Significance and management of acetic acid bacteria culture collections

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Abstract

Acetic acid bacteria (AAB) are obligate aerobic microorganisms which have large significance in human life. Traditionally, AAB species have been used to produce fermented food and beverages thanks to their ability to oxidize ethanol to acetic acid. Moreover, in the last decades, they have been extensively investigated for other industrial biotechnology applications as the development of processes for high-value products or biosensors. The potential exploitation of AAB diversity requires the existence of microbial culture collections, which are able to supply not only strains but essential data for fundamental microbial research. Therefore, microbial collections can be helpful to provide critical insights into AAB physiology and metabolism as well as integrate sequence data with transcriptional and functional studies to better define complex traits and develop new potential microbial processes.

This article reviews the significance of microbial collections, with an overview of the well-known European Biological Resources Centers (BRCs) collecting AAB, and provides an insight into their cultivability and metabolic activity. It also discusses appropriate techniques in preserving *authentic* strains, quality control implications, databases and BRC networking as well as connections among collections and stakeholders.

Introduction

Microbial culture collections are fundamental biological resource centers able to supply more than just strains but essential data related to the genome, transcriptome, proteome and metabolome of the microorganisms collected. In a few words, culture collections hold biological material and related information, the first is meaningless without the second. They can offer services helpful for several purposes such as research, teaching, screening assay, knowledge-based bioeconomy as well as biotechnological applications. Generally, microbial collections have the crucial role to maintain and provide the *authenticated biological material* which can include microorganisms as well as associated genomic materials (*e.g.* purified DNA, DNA sequences, cloned genes, plasmids and vectors). Additionally they maintain microorganisms cited in scientific papers that can be used for further study, can serve as repositories for strains as patent deposits and providers of safe and confidential services to store key organisms for research and industry.[1]

The demands upon microbial collections can be different according to the type of microorganisms collected and their metabolic potential. Certainly culture collections and biotechnology are strongly connected, since the microorganisms maintained by them can be used as biocatalysts or sources of compounds with great relevance in research and industry. Furthermore, microbial collections have greatly contributed to the development of many techniques over time, such as cell culturing, recombinant DNA, polymerase chain reaction (PCR) techniques, DNA shuffling, site-directed mutagenesis, metabolic engineering and artificial enzyme synthesis, since they supply the *biological tools* for biochemists and molecular biologists.[2]

Acetic acid bacteria (AAB) are a group of aerobic microorganisms, with 13 genera actually recognized (*Acetobacter, Acidomonas, Ameyamaea, Asaia, Gluconacetobacter, Gluconobacter, Granulibacter, Kozakia, Neoasaia, Neokomagataea, Saccharibacter, Swaminathania, Tanticharoenia*), as reported in the List of Prokaryotic names with Standing in Nomenclature (http://www.bacterio.cict.fr Accessed 2012 July 6), which have large significance in human life. Traditionally, AAB strains have been utilized to produce fermented food and beverages, especially several kinds of vinegar, thanks to their ability to oxidize ethanol to acetic acid. Moreover, due to their metabolic uniqueness, they have been extensively investigated for production of high-value chemical products for biopharmaceutical industry. Nevertheless new insight on AAB has been recently gained in relation to the existence of some species associated with human diseases.[3-5]

The potential exploitation of AAB strain diversity requires the existence of culture collections, where successful cultivation methods as well as appropriate tools in preserving *authentic* strains are screened in order to overcome the greatest hurdles related to AAB growth in laboratory conditions. Nowadays there is a huge number of culture collections around the world. The World Data Centre for Microorganisms (WDCM; http://www.wfcc.info/ccinfo/statistics Accessed 2012 July 20) reports more than 2 million strains of a wide range of microorganisms maintained in 622 culture collection centers in 71 countries. Many public service collections have evolved into Biological Researches Centers (BRCs) which hold subcultures of *original strains* and guarantee accessibility and transparency of supply, according to all relevant regulations required by the Convention on Biological Diversity (CBD).[6]

The majority of AAB strains are maintained in well-known microbial collections established in different countries such as: the American Type Culture Collection (ATCC) in USA, the Biotec Culture Collection (BCC) in Thailand, the National Collection of Industrial Microorganisms (NCIM) in India, the Korean Collection for Type Cultures (KCTC) in Korea, the Japan Collection of Microorganisms (JCM) and the NITE Biological Resource Center (NBRC) in Japan. Considering just the European scenario, AAB strains are held in a relatively small number of BRCs (Table 1). Moreover, AAB strains are also kept in other collections some of them *non-affiliated* in official organizations, including those at universities, research institutes or companies, where knowledge of their existence is mainly due to publications, conferences or just personal contacts. These collections have usually been gathered for special purposes or specific project and sometimes they are strictly dependent on research targets, resources and facilities as well as specific interests of the scientists involved.

