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Sustainable Production of Pulque and Maguey in Mexico: Current Situation and Perspectives

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Pulque is a traditional Mexican fermented, non-distilled alcoholic beverage produced by fermenting the fresh sap (*aguamiel*) extracted from several *Agave* (maguey) species cultivated for pulque production (mainly *A. salmiana*). This beverage was produced and consumed since Pre-Hispanic times by Mesoamerican civilizations, mainly in the Mexican Central Plateau, and is one of the essential alcoholic beverages produced and consumed during several centuries in Mexico. By 2019, annual pulque production was reported in 171,482 billion liters. Nevertheless, traditional pulque production faces several significant limitations, including the disappearance of large agave plantations and the extent of time required (at least 5 years) to complete the plant maturation for aguamiel extraction; traditional production practices; and the lack of an efficient stabilization process of the fermented product resulting in low shelf life. In opposition, successful examples of sustainable cultivation of maguey species for aguamiel extraction and the fermentation process's industrialization resulted in high-quality pulque production, with international exportation certification. In this contribution, we present a review of the most relevant aspects of the history and commercial relevance of pulque, the causes that resulted in its production debacle during the first half of the twentieth century, the current situation of its traditional production, and the successful efforts of industrial production of the beverage. We describe recent results on the analysis of the physicochemical characteristics of aguamiel and on the microbiology of the beverage explored by metagenomic techniques that can be proposed as a baseline to redefine the quality criteria of the beverage and to define for the first time a microbiological core to optimize its production. We describe the relevance of maguey species for aguamiel production as a fundamental element of agroforestry and the relevance of its sustainable production, in four sustainable plantation models to maintain a stable plant population to ensure the continuous extraction of aguamiel and pulque production. Finally, we describe some successful examples of beverage industrialization and potential applications of several microorganisms isolated from aguamiel, pulque, aguamiel concentrates, and the maguey to produce high-value bioactive products.

Keywords: pulque, aguamiel, traditional fermented beverage, agave, maguey, sustainable production, process industrialization

INTRODUCTION

Pulque is a traditional Mexican non-distilled, alcoholic fermented beverage, produced by fermentation of the fresh sap or *aguamiel*, extracted from several species of *Agave* or maguey *pulquero* species (plants for pulque production) (**Table 1**; Escalante et al., 2016). Pulque is probably the most important traditional fermented alcoholic beverage produced and studied in Mexico. The archeological evidence suggests the cultivation and use of maguey for various purposes (e.g., feed and raw material for fiber production) by the inhabitants from the Tehuacan Valley (Puebla State) by 6500 BC (Lorenzo Monterrubio, 2007; Moreno-Terrazas et al., 2017). The use of specialized obsidian tools for maguey scraping in the highlands of the Central and North-Central Mexico was proposed as a common practice in the early Pre-Hispanic Middle and Late Formative Periods (500 BC—100 AD) (Parsons and Darling, 2000; Lorenzo Monterrubio, 2007). The use of ceramic vessels for pulque production, consumption, and storage was dated by 200–550 AD by the detection of hopanoids present in the cytoplasmic membrane of *Zymomonas mobilis* (one of the essential bacteria during pulque fermentation) as biomarkers detected by gas chromatography-mass spectrometry in ceramic pottery collected in the neighborhood of the ancient city of Teotihuacan, the capital of a state that controlled the Basin of Mexico (Correa-Ascencio et al., 2014). The ancient Aztec culture rediscovered the maguey plants and pulque production by 1100–1300 AD (Gonçalves de Lima, 1956). Although no information is available on the extent of the cultivated maguey plantations and pulque production by the Aztec culture, they developed a total dominion of aguamiel and pulque production establishing strict rules for consumption until the down of this Empire in 1521 (Gonçalves de Lima, 1956; Ramírez Rodríguez, 2004; Instituto Nacional de Antropología, 2012).

During the Spaniard Colony in Mexico (1535–1821), the Spaniards and Creoles controlled maguey plantations, aguamiel extraction, pulque production, sale, and consumption in sites known as *pulquerías* (a type of canteen for pulque sale and consumption). By the middle and late seventeenth century, the production and sale of pulque became a relevant economic activity by the high volumes of the beverage introduced and consumed mainly in Mexico City (estimated in 2,625,000 L) (Ramírez Rancaño, 2000; Ramírez Rodríguez, 2004; Lorenzo Monterrubio, 2007). With the development of large farms for pulque production (known as Haciendas *pulqueras*) by the end of the eighteenth century, mainly in the States of Hidalgo, Puebla, Tlaxcala, and Mexico, pulque production and commerce became the fourth-highest income of the Spaniard Colony, only after the sales tax, silver production, and coining (Ramírez Rodríguez, 2004; Lorenzo Monterrubio, 2007). Pulque production and sale decreased significantly during the Mexican Independence War (1810–1821), nevertheless, its production was not interrupted (Ramírez Rodríguez, 2004). By the second half of the nineteenth century, pulque was again the preferred beverage in the main cities of the Mexican Central Plateau. From 1866 to 1880, the new *Ferrocarril Mexicano* railway connected the larger production zones in Apan (Hidalgo State) and other relevant production centers in the States of Tlaxcala and Estado de México with Mexico City, increasing the consumption of the beverage.

By 1900 the annual pulque production in Mexico was 485.56 billion liters, according to the available data in the statistical yearbook of Mexico for 1900 (*Anuario Estadístico de la República Mexicana*) reported in Ramírez Rancaño (2000). By 1907, ∼99% of the total pulque production registered in Mexico was produced only in the States of Hidalgo, Tlaxcala, Puebla, and Estado de México resulting in a second flourishing of the Haciendas pulqueras (Ramírez Rancaño, 2000). By the end of the nineteenth and the early twentieth century, from 799 to 808 haciendas reported in Mexico, 271–279 were dedicated only to pulque production. They were vast centers for maguey cultivation, aguamiel extraction, pulque fermentation, and distribution; the larger Haciendas pulqueras had a surface up to 100 ha. The central pulque production zone located in the region of Apan comprised 250,000 ha for maguey cultivation, with an estimation of more than 100 million agave plants for pulque production (Ramírez Rancaño, 2000). This economic activity resulted in the rise of a new powerful economic and political class called "the pulque aristocracy," closely related to president Porfirio Díaz (Ramírez Rancaño, 2000; Lorenzo Monterrubio, 2007). Despite the economic relevance of pulque production and consumption, by the early twentieth century, an anti-*pulque* movement came out of concern of the negative impact of pulque consumption, attributing social unrests, and relevant health problems like alcoholism, imposing strict rules on pulque distribution and sale mainly in Mexico City.

In 1906, several Haciendas *pulqueras* (mainly in Puebla State) created the first pulque company: The *Compañía Realizadora de Pulque, Sociedad Anónima*, intending to monopolize the pulque production, distribution, and sale. In 1909, was created the *Compañía Expendedora de Pulque, SCL,* by the powerful and influential pulque producers from the Apan zone in Hidalgo, Tlaxcala, and Estado de México, including 53 large haciendas. This company controlled the production of pulque, its distribution, and sale in Mexico City. In 1910, a new company, the *Compañía Expendedora de Pulques de Pachuca, Sociedad Anónima,* promoted by the first time the industrialization of aguamiel and pulque. In 1911 and 1912, the *Compañía Expendedora de Pulque, SCL* constructed a research facility in Mexico City to study the potential health properties of aguamiel and pulque. This company developed other valuable products, such as an aguamiel concentrate, a medicinal syrup (known as *Agavan syrup*), distilled 96° alcohol, vinegar, gums, and glues, some of them sold both in Mexico as in the USA (Ramírez Rancaño, 2000; Ramírez Rodríguez, 2004).

The Revolution War in Mexico (1910–1920) had a profoundly negative impact on the structure of the Haciendas pulqueras and their agroindustry. The railways had a crucial role in the civil war, and many of them were destroyed, interrupting the transport of pulque by the *Ferrocarril Mexicano* railway from the Apan zone to Mexico City. By 1914, the triumph of the constitutionalist revolution movement by Venustiano Carranza forced the owners of the Haciendas pulqueras (closer to the defeated president Porfirio Díaz) to leave Mexico. In 1916, the production, introduction, and consumption of pulque to

TABLE 1 | *Agave* species reported for aguamiel extraction (*magueyes pulqueros*).

Scientific name	Geographic distribution reported in Mexico[a]	Common names or comments
Agave atrovirens Kraw ex Salm-Dyck	Hidalgo, Mexico City, Morelos, Nuevo León, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tlaxcala, Veracruz, Zacatecas	
Agave americana L.	Baja California Sur, Cólima, Coahuila, Chihuahua, Chiapas, Durango, Guanajuato, Hidalgo, Jalisco, México, Mexico City, Morelos, Nayarit, Nuevo Léon, Oaxaca, Puebla, Querétaro, San Luis Potosí, Sonora, Tamaulipas, Tlaxcala, Veracruz, Zacatecas	
Agave mapisaga Trel.	Guanajuato, Hidalgo, México, Mexico City, Morelos, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tamaulipas, Tlaxcala, Zacatecas	*Carrizo*
Agave salmiana Otto ex Salm-Dyck	Aguascalientes, Chiapas, Coahuila, Colima, Mexico City, Durango, Guanajuato, Hidalgo, Jalisco, México, Morelos, Nuevo Léon, Oaxaca, Puebla, Querétaro, San Luis Potosí, Tlaxcala, Veracruz, Zacatecas	*Amarillo, ayoteco, colorado, chalqueño, chino, manso, prieto, xilometl, verde, negro, cenizo* Also reported as *Agave salmiana* subsp. *tehuacanensis* or *palmilla*
Agave hookeri	Jalisco, Michoacán, Sinaloa, Sonora	
Agave inaequidens	Durango, Hialgo, Jalisco, México, Mexico City, Michoacán, Morelos, Sinaloa, Tlaxcala	
Agave marmorata Roezl	Puebla	Wild agave used for pulque production

Based on the information reported by Alfaro Rojas et al. (2007), Mora-López et al. (2011), Escalante et al. (2016), Moreno-Terrazas et al. (2017), Peralta-García et al. (2020), Trejo et al. (2020), and Álvarez-Ríos et al. (2020).
[a] Tropicos.org. Missouri Botanical Garden (2021).

