Review Article

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Approach to knowledge of the microbial bioprospecting in Venezuela

Abstract

This exploratory research was based on the review of the proceedings of the Annual Convention of the Venezuelan Association for Science Advance (AsoVAC) between 2002 and 2017 about the investigations realized in native microorganisms with biotechnological potential. In this regard, the researchers from Venezuelan universities and scientific institutions have developed lines of research to address the subject on the biotechnological utilization of microorganisms. The results indicate that the University of Oriente has the biggest contribution with respect to the evaluation of the photosynthetic microorganisms for the aquaculture food production and the biotechnologically important metabolites production. On the other hand, non-photosynthetic microorganism investigations have been directed especially to study their applicability in agrobiotechnology as biofertilizers and biocontrol agents; likewise they are candidates for the bioremediation of polluted terrestrial and aquatic ecosystems by petroleum hydrocarbon, an area very investigated by researchers at the University of Zulia due to oil exploitation that takes place within its area. Another topic of interest that stands out is the study in thermophilic and psychrophilic bacteria by the researchers from the University of Carabobo and the University of Los Andes, respectively.

Keywords: native microorganisms, microbial biotechnology, Venezuela, food biotechnology, agrobiotechnology, environmental biotechnology, proceedings of the AsoVAC

Volume 6 Issue 1 - 2019

Judith Piñero–Bonilla

Food Engineering, National Experimental University Simón Rodríguez, Venezuela

Correspondence: Judith Piñero-Bonilla, University National Experimental University Simón Rodríguez Address Sector Los Camellones, El Valle, Mérida, Mérida State, Venezuela, Zip code 5101, P.O. Box 875, Tel 58 414 7065936, Email unesrcanoabo@gmail.com

Received: November 03, 2018 | **Published:** January 07, 2019

Introduction

The structural and functional diversity of microorganisms has allowed them to colonize a wide variety of environments in all terrestrial geography by deploying various essential adaptation mechanisms and processes for the functioning of ecosystems, mainly through their participation in the biogeochemical cycles, an activity linked to the fertility of soils and aquatic ecosystems;[1] however, it is necessary to solve methodological limitations and knowledge gaps about the microbial regulation of global biogeochemical cycles.[2] Moreover, they are natural controllers of pathogens and other harmful microorganisms to human health, plants and animals populations;[3] and to continue adding benefits, its role in the mitigation of greenhouse gases and in regulating the planet climate associated with the recycling of atmospheric gases. In this sense, it has been noted that the importance of microorganisms has not been taken into account in predictive climate models suggesting for this purpose the development and application of molecular techniques to get to know the metabolic diversity that enable to assess mitigation strategies for greenhouse gas emissions.[4]

On the other hand, the search for life beyond Earth scenarios has become one of the goals of science considering that this would be represented by very simple shapes such as the microorganisms. This assessment has as starting point, the presence of microorganisms in extreme environments on Earth whose conditions have also been found in other planets of the solar system.[5] However, the exploration and investigation of the microbiodiversity in our planet continues being of interest in different types of ecosystems whose spectrum has increased thanks to the new technologies. This exploration has been focusing more on genetic potential which possess the microorganisms for biotechnological use in different scientific and technological areas. In this regard, Venezuelan researchers have explored the habitat diversity in the country carrying out studies on isolation and evaluation of the microbial potential for biotechnological purposes obtaining promising results for several years.[6] This search comprises natural and anthropic environments some of whom have been subjected to a high degradation by chemical pollution or to the effects of climate change; nevertheless, these contaminated environments have become new sources of microorganisms with important metabolic functions for the bioprocesses development of environmental and industrial interest.[7,8]

These investigations not only represent a scientific contribution to biotechnology and ecological knowledge of the Venezuelan microbiodiversity, they also represent a valuable genetic bank deposited in the strain collections which become more and more precious as environmental degradation is increased because, consequently, many beneficial species disappear. In this way, it was made an exploratory study as an approximation of the knowledge in this area based on the review of the proceedings of the Annual Convention of the Venezuelan Association for Science Advance (AsoVAC) between 2002 and 2017 about the research on photosynthetic and non-photosynthetic native microorganisms with biotechnological potential.

General scenario

The Annual Convention of the AsoVAC started in 1950 and it has as objective the dissemination of scientific, socio-humanistic and technological knowledge of Venezuela. In this sense, is a multidisciplinary event with several genres of presentation such as forums, round tables, courses, symposiums, seminars, conferences and presentations (oral and poster), latter being which meet the greatest number of work presented and published as abstracts in the

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printed and digital memories of the congress.[9] This event is organized each year in a distinct country city with the participation of different Venezuelan universities and scientific institutions, this fact can influence a greater presence of those institutions geographically next to the place of the Convention. However, in recent years certain happenings have hindered the organization of this Convention which in some cases forced to do it simultaneously in different cities as it was the case for the years 2014 and 2017. There is also a decrease in participation in this convention (Figure 1).

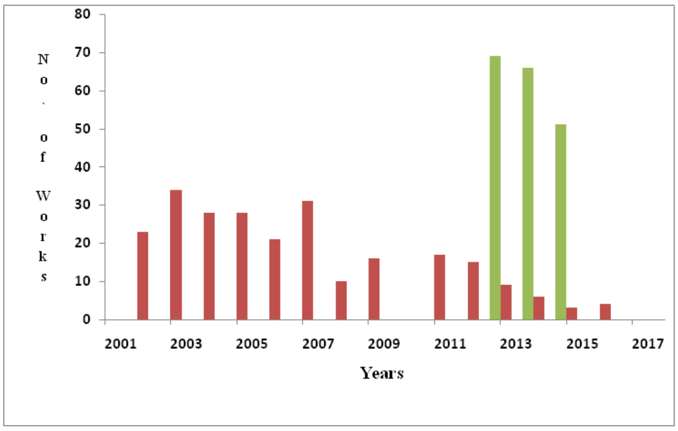

Figure 1 Works presented in the Annual Convention of AsoVAC (red) and in the Venezuelan Congress of CTI (green). The proceeding 2010 is not available.