Among these, the Food and Industrial Microbial (FIM) Collection of the Department of Life Sciences, University of Modena and Reggio Emilia, was established in 1999. It has improved over time through the activities of the food microbiology group, aiming to maintain and supply acetic acid bacteria, lactic acid bacteria and yeast strains for research as well as implementing linkages between academia and companies. Currently, FIM Collection holds about three thousands strains isolated from several matrices and, specific to this collection are about 300 AAB strains isolated from musts, wines, different kinds of vinegar and *kombucha tea*.[7-9]

Cultivability and metabolic activity of acetic acid bacteria

Several AAB are able to entry into a viable but non-cultivable status[10,11] and a task for microbial collections is to overcome drawbacks related to difficulties in cultivation and the validation of appropriate methodology for maintenance of the cultures. Actually, several media, mostly consisting of the same ingredients differently proportioned, have been developed and tested to obtain successful isolation of different AAB from specific matrices as they can have different requirements from one strain to another. For instance GYC medium (10% glucose, 1% yeast extract, 2% $CaCO_3$) has been successful used for recovering of osmotolerant AAB strains from high sugar sources such as traditional balsamic vinegar.[7] Moreover media with the addition of acetic acid and ethanol as AE (0.5% glucose, 0.3% yeast extract, 0.4% peptone, 3% (v/v) absolute ethanol, 3% (v/v) glacial acetic acid) and RAE (4% glucose, 1% yeast extract, 1% peptone, 0.338% $Na_2HPO_4 \times H_2O$, 0.15% citric acid, 2% (v/v) ethanol, 1% (v/v) acetic acid) are particularly recommended for the cultivation of strains isolated from spirit vinegar fermentations which are able to produce high acetic acid concentration up to 15%.[12,13] However these media containing ethanol are not suitable for preservation as it can affect the viability of cells during long-time storage at +4°C.[14] Culture collection databases generally report the recommended media for cultivation of strains as well as the specific incubation conditions. In Table 2 the main culture media have been described according to recipes indicated in online databases of the European BRCs col-

Table 1. European Biological Resources Centers collecting acetic acid bacteria.

Acronym	Name	Country	Website URL
CIP	Collection de l'Institut Pasteur	France	http://www.crbip.pasteur.fr
BCCM/LMG	Belgian Coordinated Collections of Microorganisms/LMG Bacteria Collection	Belgium	http://bccm.belspo.be/index.php
CECT	Colección Española De Cultivos Tipo	Spain	http://www.cect.org
DSMZ	Deutsche Sammlung von. Mikroorganismen und Zellkulturen GmbH	Germany	http://www.dsmz.de/
NCCB	Netherlands Culture Collection of Bacteria	Netherlands	http://www.cbs.knaw.nl/nccb
NCIMB	National Collection of Industrial, food and Marine Bacteria	United Kingdom	http://www.ncimb.co.uk
VTTCC	VTT Culture Collection	Finland	http://culturecollection.vtt.fi/

Table 2. Main culture media for acetic acid bacteria growth.

Components (g)*	AG	GY (1)	GY (2)	GYC (1)	GYC (2)	YPG	YPM
Glucose	1.0	50	20	100.0	50	70	-
Yeast extract	5.0	5	10	10.0	10	10	5.0
Peptone	5.0	-	-	-	-	10	3.0
CaCO3	7.0	-	-	20.0	30.0	-	-
Mannitol	-	-	-	-	-	-	25.0
Glycerol	1.5	-	-	-	-	-	-
Malt extract	2.0	-	-	-	-	-	-
Agar	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Distilled water (mL)	1000	1000	1000	1000.0	1000	1000	1000.0
pH	6.8	6.5	/	6.8	/	6.0	/

*According to culture media recipes reported in online databases of the European Biological Resources Centers collecting acetic acid bacteria (see Table 1 for the websites). Accessed 2012 July 6.

lecting AAB. Important strains are required to be deposit in different collections and more than one medium can be suggested for their recovery and subculturing; an example for the AAB strains, referred to as type species, has been reported in Table 3.