Mexico City were prohibited. Nevertheless, politicians allowed its production and sale because of the millions of Mexican pesos in tax revenues associated with the pulque agroindustry (Ramírez Rancaño, 2000; Juárez de Olarte and Juárez de Olarte, 2017). In the same year the *Compañía Expendedora de Pulque, SCL* was dissolved, and the large haciendas pulqueras were seized and dismantled the extensive maguey plantations for pulque production (Ramírez Rancaño, 2000; Ramírez Rodríguez, 2004). At the end of the Revolution War, several haciendas pulqueras survived, and pulque production continued. By the 1920s, pulque introduction by train to Mexico City continued; however, the dismantlement of many haciendas by the redistribution of the land after the Revolution War, the flourishment of the brewery industry, and the anti-*pulque* movement resulted in a substantial reduction in the number of maguey plantations deteriorating the pulque industry severely. In 1929, estimated pulque production was 234 million liters; by 1930, 167 million liters and maguey plantations were reduced to ∼50,000 Ha (Ramírez Rancaño, 2000; Lorenzo Monterrubio, 2007). By 1950s, official registers reported 17,000 cultivated ha of maguey pulquero, corresponding to ∼6.8% of the cultivated surface reported in the early twentieth century. The maguey plantations in pulque-producing zones were displaced by the introduction of barley cultivation, associated with unsuccessful government efforts to rescue the pulque agroindustry. Additionally, a significant increase in beer, alcoholic distilled beverages, wine, and soft drinks production and consumption in large cities and rural locations contributed to the critical decrement in pulque production and consumption (Ramírez Rodríguez, 2004; Lorenzo Monterrubio, 2007).

Nowadays, the larger maguey plantations for aguamiel extraction and pulque production are located in the States of Hidalgo, México, Tlaxcala, and Puebla (**Figure 1A**), but are distributed in a lower extent in other states such as Mexico City, Coahuila, Oaxaca, Veracruz, and Morelos (Mora-López et al., 2011; Escalante et al., 2016; Moreno-Terrazas et al., 2017; Chacón-Vargas et al., 2020; Rocha-Arriaga et al., 2020; Servicio de Información Agroalimentaria y Pesquera, 2021). From 1984 to 2019, pulque production showed two maximum production peaks by 1987 (∼550 million liters, produced in Estado de México) and 2013 (∼504 million liters, produced in Hidalgo), but in 2019 were reported the production of ∼171 million liters (a decrement of ∼66% of the maximum production peaks) (**Figure 1B**). Remarkably, Hidalgo State is the central production zone of pulque, providing an average of 73% of the pulque's national production in the last 10 years (Servicio de Información Agroalimentaria y Pesquera, 2021).

Despite the historical and economic relevance of pulque, the current traditional production of the beverage faces main significant limitations such: the considerable time required by the *Agave* species used for pulque production (at least 5 years) to complete their maturation for aguamiel extraction; the production process remains practically without changes since the golden age of production (late nineteenth and early twentieth century); the lack of an updated Mexican norm for aguamiel and pulque production quality and their microbiological, sensorial, and physicochemical properties; and the reluctance of traditional producers and consumers to change consumption habits from the fresh fermented beverage to canned or bottled options. In this review, we describe the current procedures for traditional aguamiel extraction and pulque production methods and how recent results on the analysis and stability of the physicochemical profile of aguamiel and the microbiology of the beverage led to propose a new baseline to redefine the quality criteria and to define for the first time a microbiological core to optimize its production; and the relevance of the sustainable cultivation of the maguey species used for aguamiel production as a fundamental element of an agroforestry system. In addition, we describe some successful examples of beverage industrialization resulting in high-quality pasteurized bottled or canned pulque production. Finally, we review the potential applications of several microorganisms isolated from aguamiel, pulque, aguamiel concentrate syrups, and the use of the maguey as the source of bioactive compounds and other high-value products.

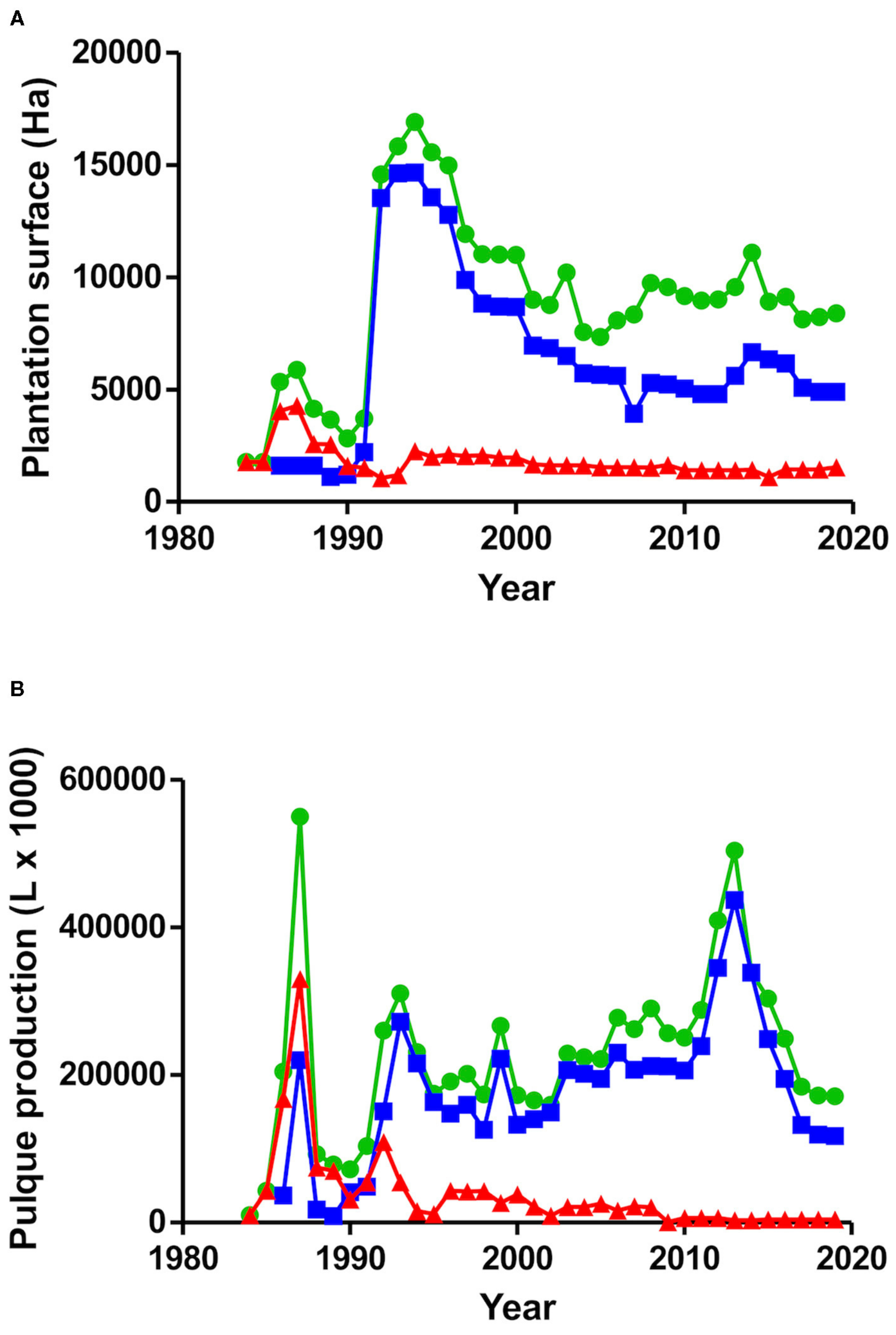

FIGURE 1 | Plantation surface of *maguey pulquero* plantation and *pulque* production. **(A)** Reported cultivated surface (ha) for *maguey pulquero*. **(B)** Reported *pulque* production associated to maguey plantations on basis of the available information at the Anuario Estadístico de la Producción Agrícola, Servicio de Información Agroalimentaria y Pesquera (2021). • National; ■ Hidalgo; ▲ Estado de México.

AGUAMIEL EXTRACTION AND PULQUE FERMENTATION PROCESSES

The pulque fermentation process starts with the selection of mature plants for aguamiel extraction with an age between 5 and 15 years (Ramírez Rodríguez, 2004; Lorenzo Monterrubio, 2007; Escalante et al., 2012, 2016; Peralta-García et al., 2020) and comprises four common steps in all the producing zones: (1) castration, (2) *cajete* scraping and aguamiel extraction, (3) seed preparation, and (4) fermentation (Ramírez

Rodríguez, 2004; Lorenzo Monterrubio, 2007; Escalante et al., 2016).

Maguey Castration

Selected mature plants are castrated by destroying the embryonic floral peduncle surrounding the floral bud (or *quiote*) (**Supplementary Figure 1**). During this operation, the plant's central leaves (heart*, meloyote*, or *cogollo*) are extracted with a pointed and sharp instrument (e.g., a pray bar), leaving a cavity known as *cajete*. The walls are then scraped with a metal scraping tool (a metal instrument like a large spoon) to shape the cavity (**Figures 2A–C**). The final cavity is covered traditionally with folded maguey leaves or with a large stone. This operation is usually performed in early spring or autumn fall. After this operation, the plant enters a maturation period ranging from 3 months to 1 year to promote the preservation of the treated plant's carbohydrate reservoir for aguamiel production (Nieto Aquino et al., 2016; Peralta-García et al., 2020). Immediately, a second scraping process is performed to enlarge the cavity, open the vessels, promoting the aguamiel outflux and its accumulation in the cavity. The castration process is performed traditionally by a person known as *tlachiquero,* who has a vast, in-depth knowledge of the maguey species for aguamiel production. The tlachiquero determines the precise moment for castration in mature plants, avoiding the floral bud's development. This a critical moment because if the floral peduncle (or *quiote*) grows, the plant is not useful for aguamiel production and dies after the inflorescence raises (Ramírez Rodríguez, 2004; Lorenzo Monterrubio, 2007; Narváez Suárez et al., 2016; Nieto Aquino et al., 2016; García Mendoza et al., 2017; Moreno-Terrazas et al., 2017; Álvarez-Duarte et al., 2018; Peralta-García et al., 2020). **Figure 2** illustrates the main activities involved in the extraction of aguamiel and pulque fermentation, and **Figure 3** shows a schematic representation of the stages for pulque production.

Cajete Scraping and Aguamiel Extraction

After the maturation period, the aguamiel extraction process is performed daily, twice (at morning and dusk), during the maguey's producing life until the plant dies. For this operation, the aguamiel accumulated in the cajete is extracted traditionally by oral suction using a large, dried gourd known as *acocote* (**Figures 2D–F**), or a device made with a hose or a plastic tube connected to a plastic soda bottle. In some rural regions, aguamiel is extracted with a plastic container, such as a cup, but some modern producers can use a pump for extraction. After aguamiel extraction, the cajete is scraped again, discarding the extracted plant material (or *metzal*) (**Figure 2G**). This action promotes the outflux of aguamiel during the day and repeats the operation at dusk. Collected aguamiel is transferred into plastic or wood containers (**Figures 2E,F**) and transported by the tlachiquero to the fermentation facilities known as *tinacal* (literally house of the tubs or vats), a facility (usually a large room or a specific zone) for aguamiel fermentation in vats, wood, or plastic containers (**Figures 2H–K**). The amount of aguamiel produced varies on the age, size (large plants can measure more than 5 m in diameter), and production lifetime (3–9 months) of the plant (Nieto Aquino et al., 2016; Álvarez-Duarte et al., 2018; Álvarez-Ríos et al., 2020; Peralta-García et al., 2020; Trejo et al., 2020). Ortiz-Basurto et al. (2008), reported an initial aguamiel production of 0.4 L/day during the first days of production. Then, the production volume increased to 6 L/day for several months, and final production of 0.4 L/day before the plant dies. Other authors reported a daily aguamiel production by several maguey species ranging from 2.5 L/day (in wild maguey plants such as *A. marmorata*) up to 10 L/day (for large plants of *A. salmiana* var. s*almiana)* in intensive maguey plantations, with a maximum total production between 1,500 and 1,960 L during the productive life of the plant (Tovar et al., 2008; Álvarez-Ríos et al., 2020).

