influence global supply flows, the world's largest sugar exporter sets a benchmark that few countries can match. For smaller Latin American producers, the alternative is not to fight for a larger share of the commodity market but to find their own segments.

Organic sugar production support sugar farmers in Guatemala.
PHOTO CREDIT SLOW FOOD INITIATIVE

## A Big Divide in the Paraguay Sugar Industry

Paraguay's sugar industry is increasingly defined by a tale of two markets. While organic sugar producers are rapidly expanding their presence in premium export markets, conventional mills find their business under pressure from weak prices, raw material shortages, and trade barriers.

Around 95% of the organic sugar in Paraguay is exported, said Andrés González Aguilera, a sugar industry analyst with ABC Rural, a think tank.

"Most of our organic sugar is exported because domestic consumers still overwhelmingly prefer white sugar, with purchasing decisions driven more by volume than by demand for differentiated, premium-quality products," Aguilera said.

The key export market is the US, where the demand for organic sugar has been booming in recent years.

World Bank data show that Paraguay exported 34,935 metric tons of raw cane sugar worth $27 million in 2024. Exports of other cane and beet sugars totaled 22,535 metric tons, with a value of $16.3 million.

Roughly half of raw cane sugar and almost all other cane and beet sugars land on the US market, though European countries like Switzerland, Germany and France, have also been expanding purchases of Paraguayan sugar in the last few years.

The figures may appear modest in the context of the global sugar trade, but they carry considerable weight for a country the size of Paraguay.

The organic sugar niche has emerged as a lifebuoy for Paraguay's sugar industry in the last few years.

Currently, sugar mills in the country operate at 60% to 70% capacity utilization ratio – a potentially dangerous zone, according to Aguilera. As a result, sugar farmers are paid around 190,000 guaraníes ($0.032) per metric ton of sugarcane delivered to the plant, a figure that has remained virtually unchanged for four years.

According to analysts, without the popularity of Paraguayan organic sugar, which is sold at a significant premium to conventional sugar on foreign markets, the financial situation in the industry would be noticeably worse.

## Guatemala: Moving Up the Value Chain

While Paraguay has built its export strategy around organic and specialty sugars, Guatemala has taken a different route to compete in global markets. The country has developed a highly export-oriented industry focused on high-quality raw sugar for international buyers, combining competitive production costs with sustainability credentials.

"[Paraguay] combines meaningful export capacity with very high agricultural and industrial efficiency. The country has also expanded refinery capacity, enabling it to sell more refined and direct-consumption sugar instead of relying only on lower-value raw sugar exports," commented Nandini Roy Choudhury, analyst with Future Market Insight, a think tank.

Guatemala's export strategy is increasingly shifting toward higher-value refined sugar. Total sugar exports are projected to reach 1.685 million metric tons in 2026/27, with refined sugar accounting for approximately 70% of shipments at 1.185 million tons, compared with around 500,000 tons of raw sugar, as estimated by Commodity Board, a commodity information service. The shift builds on the previous season, when refined sugar already made up 68% of exports.

Guatemala carved out a niche in the global sugar market.
PHOTO CREDIT CV INTERNATIONAAL

The analysts note high demand from wealthy markets such as the United States, Taiwan and Canada and sustainability certifications —


BROADBENT CENTRIFUGALS

**Batch & Continuous Centrifugals for Cane & Beet Sugar Dextrose/Sweeteners**

- Single Machines
- Complete Batteries
- Ancillary Equipment
- Conversions & Spares for Broadbent & Other Brands of Centrifugals

Decanter Centrifugals for Cane and Beet Muds & Ethanol Stillage Dewatering

**BROADBENT INCORPORATED**
7404 Sand St.
Ft. Worth, Texas 76118
Tel: 817-595-2411
jcastaneda@broadbent-americas.com
www.broadbent.co.uk

***150 years of Centrifuge Technology***

## SugarSites

**BROADBENT, INC.**
broadbent.co.uk

**ED&F MAN**
edfman.com

**JOHN DEERE THIBODAUX, INC.**
johndeere.com

**POSTLE INDUSTRIES**
postle.com

**SAISIDHA ENGINEERING INDUSTRIES PVT. LTD.**
saisidha.com

**SHRIJEE**
shrijee.com

**WELDING ALLOYS**
welding-alloys.com

**WESTERN STATES MACHINE CO.**
westernstates.com

**Add your Business Listing to our SugarSites!**
**Call 504-482-3914 Today!**

Pareguay is establishing modern sugar industry infrastructure.
PHOTO CREDIT DIALOGUE EARTH

including ISO 14001, Bonsucro and Rainforest Alliance — as the key factors allowing Guatemalan producers to strengthen their ability to compete on quality, traceability and value rather than volume alone.

Guatemala's strongest advantage is operational efficiency, Choudhury agreed, adding that according to the USDA, Guatemala ranked as the world's second-most efficient sugarcane producer and fourth in overall sugar production efficiency in the 2023/24 marketing year.

"Its mills also produce refined sugar, molasses and renewable electricity, supporting better utilization of the cane crop and improving overall industry economics."

## When Size Truly Matters

Both Paraguay and Guatemala can potentially be more responsive than very large commodity systems when serving specialised customers, analysts believe.

"They may offer dedicated production runs, specific certifications, customized crystal sizes, specialty packaging or identity and preserved supply chains that would be less attractive to a producer focused primarily on bulk volumes," Choudhury said.

At the same time, their vulnerabilities are equally clear. "Smaller exporters have less ability to absorb crop failures, freight disruption, certification costs or the loss of a major customer. Premium markets are also limited in size and can become oversupplied. Certification does not guarantee a buyer, and producers may sometimes have to sell certified output at conventional market prices," Choudhury added.

Observers also believe that successful examples of Paraguay and Guatemala are already encouraging other countries to follow a similar path. Across Latin America, producers that lack the scale to compete with Brazil are increasingly exploring ways to capture value through differentiation. As a result, Colombia is focusing on sustainable and specialty sugars, Peru is trying to leverage its reputation in premium agricultural exports, while Costa Rica's environmental credentials provide a foundation for niche markets.

The common strategy is the same: compete on quality, traceability and sustainability rather than tons.

"The strategic lesson is that smaller origins should not attempt to become miniature versions of Brazil," Choudhury said. "Their strongest opportunity is to build defensible value around provenance, certification, product quality, traceability and customer-specific supply arrangements — areas in which scale alone does not determine competitiveness."

# Environmental Impact Index and Efficacy of Pre-Emergent Herbicides for the Management of *Sicyos Polyacanthus* in Sugarcane in Tucumán, Argentina

## INTRODUCTION

*Sicyos polyacanthus* is a species in the Cucurbitaceae family, an annual climber native to the Tucumán-Bolivian jungle, known for rapid growth, high aggressiveness, multiple emergence cohorts, and adaptation to green cane management. In Tucumán sugarcane systems, it has become one of the main troublesome weeds, with a high capacity to interfere with crop development, cause productivity losses, and hinder harvesting (Sánchez Ducca et al., 2020). Its complex biology intensifies the challenges for chemical management and favors the persistence of infestations. Pre-emergent herbicides are a fundamental tool for early management of this weed. However, selecting these products should be based not only on control effectiveness but also on their environmental impact as part of a sustainable production system. The Environmental Impact Quotient (EIQ), proposed by Kovach (Kovach, 1992) is one of the most widely used tools. This indicator integrates twelve parameters related to a pesticide's toxicity and environmental behavior: cutaneous, chronic, and systemic toxicity; toxicity to beneficial fish, birds, bees, and arthropods; leaching potential; surface mobility; and degradation times in soil and on leaf surfaces (Carbonaria et al., 2021). The EIQ synthesizes multiple dimensions of environmental risk into a single value, facilitating comparison between

CONT. PG. 14

BY M. FERNANDA BARCELÓ [1], AGUSTÍN SÁNCHEZ DUCCA [2], PABLO D. VARGAS [3], SEBASTIÁN SABATÉ [1]
[1]Sección manejo de malezas (EEOAC), [2]Facultad de Agronomía, Zootecnia y Veterinaria (UNT),
[3]Ensayista privado E-mail: fbarcelo@eeaoc.org.ar