In this context, the results extracted from the proceedings of the Annual Convention of the AsoVAC show that biotechnological investigations have focused on four main areas: firstly, the industrial biotechnology, specifically with application in the food industry. Secondly, the environmental biotechnology dominated research on bioremediation of petroleum hydrocarbon polluted environments (PH), followed by agricultural biotechnology with the production of biofertilizers and biocontrol agents. Finally, the biotechnology in human health has few jobs in diagnosis and production of bioactive substances among other studies, although this area can share research with food biotechnology on functional foods and nutraceuticals (Table 1).

In addition, it can also analyze the institutional participation, noting an increased number of investigations carried out by the University of Zulia (LUZ), the University of Carabobo (UC) and the University of Oriente (UDO) and among them LUZ and the UDO have the biggest contribution of studies on photosynthetic microorganisms (cyanobacteria and microalgae), without considering the research jointly with other institutions (Table 2). These results reflect the location of each institution to have the advantage of possessing a rich aquatic microbiodiversity for research. Thus, LUZ has a great reservoir of water coming from the Lake Maracaibo and the Caribbean Sea. On the other hand, the UDO also has the biodiversity of the Caribbean Sea and the Atlantic façade among other water resources.

In the same way, the proximity to places with high oil activity generating a significant contamination of soils and waters offer to LUZ the opportunity to carry out studies on indigenous microbial potential (photosynthetic and non-photosynthetic) to degrade PH, its derivatives and heavy metals, including ecotoxicological studies (15.2%). While the UDO has principally explored the marine photosynthetic microbiodiversity as food and drug resource (6.6%) (Table 2).[10]

Table 1 Studies on photosynthetic and non-photosynthetic microorganisms presented in the Annual Convenction of AsoVAC (2002–2017) and in the Venezuelan Congress of CTI (2012–2014). It Include the total works on Biotechnology of autochthones and commercial microorganisms

Researches	WORKS			
	AsoVAC		CTI	
	FM	NFM	FM	NFM
Food biotechnology	23	21	04	10
Biomass production (SCP)	23	05	04	01
Fermented foods production		07		01
Probiotics		09		07
Food conservation				01
Food and drug biotechnology: metabolites production	30	53	03	12
Enriched biomass	25			
Bioactives compounds				
Bacteriocins (food conservation)		04		01
Pharmacological use	05	11	01	02
Enzymes		29		05
Biosurfactants		05		01
Others		04	02	03
Environmental biotechnology	36	28	08	21
Heavy metals biosorption	13		07	
Bioremediation:				
Hydrocarbon biodegradation	08	25		18
Pesticides biodegradation		02		
Wastewater treatment	12		01	02
Ecotoxicological studies	03			
Others (biodegradation)		01		01
Energetic and mining biotechnology	02	11	01	09
Biofuel production	02	02	01	07
Improvement of oil		03		01
Bioremediation and improvement of oil		04		
Bioleaching		02		
Bioelectricity generation				01
Agricultural biotechnology		32*		104*
Biocontrol agents		16		54
Biofertilization		15		46
Microbial culture collections			01	06
Others	04	05	03	04

*It include integrated studies on biocontrol and biofertilization agents.

Citation: Piñero–Bonilla J. Approach to knowledge of the microbial bioprospecting in Venezuela. *J Appl Biotechnol Bioeng.* 2019;6(1):1–14.
DOI: 10.15406/jabb.2019.06.00168

Table 2 Participating institutions in the congress of AsoVAC (2002–2017) and CTI (2012–2014). It includes the total works on biotechnology of autochthones and commercial microorganisms

	Works (%) AsoVAC Convention			Works (%) CTI Congress		
Institutions	Microorganisms		Total	Microorganisms		Total
	F	NF		F	NF	
LUZ	25,4	9,4	34,8	4,9	2,7	7,6
UC		12,7	12,7	1,1	2,2	3,2
UDO	8,2	1,6	9,8	1,6	2,7	4,3
UCV		7,0	7,0		14,6	14,6
ULA		6,2	6,2		2,7	2,7
USB		5,3	5,3		0,5	0,5
UNEFM		2,5	2,5	1,1	6,0	7,0
IDEA		2,1	2,1		4,3	4,3
UCLA					4,9	4,9
UNET					3,8	3,8
INIA					6,5	6,5
Others	5,3	14,3	19,6	2,2	38,4	40,5

F, Photosynthetics; NF, Non-photosynthetics.

In the case of the UC, researchers from the Chemistry and Biology departments of the Faculty of Science and Technology (FACYT) took advantage of thermal springs at Las Trincheras (Carabobo State) to isolate and study several thermophilic bacteria with potential for the production of valuable enzymes for industrial use.[11–25] Similarly, the group of research of the Laboratory of Molecular Microbiology and Biotechnology (Biology Department, Faculty of Sciences, University of Los Andes (ULA), Merida State) has explored the microbiodiversity of the Sierra Nevada glaciers in this city with very promising results in the isolation of psychrophilic bacteria as a novel source of bioactive compounds and biofertilizers of soils;[26–28] this microbial group may be threatened by climate change that has affected Andean Mountain systems (Table 3).[8]

In general, the papers presented at the Annual Convention of the AsoVAC have applications in different biotechnological areas concentrating most of the works on bioremediation, on agricultural and food production (Table 1). Moreover, many of the organisms studied are natives of different Venezuelan ecosystems; of the total of presented research, the studies on isolation and evaluation of indigenous microorganisms represent 83.3%, the photosynthetics 38.4% and the non-photosynthetics 44.9%; the work done with bacterial strains predominate in the second group (39.6%).