Seed Preparation

Traditional tinacales contained large vats for aguamiel fermentation made initially of cow leather, where the shaved hairy side faces the inner side of the vat; these vats were replaced by wood and more recently by masonry, fiberglass, or plastic vats, some of them with a larger volume up to 1,000 L (Lorenzo Monterrubio, 2007; Moreno-Terrazas et al., 2017; Álvarez-Ríos et al., 2020). The operator and administrator of the tinacal, known as *mayordomo* (butler), had a higher status among workers in the former Haciendas pulqueras and was responsible for the seed preparation and pulque fermentation (Ramírez Rodríguez, 2004; Lorenzo Monterrubio, 2007). The seed is the starting material or inoculum for the fermentation of freshly collected aguamiel. The seed preparation process varies between pulque producing zones, but generally, for its preparation, 10–50 L of the best quality aguamiel is fermented in a small vat used only for seed preparation by pouring a volume of previously fermented aguamiel. The seed container is covered, and the fermentation develops at room temperature during 1–4 weeks until an upper layer called *zurrón* appears (Moreno-Terrazas et al., 2017). The seed is used to start a small volume fermentation for pulque production or prepare a second inoculum or seed known as *pie de cuba*. The pie de cuba is prepared by pouring into a new vat, one-fourth of the first seed, and three-fourths of high-quality fresh collected aguamiel (Crist, 1939; Moreno-Terrazas et al., 2017). The name used to refer to the seed varies among producing zones and practices: *Pie de pulque, pulque fuerte, asiento,* or *nancle* (Álvarez-Ríos et al., 2020).

Pulque Fermentation

The proper fermentation process for pulque production starts in a second fermentation vat where a volume of the pie de cuba or second seed is poured; then, freshly collected aguamiel is poured twice daily (in the morning and dusk) until it fills the vat. Then, the content of this vat is divided into two halves, and each half is poured into two new containers, respectively, starting the process again, now in two vats; then freshly collected aguamiel is poured twice daily until the vats fill. The mayordomos determine the fermentation time based on the development of characteristic sensorial properties, such as the alcohol content, acid notes, foam, the development of a characteristic viscosity, and tasting the fermented beverage (Escalante et al., 2016). A more extensive fermentation time, as 36 h, is reported as common practice for pulque production,

FIGURE 2 | Aguamiel collection and relevant traditional pulque production operations. **(A)** Metallic scraping tools or *raspador.* **(B)** Scraping process of the cajete's wall. **(C)** A close view of *the cajete* with accumulated aguamiel. **(D–F)** Aguamiel extraction by the tlachiqero using the traditional acocote and the sap's transference into a plastic container for transport to the tinacal. **(G)** Residual wall material or *metzal* in a plastic bucket after the scraping of the cajete. **(H–K)** Diversity of tinacales, fermentation vessels and vats. Images **(A,B,F,G–K)** were kindly provided by Mrs. E. Velázquez Gutiérrez.

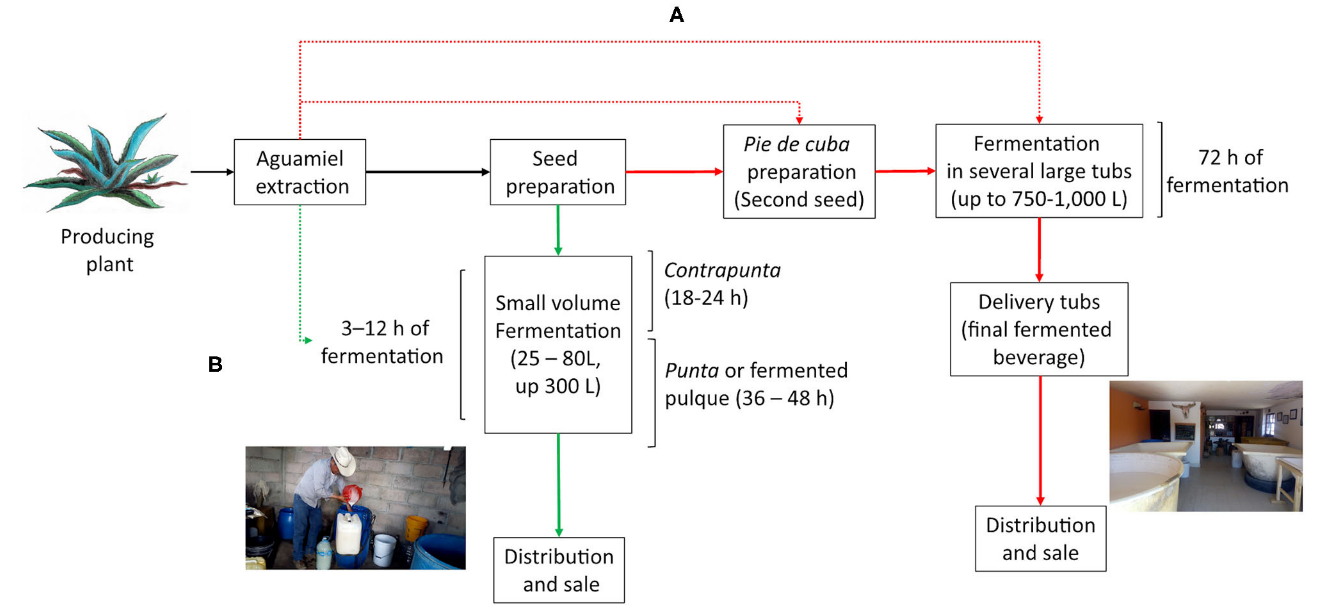

FIGURE 3 | Traditional pulque production flowchart. **(A)** Medium-large volume production process as described in section Types of Pulque and Properties. Dotted lines indicate pouring of freshly collected aguamiel to feed the pie de cuba and the fermentation tanks. **(B)** Small volume production process as described in section Types of Pulque and Properties. Dotted lines indicate freshly collected aguamiel to feed the fermentation tank. *Contrapunta* and *punta* are colloquial terms used by pulque producers to refer to an intermediate fermentation stage (contrapunta) and the final fermented pulque fermented (*punta*). This flowchart was elaborated from information reported in Crist (1939), Ramírez Rancaño (2000), Ramírez Rodríguez (2004), Escalante et al. (2016), Moreno-Terrazas et al. (2017), Álvarez-Ríos et al. (2020), and Rocha-Arriaga et al. (2020).

including seed preparation and further fermentation in vats as reported from three locations of Hidalgo State (Zempoala, Tepeapulco, and Epazoyucan) (Rocha-Arriaga et al., 2020). For a large-scale production process, the fermentation takes up to 72 h and is performed in several vats (up to 10) of 750–100 L each one. Larger producers deliver and sale the fermented pulque to pulquerías or restaurants offering this beverage (Álvarez-Ríos et al., 2020). In the traditional fermentation process, the final fermented pulque is not subjected to a stabilization process (e.g., pasteurization) to stop the microbial activity responsible for the fermentation. Small traditional pulque producers perform all the above-described stages and sold the fermented pulque directly from the tinacal during the day or in local markets (Ramírez Rancaño, 2000; Ramírez Rodríguez, 2004; Escalante et al., 2012, 2016; Moreno-Terrazas et al., 2017).

AGUAMIEL AND PULQUE SENSORIAL AND PHYSICOCHEMICAL PROPERTIES

Aguamiel Properties

Freshly collected aguamiel is a lightly amber, thick, sweeter, fresh plant flavored, and slightly acid sap (Escalante et al., 2016; Peralta-García et al., 2020). The Mexican norm (Norma Mexicana) defining the sensorial properties and quality criteria for aguamiel was published in 1972: NMX-V-022-1972 (*hidromiel, aguamiel*) (Secretaría de Economía, 1972a), and is still in force today. This norm defines the aguamiel as a light amber liquid with a characteristic smell and taste and is used as the fermentable substrate for pulque production. It also defines aguamiel as the "juice" obtained by scraping the cajete or the central cavity from maguey pulquero plants of the genus *Agave* and cultivated mainly in the States of México, Hidalgo, and Tlaxcala. The norm defines two types of aguamiel, Type I (high-quality aguamiel with high sugar content, used for seed preparation) and Type II (lower quality aguamiel, lower sugar content used for pulque production).

This norm establishes that acceptation or rejection of an aguamiel batch for pulque production requires the minimal determination of the pH (6.6–7.5 for Type I and 4.5 for Type II) and total reducing sugars (8–12 g/100 mL for Type I and 6.0 g/100 mL for Type II), determined in a sample of 100 mL for each aguamiel container. These analyses should be determined in the tinacal previously to deliver the freshly collected aguamiel for fermentation. The norm also establishes that high-quality aguamiel or Type I should be collected 15 days after the first cajete's scraping in the producing plant and during the next 30–60 days. Both Type I and Type II aguamiel should not show advanced fermentation.

Types of Pulque and Properties

As shown in **Figure 3**, two primary processes for pulque production are performed by traditional producers: A medium-large volume production process (**Figure 3A**) including the steps of aguamiel extraction, preparation of seed with high-quality aguamiel, the preparation of a second seed or pie de cuba, and the final fermentation process in large vats or tinacales. In this process, the fermentation in the vat is a feed-batch process, where collected aguamiel is continuously poured into the fermentation vat twice daily (morning and dusk) for several days until the producer (mayordomo) determines the "end" of the process. A fermentation time of 72 h was reported for process in vats of 750–1,000 L. Then, the complete batch is usually transferred into wood barrels and delivered for sale. The second process is performed at smaller volume containers (**Figure 3B**), comprising aguamiel extraction, preparation of a seed, transference into a plastic container or wood barrels to start the fermentation, where twice daily collected aguamiel (morning and dusk) is poured and fermented. After several hours of fermentation, the beverage is withdrawn for *in situ* sales or distribution, usually in small plastic containers. Fermentation times vary from 3 to 12 for traditional producers, but the fermentation can extend up to 48 h. When the producer considers that the fermentation has finished, the beverage is delivered for sale. The remaining fermented product in the container is feed with new aguamiel, and the process is maintained for several days until the producer decides to discard the remaining beverage, then prepares a new seed and start a new fermentation process (Lorenzo Monterrubio, 2007; Escalante et al., 2016; Narváez Suárez et al., 2016; Moreno-Terrazas et al., 2017; Rocha-Arriaga et al., 2020).