# Índice de Impacto Ambiental en Campo y Eficacia de Herbicidas Pre-Emergentes para el Manejo de *Sicyos Polyacanthus* en Caña de Azúcar en Tucumán, Argentina

## INTRODUCCIÓN

*Sicyos polyacanthus* es una especie de la familia Cucurbitácea, trepadora anual, nativa de la selva tucumano–boliviana, caracterizada por su rápido crecimiento, elevada agresividad, numerosas cohortes de emergencia y su adaptación al manejo de caña verde. En los sistemas cañeros de Tucumán se ha convertido en una de las principales malezas problema, con alta capacidad de interferir con el desarrollo del cultivo, provocar pérdidas de productividad y dificultar las labores de cosecha (Sánchez Ducca *et al.*, 2020). Su biología compleja intensifica los desafíos para el manejo químico y favorece la persistencia de infestaciones. El uso de herbicidas pre-emergentes constituye una herramienta fundamental para el manejo temprano de esta maleza. Sin embargo, la selección de estos productos no solo debe fundamentarse en su eficacia de control, sino también en su impacto ambiental como parte de un sistema de producción sostenible.

El Cociente de Impacto Ambiental **(EIQ, en inglés)**, propuesto por Kovach (Kovach, 1992) es una de las herramientas más usadas. Este indicador integra doce parámetros vinculados a toxicidad y comportamiento ambiental del plaguicida: toxicidad cutánea, crónica y sistémica; toxicidad para peces, aves, abejas y artrópodos benéficos; potencial de lixiviación; movilidad en superficie; y tiempos de degradación en suelo y superficie foliar (Carbonaria *et al.*, 2021). El EIQ permite sintetizar múltiples dimensiones del riesgo ambiental en un valor único, facilitando la comparación entre alternativas de manejo.

A pesar de su importancia, no se conocen estudios que relacionen la eficacia de herbicidas para el control de *S. polyacanthus* y el posible impacto ambiental. Por lo tanto, este estudio es de gran relevancia para diseñar estrategias de manejo más efectivas y ambientalmente amigables de esta

CONT. PÁG 15

CONT. FROM PG. 13

management alternatives.

Despite their importance, no studies link herbicide efficacy for controlling *S. polyacanthus* with potential environmental impacts. Therefore, this study is highly relevant to designing more effective, environmentally friendly management strategies for this weed by identifying pre-emergent herbicides for control and, in turn, estimating their environmental impact in the field using the EIQ index.

**Table 1:** Treatments evaluated for pre-emergency control of *S. polyacanthus*

| Field Environmental Impact Index (EIQ) | Environmental Impact Category |
|---|---|
| < 5 | Very low impact |
| 5-25 | Low impact |
| 25-45 | Medium-high impact |
| > 45 | Very high impact |

**Tabla 2:** Kovach (1992) Environmental Impact Classification

| Treatment | Herbicide | Doses (liters or kg ha$^{-1}$) |
|---|---|---|
| 1 | Atrazine 90% + acetochlor 90% | 2,0 + 2,0 |
| 2 | Amicarbazone 70% | 1,0 |
| 3 | Isoxaflutole 45% + indaziflam 15% | 0,3 |
| 4 | Flumetsulam 12% | 1,5 |
| 5 | Diclosulam 84% | 0,060 |
| 6 | Untreated check | |

## METHODOLOGY USED

The study was carried out during two consecutive agricultural seasons (2023/2024 and 2024/2025) in two commercial sugarcane lots with a history of high infestation of *S. polyacanthus*, located in the department of Cruz Alta, Tucumán, Argentina. In the first campaign, the trial was carried out in the town of Esquina; the crop corresponded to the variety TUC 06-7 at the cane plant stage, while in the second campaign, the evaluations were

CONT. PG. 16

Picou Group
SERVICES
CANADA

Over 40 Years of Engineering and Construction Experience within the Sugar Industry. Partnering with Sugar Refineries across North America in Capital and Maintenance Projects!

Our Capabilities Include:

- Mechanical Construction
- Civil Construction
- EPC Project Delivery
- Boiler Services
- Fabrication & Shop Work

Contact Picou Group Today!
Phone: 504-875-4562
Email: info@picougroup.com

Our Vision
Your Future

www.picougroup.com

CONT. DE LA PÁG. 13

maleza, identificando herbicidas pre-emergentes para su control y a su vez estimar su impacto ambiental mediante el índice EIQ en campo

## METODOLOGIA USADA

El estudio se llevó a cabo durante dos campañas agrícolas consecutivas (2023/2024 y 2024/2025) en dos lotes comerciales de caña de azúcar con historial de alta infestación de *S. polyacanthus*, ubicados en el departamento Cruz Alta, Tucumán, Argentina. En la primera campaña el ensayo se realizó en la localidad de Esquina, el cultivo correspondía a la variedad TUC 06-7 en edad de caña planta, mientras que en la segunda campaña las evaluaciones se realizaron en la localidad de Los Porceles, el cultivar fue TUC 95-10 y la edad caña soca 1. Para cada ensayo se utilizó un diseño experimental en bloques completos al azar con tres repeticiones. Cada unidad experimental tuvo una superficie de 24 $m^2$.

CONT. PÁG 17

**Tabla 1:** Tratamientos evaluados para control pre-emergente de *S. polyacanthus*

| Índice de impacto ambiental en campo (EIQ) | Categoría de impacto ambiental |
|---|---|
| < 5 | Muy bajo impacto |
| 5–25 | Bajo impacto |
| 25–45 | Medio-alto impacto |
| > 45 | Muy alto impacto |

**Tabla 2:** Clasificación de impacto ambiental de Kovach (1992)

| Tratamiento | Herbicida | Dosis (litros o kg $ha^{-1}$) |
|---|---|---|
| 1 | Atrazina 90% + acetoclor 90% | 2,0 + 2,0 |
| 2 | Amicarbazone 70% | 1,0 |
| 3 | Isoxaflutole 45% + indaziflam 15% | 0,3 |
| 4 | Flumetsulam 12% | 1,5 |
| 5 | Diclosulam 84% | 0,060 |
| 6 | Testigo sin herbicida | |

CONT. FROM PG. 14

carried out in the town of Los Porceles; the cultivar was TUC 95-10 and ratoon 1. We used a randomized complete block experimental design with three replications for each trial. Each experimental unit had an area of 24 $m^2$.

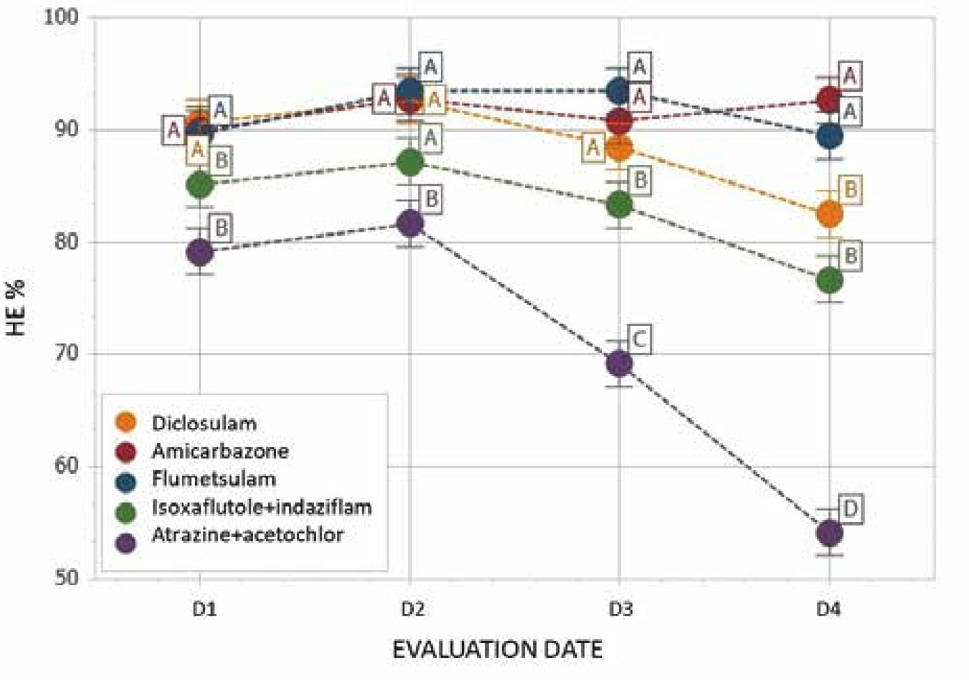
Before installing the trials, a selective application was carried out with no residual effect to eliminate emerged *S. polyacanthus* plants and avoid interference with the evaluation of pre-emergent treatments. Fluroxypyr 48% (0.3 l $ha^{-1}$) was used in a mixture with bromoxynil 34.9% (1.5 l $ha^{-1}$). The pre-emergent treatments (Table 1) were applied using an experimental $CO_2$-pressurized backpack sprayer equipped with a six flat fan nozzles (TT11002) boom, spaced 50 cm apart. The application volumes were 130.0 and 110.0 l $ha^{-1}$. The applications were carried out during the crop phenological stage of tillering.