By contrasting these results with those obtained in the Venezuelan Congress of Science, Technology and Innovation between 2012 and 2014 (event organized by the National Centre of Science, Technology and Innovation (ONCTI)), it can see important differences in the institutional presence.[29,30] 38.4% of the work corresponds mostly to joint investigations between various institutions; however, in this group stand out the participation of the National Institute for Agricultural Research (INIA) with 12.4% of the studies, this entity is attached to the Ministry of the Popular Power of Agriculture and Lands. In this regard, INIA researchers share the authorship of his works mainly with those pertaining to the faculties of Agronomy of the UCV, LUZ, UCLA, UNEFM and UNET, highlighting studies in the area of biocontrol and biofertilization with the use of commercial and native microorganisms (Tables 1 & 2). Additionally, the INIA has a microbial culture collection and production of bio-inputs.[31,32]

Table 3 Extremophile microorganism sources in Venezuela with biotechnological potential

Microorganisms	Researches
Thermophile Bacteria	
Bacillus stearothermophilus	Production of exochitinases, endoglucanasas, lipases, amylases and proteases; improvement of oil[11–15]
Bacillus sp.	Production of proteases[16]
Brevibacillus brevis	Production of xylanases[17]
Geobacillus pallidus	Production of xylanases (β-xylosidase)[18,19]
Geobacillus stearothermophilus	Production of β-xylosidase, amylases and cellulases[20–22]
Sulfur-reducing thermophile bacteria	Bioremediation and improvement of oil[23,24]
Non specified strain Gram+	Production of alcaline phosphatase[25]
Psychrophile and psychrotolerant Bacteria	
Actinobacteria and Proteobacteria	Isolation, identification and characterization[26]
Janthinobacterium, Iodobacter and *Duganella*	Production of violacein (biological activity)[27]
Pseudomonas	Inorganic phosphate solubilizing, production of the phytohormone indol-acetic acid and antimicrobial compounds, detection of siderophores[28]

This scientific work has generated a significant amount of biotechnology information which has involved the preservation of microorganisms in different types of collections, usually administered under the criteria established by the same participating researchers in their respective research units, invaluable spaces of preservation of genetic material for different purposes, often academics. In this way, the UDO has the collection of permanent cultivation of microalgae in the Aquaculture Laboratory of the Oceanographic Institute of

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Venezuela;[10] similarly, LUZ has the collection of microalgae from the Laboratory of Photosynthetic Microorganisms (Biology Department, Faculty of Experimental Sciences, Zulia State).[33] The Venezuelan Center for Microorganisms Collection (CVCM) is recognized at the national level belonging to the Institute of Experimental Biology (IBE) of the UCV whose services include the deposit and conservation of microbial strains of researchers who request it; this center is registered and is governed by the performance standards established by the World Federation for Culture Collections (WFCC) and it belongs also to the Latin American Federation for Culture Collections.[34]

Photosynthetic microorganisms

Food production

The studies leading to the search of photosynthetic and non-photosynthetic microorganisms have brought great findings on two important topics in microbial ecology such as biodiversity and metabolic activity in natural and anthropic environments. In this way, biotechnology has taken advantage of the different microbial metabolic strategies optimizing the culture processes among which the biomass production is priority for different purposes (Table 1). On this aspect, the valuation of the photosynthetic microorganisms as food source has included studies on growth kinetics under different physicochemical and nutritional conditions to optimize the enriched biomass production with proteins, lipids (especially polyunsaturated fatty acids), pigments and carbohydrates (exopolysaccharides) that improve their food quality. To this objective several species of microalgae and cyanobacteria usually intended as a source of live food for marine or freshwater organisms under cultivation (fishes, crustaceans and molluscs) has been evaluated (Table 4).[35–55]

Table 4 Investigations carry out on photosynthetic microorganisms: food and drug biotechnology[35–75]

Cyanobacteria	**Microalgae**
Food production	
Source not specified: *Anabaena* sp., *Nostoc* sp. *Spirulina platensis*, *S. Subsalsa*, *Synechococcus* sp.	Marine ecosystems: *Chaetoceros* sp., *Chroomonas* sp., *Skeletonema* sp., *Tetraselmis* sp., *T. chuii*, *T. tetrathele*, Hypersaline environments: *Dunaliella salina*, *D. viridis* Freshwater: *Chlorella* sp., *C. sorokiniana*, *C. vulgaris*, **Selenastrum capricornutum*, **Pseudokirchneriella subcapitata* Source not specified: *Nannochloris oculata*, *Navicula* sp. y *Nizschia* sp. (mixed culture), *Muelleriopsis limbata*,
Enriched biomass production	
Marine ecosystems: *Limnothrix* sp., *Oscillatoria* sp., *Phormidium* sp. Source not specified: *Nostoc* sp., *Synechococcus* sp., *Synechocystis minuscula*	Marine ecosystems: *Chroomonas* sp., *Rhodosorus marinus* Hypersaline environments: *D. salina*, *D. viridis* Savannas: *D.salina*
Bioactive compounds production	
Marine ecosystems: *Limnothrix* sp., *Oscillatoria* sp., *Phormidium* sp. Source not specified: *Nostoc* sp., *Spirulina subsalsa*	