The physicochemical and sensorial properties of fermented pulque are described in the Mexican norm (Norma Mexicana) NMX-V-037-1972 (Pulque—handled in bulk) (Secretaría de Economía, 1972b). This norm is still in force and describes the bulk handled pulque as the fermented beverage, with lower alcohol content, white color, not clarified, and viscous beverage produced from the fermentation of aguamiel extracted from maguey pulquero. It also defines the seed and pie de punta (described above) and the pulque's industrial production.

The norm defines two types of pulque, Type I (seed and pie de cuba) and Type II or commercial pulque. Relevant physicochemical properties for Type I are a final pH $>$3.5–4.0; sugar content of 0.10–0.8 g/100 mL of reducing sugars, expressed as equivalents of glucose; and 6–9% of alcohol content (v/v). For Type II, the norm defines a final pH of 3.0–4.0; sugar content of 0.20–0.50 g/100 mL of reducing sugars; and alcohol content of 4.0–0.6% (v/v). The norm defines the *biochemical properties of pulque* as a beverage resulting "from the equilibrated fermentative activity of the associated microbiota without evident alterations in the sensorial properties affecting its final quality." Sensorial properties described are a white color beverage for both types of pulque and "*sui generis*" taste and smell. The norm defines the quality criteria and sampling procedure for acceptance or rejection of a batch of pulque. These analyses were performed in the former customs facilities for pulque control in Mexico City, such as the customs facilities in the railway station of Pantaco (North of Mexico City), where pulque transported by train from the producing zones of Hidalgo and Tlaxcala was analyzed before final delivery to the sale sites. The norm establishes as mandatory that if a sampled 250 L cask of pulque was rejected, the entire batch should be disposed into the drainage. The former railway line for pulque transport to Mexico City and the custom of Pantaco closed in 1993 by high operating costs (Ramírez Rodríguez, 2004). Small traditional pulque producers have their quality criteria for the final fermented beverage and vary according to the producer; these criteria are mainly based on their own traditional tasting experience (Escalante et al., 2016).

STUDIES ON AGUAMIEL AND PULQUE'S MICROBIOLOGY: DEFINITION OF THE MICROBIAL CORE FOR THE FERMENTATION AND PROPOSING THE DEVELOPMENT OF INOCULUM FOR THE BEVERAGE PRODUCTION

Earlier studies on the microbiology of aguamiel and pulque described the isolation of *Zymomonas mobilis*; the lactic acid bacteria (LAB) *Leuconostoc mesenteroides, L. dextranicum, Lactobacillus* sp.; and the yeast *Saccharomyces cerevisiae* in aguamiel and pulque samples (Sánchez-Marroquín and Hope, 1953; Sánchez-Marroquín and Echegaray, 1954). These microorganisms were used as a mixed inoculum to start a controlled fermentation with sterilized aguamiel as substrate (inoculum 5% v/v, pH 5.0, 28°C). The sequential inoculation with the LAB followed by *S. cerevisiae* resulted in a high-quality fermented beverage (final alcohol content = 5.43°GL; final pH = 4.6, and total acidity (lactic acid) = 0.348) (Sánchez-Marroquín and Hope, 1953). Further attempts included *Z. mobilis* in the mixed inoculum, proposing the use of these microorganisms as a starter to ferment sterilized aguamiel to produce a good quality and a hygienic beverage (Sánchez-Marroquín and Hope, 1953; Sánchez-Marroquín et al., 1957, 1967).

Recent studies reported a high diversity of bacteria and yeasts both in aguamiel and pulque. A complete compilation of the microorganisms isolated and identified in aguamiel and pulque was reported previously, indicating the presence of a complex bacterial and yeast diversity (Lappe-Oliveras et al., 2008; Escalante et al., 2016). Nevertheless, recent non-culturable and metagenomic analysis of aguamiel and pulque from different geographical origins (Villarreal Morales et al., 2019; Escobar-Zepeda et al., 2020; Peralta-García et al., 2020; Rocha-Arriaga et al., 2020), as during a 6-h laboratory pulque fermentation (Chacón-Vargas et al., 2020), revealed the presence of non-previously reported bacterial and yeasts diversity and provided relevant biochemical and metabolic traits associated to aguamiel and the process of fermentation. Remarkably, only two reports correlate the microbial diversity dynamics with the biochemical y physicochemical properties of aguamiel (Peralta-García et al., 2020) and during the fermentation process (Chacón-Vargas et al., 2020).

Biochemistry and Microbiology of Aguamiel

Aguamiel quality and sugar composition during the plant producing lifetime has been analyzed to propose a baseline of quality for standardization purposes (Ortiz-Basurto et al., 2008; Peralta-García et al., 2020). The study of the production lifetime (3 months) of three plants of *A. salmiana* from the location of Tecamachalco (18° 53′ N, 97° 44′ W; altitude 2,020 m), in Puebla State, showed that aguamiel composition has no relevant differences among samples collected at different time points during the producing life of the studied plants. Samples had a relatively constant pH (∼4.5) and a dry matter (11.5 wt %) content composed mainly by 74 wt % sugars, including 8.8% sucrose; 26.5% glucose; 32.4% fructose; and 10.2% fructooligosaccharides (FOS) with a high degree of polymerization (DP), mainly inulin-type fructans and agavins with few β-fructofuranosyl units linked by β1-2 and β2-6 linkages. These authors concluded that aguamiel is a stable raw material for pulque production suitable for the standardization of pulque industrial production (Ortiz-Basurto et al., 2008).

The analysis of sugar composition in aguamiel collected from three plants from the pulque producing locality of Huitzilac, Morelos (19°01′42′′ N 99°16′02′′ O; altitude 2,561 m), showed variation during the producing lifetime (4–9 months) among three analyzed plants: *A. mapisaga* (two plants: 1 and 2) and *A. salmiana* (one plant: 3) (Peralta-García et al., 2020). These authors reported for the first time the characterization of the sap accumulated in the cajete immediately after the scraping of the cavity (15–20 mL collected immediately after scraping, named as *fresh aguamiel*) and the accumulated sap 7 h after scraping, which is collected for pulque production. Remarkably, in the first 9–13 weeks of the producing lifetime, the fresh sap's pH was neutral to slightly alkaline (7.0–10), but the accumulated aguamiel was slightly acid (5.0–3.5) among the three plants. The fresh sap contains just sucrose and FOS but not fructose or glucose (resulting from the hydrolysis of sucrose). During the production lifetime, sucrose content remains relatively constant in the fresh aguamiel but decreases between 30 and 50% after the accumulation time, associated with the presence of glucose and fructose. Nevertheless, fructans showed the highest concentration in fresh aguamiel between the first third and the midpoint of the production lifetime with a full FOS content between weeks 9 and 15 (Peralta-García et al., 2020).

FOS analysis in fresh and accumulated aguamiel of the three analyzed plants showed three types of fructan profiles: Type I found in the three studied plants during the early sap accumulation period after scraping. These FOS showed a close related profile to inulin or isomalto-oligosaccharides, different to the FOS of the plant, possibly synthesized by the glycosyltransferase activities of microorganisms present both in the plant as in the sap. These FOS were proposed to be partially or hydrolyzed or consumed by the sap's microbiota. Type II was found in aguamiel collected from plants at intermediate and late production times, identified as a complex mixture of oligosaccharides such as 1-kestose and 6-kestose signals, proposed as the early intermediates of the synthesis of inulin or levan, respectively. These FOS's presence was proposed due to the hydrolysis of agavins or by enzymatic synthesis mediated by microbial fructosyl transferase enzymes. Type III, corresponding to FOS found in the plant's intermediate and late producing lifetime, with a similar profile to that of the FOS detected in fresh sap after scraping and diluted as they accumulated in the cavity. FOS identified in aguamiel Types I and II have a β2-1 or β2-6 structure, respectively, a different structure from that α1-6 type corresponding to dextran, the primary polymer present in fermented sap. These authors also estimated an aguamiel production of 676.6 L for plant 1, 662.3 L for plant 2, and 334 L for plant 3; total sucrose present in aguamiel was estimated at 70.6 Kg for plant 1, 61.5 Kg for plant 2, and 36.8 Kg for plant 3; and fructans were estimated at 3.0 Kg for plant 1, 1.7 Kg for plant 2, and 2.1 Kg for plant 3 (Peralta-García et al., 2020). These authors proposed that the described FOS profile could be considered a marker for high-quality aguamiel as the substrate for pulque production and suggest this profile as the baseline for quality control purposes. Remarkably, the DP of the FOS found in producing plants from Huitzilac, Morelos (two plants of *A. mapisaga* and one of *A. salmiana*) agrees with the FOS profile reported for three plants of *A. salmiana* previously studied from the location of Tecamachalco, Puebla (Ortiz-Basurto et al., 2008). Finally, the analysis of the bacterial diversity present in fresh sap by a metagenomic approach showed a significant abundance at the genus level for *Leuconosto*c (46.08%), *Zymomonas* (35.98%), *Acetobacter* (5.0%), *Lactococcus* (4.67%), and *Acinetobacte*r (3.22%). Among these bacteria, *Leuconostoc* and *Zymomonas* are proposed to synthesize additional FOS detected in aguamiel by their fucosyltransferase enzymes (Peralta-García et al., 2020).