The herbicide effect (HE) was evaluated on four dates after application in each locality: Esquina at 17, 27, 41, and 56 days after application (DAT), and Los Porceles at 11, 28, 43, and 63 DAT. A scale was used that classifies the level of control into six categories: poor (0–40%), fair (41–60%), sufficient (61–70%), good (71–80%), very good (81–90%), and excellent (91–100%) (ALAM, 1974).

The data were analyzed using NavureTeam software (2023). A linear model with fixed effects of year, treatment, and date of evaluation, its interactions and a random effect of plots was used. For mean comparisons, DGC as a posteriori (Di Rienzo et al., 2002) was used. We did not include the herbicide-free control treatment in the analysis; since it was used only as a reference for HE evaluations.

The values of the field environmental impact index (field EIQ) of the treatments were obtained using the online calculator developed by Cornell University (Grant, 2010-2020), based on the equation proposed by Kovach (1992); and, the classification of the environmental impact was based on the scale suggested by Kovach (1992) with four categories (Table 2), which establishes the following categories:

**Figure 1:** Herbicidal effect (HE) of the different treatments on *S. polyacanthus* for the two campaigns evaluated. Different letters indicate statistically significant differences ($p<0.0001$).




## RESULTS AND DISCUSSION

The results showed marked differences in herbicidal effects (HE) among the treatments evaluated (Figure 1). The mixture of atrazine + acetochlor (T1) presented the lowest control efficiency, with HE values that progressively decreased from 78% for the first evaluation date to approximately 55% at the end of the trials, which reflects a good initial control, according to the ALAM scale, with loss of residuality over time, reaching an unsatisfactory level of control; suggesting that a good initial control does not guarantee a prolonged protection of this weed, with successive emergence of new populations; therefore, the prolonged action of the herbicide constitutes a key attribute for management.

The combination isoxaflutole + indaziflam (T3) showed very good initial control (85% for F1), with a slight decrease toward the end of the evaluation period, ending in the good-control category. Although this treatment showed superior efficacy to the mixture atrazine + acetochlor, the decrease in herbicidal effect in the latest evaluations suggests lower residuality than in the best treatments.

Flumetsulam (T4), amicarbazone (T2), and diclosulam (T5) were the best performers during the evaluation period. The T4 and T2 treatments achieved excellent levels at all four dates evaluated, while T5 declined toward the end of the period, although it maintained very good control. The high efficacy and persistence observed in these herbicides represent an agronomic advantage, since they allow maintaining low levels of emergence for a prolonged period, reducing the need for complementary interventions and favoring a more efficient management of the weed.

The field evaluation of environmental impact using the EIQ index

CONT. PG. 18

CONT. DE LA PÁG. 15

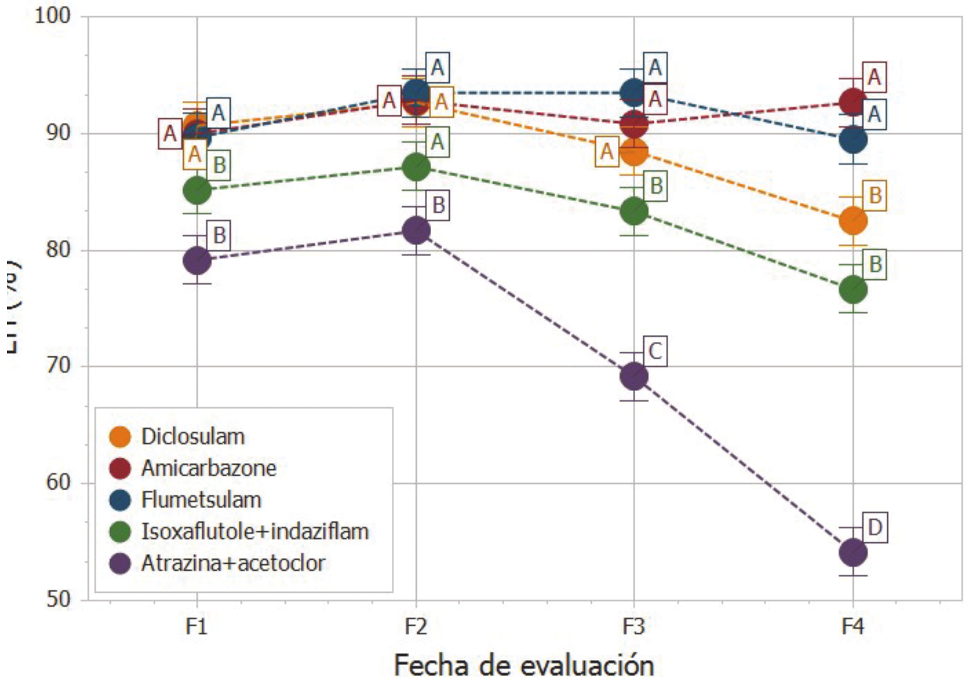
Previo a la instalación de los ensayos se realizó una aplicación selectiva y sin efecto residual con el fin de eliminar plantas emergidas de *S. polyacanthus* y evitar interferencias en la evaluación de los tratamientos pre-emergentes. Para ello se utilizó fluroxipir 48% (0,3 l×ha$^{-1}$) en mezcla con bromoxinil 34,9% (1,5 l ha$^{-1}$). Los **tratamientos pre-emergentes** (Tabla 1) se aplicaron mediante una mochila experimental presurizada con $CO_2$, equipada con una barra de seis picos de abanico plano TT11002, espaciados a 50 cm. El volumen de aplicación fue de 130,0 y **110,0 l ha$^{-1}$**. Las aplicaciones se realizaron con el cultivo en **estado fenológico de macollamiento.**

Se evaluó el efecto herbicida (EH) en cuatro fechas después de la aplicación de cada localidad: Esquina 17, 27, 41, 56 Días después de la aplicación (DDA) y Los Porceles 11, 28, 43, 63 DDA. Se utilizó una escala que clasifica el nivel de control en seis categorías: pobre (0–40%), regular (41–60%), suficiente (61–70%), bueno (71–80%), muy bueno (81–90%) y excelente (91–100%) (ALAM, 1974).

Los datos fueron analizados con el software NavureTeam (2023). Se utilizó un modelo lineal con efectos fijos de año, tratamiento y fecha de evaluación y sus interacciones y efecto aleatorio de parcelas. Para la comparación de medias se usó a posteriori DGC (Di Rienzo *et al.*, 2002). El tratamiento testigo sin herbicida no fue incluido en el análisis, se utilizó como referencia para las evaluaciones de EH.