*In this work the scientific names of the species were maintained as they appear in the abstracts of the AsoVAC Convention. Some of these have changed, for example, *Selenastrum capricornutum* was modified to *Pseudokirchneriella subcapitata* .[55]

On the other hand, the production of enriched biomass with metabolites of agrifood, cosmetic, clinical or pharmaceutical interest increases the value added of this organism group.[56–75] On this subject, investigations have been focused mainly on evaluating the production of bioactive substances that are especially pigments carotenoids and the phycobiliproteins. In this sense, *Dunaliella salina* (halotolerant microalgae from salty lagoons), is a source of vitamin A and the cause of the coloration in the red flamingo (*Phoenicopterus ruber*).[47] Moreover, the phycobiliproteins has been used in immunoassays and fluorescent microscopy for diagnostics and biomedical research.[75] Among the cyanobacteria with these properties is *Phormidium* sp., *Oscillatoria Limnothrix sp*., *Spirulina subsalsa,* and Nostoc strains some of which are cultivated around the world (Table 4).[71–75] In this way, the photosynthetic microorganisms become an excellent source of beneficial functional foods for human and animal consumption because they can synthesize bioactive substances in addition to their nutritional properties.[42]

The Venezuelan coasts offer interesting hypersaline systems, source of halophilic or halotolerant microorganisms. Among the most studied are the salt beds of Araya (Sucre State) and Coche (Nueva Esparta State) in the northeast of Venezuela; then include the salt beds Las Cumaragüas (Paraguaná Peninsula, Falcon State) and Las Peonias (Zulia State). Several strains of *D. salina* and *D. viridis* have been isolated from these environments, however, the first is the more attractive species due to its ability to produce carotenoid pigments of food and pharmaceutical interest.[56–58,60,61] *D. salina* has also been studied for food purposes,[47] ecotoxicological analysis[76] and as a potential input for the production of biodiesel.[77] In this sense, some researchers have evaluated the favorable physicochemical conditions for the development of *D. salina* and *Spirulina platensis* cultures with optimal performance and high nutritional value for the consumption human, which has led to propose its cultivation in Venezuela.[47,48]

Bioremediation

Although less frequent, it has also been evaluated the ability of microalgae and cyanobacteria for the bioremediation of PH-contaminated wáter.[78–81] However, cyanobacteria have been the chosen candidates on heavy metals biosorption research (Cu, Fe, Ni, Pb and Zn) in aquatic ecosystems (Table 5).[82–91] Furthermore, the microbial mats are a type of particular natural consortium of ecological relevance that constitute microhabitats of special scientific interest since they can grow on natural, degraded or contaminated soils.[80]

Citation: Piñero–Bonilla J. Approach to knowledge of the microbial bioprospecting in Venezuela. *J Appl Biotechnol Bioeng.* 2019;6(1):1–14.
DOI: 10.15406/jabb.2019.06.00168

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Table 5 Investigations carry out on photosynthetic microorganisms: environmental biotechnology[78–100]

Cyanobacteria	Microalgae
Metal heavys biosorption	
Marine ecosystems: *Limnothrix* sp., *Oscillarotoria* sp., *Phormidium* sp. Source not specified: *Pseudanabaena* sp.	
Bioremediation	
Marine ecosystems: *Limnothrix* sp. Source not specified: *Synechococcus* sp.	Port of Lanchas: *Chlorella* sp. Oil pit: *Chlorella* sp y *Chlorococcum* sp. (mixed culture)
Microbial mats of coastal sediments polluted by petroleum hydrocarbon: Microalgae: *Chorella*, *Scenedesmus*, *Merismopedia*, *Chlamydomonas*, *Ankistrodesmus* and *Nitzschia*. Cyanobacteria: *Spirulina*, *Anabaena*, *Oscilatoria*, *Westiellopsis*, *Synechocystis* and *Synechococcus*	
Wastewater treatment	
Source not specified: *Synechocystis* sp.	Freshwater: *Chlorella* sp. Source not specified: *Scenedesmus* sp.
	Floating aquatic plant system: *Scenedesmus* sp. y *Chlorococcum* sp.
	Constructed wetland: *Chlorophyta* sp. and *Stauronesis* sp.
Macrophyte- based constructed wetland: Cyanobacteria: *Oscillatoria* sp., *Microcystis* sp. *Arthrospira* sp., *Synechocystis* sp. Microalgae: *Kirchneriella lunaris*, *Chlorella* sp., *Scenedesmus* sp., *Navicula* sp., *Nitzschia* sp., *Euglena* sp. and *Trachelomonas* sp., photosynthetic bacteria *Thiopedia* sp	
Ecotoxicological studies	
	Marine ecosystems: *Chaetoceros* sp., *Tetraselmis muelleri* Hypersaline environments s: *D. salina* Oil pit: *Chlorella* sp.
Biofuel production	
	Marine ecosystems: *Chaetoceros* sp., *Chlorella capsulata*, *Skeletonema* sp., *Tetraselmis* sp. Hypersaline environments: *D. Salina*

Another consideration is that mixed and axenic cultures of cyanobacteria and microalgae are particularly beneficial in systems of wastewater treatment for its ability to remove nitrogen and phosphorus compounds, so the understanding of its dynamics is valuable information for the management and design of these systems.[92–100] In addition, *Scenedesmus* sp. can be used with a dual purpose: on the purification of wastewater from food industry that constitute at the same time an appropriate culture medium for the production of protein-rich biomass as a source of food (Table 5).[100]