Biochemistry and Microbiology of Pulque Fermentation

Chacón-Vargas et al. (2020), analyzed by a metagenomic shotgun sequencing approach the diversity present in overnight accumulated aguamiel (collected at daybreak) and in overnight fermented pulque from the locality of Huitzilac, Morelos, and during a laboratory fermentation of 6 h started by mixing a sample of the daybreak collected aguamiel and a sample of the overnight fermented pulque. Their results showed a great bacterial diversity determined by the Simpson's diversity (D) and Shannon's (H) diversity indexes in aguamiel, compared to that in fermented pulque and during three fermentation times analyzed (0, 3, and 6 h). Aguamiel showed a D = 9.03 and H = 2.86. At the genus level, the highest diversity was composed of *Acinetobacter* (21.95%), *Leuconostoc* (13.92%), *Lactococcus* (13.72%), *Zymomonas* (4.77%), and *Lactobacillus* (0.97%). At the species level, the highest diversity was composed by *Lactococcus plantarum* (8.50%), *Z. mobilis* (4.78%), *A. nectaris* (2.68%), *L. gelidum* (1.67%), *L. citreum* (1.68%), *L. piscium* (1.65), *A. buissieri* (1.59%), and *L. lactis* (0.97%). Remarkably, the yeast *Saccharomyces* was present in a lower percentage (0.03%). Diversity at T0 (start of the laboratory fermentation by mixing 2:3 L of aguamiel:pulque, and maintained at 23°C) showed a D = 5.88 and H = 2.43. The highest diversity at the genus level was composed of *Lactococcus* (19.52%), *Leuconostoc* (17.34%), *Zymomonas* (12.57%), *Saccharomyces* (10.79%), *Acinetobacter* (5.73%), and *Lactobacillus* (4.94%). At species level: *Z. mobilis* (12.57%), *L. plantar*um (12.11%), *L. citreum* (2.44%), *S. cerevisiae* (2.42%), *Lactobacillus sanfranciscensis* (2.22%), *L. gelidum* (1.77%), *L. citreum* (1.68%), and *L. lactis* (0.79%). At T3, D = 4.19 and H = 2.21. At the genus level, diversity was composed of *Zymomonas* (19.91%), *Saccharomyc*es (19.08%), *Leuconostoc* (12.38%), *Acinetobacter* (5.45%), and *Lactobacillus* (3.61%). At the genus level, *Z. mobilis* (19.91%), *L. plantarum* (7.08%), *S. cerevisiae* (4.05), *L. sanfraciscensis* (1.41%), *L. piscium* (1.31%), *L. geliudum* (1.29%), and the yeast *S. eubayanus* (0.99%). At T6, D = 3.71 and H = 2.14. Diversity at genus level was *Zymomonas* (22.27%), *Lactococcus* (11.73%), *Acinetobacter* (7.17%), *Lactobacillus* (7.08%), and *Saccharomyces* (5.65%). At the species level: *Z. mobilis* (22.27%), *L. plantarum* (7.33%), *L. sanfranciscensis* (2.2%), *L. gelidum* (1.72%), *L. piscium* (1.39%), S. *cerevisiae* (1.25%), and *L. citreum* (1.2%). Finally, fermented pulque had a D = 4.10 and H = 2.13. At the genus level *Z. mobilis* (21.48%), *Leuconostoc* (14.30%), *Saccharomyces* (13.51%), *Lactococcus* (13.03%), *Lactobacillus* (7.53%), and *Acinetobacter* (2.85%). At the species level, the highest diversity was composed by *Z. mobilis* (21.48%), *L. plantarum* (8.11%), *L. sanfranciscensis* (3.71%), *S. cerevisiae* (2.86%), *L. piscium* (1.57%), *L. gelidum* (1.47%), and *L. citreum* (1.04%).

Rocha-Arriaga et al. (2020), explored the microbial diversity during a traditional fermentation process, including the analysis in the collected aguamiel (0 h), in the middle stage of the fermentation (18 h) (known by producers as *contrapunta*), and in the final fermented pulque (36 h) (also known as *punta*) from three locations of Hidalgo State (Epazoyucan 20°01′ 03′′ N; 98° 38′ 11′′ W, an altitude of 2,456 m; Tepeapulco 19° 47′ 06′′ N; 99° 33′ 11′′ W, altitude 2,508 m; and Zempoala 19° 48′ and 20° 03′ N; 98° 50′ W and altitude of 2,400–2,900 m) by sequencing of the 16S rDNA V3-V4 regions for bacterial diversity and ITS sequencing for yeasts diversity. Contrary to the results by Chacón-Vargas et al. (2020), these authors found a lower diversity of OTUs in aguamiel (H = 1.47, D = 0.39), an important increment at the middle stage (contrapunta, 12 h of fermentation, H = 2.5, D = 0.61), and a further decrement in diversity in fermented pulque (36 h of fermentation H = 1.58, D = 0.45). These authors identified in all the studied stages as the most abundant OTUs *Sphingomonas, Acetobacter, Lactobacillus, Acinetobacter, Enterobacter, Gluconobacter, Halomicronema, Lactococcus, Leuconostoc, Marivitia, Serratia*, and *Weissella*. Among these, the most abundant OTUS found in analyzed sap were *Sphingomonas, Lactobacillus*, and *Acinetobacte*r; in contrapunta, the most abundant OTUs were *Acinetobacter, Sphingomonas*, and *Lactobacillus;* whereas the most abundant OTUs in pulque were *Sphingomonas, Lactobacillus*, and *Acetobacter*. Remarkably these authors did not find *Zymomonas* in the analyzed samples. Regarding yeasts diversity, the most abundant yeasts identified in all production stages were *Candida zemplinina* (25% in sap, 1.7% in contrapunta, 17.98% in pulque); *Clavidospora lusitaniae* (23% in sap, 16% in contrapunta, 9% in pulque), and *Candida stellate* (0.6% in sap, 32.96% in contrapunta, 6.19% in pulque), but *Saccharomyces* was found as the 9th most abundant yeast in all samples (0.74% in sap, 13.31% in contrapunta, 8.49% in pulque) (Rocha-Arriaga et al., 2020). The absence of *Zymomonas* in the analyzed samples by these authors is a relevant result as this bacteria has been reported in pulque samples since the earlier studies of Linder on the microbiology of aguamiel and pulque between 1923 and 1924 (described as *Thermobacterium mobile*) (Swings and De Ley, 1977) and by Sánchez-Marroquín and Hope (1953). *Zymomonas* sp. was also reported in studied pulque samples from Oaxaca State, outside of the main producing zone of Hidalgo State (Valadez-Blanco et al., 2012).

Remarkably, *Sphingomonas,* the most abundant genus reported in aguamiel and pulque fermentation identified by Rocha-Arriaga et al. (2020), was reported in the analysis of a mixed pulque sample of 24 and 48 h of fermentation from Huitzilac, Morelos by a whole metagenomic sequencing strategy, showing a relative abundance of 2.47% (Escobar-Zepeda et al., 2020), and also reported by Chacón-Vargas et al. (2020), but detected in a lower abundance (above 0.1% in T6 and fermented pulque samples).

The studies on the microbiology of aguamiel and during the fermentation process showed high microbial diversity with common microbial groups among studied samples independently of the plant's geographical origin, species, and local variations involved in the fermentation process. Nevertheless, a systematic and standardized methodology is required to provide information on the relevance of the significant and minor microbial groups present in aguamiel and pulque fermentation from diverse geographical origins. This information should be correlated with the sap and fermented pulque's main physicochemical characteristics to propose a microbial core responsible for the beverage's main sensorial properties to define a mixed starter culture to standardize the fermentation process.

TOWARD THE DEFINITION OF A MICROBIAL STARTER CULTURE FOR PULQUE PRODUCTION

A starter culture is defined as a selected microbial preparation developed to increase the efficiency of a fermentation process, providing a quick start of the process, reducing the risk of fermentation failure, and improving safety, stability, and the final sensorial value of the fermented product. The production of fermented products with a superior quality depends on the presence, growth, and metabolic activity of specific microorganisms (Vinicius De Melo Pereira et al., 2020). It is necessary to establish a microbiological core and a new physicochemical and sensorial definition of aguamiel and pulque to improve and standardize the fermentation process. Escalante et al. (2016) proposed that fermented pulque's final sensorial properties are defined by the simultaneous development of four fermentation types: (i) An alcoholic fermentation performed by *Z. mobilis* and *S. cerevisiae* to ferment available sugars in aguamiel (sucrose, fructose, and glucose), resulting in ethanol production. (ii) A lactic acid fermentation developed mainly by LAB including several species of *Leuconostoc, Lactobacillu*s, and *Lactococcus,* which result in the production of lactic acid, acetic acid, and possibly other minor metabolic products such acetoin, diacetyl, and butanediol. (iii) An acetic acid fermentation by acetic acid bacteria such as *Acetobacter* and *Gluconobacter*. (iv) The production of exopolysaccharides like dextran and levan by *L. mesenteroides* and *Z. mobilis,* respectively, is responsible for developing the fermented beverage's characteristic viscosity. Nevertheless, recent metagenomic reports on the diversity of pulque from Huitzilac (Morelos) and three Hidalgo State locations showed that *S. cerevisiae* was detected as a minor microorganism in aguamiel as in the fermentation process. Likewise, the acetic acid bacteria *Acetobacter* and *Gluconobacter*, previously reported (Escalante et al., 2004, 2008), were not detected or were detected as a minor microorganism by the two metagenomic approaches (Chacón-Vargas et al., 2020; Rocha-Arriaga et al., 2020).

A remarkable effort to propose a microbial core with aguamiel and pulque fermentation's physicochemical properties was also reported (Chacón-Vargas et al., 2020). These authors correlated the temporal patterns of the major genera and species present at $\geq$1% in the aguamiel and at least one fermentation stage and their association with the significant fermentative products of pulque such as lactic and acetic acids and ethanol from available sugars (sucrose, fructose, and glucose). Results showed that *Acinetobacter, Lactococcus*, and *Leuconostoc* were highly abundant in the sap (>13%). Both *Lactococcus* and *Leuconostoc* showed a slight fluctuation during the 6-h fermentation but were present at the same abundance in fermented pulque. Nevertheless, *Acinetobact*er decreased during the fermentation process (6 h) but increased to 2.85% in overnight fermented pulque. The abundance of this bacteria was proposed as positively associated with glucose content and negatively with fructose (resulting from the hydrolysis of sucrose or FOS) and ethanol. The abundance of *Zymomonas* correlated positively with the increased production of ethanol, lactate, and an increment in fructose concentration, and negatively respect to sucrose content. Regarding *Lactobacillus* abundance, it was positively correlated with lactate and ethanol production. Remarkably, *Saccharomyces* showed lower abundance in aguamiel (0.033%), fluctuated during the 6-h fermentation, and increased to 13.51% in fermented pulque. As stated above, a relevant conclusion from this work was that the abundance of *Saccharomyces* did not correlate significantly with sugar or ethanol abundance, focusing on the relevance of *Z. mobilis* in ethanol production during the fermentation of pulque (Chacón-Vargas et al., 2020). It is relevant to highlight that *Acinetobacter* was detected by both metagenomic approaches as one of the most abundant genera in aguamiel, during the fermentation process, as in the final fermented product (Chacón-Vargas et al., 2020; Rocha-Arriaga et al., 2020). The genus *Acinetobacter* has been reported in traditional fermented foods such as the *chicha* from Brazil, the *koko* from Ghana, and the *chikwangue* from Zaire (Tamang et al., 2016). This bacterium was previously reported in aguamiel and pulque samples from Huitzilac, Morelos, but in a lower proportion (Escalante et al., 2008; Escobar-Zepeda et al., 2020). *A. nectaris* (reported by Chacón-Vargas et al., 2020) is a Gram-negative bacterium reported as the most abundant bacteria found in the floral nectar of some cultivated plant species from Northern Israel and wild plants in the Southern Spanish Mediterranean Sea. This bacterium can grow on glucose and oxidizes D-fructose and sucrose. Remarkably, produces levan from sucrose by a periplasmic levansucrase (Álvarez-Pérez et al., 2013; González-Garcinuño et al., 2017). High sugar content in aguamiel supposes a suitable environment for this bacterium.