Los valores del índice de impacto ambiental en campo (**EIQ campo**) de los tratamientos se obtuvieron utilizando la **calculadora en línea desarrollada por la Universidad de Cornell (Grant, 2010-2020)**, basada en la ecuación propuesta por Kovach (1992); y, la clasificación del impacto ambiental se basó en la escala sugerida por Kovach (1992) con cuatro categorías (Tabla 2), que establece las siguientes categorías:

**Figura 1:** Efecto herbicida (EH) de los distintos tratamientos sobre *S. polyacanthus* para las dos campañas evaluadas. Letras distintas indican diferencias estadísticamente significativas (p<0,0001).




## RESULTADOS Y DISCUSIÓN

Los resultados mostraron marcadas diferencias sobre el efecto herbicida (EH) entre los tratamientos evaluados (Figura 1). La mezcla Atrazina + Acetoclor (T1) presentó la menor eficiencia de control, con valores de EH que disminuyeron progresivamente desde 78% para la primera fecha de evaluación hasta aproximadamente 55% para el fin de los ensayos, lo cual refleja un control inicial bueno, según la escala de ALAM, con pérdida de residualidad a través del tiempo, llegando a un nivel insatisfactorio de control; sugiriendo que un buen control inicial no garantiza una protección prolongada de esta maleza, con emergencias sucesivas de nuevas poblaciones, por lo que la acción prolongada del herbicida constituye un atributo clave para el manejo.

La combinación Isoxaflutole + Indaziflam (T3) registró un **muy buen control** inicial (85% para F1), con un leve descenso hacia el final del período evaluado, finalizando en la categoría de buen control. Si bien este tratamiento mostró una eficacia superior a la mezcla Atrazina + Acetoclor, la disminución del efecto herbicida hacia las últimas evaluaciones sugiere una residualidad menor que la observada en los tratamientos más destacados.

Los tratamientos de Flumetsulam (T4), Amicarbazone (T2) y Diclosulam (T5) fueron los de mejor desempeño durante el periodo evaluado. Los tratamientos T4 y T2 lograron niveles de excelencia en las cuatro fechas evaluadas, mientras que T5 evidenció una reducción hacia el final del periodo, aunque mantuvo un muy buen control. La elevada eficacia y persistencia observadas en estos herbicidas representa una ventaja agronómica, ya que permiten mantener bajos niveles de emergencia durante un periodo prolongado, reduciendo la necesidad de intervenciones complementarias y favoreciendo un manejo más eficiente de la maleza.

La evaluación del impacto ambiental mediante el índice EIQ a campo mostró diferencias marcadas entre las estrategias de manejo analizadas. El tratamiento con Atrazina + Acetoclor

CONT. PÁG 19

CONT. FROM PG. 16

showed marked differences among the management strategies analyzed. Treatment with Atrazine + Acetoclor registered the highest environmental impact, EIQ 128.2, and was classified within the category of very high impact.

On the other hand, treatments without atrazine showed lower EIQ values. Amicarbazone achieved an EIQ of 21.2, classified as low impact. The lowest values corresponded to treatments with isoxaflutole + indaziflam (EIQ 4.1), flumetsulam (EIQ 3.5), and diclosulam (EIQ 1.6), all within the very low impact category, evidencing a significantly more favorable environmental profile.

While the mixture with atrazine significantly increased the EIQ value, amicarbazone, indaziflam + isoxaflutole, flumetsulam, and diclosulam were positioned as alternatives with lower environmental impact, offering an important advantage for sustainable management strategies. In this sense, the results show that greater agronomic efficiency was not associated with a greater environmental impact. On the contrary, the treatments with the best performance coincided with the lowest EIQ values, showing that it is possible to reconcile effective control of *S. polyacanthus* with environmental sustainability criteria. The combination of the control index with the environmental indicators constitutes an objective tool to guide more responsible management decisions in sugarcane, contributing to optimizing weed control and reducing the environmental risks associated with the use of herbicides.

## REFERENCES

*Asociación Latinoamericana de Malezas (ALAM) (1974). Recomendaciones para el control de malezas en América Latina. Asociación Latinoamericana de Malezas.*

*Carbonaria Caio A., Velini Edivaldo D. 2021. Risk assessment of herbicides compared to other pesticides in Brazil. Advances in WeedScience 39 (e21202032). https://doi.org/10.51694/AdvWeedSci/2021;39:00006*

*Di Rienzo, J. A., Guzmán, A. W., &Casanoves, F. (2002). A multiple-comparisons method based on the distribution of the root node distance of a binary tree.Journal of agricultural, biological, and environmental statistics, 7(2), 129-142.*

*Grant, J. A. (2010-2020). Calculator for Field Use EIQ (Environmental Impact Quotient). New York State Integrated Pest Management Program, Cornell Cooperative Extension, Cornell University. https://eiq.cornell-ipm.org*

*Kovach, J., Petzoldt, C., Degni, J., &Tette, J. (1992). A method to measure the environmental impact of pesticides. NY State IPM Program, Cornell University.*

*Navure Team (2023). Navure (4.0.1): A data-science-statistic oriented application for making evidence-based decisions. URL http://www.navure.com*

*Sánchez Ducca, A.; Vargas, P. D.; Sabaté, S.; Romero, E. R. (2020). Relevamiento sobre el manejo de malezas en caña de azúcar en la provincia de Tucumán, encuesta 2018. Avance Agroindustrial, 41(1), 24–28.*

***We're truly thankful for your support.***

The Sugar Journal community is at the heart of everything we do. Inside, you'll find fresh updates, news, latest research, and crucial ways to keep our industry connected!

Keep the Sugar Journal experience coming by subscribing online. Choose a printed edition for your doorstep or enjoy the digital version in your inbox. Visit SugarJournal.com to unlock your next issue and exclusive content.

Sugar Journal

**sugarjournal.com**

CONT. DE LA PÁG. 17

registró el mayor impacto ambiental, EIQ 128,2, clasificándose dentro de la categoría de impacto muy alto.

Por otro lado, los tratamientos sin atrazina mostraron valores de EIQ menores. Amicarbazone alcanzó un EIQ de 21,2 clasificado como bajo impacto. Los valores más bajos correspondieron a los tratamientos con Isoxaflutole + Indaziflam (EIQ 4,1), flumetsulam (EIQ 3,5) y diclosulam (EIQ 1,6), todos dentro de la categoría de impacto muy bajo, evidenciando un perfil ambiental significativamente más favorable.

Mientras que la mezcla con atrazina incrementó notablemente el valor del EIQ, los herbicidas Amicarbazone, Indaziflam + Isoxaflutole, Flumetsulam y Diclosulam se posicionaron como alternativas de menor impacto ambiental, ofreciendo una ventaja importante para estrategias de manejo sustentable. En este sentido, los resultados evidencian que la mayor eficacia agronómica no estuvo asociada a un mayor impacto ambiental. Por el contrario, los tratamientos de mejor desempeño coincidieron con los menores valores de EIQ, lo que demuestra que es posible compatibilizar un control efectivo de *S. polyacanthus* con criterios de sostenibilidad ambiental. La combinación del índice de control con los indicadores ambientales constituye una herramienta objetiva para orientar decisiones de manejo más responsables en caña de azúcar, contribuyendo a optimizar el control de la maleza y reducir los riesgos ambientales asociados al uso de herbicidas.