Photosynthetic microorganisms

Food production

Unlike the photosynthetic microorganisms, the non-photosynthetic shows greater diversity in its application. Although they are also used as food source, studies conducted for the production of single-cell protein (SCP) include the development of enriched products with microbial biomass usually using submerged fermentation of organic wastes or byproducts of low or no commercial value as carbon and energy source. The utilization of these wastes tends to be a common practice in the obtaining of biomass from non-photosynthetic microorganisms. However, they generally are pretreated before being used as fermentation substrates depending on their nature; particularly the lignocellulosic residues are predigested with organic solvents, acid or enzymatic hydrolysis that release fermentable sugars for the metabolites production or to enhance the digestibility of the biomass obtained for animal feed (Table 6).[101–116]

Table 6 Utilization of industrial byproducts as fermentation substrates

Microorganisms (source)	Agroindustrial byproducts	Fermentation products
Hot springs: *Geobacillus stearothermophilus*	Paper, napkins, ground grass	Enzymes[101]
Comercial: *Saccharomyces cerevisiae* ATCC-4921. Mosto de uvas: *S. cerevisiae* LGBM-26.	Pretreated urban paper (acid hydrolysis).	Bioethanol[102]
Source not specified: *S. cerevisiae*	Pretreated mesocarp orange (*Citrus sinensis*) (acid hydrolysis)	SCP-enriched biomass for animal food[103,104]
Source not specified: Thermotolerant yeasts	Pretreated *Pinus radiata* wood chips (organosolv-enzymatic hydrolysis)	Bioethanol[105]
Poultry manure: *Nocardia sp.* EP3-MC3	Integral wastes of orange (*Citrus sinensis*) not pretreated	SCP-enriched biomass[106,107]

DOI: 10.15406/jabb.2019.06.00168

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Table Continued....

Microorganisms (source)	Agroindustrial byproducts	Fermentation products
Corn grains and sunflower seeds, respectively: *Aspergillus niger* ANM-1, *A. niger* ANG ()	Cocoa husks (ANM-1)	Proteins, xylanases, simple sugars[108]
	Wheat bran, wet paste, potatos, starch, cellulose, *CMC (ANM-1)	Enzymatic aditives for monogastric[109] diet (CMCases, 1,4-β-endoglucanases)
	Hydrolysis of bovine, ovine and caprine whey proteins by proteases of *A. niger*	Antihypertensive and antioxidants bioactive-peptides[110]
	Banana peel (*Musa sapientum*)	1,4-β-endoglucanases and fermentable sugars[111]
Poultry wastes: *Kocuria rosea*	Bristles and hairs of pig	SCP-enriched biomass, carotenoids and proteases[112]
	Proteo-chitinous wastes of the shrimp industry	protein hydrolysates,[113] N-acetyl-glucosamine and chitosan for animal food and pharmaceutical industry
	Feathers and pig's hairs (keratin), shrimp wastes (chitin)	Proteases[114]
	Feathers	Carotenoids[115]
	Feathers	Keratinases[116]

CMC, carboximetilcellulose; CMCases, carboximetilcelulases.

Lactic acid bacteria (LAB)

LAB have been used in the manufacture of processed dairy products and cheeses for the production of organic acids, bacteriocins (antimicrobial proteins), components of the flavor or as probiotics (Table 7).[117–128] In addition, *Lactobacillus casei* has the ability to hydrolyze casein, an activity which makes it a potential source of proteases.[129] In order to expand the applications of this group of bacteria, Zambrano and Maldonado[130] isolated and characterized autochthonous bacteria to produce a mature cheese (cheddar) obtaining favorable results.

On the other hand, bacteriocins have shown potential to inhibit or slow the growth of pathogenic microorganisms present in some foods. Thus, the investigations have been directed to the pursuit of these bacteriocins in the *Enterococcus* genus as a response to the growing rise of antibiotic-resistant bacteria. With this aim, several species of bacteria have been isolated and evaluated from milk and cheese.[117–120] Studies on functional foods supplemented with probiotics are also a promising area of biotechnology research. *Lactobacillus* and *Bifidodacterium* are two of the most widely used microbial groups in the food industry, particularly in the production of dairy products, whose aspects to be considered include evaluation of the methods of counting.[131–133] In this sense, Biomon et al.,[121] assessed the incorporation of *Lactobacillus fermentum* isolated from guayanes cheese in the food rations for rats in order to determine the effect on the development of salt-sensitive hypertension in these animals. The results showed that this bacterium may act as a probiotic in the alternative treatment of hypertensive patients.

Yeasts

Yeasts have wide application not only in the preparation of fermented foods and beverages but also in the production of biomass by its nutritional characteristics and probiotic effect for animal consumption.[134] For example, oenology is a highly relevant biotechnology area so that the yeasts are given special attention. For this purpose, Medina et al.,[122,123] isolated and evaluated the sugarcane (*Saccharum officinarum*) must yeasts of the Zulian region obtaining tolerant strains to high temperatures and concentrations of sugars and ethanol, desirable features in the selection of strains–initiating the fermentation (Table 7).