The metabolic activities shown in **Figures 4**, **5**, resulting in the production of ethanol, lactic acid, acetic acid, CO_2, dextran, and levan, could be considered as a basal line for the development of a well-defined inoculum for pulque fermentation, composed by *Z. mobilis, Leuconostoc, Lactobacillus, Lactococcus, Acinetobacter,* and possibly *S. cerevisiae*. The use of these well-adapted microorganisms to the physicochemical conditions in aguamiel and pulque represent an opportunity to reduce some stages during the fermentation process, the fermentation time, and increasing reproducibility of the process. It is necessary to determine which species of *Leuconostoc* and *Lactobacillus* could be considered essential for a precise mixed inoculum development for controlled pulque production. According to Chacón-Vargas et al. (2020), *L. plantarum* was the second most abundant bacterium after *Z. mobilis* in the sap, during a 6-h fermentation, as in 12-h fermented pulque. In contrast, *Lactobacillus sanfranciscensis* was the most abundant lactobacilli detected by these authors, but other *Leuconostoc* species such as *L. citreum, L. kimchi*, and *L. mesenteroides* have been detected in aguamiel and pulque from Huitzilac as main microorganisms (Escalante et al., 2004, 2008; Escobar-Zepeda et al., 2020).

Recent metagenomic, physicochemical, and metabolic characterization of aguamiel and pulque fermentation process has provided valuable information to propose for the first time the basis for a new definition of the physicochemical and biochemical quality of the aguamiel used as a substrate for pulque production and the development of a defined mixed inoculum suitable to improve the quality, stability, and end products of the pulque fermentation process. It is necessary to use this valuable information to propose a new Mexican norm (or to update the existing ones) to define the quality and sensorial properties for aguamiel and pulque, based on precise analytical criteria and the standardization of the fermentation process under controlled production conditions.

RELEVANCE AND CURRENT SITUATION OF THE CULTIVATION OF MAGUEY FOR PULQUE PRODUCTION AND DERIVATIVE TRADITIONAL AND VALUABLE PRODUCTS

Cycle of Life of Maguey Pulquero

The agaves are plants with unique biological and ecological characteristics. These plants, known as maguey or *mezcales*, are endemic to the American Continent and are members of the Family Asparagaceae, Subfamily Agavoideae, genus *Agave,* comprising ∼210 species (Colunga-GarcíaMarín et al., 2007; The Angiosperm Phylogenetic Group, 2009). In Mexico, the genus is widely distributed, covering 76% of its territory with 61% of endemic species. Showed a tremendous significant diversification as they grow from the sea level up to 3,000 m altitude (García Mendoza, 2011; García Mendoza et al., 2017, 2019).

The agaves for pulque production are plants of vital aspect, perennial, formed by rosettes of lanceolate leaves arranged in

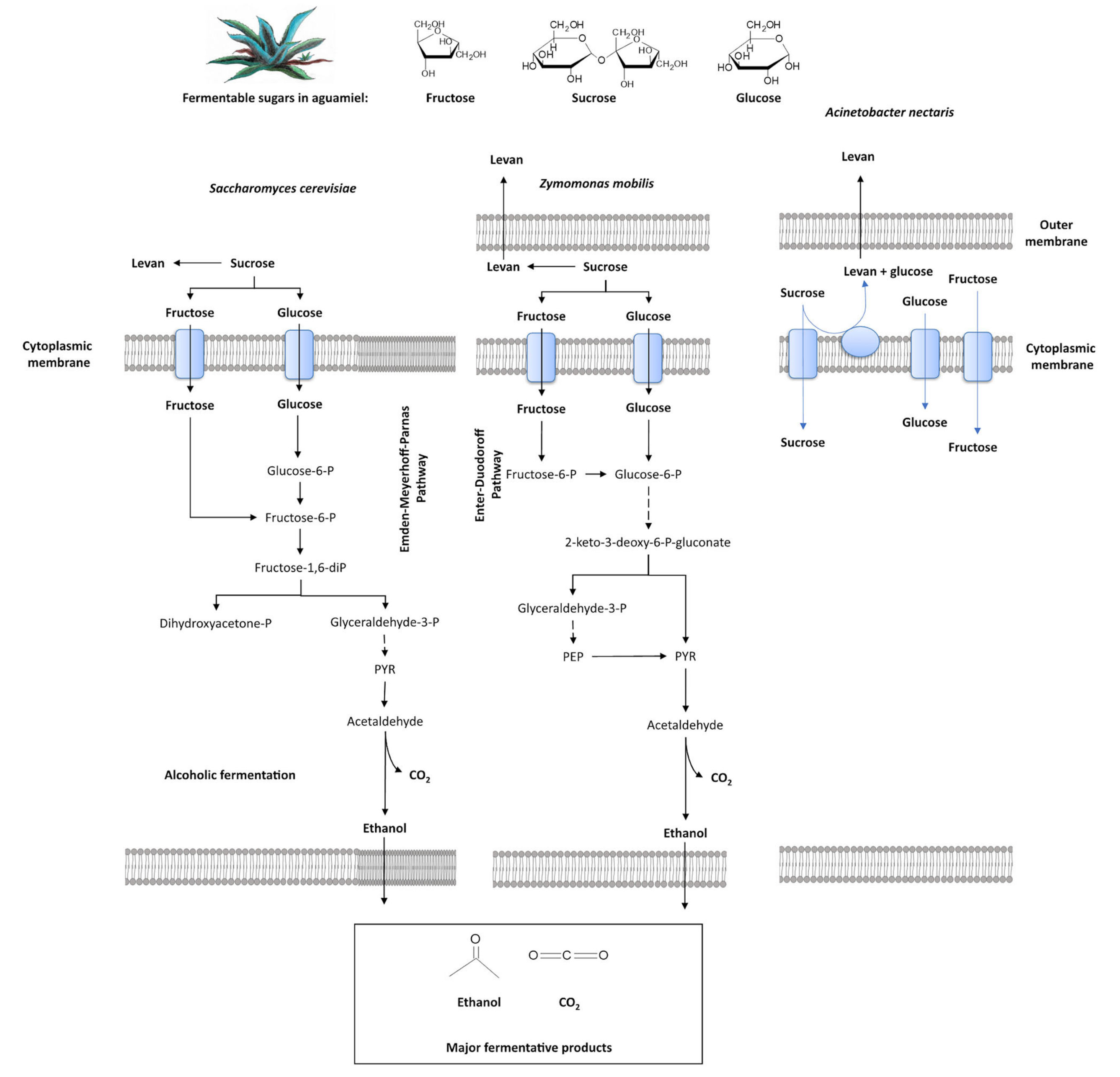

FIGURE 4 | Schematic representation of key metabolic pathways from the possible microbial essential core for pulque fermentation: The role of *Saccharomyces cerevisiae, Zymomonas mobilis,* and *Acinetobacter nectaris.* Sugar transporter systems are shown as blue structures in the cytoplasmic membrane. Continue arrows represent single metabolic enzyme-catalyzed reactions. Dotted lines represent several enzymatic reactions. Adapted from Swings and De Ley (1977), Sahm et al. (2006), Álvarez-Pérez et al. (2013), Walker and Stewart (2016), Weir (2016), Yang et al. (2016), Jansen et al. (2017), Chacón-Vargas et al. (2020), Rocha-Arriaga et al. (2020), and Carvalheira et al. (2021).

a spiral, succulent, fibrous and thick, thorny in the margins of the apex. It has a panicle inflorescence of 5–12 m, with greenish-yellowish flowers (or *gualumbos*) (García-Mendoza, 2002). These plants have a sexual reproduction by seeds resulting from pollination of the inflorescence and vegetative propagation by transplanting of stoloniferous shoots (named *hijuelos* or *matecuates*) of the mother plant; from bulbils, which are seedlings born from leaves, buds, rhizomes, and by micropropagation (**Supplementary Figure 2**; García Mendoza, 2007; García Mendoza et al., 2017; Torres-García et al., 2019). The sexual reproduction of this plant is semelparous or monocarpic, which indicates that the plant dies after a unique event of sexual reproduction (García Mendoza, 2007). The maguey cultivation cycle comprises the following stages

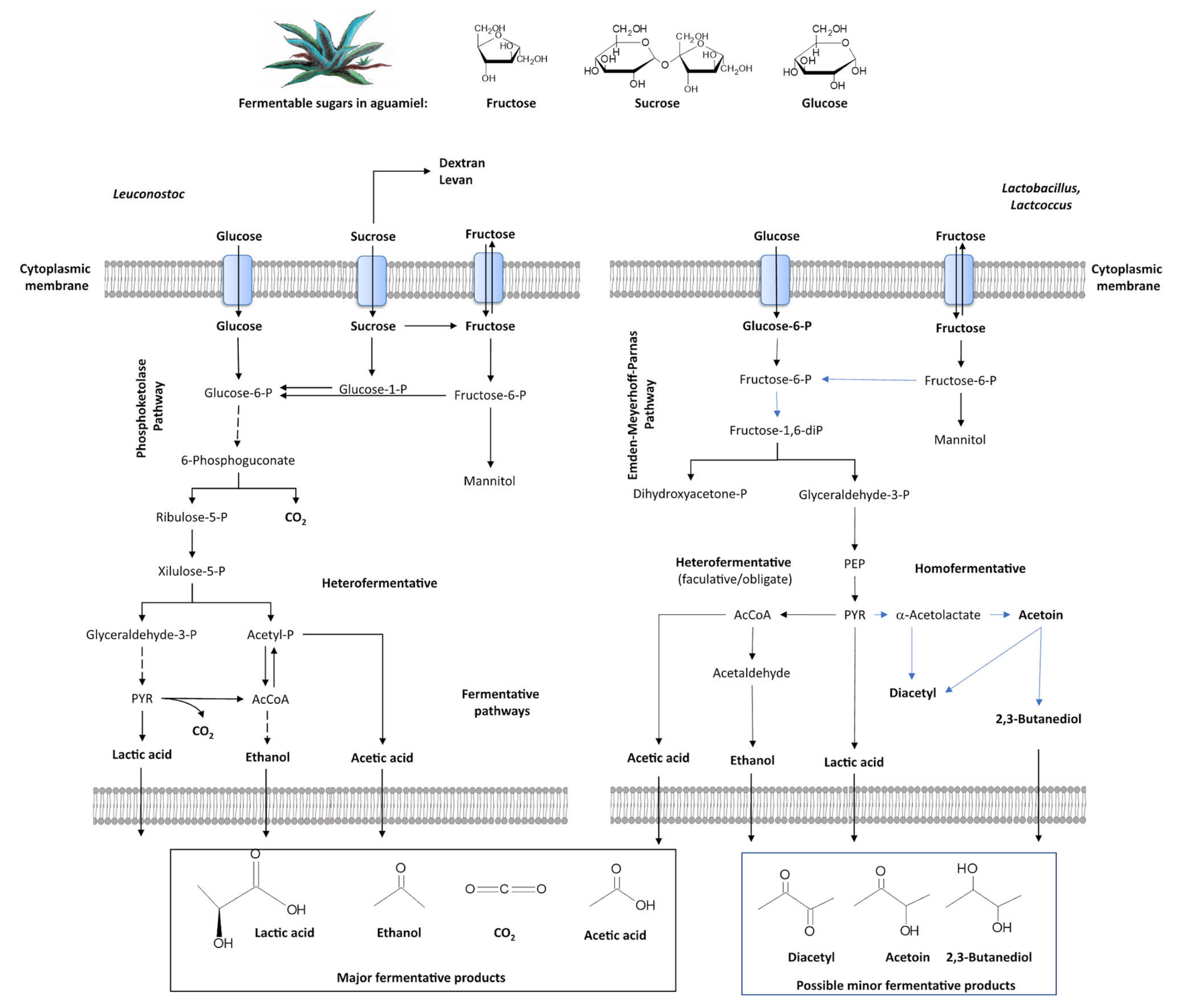

FIGURE 5 | Schematic representation of key metabolic pathways from the possible microbial essential core for pulque fermentation: The role of *Leuconostoc, Lactobacillus,* and *Lactococcus*. PEP, phosphoenolpyruvate; PYR, pyruvate. Sugar transporter systems are shown as blue structures in the cytoplasmic membrane. Continue arrows represent single metabolic enzyme-catalyzed reactions. Dotted lines represent several enzymatic reactions. Adapted from Dols et al. (1997), Melchiorsen et al. (2001), Zotta et al. (2017), Chacón-Vargas et al. (2020), Rocha-Arriaga et al. (2020), and Carvalheira et al. (2021).