## BIBLIOGRAFIA

*Asociación Latinoamericana de Malezas (ALAM) (1974). Recomendaciones para el control de malezas en América Latina. Asociación Latinoamericana de Malezas.*

*Carbonaria Caio A., Velini Edivaldo D. 2021. Risk assessment of herbicides compared to other pesticides in Brazil. Advances in WeedScience 39 (e21202032). https://doi.org/10.51694/AdvWeedSci/2021;39:00006*

*Di Rienzo, J. A., Guzmán, A. W., &Casanoves, F. (2002). A multiple-comparisons method based on the distribution of the root node distance of a binary tree. Journal of agricultural, biological, and environmental statistics, 7(2), 129-142.*

*Grant, J. A. (2010-2020). Calculator for Field Use EIQ (Environmental Impact Quotient). New York State Integrated Pest Management Program, Cornell Cooperative Extension, Cornell University. https://eiq.cornell-ipm.org*

*Kovach, J., Petzoldt, C., Degni, J., &Tette, J. (1992). A method to measure the environmental impact of pesticides. NY State IPM Program, Cornell University.*

*Navure Team (2023). Navure (4.0.1): A data-science-statistic oriented application for making evidence-based decisions. URL http://www.navure.com*

*Sánchez Ducca, A.; Vargas, P. D.; Sabaté, S.; Romero, E. R. (2020). Relevamiento sobre el manejo de malezas en caña de azúcar en la provincia de Tucumán, encuesta 2018. Avance Agroindustrial, 41(1), 24–28.*

# Microbial Isolates from North American Sugar Beet Factory Juices and Biofilms

BY YUNCI QI [1], EVAN TERRELL [2], GILLIAN O. BRUNI [2], ZIANAB YASSIN [2], AND SHYAM L. KANDEL [3]

## 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 ef- forts to reduce microbial contamination and improve factory sanitation, but these approaches also have limitations such as potential culture bias and low throughput (Abdel-Rah- man 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 (H2S, H2, CO2), 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 (Ab- del-Rahman et al., 2023; Galié et al., 2018)).

Recent advances in high-throughput sequencing have great- ly 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 understudied 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 microbi-

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

al 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 over- night 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 great- er 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 re- move 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 tenden- cy 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'-AGAGTTTGA TCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTAC GACTT-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 Ver- sion 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 identi- fied and thus the ITS1F (5'-CTTGGTCATTTAG AGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCAT CGATGC-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 pre- cultures 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 be- came 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 120g/L sucrose in 250 mL culture flasks to OD600 of 0.05. The flask cultures were shaken at 250 RPM for 24 hours at 28°C OD600 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 inter- mediate 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 poly- saccharide 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.

**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.*

## 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 iso- lates of the most abundant microbial con-

**Figure 1.** Microbial isolates tallied by genus from sugar beet factories in North America. Gram-positive bacteria are indicat- ed 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*.

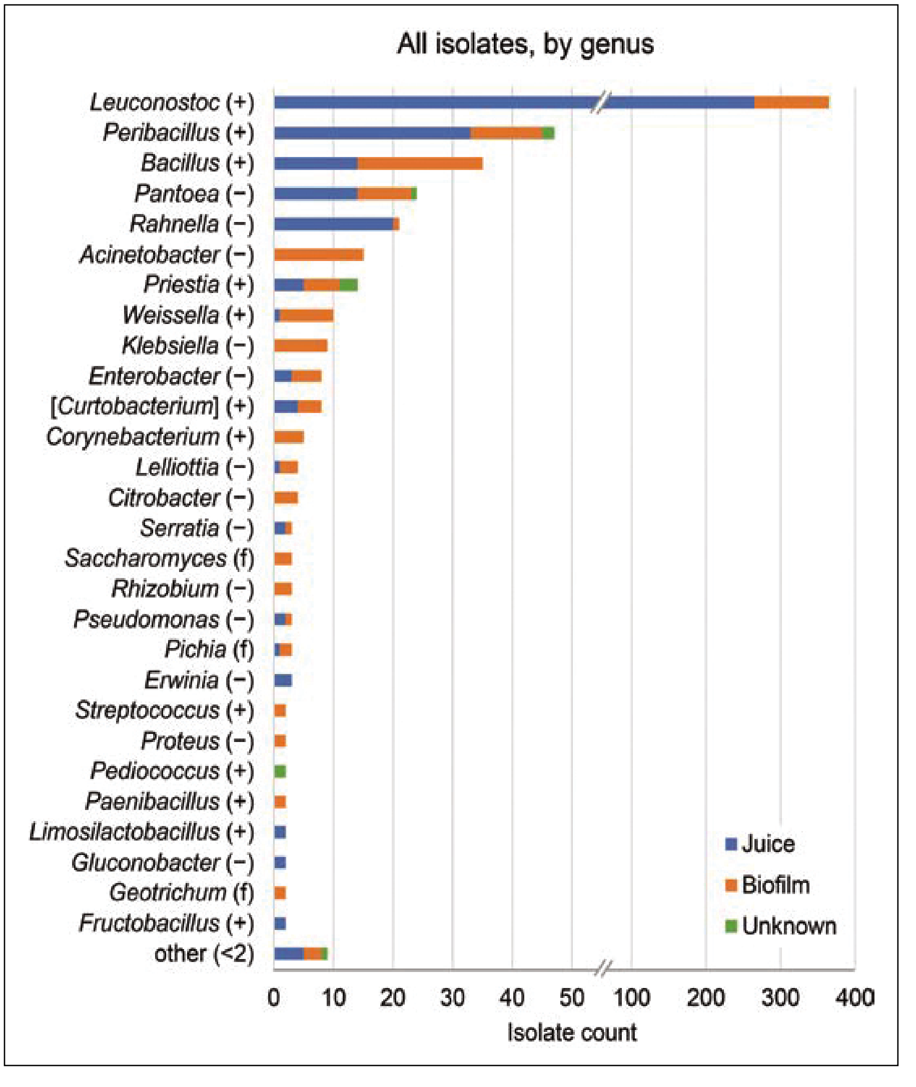
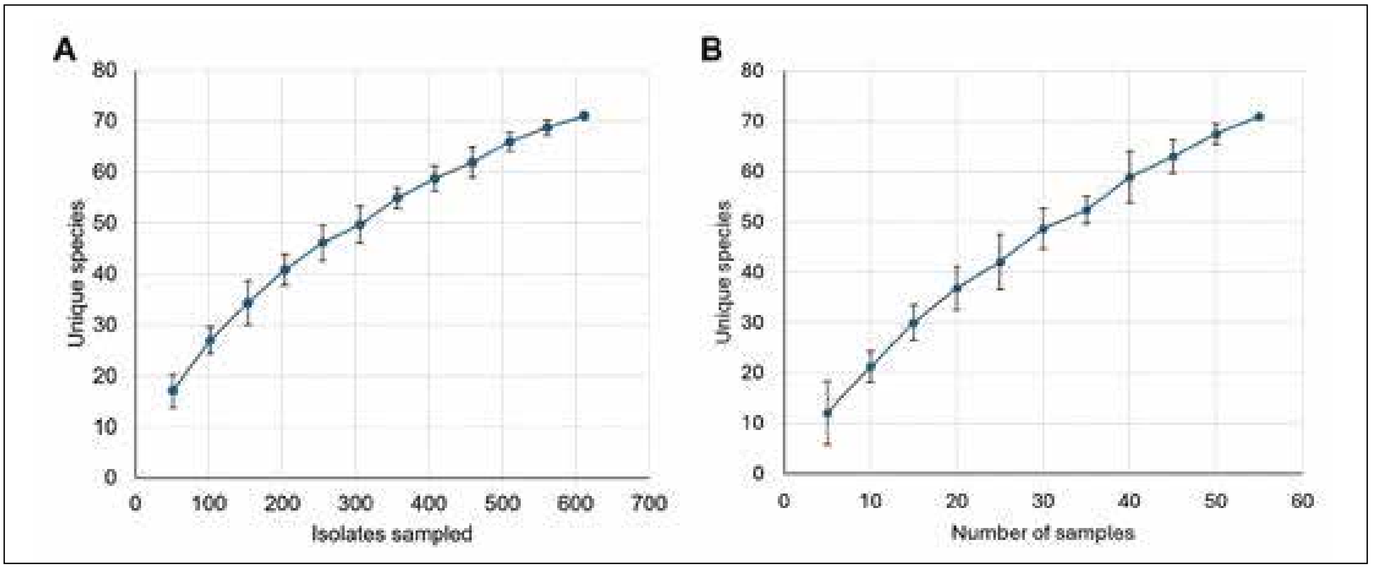


**Figure 2.** Rarefaction analysis estimating the isolation rate of novel taxa from sugar beet factories in North America. Each point represents the average of 10 random subsamples at intervals of A) isolates and B) factory samples. Error bars denote the standard deviation.