Table 7 Investigations realized on non-photosynthetic autochthones microorganisms: food and drug biotechnology

Microorganisms (Sources)	Researchs
Milk and cheese from buffalo (*Bubalus bubalis*): bacteriocinogenic lactic acid bacteria (LAB): *Aerococcus viridans, Enterococcus faecium, E. faecalis, Pediococcus acidilactici*	Bacteriocins production[117,118]
Foods: *E. faecalis*	Bacteriocins production (control of *Staphylococcus*)[119]
Artisanal white Cheese: *E. faecalis*	Bacteriocins production (control of *Listeria monocytogenes*)[120]
Guayanes cheese: *Lactobacillus fermentum* (LAB)	Probiotic[121]
Fermented sugarcane juice (*Saccharum officinarum*): *Candida intermedia*, *C. tropicalis*, *Dekkera anomala*, *Kluyveromyces marxianus, Pichia anomala*, *P. fermentans*, *P. guilliermondii, Torulaspora delbrueckii* y *Zigosaccharomyces fermentati/Z. cidri*.	Fermented beverages elaboration[122,123]
Fermented cacao (*Teobroma cacao*): acetic acid bacteria: *Acetobacter cibinongensis, A. cerevisiae* y *A. estunensis*	Cacao fermentation[124]
Mangrove roots (*Rhizophora mangle): Penicillium* sp., *P. citrinum*, *Aspergillus niger*, *Trichoderma viride*, *Syncephalastrum racemosum*, *A. ochraceus*	Bioactive compounds production (Antimicrobial agents)[125,126]
Soil: *Streptomyces sp.*	Bioactive compounds production (antibacterians) [127]
Endophytic fungi: *Xylaria* spp.	Bioactive compounds production (Antimicrobial agents)[128]

Production of bioactive metabolites

The non-photosynthetic microorganisms produce a wide range of bioactive substances with different functions; however, a larger number of investigations have been made with the purpose of finding antimicrobial compounds as consequence of the increasing rate of antibiotic resistance which demand new drug products to combat them. In this sense, the isolation and characterization of new microbial strains producing these metabolites is an important and attractive area for research, especially the study of the actinomycetales traditionally known as an important antibiotic source very abundant in the soil which can isolate,[127] just as marine and endophytic fungi with antibacterial and antifungal activities (Table 7).[125–128]

Production of enzymes

Non photosynthetic mesophile microorganisms constitute an important source of enzymes of wide application in the chemical, textile, paper, food and pharmaceutical industry. Enzymatic applications include the sugars production mainly from cellulosic wastes for the further fermentation and conversion in biofuels, organic acids, antibiotics and other enzymes (Table 6);[108–116] on the other hand, the whey is an important byproduct from cheese manufacture due to the presence of bioactive peptides (antihypertensive, antioxidant, antithrombotic, immunomodulatory, antibacterial), which can be utilized as functional ingredient in the food or pharmaceutical industry through the employ of microbial proteases.[110]

In this sense, the commercial and native species of the *Aspergillus* genus have been very studied; such is the case of the production of glucose oxidase from *Aspergillus niger* whose applications include preparation of glucose assay kits[135] and the development of antioxidant additives in the food preservation.[136] This enzyme is industrially used, so the studies leading to the optimization of culture media to improve its production is one of the objectives of research.[137–139] *A. niger* is also a producer of Hydrolase (β-1,4-Endoglucanases, α-amylases, glucoamylases, xylanases and phytase) that can be incorporated in the monogastric animal diets as enzymatic additives to improve their feed conversión (Table 6).[109]

Agroforestry applications

Biofertilization and biocontrol are important biotechnological applications in the agricultural sector. Both biotechnological processes bring significant benefits to agriculture since it means the decline in the application of chemical inputs highly polluting for the environment or harmful to human health; likewise, it represents a contribution to agroecological practices with emphasis on sustainable agriculture. In this sense, the use of commercially known and native microorganisms in important crops from the production and consumption point of view around the world has been research (Table 8).[140–158]

Table 8 Investigations realized on non-photosynthetic autochthones microorganisms: agrobiotechnology

Microorganisms (sources)	Researchs
Soils: *Bacillus thuringiensis*	Pest biocontrol, chitinases production[140–146]
Cacao plantation: Entomopathogenic fungi	Pest biocontrol[147]
Rhizosphere: wild strain *Pseudomonas fluorescens*	Biocontrol and biofertilization (gluconic acid production)[148]
Soil and rabbit manure: diazothrophics bacteria phylogenetically related with *Burkholderia vietnamiensis* and *Paenibacillus sabina*	Nitrogen fixation[149]
Root nodules of *Calopogonium* sp.: *Rhizobium spp.*	Nitrogen fixation[150,151]
Rhyzosphera and roots of rice plants (*Oryza sativa*): Plant growth-promoting bacteria	Nitrogen fixation, inorganic phosphate[152] solubilizing and auxins production
Bog-iron ore: Pantoea, Burkholderia, Serratia, Ralstonia y Enterobacter	Inorganic phosphate solubilizing[153–155]
Rhyzospheric soil and roots of wild and cultivated plants: plant growth-promoting bacteria (Gram-negatives)	Nitrogen fixation and inorganic phosphate solubilizing[156,157]
Rhyzospheric soil and roots of dry forest plants: *Arbuscular mycorrhizae*: Glomus, Acaulospora, *Scutellospora y gigaspora*	Degraded forest recovery[158]

Biopesticides

Bacillus thuringiensis (producer of cry toxins) and *Beauveria bassiana* are the most known and used species, they are employed in several countries including Venezuela for the control of insect pests of agricultural crops as well as of those considered biological and mechanical vectors of human and animal public health interest. In this sense, strains adapted to the conditions of the Venezuelan tropics have been isolated and selected.[140–147] On the other hand, several rhizobacteria of wild and cultivated plants interact with various microorganisms exhibiting chemical defense mechanisms such as the production and excretion of gluconic and 2-cetogluconic acids that acidify the soil and in this way they inhibit the growth of pathogen microorganisms with the additional advantage of dissolving the inorganic phosphorus of soil.[148]