(**Supplementary Figure 2A**; Ramírez Rodríguez, 2004; Nieto Aquino et al., 2016): (i) Reproduction. Young plants from germinated seeds, consolidated seedlings (2–6 months), and the stoloniferous shoots of 1-year-old and a height of 50-60 cm are cultivated in a greenhouse. Young plants are transplanted to the field when they reach 1 m in height or are 1–2 years old (**Supplementary Figure 2B**). (ii) Transplanting of stoloniferous shoots. When the plants reach adulthood (up to 8 years) produce several *hijuelos*. Vegetative propagation by transplanting the stoloniferous shoots led to the exchange of different species or varieties of plants from a diverse geographic origin, resulting in maguey plantations with mixed populations (Torres-García et al., 2019; Álvarez-Ríos et al., 2020). (iii) Physiological maturity: The adult plants are ready for flowering, their growing activity decreases, and channel resources for the rise of the floral bud. It is in this phase where the preparation for the extraction of aguamiel (castration) takes place. This process lasts from at least 5 years depending on the species (García Mendoza, 2007; Nieto Aquino et al., 2016; Álvarez-Ríos et al., 2020).

The use and management of these plants for thousands of years have resulted in an artificial selection process, with gigantism being the most evident result. In the case of *A. mapisaga*, these plants can reach up to 4 m in height, representing higher mead yields. Other effects of artificial selection are reducing morphological mechanisms (e.g., smaller size and lesser number of teeth or lateral spines) and chemical defense mechanisms, making these plants easier to manipulate. The propagation by offsprings over sexual reproduction resulted in the development of a stable genotype maintained for several generations, but with a decreased genetic diversity, larger population structure, and less gene flow, in addition to increasing its vulnerability to diseases (Álvarez-Duarte et al., 2018; Torres-García et al., 2019).

The Maguey Pulquero in the Agroforestry Systems and Large Plantations for Aguamiel and Pulque Production

The agroforestry systems are spaces where wild and agricultural diversity coexist (Torres-García et al., 2019). In Mexico, agaves for aguamiel production have been used since Pre-Hispanic times with this purpose in an agricultural model known as *metepantle* (*metl* = agave and *pantli* = wall). The metepantle is a cultivation terrace characterized by having a soil containment board where magueys are planted. The space between the board or rows of cultivated magueys retains the soil and is used to grow other crops in zones with orography of steep slopes and high erosion, mainly in the States of México, Tlaxcala, Hidalgo, and Oaxaca (Álvarez-Duarte et al., 2018; Torres-García et al., 2019; Álvarez-Ríos et al., 2020; Viniegra-González, 2020). Agave plantations in the model of the metepantle are essential components of the agroforestry systems, as these plants retain soil and humidity, promoting the infiltration of water into the soil. If the plants bloom, they attract various pollinators such as bees and various species of nectar feeders, promoting pollination. These systems are also habitats for different species of animals (Torres-García et al., 2019).

Current maguey plantation for pulque production comprises four models classified based on the type and intensity of management to which they are subjected, the diversity and number of plants cultivated and managed for production, and the amount of aguamiel and pulque produced (Álvarez-Ríos et al., 2020):

a. *Intensive maguey plantation.* This model includes private or communal properties where magueys are grown at a high density. An example of this plantation model is located in Nanacamilpa, Tlaxcala State. This high-density cultivation area comprising 44 ha with 2,500 plants per ha, of which 500 plants are used for aguamiel production, yielding 2,500–3,000 L/day. The magueys used belongs to the varieties *ayoteco* and *chalqueño* with a maturity age of 12–14 years; and *manso* and *púa larga*, with a maturity age of 8–10 years. The aguamiel production life depends on the maguey's size: For larger plants, it is 5–7 months, and for smaller ones, 4–6 months. Pulque production is carried out in 10 fiberglass vats with a capacity of 750–1,000 L each, and the fermentation process takes 72 h. The producer uses sensorial criteria to define the fermentation's end, and the naturally fermented pulque is mixed with fruits and pasteurized to kill associated microbiota and stop the fermentation. The pasteurized product is canned and exported. This production model produces up to 20,000 L of pulque monthly.

b. *Remaining maguey plantations*. This small system (<1 ha) corresponds to the remaining maguey plantations enclosed in urban zones, represented by a maguey plantation in the west of Mexico City (municipality of Cuajimalpa). This plantation is an example of a familiar plantation where the magueys are planted every four meters in rows. The plants have a maturity age of 10 years and correspond to the varieties *chalqueño, manso*, and *mano larga.* In this plantation, 15 plants are used for production, yielding 68–80 L/day for 3 months. Pulque fermentation is performed in 25 L buckets, and the fermentation time is 3–6 h. The pulque is produced for self-consumption or local sale.

c. *Metepantle.* An example of this system is located in the Rancho la Coyotera, municipality of Zacualtipán de Angeles, Hidalgo State. The magueys are planted in staggered rows with native vegetation on a surface of 20 ha. The planted magueys include eight varieties, of which the most abundant are the *manso de zoqui* and a wild variety or *corriente*. In minor proportion the varieties *penca ancha, púa larga, penca larga, espina china, corriente cenizo*, and *corriente colorado.* Thirty-five plants with an age of 8 years are used for production, yielding 30–100 L/day. The fermentation is performed in an 80 L plastic vessel located in a tinacal, and the fermentation process takes 2 h. The product is sold locally, by order, in neighboring localities, or producers' fairs in large cities.

d. *Bordering plants.* This plantation is a standard system in the rural landscape where the magueys border a crop or grazing surfaces (2–4 ha) functioning as living fences to delimit and protect plots of land, forming small agave communities, or bordering roads. One example of this model is located in the municipality Santiago Undameo, Michoacán State. The maguey species planted are the varieties *verde, negro, cenizo,* and *tarimbaro*. Twenty plants are used for aguamiel production at the age of 10 years old, with a producing life of 4 months, yielding 30–60 of L aguamiel daily. The fermentation is performed in 80 L plastic vessels located in the producers' house. In this location, the collected aguamiel is boiled for several minutes to be drunk or fermented. The fermentation occurs in up to 80 L containers for 3 h and is sold in local markets or nearby towns.

e. *Wild maguey*. This system remembers the ancient maguey utilization for pulque production as it uses wild agave species. The example of this system is located in San Juan Raya and Zapotitlán Salinas, municipality of Zapotitlan, Puebla State, where the wild agave species of *A. marmurata* Rozel is used for aguamiel production in the wild, by transplanting offsprings or by the transplantation of the entire young plants closer to the producer's home. The plants used for aguamiel extraction are 12–15 years old and produce 1.5 L/day for 2 months. In this system, 35 plants are used for production, and aguamiel is collected three times daily. The fermentation is performed for 3 h in 25 L buckets located inside the house's producer, and the fermented beverage is sold.

Each one of the systems described above maintains self-sustaining plant cultivation practices, trying to maintain a stable plant population by transplanting offspring to ensure new plants' availability. Sexual reproduction of plants is not allowed in any system (Álvarez-Ríos et al., 2020). Nevertheless, maguey cultivation for pulque production faces different situations that have resulted in a significant decrease of plants in some regions, where pulque production has practically disappeared. A fundamental problem for pulque producers is the lack of regulation for clandestine activities such as *desmixiotado*, an illegal activity that consists of removing the cuticle (*mixiote*) of the leaves or *pencas* or the mutilation of the entire leaves of the plant for cuisine purposes, which significantly affects its development (José-Jacinto and García Moya, 2000; Álvarez-Duarte et al., 2018). The extraction of two species of lepidopteran caterpillars or maguey worms (see below), which have a high gastronomic value, results in the plant's destruction. The disappearance of the maguey due to its irrational or clandestine use, the scarce and inadequate propagation strategies, and the lack of interest in pulque production by new generations have seriously affected the pulque agroindustry in some regions, becoming its production as a secondary economic activity (Álvarez-Duarte et al., 2018).

Production of Traditional and New Products From Maguey, Aguamiel, and Pulque

The cultivation and propagation of maguey pulquero are activities performed mainly to extract aguamiel for pulque production. However, the plant's leaves are used to extract fibers (*ixtle*) to produce bags, ropes, brush bristles, and broom fibers. The dry leaves and the dry plant's pine are used as fuel, and the flowers of the inflorescence (*gualumbos*) are the main ingredient of several dishes in traditional Mexican cuisine. The caterpillars from the lepidoptera *Comadia redtenbacheri* (red worm or *chunicuil*) and *Aegiale hesperiaris* (white worm or *escamol*) infecting the leaves, roots, and the pine of the plant are highly appreciated in the traditional and gourmet Mexican cuisine (García Mendoza, 2007; Narváez Suárez et al., 2016; Torres-García et al., 2019). Additionally, the scraped bagasse or metzal from the cajete during aguamiel extraction is a rich-sugar material (containing sucrose, glucose, fructose, and FOS) used as fodder (**Supplementary Figure 3**). The total mass of extracted metzal during the aguamiel producing life of a plant varies from 21.5 to 37.2 Kg (Peralta-García et al., 2020).