taminants of beet sugar juice and biofilms, rather than a comprehensive pro- filing 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 bio- film- (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 bio- film 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; bio- film, 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 polysac-

**Table 4.** Participating factories and their locations and parent companies.

| 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 |
| *Peribacillus* | 51-4 | Biofilm | Mucoid | 2.00 ± 0.07 | 2.06 ± 0.08 |
| *Peribacillus* | 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 |
| *Peribacillus* | 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 ± 0.0 |
| *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 |

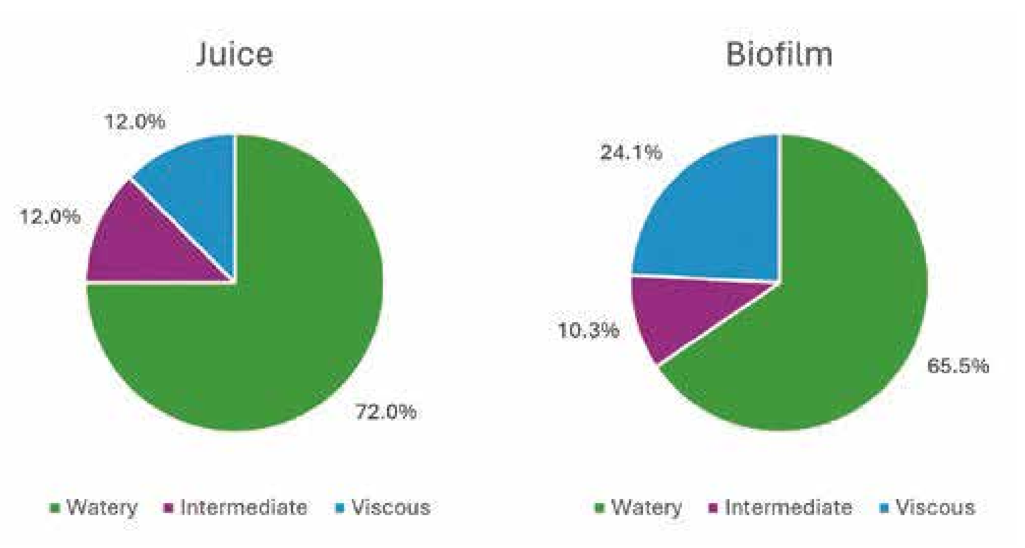
charide solutions (Evageliou, 2020; Xu et al., 2009).

## 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 (Ab- del-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 result- ed 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 recontamination, 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.

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




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 *a*-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.

## ACKNOWLEDGEMENTS


We thank the staff at each of the sugar beet factories for participating through collection and preservation of juice and biofilm samples. We also thank Ms. Sarah Newton, Ms. Heidi Giles, and Ms. Anna Murphy of the Beet Sugar Development Foundation for coordinating factory sampling and for administrative support. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by USDA. USDA is an equal opportunity provider and employer.


## FUNDING


This work was supported by the U.S. Department of Agri- culture (USDA), Agricultural Research Service (ARS), under project number 6054-41000-114-000-D. This work was also supported by the Beet Sugar Development Foundation project number 501 to G.B.

## LITERATURE CITED

*Abadam, V. (2023). Sugar and Sweeteners Outlook: December 2023. .*

*Abdel-Rahman, E. A., Frankenfeld, T., Saleh, A. S. M., & Floter, E. (2023). Reduction of the microbiological action during sugar beet extraction with focus on nitrite contamination. Assiut Journal of Agricultural Sciences, 54(2), 108-126. https://doi.org/10.21608/ajas.2023.196364.1236 Amberg, D. (2005). Methods in yeast genetics: A Cold Spring Harbor Laboratory course manual. In: Cold Spring Harbor Laboratory Press.*

*Arvanitis, N., Kotzamanidis, C. Z., Skaracis, G. N., & Karagouni, A. D. (2004). The effectiveness of commercial antimicrobial compounds against saccharolytic microorganisms isolated from a beet sugar production line. World Journal of Microbiology and Biotechnology, 20(3), 291-296. https://doi.org/10.1023/B:WIBI.0000023837.73558.35*

*Asadi, M. (2007). Beet Sugar Handbook. John Wiley and Sons, Inc.*

*Ayers, A. R., Ayers, S. B., & Goodman, R. N. (1979). Extracellular polysaccharide of Erwinia amylovora: a correlation with virulence. Applied and Environmental Microbiology, 38(4), 659-666. https://doi.org/10.1128/aem.38.4.659-666.1979*

*Bill, M., Eide, J. D., Fugate, K. K., Bolton, M. D., Kandel, H. P., & Kandel, S. L. (2024). Continental-scale insights into the sugarbeet diffusion juice microbiomes. Microbiology Spectrum, 0(0), e01093-01024. https://doi.org/10.1128/spectrum.01093-24*

*Borji, A., Borji, F.-E., & Jourani, A. (2019). Sugar Industry: Effect of dextran concentrations on the sucrose crystallization in aqueous solutions. Journal of Engineering, 7987369. https://doi.org/10.1155/2019/7987369*

*Breakwell, D., Woolverton, C., MacDonald, B., Smith, K., & Robison, R. (2017). Colony Morphology Protocol. In (pp. 1-7): American Society for Microbiology.*

*Bruni, G. O., Qi, Y., Klasson, K. T., Lima, I. M., & Terrell, E. (2022). Isolation and analysis of microbial contaminants from Louisiana raw sugarcane factories. International Sugar Journal, 124(1485), 530-538. https://internationalsugarjournal.com/paper/isolation-and-analy- sis-of-microbial-contaminants-from-louisiana-raw-sugarcane-factories/*

*Cabrera, E., Welch, L. C., Robinson, M. R., Sturgeon, C. M., Crow, M. M., & Segarra, V. A. (2020). Cryopreservation and the freeze-thaw stress response in yeast. Genes (Basel), 11(8). https://doi.org/10.3390/genes11080835*

*Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in mark- er-gene data analysis. The ISME Journal, 11(12), 2639-2643. https://doi.org/10.1038/ismej.2017.119*

*Cramer, H. (1946). Mathematical Methods of Statistics. Princeton University Press.*

*De Angelis, M., & Gobbetti, M. (2016). Lactobacillus spp.: general characteristics. In Reference Module in Food Science. Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-08-100596-5.00851-9*

*DeMan, Rogosa, & Sharpe. (2003). DeMan, Rogosa and Sharpe (MRS) agar. In J. E. L. Corry, G. D. W. Curtis, & R. M. Baird (Eds.), Progress in Industrial Microbiology (Vol. 37, pp. 511-513). Elsevier. https://doi.org/https://doi.org/10.1016/S0079-6352(03)80066-8*

*Díaz-Montes, E. (2021). Dextran: sources, structures, and properties. Polysaccharides, 2(3), 554-565. https://www.mdpi.com/2673-4176/2/3/33*

*English, W. (2020). Long term storage of sugar beets and the role of temperature Swedish University of Agricultural Sciences, SLU. Alnarp, Sweden.*

*Ernst, L., Offermann, H., Werner, A., & Wefers, D. (2024). Comprehensive structural characterization of water-soluble and water-insoluble homoexopolysaccharides from seven lactic acid bacteria. Carbohydrate Polymers, 323, 121417. https://doi.org/https://doi.org/10.1016/j. carbpol.2023.121417*

*Evageliou, V. (2020). Shear and extensional rheology of selected polysaccharides. International Journal of Food Science & Technology, 55(5), 1853-1861. https://doi.org/https://doi.org/10.1111/ijfs.14545*

*Galié, S., García-Gutiérrez, C., Miguélez, E. M., Villar, C. J., & Lombó, F. (2018). Biofilms in the food industry: health aspects and control methods [Review]. Frontiers in Microbiology, 9. https://doi.org/10.3389/fmicb.2018.00898*

*Haynes, W. C., Wickerham, L. J., & Hesseltine, C. W. (1955). Maintenance of cultures of industrially important microorganisms. Applied and Environmental Microbiology, 3(6), 361-368. https://doi.org/https://doi.org/10.1128/am.3.6.361-368.1955*