A common metabolic activity to AAB is the incomplete oxidation of alcohols or sugars. Most of them, except *Asaia* sp., are capable of oxidising ethanol to acetic acid, however *Saccharibacter* sp. and *Granulibacter* sp. may produce only negligible or very little acetic acid from ethanol. Furthermore, some AAB are able to carry out the oxidation of acetic acid to CO_2 and H_2O. Specifically, the overoxidation of acetic acid is a feature of *Acetobacter* (*A.*), *Gluconacetobacter* (*Ga.*) and *Acidomonas* strains whereas it is lacking in *Gluconobacter* (*G.*), *Saccharibacter* and *Neoasaia* and weak in *Asaia*, *Kozakia*, *Swamina-thania*, and *Granulibacter*.[15]

Different AAB strains show variation in their metabolism of individual carbohydrates, but those most preferentially used are glucose, galactose, mannose, ribose, xylose, arabinose, erythritol, mannitol, and sorbitol.[16] Species of *Acetobacter* and *Gluconobacter* lack a functional Embden-Meyerhof Parnas pathway and, consequently, are unable to metabolize hexose sugars by this route.[17] Hexose and pentose sugars, after initial phosphorylation, are oxidatively metabolized by the hexose monophosphate pathway to acetic and lactic acids which, in the case of *Acetobacter* species, can be further oxidized to carbon dioxide and water *via* the tricarboxylic acid cycle. The carbohydrate metabolism of *G. oxydans* has been extensively investigated since its incomplete oxidation of various sugars and sugar alcohols leads to the accumulation of industrially significant substances, such as sorbose, dihydroxyacetone, gluconic acid and ketogluconic acids in the culture medium.[17-20]

Two types of enzyme systems, which differ in location and function within the cell, are capable of dehydrogenation reactions,[21] consequently sugars, alcohols and polyols are oxidized *via* two alternative pathways.[16] The first type of enzyme systems involve dehydrogenases located in the cytoplasmic membrane that catalyze the nonphosphoryla-tive oxidation of sugars, polyols, aliphatic and cyclic alcohols to almost quantitative yields of oxidation products which are easily released into the medium *via* porines in the outer membrane.[21,22]

The second type of enzyme systems is characterized by NAD(P)$^+$-dependent enzymes, which are believed to participate in the synthesis of biosynthetic precursors and are involved in the maintenance of cells in the stationary growth phase.[21] The substrate oxidized and the resulting intermediates are phosphorylated and further metabolized *via* the pentose phosphate pathway.[20]

Preservation techniques and quality control: implications and strategies

AAB preservation in culture collections has the primary purpose to maintain them in a viable state without morphological, physiological or genetic change. Nevertheless, marked genetic instability has been observed in these bacteria, which causes spontaneous mutations at high frequencies leading to deficiencies in various physiological properties, such as acetic acid resistance and ethanol oxidation.[23] An insertion sequence element, IS1380, was identified as a major factor of the genetic instability, which causes insertional inactivation of the gene encoding cytochrome c, an essential component of the functional alcohol dehydrogenase complex.[24] In addition, an insertion sequence, named IS1452, in the ADH subunit III *(adhS)* gene was found to cause mutations at high frequencies in spontaneous *A. pasteurianus* mutants, deficient in ethanol oxidation.[25] Despite genetic background of the AAB instability is still not well investigated, it has significant influence on industrial use of these microorganisms. Due to the existence of hypermutable AAB isolated from nature,[26] continuous growth techniques are not recommended for preservation but mainly used just for short-term maintenance of the strains. In fact these techniques, which initially provide optimum growth conditions and involve frequent transfer from depleted to fresh nutrient sources, usually allow deterioration and loss of properties during storage.[27] Long-term maintenance of the strains

Table 3. Strains referred to as type species of AAB collected by European Culture Collections.