Biofertilizers

Rhizobacterial and endophytic microorganisms have shown several benefits in addition to protection against pathogens, such as the production of the plant growth-promoting substances and the supply of essential nutrients. In the latter case, the fixation of atmospheric nitrogen, inorganic phosphate solubilizing and production of siderophores stand out; for this reason, the study of plant growth promoting strains is relevant to the soil fertility so the formulation and application of inoculants with these characteristics could optimize the nutritional capacity of them and promote the ecological agriculture.[149–157]

Among the different food items produced in Venezuela, legumes and cereals such as the rice (*Oryza sativa*) and the corn (*Zea mays*) are very important for its nutritional value.[152,156,157–160] Additionally,

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legumes are interesting fodder; as a result, some researchers have prioritized the pursuit of plant growth-promoting bacteria whose most known and studied species belong to the *Rhizobium* genus, an endosymbiotic bacteria associated to the radical structures of legumes, crops that enrich the nitrogen content of soil, thus increasing its fertility.[150,151] With this interest, Venezuelan researchers have explored the microbiodiversity associated with the rhizosphere and roots of these crops finding several strains that expand the spectrum of this kind of agrobiotechnology local resource as a source of inoculants.[161–169]

Environmental biotechnology

The bioremediation of polluted envorinments with xenobiotic compounds especially by petroleum hydrocarbon is one of the most important biotechnological applications of microorganisms in environmental matters; as a consequence, the aromatic compounds acquire relevance because they are partially responsible for the low combustion of hydrocarbons, in addition to being highly recalcitrant, carcinogenic and mutagenic compounds persisting in contaminated environments.[170–181] like this, photosynthetic and non-photosynthetic microorganisms are equally important in the environmental recovery, however, the first have been studied for their potential on the heavy metals biosorption[82–91] while the latter are evaluated for the bioremediation by their ability to biodegrade hydrocarbons and their derivatives (Table 9). Despite this capability, microalgae may be sensitive to these chemicals pollutants in aquatic environments which affect the growth and pigment synthesis, reason for which they are used to carry out ecotoxicological studies (Table 5).[176,178,179]

Table 9 Investigations realized on non-photosynthetic autochthones microorganisms: environmental and mining biotechnology

Microorganisms (sources)	Researchs
Agricultural soils and coal mining activity soils: degrading pesticides bacteria (*DDT and parathion)	Bioremediation[161,162]
Oil pit: (1) Gram-negative strains: *Pantoea agglomerans*, *Sphingobacterium thalpophilum* , *Actinobacillus* sp. (2) Gram-negative mixed bacterial culture	Bioremediation[163–166]
Soils by PH: (1) *Pseudomonas* sp. (2) *Bacillus sp., P. aeruginosa,*	Biosurfactants production[167–169]
Polluted Soils/sediments by PH: (1) *Staphylococcus spp., Micrococcus spp.* (2) *P. alcaligenes*	Bioremediation[170,171]
Polluted Waters by PH: (1) *Serratia, Alcaligenes, Vibrio, Aeromonas, Pseudomonas,Morococcus, Acinetobacter, Flavobacterium, Enterobacter, Citrobacter.* (2) *Escherichia coli.* (3) Bacterias sulforreductoras: *Desulfovibrio desulfuricans, D. vulgaris, D. termitidis.* (4) *Pseudomonas aeruginosa, P. stutzeri, P. fluorescens, P. syringae.*	Bioremediation[172–174]
Hydrocarbon mixture wastes: Consortium: *Pseudomonas sp.* and *Serratia sp.*	Bioremediation[175]
Polluted Environments by PH, white rot and biterness of *Aloe vera*: *Aspergillus oryzae, A. nidulans y A. versicolor*	Bioremediation[176]
Iron ore associated microflora: *Aspergillus niger* HNA-1	Bioleaching[177]

*DDT, dichlorodiphenylthrichloroethane.

Hydrocarbonoclastic bacteria have shown that they can be used not only in bioremediation processes but also as agents of improvement of the properties of heavy or extra-heavy oil (viscosity, density, and API grades) to optimize its extraction, transport and refining, and hence obtain high economic benefits;[180] in this case, an alternative involves the utilization of thermophilic enzymes for its greater stability at high temperatures.[181] Moreover, the improvement may include the use of sulfur-reducing bacteria to eliminate the sulphur present in the oil that is released during its combustion in the form of highly pollutant sulphur dioxide which is one of the components in acid rain.[24]

Bioremediation

Hydrocarbons biodegradation is usually accompanied by the production of surfactants that emulsify these hydrophobic substrates improving its bioavailability and consequently the contact between the bacteria and the oily phase; the best known surfactants were obtained of the *Pseudomonas* genus some of whose species have been isolated from soils, sediments and water contaminated with oil from Lake Maracaibo (Zulia State);[167,168,171,175] however, it can also be produced by bacteria of the *Bacillus* genus when grow in soluble substrates.[169]

One of the objectives includes the study of catabolic routes of some aromatic compounds in species such as *Pseudomonas putida*;[180] this information is useful for the microbial consortia design in the bioremediation processes development of hydrocarbon polluted environments which involves strains producing biosurfactants as *P. aeruginosa*.[182] The utilization of these bacterial or fungal consortia (natural or artificial) in bioremediation processes tends to be more efficient in the degradation of hydrocarbons than the axenic cultures.[166,175, 176]