*Hector, S., Willard, K., Bauer, R., Mulako, I., Slabbert, E., Kossmann, J., & George, G. M. (2016). Diverse exopolysaccharide producing bacteria Isolated from milled sugarcane: implications for cane spoilage and sucrose yield. PLoS ONE, 10(12), e0145487. https://doi.org/10.1371/journal.pone.0145487*

*Heuer, H., Krsek, M., Baker, P., Smalla, K., & Wellington, E. M. (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Applied and Environmental Microbiology, 63(8), 3233-3241. https://doi.org/doi:10.1128/aem.63.8.3233-3241.1997*

*Holland, G., Buckley, M., & Frampton, S. (1990). Experiences in the use of biocides for microbiological control in the raw sugar factory. . Technical Conference-British Sugar Plc, Peterborough GB,*

*Jeon, E., Kim, H., Kim, G. B., & Lee, D. (2023). A review of bacterial biofilm formation and growth: rheological characterization, techniques, and applications. Korea-Australia Rheology Journal, 35(4), 267-278. https://doi.org/10.1007/s13367-023-00078-7*

*Kohout, C. K., Ukowitz, C., Reiter, D., Zitz, U., Moder, K., Emerstorfer, F., Hein, W., Kneifel, W., & Domig, K. J. (2020). Bacterial growth dy- namics and corresponding metabolite levels in the extraction area of an Austrian* *sugar beet factory using antimicrobial treatment. J Sci Food Agric, 100(6), 2713-2721. https://doi.org/10.1002/jsfa.10303*

*Kusstatscher, P., Cernava, T., Harms, K., Maier, J., Eigner, H., Berg, G., & Zachow, C. (2021). On-field microbial community influences post- harvest root rot in sugar beets. Acta Horticulturae, Leuven, Belgium.*

*Kusstatscher, P., Zachow, C., Harms, K., Maier, J., Eigner, H., Berg, G., & Cernava, T. (2019). Microbiome-driven identification of microbial indicators for postharvest diseases of sugar beets. Microbiome, 7(1), 112. https://doi.org/10.1186/s40168-019-0728-0*

*Lee, Y.-e., Jain, M. K., Lee, C., & Zeikus, J. G. (1993). Taxonomic distinction of saccharolytic thermophilic anaerobes: description of Ther- moanaerobacterium xylanolyticum gen. nov., sp. nov., and Thermoanaerobacterium saccharolyticum gen. nov., sp. nov.; reclassification of Thermoanaerobium brockii, Clostridium thermosulfurogenes, and Clostridium thermohydrosulfuricum E100-69 as Thermoanaerobacter brockii comb. nov., Thermoanaerobacterium thermosulfurigenes comb. nov., and Thermoanaerobacter thermohydrosulfuricus comb. nov., respectively; and transfer of Clostridium thermohydrosulfuricum 39E to Thermoanaerobacter ethanolicus. International Journal of System- atic and Evolutionary Microbiology, 43(1), 41-51. https://doi.org/10.1099/00207713-43-1-41*

*Majumdar, R., Strausbaugh, C. A., Galewski, P. J., Minocha, R., & Rogers, C. W. (2022). Cell-wall-degrading enzymes-related genes orig- inating from Rhizoctonia solani increase sugar beet root damage in the presence of Leuconostoc mesenteroides. International Journal of Molecular Sciences, 23.*

*McGinnis, R. A. (1982). Beet-Sugar Technology (3 ed.). Beet Sugar Development Foundation.*

*Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J. H. M., Piceno, Y. M., DeSantis, T. Z., Andersen, G. L., Bakker, P. A. H. M., & Raaijmakers, J. M. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science, 332(6033), 1097-1100. https://doi.org/doi:10.1126/science.1203980*

*Passerini, D., Vuillemin, M., Ufarté, L., Morel, S., Loux, V., Fontagné-Faucher, C., Monsan, P., Remaud-Siméon, M., & Moulis, C. (2015). Inventory of the GH70 enzymes encoded by Leuconostoc citreum NRRL B-1299 – identification of three novel α-transglucosylases. The FEBS Journal, 282(11), 2115-2130. https://doi.org/10.1111/febs.13261*

*Pollach, G., Hein, W., Leitner, A., & Zöllner, P. (2002). Detection and control of strictly anaerobic, spore-forming bacteria in sugarbeet tower extractors. Sugar Industry, 127(7), 530-537.*

*Purama, R. K., Goswami, P., Khan, A. T., & Goyal, A. (2009). Structural analysis and properties of dextran produced by Leuconostoc mes- enteroides NRRL B-640. Carbohydrate Polymers, 76(1), 30-35. https://doi.org/https://doi.org/10.1016/j.carbpol.2008.09.018*

*Qi, Y., Bruni, G. O., & Klasson, K. T. (2023). Microbiome analysis of sugarcane juices and biofilms from Louisiana raw sugar factories. Microbiology Spectrum, 11(3), e04345-04322. https://doi.org/doi:10.1128/spectrum.04345-22*

*Raup, D. M. (1975). Taxonomic diversity estimation using rarefaction. Paleobiology, 1(4), 333-342. https://doi.org/10.1017/ S0094837300002633*

*Robles-Gancedo, S., López-Díaz, T. M., & Otero, A. (2009). Microbiological counts during beet sugar extraction. J Food Prot, 72(6), 1332- 1337. https://doi.org/10.4315/0362-028x-72.6.1332*

*Sanders, H. L. (1968). Marine benthic diversity: a comparative study. The American Naturalist, 102(925), 243-282.*

*Šereš, Z., Maravić, N., Rakić, D., Dokic, L., Nikolić, I., Šoronja-Simović, D., & Djordjević, M. (2017). Application of biocides in the process of sucrose extraction from sugar beet: effect on sucrose content, number of Leuconostoc colonies and wet pulp characteristics. LWT - Food Science and Technology, 75. https://doi.org/10.1016/j.lwt.2016.08.038*

*Smith, D. P., & Peay, K. G. (2014). Sequence depth, not PCR replication, improves ecological inference from Next Generation DNA Se- quencing. PLoS ONE, 9(2), e90234. https://doi.org/10.1371/journal.pone.0090234*

*Soliman, E. A. A. (2007). Investigations on the influence of dextran during beet sugar production with special focus on crystal growth and morphology Technical University of Berlin]. Berlin, Germany.*

*Solomon, S. (2009). Post-harvest deterioration of sugarcane. Sugar Tech, 11(2), 109-123. https://doi.org/10.1007/s12355-009-0018-4*
*Strausbaugh, C. A. (2016). Leuconostoc spp. associated with root rot in sugar beet and their interaction with Rhizoctonia solani. Phytopathology, 106 5, 432-441.*

*Strausbaugh, C. A. (2018). Incidence, distribution, and pathogenicity of fungi causing root rot in Idaho long-term sugar beet storage piles. Plant disease, 102(11), 2296-2307. https://doi.org/10.1094/pdis-03-18-0437-re*

*Strausbaugh, C. A., Eujayl, I. A., Panella, L. W., & Hanson, L. E. (2011). Virulence, distribution and diversity of Rhizoctonia solani from sugar beet in Idaho and Oregon. Canadian Journal of Plant Pathology, 33(2), 210-226. https://doi.org/10.1080/07060661.2011.558523*

*Tallgren, A. H., Airaksinen, U., von Weissenberg, R., Ojamo, H., Kuusisto, J., & Leisola, M. (1999). Exopolysaccharide-producing bacteria from sugar beets. Applied and Environmental Microbiology, 65(2), 862-864. https://doi.org/10.1128/AEM.65.2.862-864.1999*

*Vartoukian, S. R., Palmer, R. M., & Wade, W. G. (2010). Strategies for culture of 'unculturable' bacteria. FEMS Microbiology Letters, 309(1), 1-7. https://doi.org/10.1111/j.1574-6968.2010.02000.x*