The use of maguey plants as raw material for the production of several of the byproducts mentioned above or as a food ingredient is only carried out on a local scale with a low commercial impact profile, leading to the extraction of aguamiel for pulque production as the main product obtained from the species of maguey pulquero. Narváez Suárez et al. (2016), studied the producing maguey plantations and pulque production zones of Zempoala, Singuilucan, and Epazoyucan (Hidalgo, State); Españita and Nanacamilpa (Tlaxcala State), Texcoco and Tepetlaoxtoc (Estado de México); Tepeyahuaco (Puebla State); and Perote (Veracruz State), to determine whether its cultivation still represents a viable economic activity. These authors conclude that the sale of aguamiel, pulque, red, and white worms, and the sale of the entire cut leaves for barbecue preparation are the activities with higher economic value for these rural agricultural locations. Finally, they consider the production of concentrated aguamiel syrups, candies, pulque distillates, and rural *pulque tourism* as relevant products/activities with higher economic value for these communities (Narváez Suárez et al., 2016).

Nowadays, the industrial production of canned and bottled pulque is an important economic activity in the main pulque-producing zones. As the USA and Europe as primary markets, several brands are successful examples of the pulque industrialization process incorporating tank-fermentation homogenization, pasteurization of the final product, quality control, and bottled or caning for final sale. Relevant examples are the *Corporativo Maguey San Isidro* established in 2003 in Tlaxcala State, with Pulque del Razo Group, which produces about 300,000 units/month of canned pulque brand *Pulque Hacienda 1881*, a product with certification from the Foods and Drugs Administration (FDA), USA. Pulque del Razo also provides high-quality pulque to 70% of the pulquerías in Mexico City and produces byproducts such as aguamiel concentrated syrups, inulin (inulin-type fructans), pulque distillates, flavorings, and sugars. Additionally, the *Corporativo Maguey San Isidro* offers pulque tourism to the *Rancho San Isidro*. This company possesses an own sustainable production of maguey varieties for pulque production by propagating and plantation of the varieties *manso, púa larga, ayoteco*, and *chalqueño* (corpmasir.com). *La Flor Pura* company established in 2009 in the Estado de México, produces bottled aguamiel and pulque with *La Flor Pura*'s brand (pulquelaflorpura.com). The most recently established company was *Embotelladora Pulquemanía* (established in 2020 in the city of Texcoco, Estado de México), producing bottled pulque with the brand *Pulque Penca Larga* with a monthly production of 260,000 bottles from aguamiel collected from the locality of Nanacamilpa, Tlaxcala State. This brand is also certified by the FDA for exportation to the USA (pencalarga.com).

Potential New Products From Maguey, Pulque, and Aguamiel

Several studies have focused on the potential probiotic benefits of specific bacteria isolated from aguamiel and pulque: The *Leuconostoc mesenteroides* strain P45 showed higher antimicrobial activity against pathogenic bacteria such as EPEC *Escherichia coli, Listeria monocytogenes, Salmonella* Typhi, and *S.* Typhimurium, both *in vitro* and *in vivo* mice models, compared to the antimicrobial activity shown by a commercial probiotic bacteria (Giles-Gómez et al., 2016); the *Lactobacillus sanfranciscensis* strain LBH1068 improved mice health by a significant reduction of weight loss, decreasing the gut permeability, and cytokine modulation (Torres-Maravilla et al., 2016); the isolated LAB identified as *Lactobacillus* sp. and *Pediococcus* sp. showed antimicrobial activity *in vitro* against *Staphylococcus aureus* and *Helicobacter pylori* (Cervantes-Elizarrarás et al., 2019). Other relevant biological activities have been identified in other bacteria isolated from aguamiel concentrated syrups (agave sap concentrate), including the potential *in vitro* anticancer enhancement effect of the isolated bacteria *Gordonia* sp. and *Arthrobacter globiformis;* and the ability to survive and grow in the colonic environment and the production of short-chain fatty acids by microorganisms such as *Lactobacillus* and *Leuconostoc* suggest their potential use as probiotics (Figueroa et al., 2017, 2021).

Several reports also showed the potential biological activity of byproducts from the maguey. The *in vitro* activity of fructans with high molecular weight and branched fructans extracted from a 6-year-old plant of *A. angustifolia* as a growth-promoting agent of several *Bifidobacterium* and *Lactobacillus* species suggest a potential prebiotic activity (Velázquez-Martínez et al., 2014). Recent studies have focused on the potential properties of agave bagasse from *A. salmiana* as a source of bioactive compounds such as steroidal saponins with potential biological activities such as antifungal, anticarcinogenic, or anti-inflammatory (Santos-Zea et al., 2020), and the role of polysaccharides present in the bagasse of this maguey species as a natural emulsion stabilizer and enhancer of topical anti-inflammatory activity of indomethacin (Jiménez-Rodríguez et al., 2021). These reports open an area of opportunity to use different bacterial strains isolated from aguamiel, pulque, or byproducts such as aguamiel concentrates as probiotic agents that can be incorporated into the formulation of functional foods and beverages, using just aguamiel or a diluted preparation of concentrated aguamiel syrups as a substrate for their growth or formulation.

Finally, the maguey plantation model of meteplantle was proposed as a potential bioresource for the production of several products with higher economic added value, including the use of aguamiel for the production of a maguey beer, the use of the fiber ixtle as raw material for the production of biodegradable food bags, or the use of aguamiel as a substrate for the production of lactic acid as food acidulant or as raw material for the production of the valuable polymer polylactate (PLA), as possible alternative commercial scenarios. Particularly for lactic acid production from aguamiel as substrate by industrial fermentations, Viniegra-González (2020) projects a scenario where lactic acid production for PLA synthesis could consume 3 billion liters of aguamiel to be produced in 100,000 ha. Nevertheless, this scenario does not contemplate a sustainable system of replacing mature magueys for aguamiel production. In any case, it is a potential scenario that, with adequate investment, infrastructure conditions, and sustainable maguey replacement, would require at least 20 years for its development (Viniegra-González, 2020).

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Pulque production and maguey cultivation are essential elements of identity, millenary tradition, and contextualized in a close-related environmental, alimentary, social, religious, cultural, and productive relationship as they form part of the same production system, mainly in rural communities. Today there is a general perception that pulque is a popular beverage that has practically disappeared from urban markets and just sold in pulquerías, some restaurants, and rural locations. Although it is a preferred beverage in rural areas, its traditional production is in a critical situation and at risk of disappearing for different reasons, such as the irrational use of maguey by producers, inadequate or inexistent propagation strategies, displacement of its cultivation by other agricultural products, its use for other activities such as desmixiotado, mutilation of leaves, or the collection of maguey worms, or because of a lack of interest in continuing with this activity. All these situations have significantly affected the economy and food sufficiency of several rural communities.

However, another scenario in which different producers have successfully industrialized the production of pulque and marketed canned or bottled, reaching a production of more than 1,300,000 bottles per year, equivalent to more than 1,138,000 L of pulque (just for one producing company), have shown the successful development of this agroindustry. Remarkably, some of these companies have their own maguey propagation and cultivation practices, but others buy an average of 5,000 L of aguamiel per day, generating a permanent market and an economic benefit to aguamiel producers. The beverage's industrial production has made it possible to standardize the traditional fermentation process (extraction of aguamiel, preparation of the seed, and fermentation), incorporating quality standards and the final product's pasteurization process. These operations solve an ancestral problem with the production of pulque and stop the fermentation process, increasing the shelf life of the final product, which in some cases has achieved international quality standards. Similarly, some companies sustainably cultivate the maguey varieties used to produce aguamiel by propagating and planting to avoid a shortage of plants and ensure continuous beverage production. However, it is paradoxical that regular pulque consumers do not like industrialized pulque despite some international certifications.

This situation has a relevant impact on the pulque agroindustry, where two cultural visions of pulque production are opposed. The cultivation of maguey pulquero for its traditional production represents the continuity and conservation of culture and traditional knowledge. It also provides environmental benefits in the agroforestry system, such as reducing soil erosion, conserving native flora and fauna, capturing carbon, and recharging groundwater. Nevertheless, this activity represents a minor economic activity for many traditional producers due to the lower volume production and market. Although the industrial production of pulque represents an economic opportunity for traditional aguamiel producers as providers of the sap, they must comply with the quality standards required for the producer, resulting in sometimes opposite to the traditional production customs. It is necessary to promote in the Mexican market the canned or bottled pulque. Current pulque production in Mexico is ∼0.1% of the current beer market reported for 2020 (Instituto Nacional de Estadística Geografía e Informática, 2021). The possibility of increasing the Mexican market to canned or bottled pulque at least at the same level of the exported production would generate, for example, a greater demand for aguamiel. It would result in this agroindustry's reactivation in many rural communities where it is currently considered a secondary economic activity.

The species of agaves for aguamiel production, the collected sap, and traditional pulque fermentation represent an invaluable resource from the genomic, metabolic, and microbiological point of view, resulting as a strategic activity the elucidation of the natural microbiota responsible for the fermentation among different producing zones and the long-term maintenance of stocks of these microbial cultures. The microbiological and functional studies of the associated microbiota have provided valuable information to propose a defined culture starter to optimize the fermentation. It has also provided the scientific basis of some of the traditional health benefits associated with the beverage's consumption, proving the source for developing new potential probiotic and functional foods or beverages and developing future bio-industries.

AUTHOR CONTRIBUTIONS

All the authors contributed equally in the preparation and review of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2021.678168/full#supplementary-material

Supplementary Figure 1 | Pulque castration process. The *tlachiquero* selects the proper mature plants on the basis of the thinness of the central leaves (heart or *meloyote*). **(A)** The *tlachiquero* uses a sharpened knife to cut off some leaves surrounding the heart. **(B,C)** Cutting of the heart. **(D–F)** Destruction and extraction of the remaining floral bud with a sharpened pray bar. **(G)** Scraping of the wall of the resulting cavity with a scraping tool or *raspador* to shape the final size of the *cajete*. **(H)** Once the cavity was completed, it is covered with leaves to start the maturation process. Images kindly provided by Mrs. E. Velázquez Gutiérrez.

Supplementary Figure 2 | The cycle of life of maguey for pulque production. **(A)** Seedling, (b) adult plant, (c) flowering, (d) flower, (e) seeds, (f) bulblet, and (g) stolon. Sexual reproduction by seeds resulting from pollination of the inflorescence (c–e, a). Vegetative propagation from bulblets (f) or by transplanting stoloniferous shoots or *hijuelos* of the mother plant (g). **(B)** Vegetative propagation by transplanting stoloniferous shoots of 1-year-old and a height of 50–60 cm (a) are transplanted to a greenhouse. Young plants are transplanted to the field when they reach 1 m in height or are 1–2 years old (b) to complete its growth for aguamiel production (c) (García Mendoza, 2007; Nieto Aquino et al., 2016; Álvarez-Ríos et al., 2020).

Supplementary Figure 3 | Main products obtained from the maguey, aguamiel, and pulque. *Gualumbos, mixiote,* maguey worms, and leaves (*pencas*) are used in traditional and gourmet Mexican cuisine. *Ixtle* fibers are used for the production of bags, ropes, brush bristles, and broom fibers.

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