In the same line of investigation, some authors have explored contaminated waters and soils by petroleum in Zulia State in search of aromatics-degrading bacteria and resistance to heavy metals (mercury, chromium, cadmium and nickel) in order to determine its potential for bioremediation.[170,172,174] However, despite having obtained promising results, is also cause for alarm because isolates including important pathogenic species for man with implications for public health which have shown resistance to antibiotics tested.[7]

On the other hand, it was evaluated the use of sulfur-reducing bacteria for the recovery of environments contaminated by industrial effluents which contain high concentrations of toxic sulfates to living organisms; this alternative have ecological advantages and low cost. Studies for the evaluation of these bacteria include *Desulfovibrio desulfuricans, D. vulgaris* and *D. termitidis* isolated from Maracaibo Lake which additionally can remove heavy metals.[147] With the same aim, González & Wilkesman[24] determined the oxygenase activity to degrade dibenzothiophene as carbon and energy source in thermophilic strains isolated from the hot springs at Las Trincheras.

Mining: bioleaching

The high content of phosphorus in iron ore (>0.08%) raises industrial costs for its dephosphorization in obtaining high quality derivatives; as a result, it produce large amounts of ore rejected producing an important environmental liability. In this sense, may be

Citation: Piñero–Bonilla J. Approach to knowledge of the microbial bioprospecting in Venezuela. *J Appl Biotechnol Bioeng.* 2019;6(1):1–14.
DOI: 10.15406/jabb.2019.06.00168

applicable biotechnology alternatives that consist of iron ore leaching to reduce the phosphorus content using axenic cultures of *Aspergillus niger* HNA-1 isolated from the iron ore associated microflora (Table 9).[177]

Conclusion

Documentary search based on the AsoVAC memories is an approach to the research lines developed by the Venezuelan investigators on bioprospecting; thus, it underestimates the work in microbial biotechnology carried out by some institutions,[183] especially in non-photosynthetic microorganisms. In this regard, the consultation of other documentation sources demonstrates this investigative work, for example, Institutional Repository Saber ULA[184] where it can find information published by researchers of the Laboratory of Microbial Biotechnology (Faculty of Sciences). In general, papers in microbial biotechnology of the AsoVAC Convention between 2002 and 2017 have applications in different areas concentrating most of the studies on the environmental biotechnology, agricultural and food production.

In this sense, the agroecological crop management should the most immediate potential application through the formulation of biocontrol and biofertilization supplies considering that food production is a priority area for Venezuela and due to the indigenous microbial richness found in the country that can replace imported commercial sources. Within this priority area, the implementation of photosynthetic organism cultures is feasible as live or processed food destined to aquaculture or for human consumption; this practice would directly benefit existing populations along the Venezuelan coasts. In addition, photosynthetic organisms have added value as a source of important bioactive substances and functional foods.

On this aspect, there are few algae-producing countries in Latin American to obtain products for commercial purposes, but Venezuela is not one of them;[185] however, in this country there is a large volume of information about indigenous photosynthetic microorganisms that offers the opportunity to evaluate more ambitious projects within the bioeconomy, such as the biorefineries in which microalgae and cyanobacteria biomass can integrally be utilized under matter and energy cyclic flows that generates small amounts of waste and gaseous emissions; in fact, it can promote the mitigation of greenhouse gases when attaching the production processes to the CO_2 biofixation Nevertheless, this work has big economic, technological and legal challenges.[186,187]

In terms of the legal challenges to the development of certain biotechnology areas, the obtaining of transgenic organisms is one of them. In this context, the article 9 of the law of seeds of Venezuela prohibits "the production, import, marketing, distribution, release, use, multiplication and entry of transgenic seeds".[188] Consequently, this regulation sets limits to biotechnology research, particularly in the agricultural field which has led to differing views between the Venezuelan investigators, both in favor and against GMOs.[189] However, the research of *Guglielmo-Croquer et al.,*[190] prior to the adoption of this law in which assessed the genetic improvement of coffee (*Coffea arabica*) through the introduction of the gene cry1ac (endotoxin) of *B. thuringiensis* to confer resistance to the pest *Leucoptera coffeella* does not contradict the opinion of some investigators who are in favor of the GMOs development from Venezuelan native materials.[189]

Finally, biotechnology applications have several implications in environmental decontamination, one of which involves the use of agroindustrial wastes of low or no commercial value for production of protein-rich foods intended for animal consumption. On the other hand, despite numerous investigations on the use of microorganisms on bioremediation of petroleum hydrocarbon polluted environments, more studies are required for its implementation, especially in the biosecure consortia design. However, in aquatic environments such as Maracaibo Lake, it adds the pollution with industrial, hospital and domestic waste water, a source of pathogens to man requiring additional controls as well as the recovery with hydrocarbonoclastic microorganisms.

Climate change together with the general deterioration of the terrestrial and aquatic ecosystems by pollution are altering the microbial community structure-function relationships and destroying beneficial populations to give way to those harmful to humans and other forms of life on Earth.[4,8] This microbial loss also affects biotechnology opportunities to reduce the richness of this resource which has to develop important biological processes. This scenario reveals the importance of microbial collections which constitute an important resource for the research development giving them as Emerson and Wilson say "a home to the microbial diversity".

Acknowledgments

To the Chapter Merida of the AsoVAC for providing me the printed proceedings not available in digital format of the Annual Convention.

Conflicts of interest

The author declares that there is no conflict of interests regarding the publication of this review article.

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Citation: Piñero–Bonilla J. Approach to knowledge of the microbial bioprospecting in Venezuela. *J Appl Biotechnol Bioeng.* 2019;6(1):1–14.
DOI: 10.15406/jabb.2019.06.00168

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