*Walters, W., Hyde, E. R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J. A., Jansson, J. K., Caporaso, J. G., Fuhrman, J. A., Apprill, A., & Knight, R. (2016). Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal Internal Transcribed Spacer marker gene primers for microbial community surveys. mSystems, 1(1). https://doi.org/10.1128/mSystems.00009-15*

*Wiegel, J., & Ljungdahl, L. G. (1981). Thermoanaerobacter ethanolicus gen. nov., spec. nov., a new, extreme thermophilic, anaerobic bac- terium. Archives of Microbiology, 128(4), 343-348. https://doi.org/10.1007/BF00405910*

*Wojtczak, M., Antczak-Chrobot, A., Chmal-Fudali, E., & Papiewska, A. (2013). Determination of microbiological activity during the pro- cessing of frost damaged sugar beets. Sugar Industry, 139(4), 228-331. https://doi.org/doi.org/10.36961/si15450*

*Wolfgang, A., Temme, N., Tilcher, R., & Berg, G. (2023). Understanding the sugar beet holobiont for sustainable agriculture [Review]. Frontiers in Microbiology, 14. https://doi.org/10.3389/fmicb.2023.1151052*

*Xu, X., Li, H., Zhang, Z., & Qi, X. (2009). Hydrodynamic properties of aqueous dextran solutions. Journal of Applied Polymer Science, 111(3), 1523-1529. https://doi.org/https://doi.org/10.1002/app.29043*

*Yang, X., Xu, T. F., Cao, P., Qiao, K., Wang, L., Zhao, T., & Zhu, J. (2019). The viscosity behaviors of bacterial suspensions or extracellular polymeric substances and their effects on aerobic granular sludge. Environmental Science and Pollution Research, 26(29), 30087-30097. https://doi.org/10.1007/s11356-019-06012-1*

*Yu, L., Qian, Z., Ge, J., & Du, R. (2022). Glucansucrase produced by lactic acid bacteria: structure, properties, and applications. Fermen- tation, 8(11), 629. https://www.mdpi.com/2311-5637/8/11/629*

*Zhang, S., Wu, Z., Wang, J., Zhang, S., Zhao, S., Li, H., & Zhao, J. (2022). Nitrate and nitrite pathways and dynamic changes in bacterial communities during beet sugar processing. Journal of the Science of Food and Agriculture, 102(1), 147-155. https://doi.org/https://doi. org/10.1002/jsfa.11341*

Reprinted from Beet Sugar Development Foundation, Denver, CO 80203

USDA Agricultural Research Service

[1] National Center for Agricultural Utilization Research, Peoria, IL 61604 USA;

[2] Southern Regional ResearchCenter, New Orleans, LA 70124 USA;

[3]Beet Sugar Development Foundation, Denver, CO 80203

[4] Edward T. Schafer Agricultural Research Center, Fargo, ND 58102-2765;

*Corresponding Author Gillian O. Bruni, PhD USDA-ARS, Southern Regional Research Center

## ADVERTISERS



## UPCOMING MEETINGS & CONFERENCES

**2026**

SEPTEMBER 9–10 | **SugarBeet Expo 2026,** Springe, Germany

SEPTEMBER 23–25 | **The Mexican Sugar Technologists Meeting (ATAM), ,** Veracruz, Mexico

**LIST YOUR CONSULTANT SERVICES HERE**
**Email: jane@sugarjournal.com**

GUILHERME ROSSI MACHADO, JR., Agronomy and Breeding
rossicane@gmail.com,
Telefax: 55 19 3422 8541 | Whatsapp: +55 19 98154 7811
Piracicaba - Brazil | Tri-lingual: POR/ENG/SPA

ISABEL LIMA, Technical Consultant, American Biocarbon LLC
32525 Hwy 1, White Castle, LA 70788
225-776-5315 | Isabel.lima@americanbiocarbon.com
www.americanbiocarbon.com

# A Better Seal.

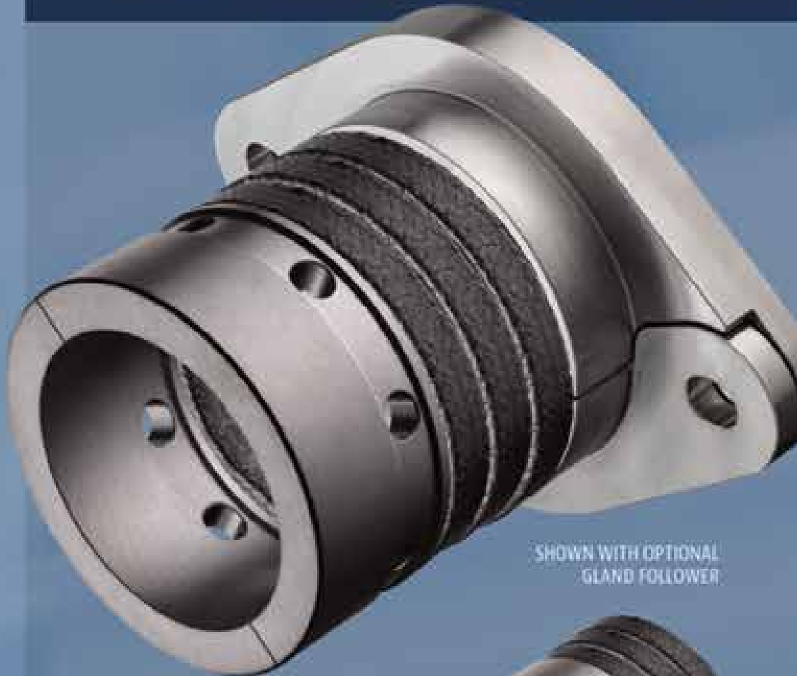
PRECISION FIT. SEALS EFFECTIVELY. LONGER LIFE.

## FOR EQUIPMENT THAT:

- Has an overhung shaft or shaft deflection
- With inconsistent or no flush
- Is old or degraded
- Pumps abrasive, slurry, or viscous mediums

The PackRyt® Sealing System is a unique stuffing box sealing arrangement that incorporates a bearing and flush channel system together with our best-in-class mechanical packing. Our patented system brings shafts into concentricity and keeps them there, significantly increasing sealing reliability.

## PACKRYT® BEARING SEALING SYSTEM



SHOWN WITH OPTIONAL GLAND FOLLOWER

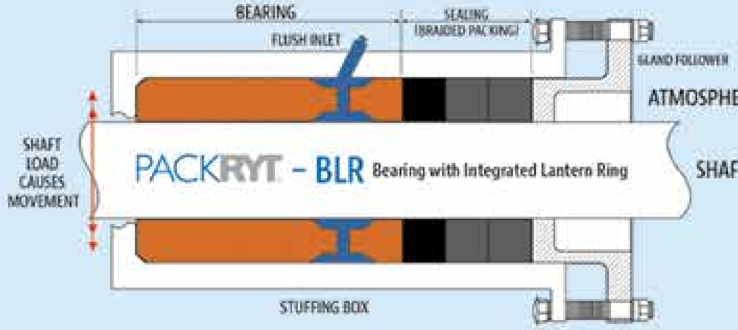
Above: Bearing with Integrated Lantern Ring

Right: Bearing for Non-flushed applications

## APPLICATIONS:

- Agitators - Horizontal or Vertical
- Massecuite Double Lobe Displacement Pump
- Vacuum Pumps
- Centrifugal Pumps
- Evaporator
- Fly Ash Pump
- Condensate Pump
- Vacuum Pan

### Patented Product




SEALRYT® CORPORATION

ROTATING SHAFT SEALING EXPERTS

SCAN to learn more.

Call us to find out why reliability and maintenance managers are moving to our patented solutions!

SEALRYT.com

64 Servistar Industrial Way
Westfield, MA 01085

Phone: 413-564-5202
Fax: 413-564-5203

Designed and Manufactured in Westfield, MA

MADE IN THE USA


WHERE INDUSTRIAL TRADITION
MEETS MODERN TECHNOLOGY
Get a quote today
WESTERN STATES
ROBERTS G-16 LINC
WESTERN STATES
www.westernstates.com
ws.sales@westernstates.com
+1 (513) 863-4758