Species	Type species*	Recommended culture media°
A. aceti	NCIMB 8621	GY (2), GYC (2)
	CIP 103111/ DSM 3508	YPM
	CECT 298/ VTT E-011873/VTT E-042567/ VTT E-97828	GYC (1), YPM
	LMG 1261/ LMG 1504	GYC (2), YPM
	NCCB 23001	YPM
G. oxydans	NCCB 23002/ NCCB 75005	GYC (2)
	NCIMB 9013	GY (2), GYC (2)
	VTT E-89365	GYC (1), YPM
	LMG 1408	GYC (2), YPM
	CIP 103106/ VTT E-97864/DSM 3503/ DSM 7145	YPM
Ga. liquefaciens	NCIMB 9136	GY (2), GYC (2)
	DSM 5603	GYC (1), YPM
	CIP 103109	YPM
	LMG 1381/ LMG 1382/ NCIMB 9136	GYC (2), YPM
Sa. floricola	LMG 23170	GY (1)
	DSM 15669	YPG
K. baliensis	DSM 14400	AG
	LMG 21812	GYC (2)
Sw. salitolerans	LMG 21291	YPM
A. bogorensis	LMG 21650/ NCIMB 13692	GYC (2)
N. chiangnaiensis	LMG 24037	YPM

*According to the List of Prokaryotic names with Standing in Nomenclature and StrainInfo.net. Accessed: 2012 July 6; °Culture media receipts are reported in Table 2.

can be achieved by techniques that are able to suspend the metabolism. Generally, these techniques involve reducing the water content available to cells by lyophilization (freeze-drying) or cryopreservation.

Freeze-drying techniques

Freeze-drying is one of the most widely used methods for long-term preservation of bacterial cultures and other microorganisms[28] (Figure 1). It is a complex process that involves the removal of water from a frozen product by sublimation.[29] The moisture remaining in freeze-dried cells is termed residual moisture. A residual moisture content of 1-3% (w/v) seems to be required for the long-term shelf life of bacteria.[28] Freeze-drying is the preferred method for transporting and storing microbial cultures as it offers many advantages over other preservation techniques, including the total sealing of the specimen and protection from infection and infestation. Cultures generally have good viability/stability and can be stored for many years. Ampoules take up little space and can be easily stored.[27] However, the method shows also some disadvantages with some isolates which fail to survive the process or have reduced viability and may show genetic change.[30] Moreover cell damage can occur during the cooling and/or drying stages because of the phase changes that can disrupt the fluid-mosaic structure of the membrane. During these processes, in fact, the liquid crystalline structure of the cell membranes may degenerate to the gel phase, causing leakage of the membranes.[27] To avoid this, suitable cryoprotectants, such as glycerol, dimethyl sulfoxide (DMSO), polyvinylpyrrolidone, skimmed milk, dextran, inositol, malt extract, mannitol, raffinose, sucrose and trehalose, have been tested at different concentrations to improve strain viability. The optimal cell concentration for freeze-drying is generally related to the protective medium used; usually, a cell concentration of 1×10^8 cells mL^{-1} can be considered appropriate, however, the higher the initial cell concentration, the longer viable cells will survive within the freeze dried sample.[31]

Several authors have evaluated the influence of cryoprotectans upon AAB survival during freeze-drying and subsequent storage when selecting a freeze-dried starter culture for vinegar making.[12,32] *Acetobacter* strains were preserved by freeze-drying for about 1 year without any loss of their original properties as starter by using 20% malt extract during the lyophilization process.[8] Nomura and co-workers[33] prepared freeze-dried cells of *A. aceti* to use them as an additive for manufacturing and processing foods. They found that the addition of 30% sucrose to the cell suspension prepared in a McIlvaine buffer (pH 6) before lyophilization was effective for preventing the decrease of the aldehyde oxidase activity during preparation and subsequent storage. Moreover, two thermoresistant AAB strains, belonging to *A. senegalensis* and *A. pasteurianus* were preserved by the freeze-drying process carried out using 20% mannitol (w/w) as cryoprotectant. Mannitol was determinant to enhance biomass preservation during storage, in fact, freeze-dried cells could be conserved at 4°C for at least 6 months without loss of viability.[34] Unfortunately, a preservation method that is satisfactory for one strain may be unsuitable for others. Certainly, preservation protocols including cell growth conditions, suspension media, cryoprotectant, and cooling rates must all be optimized as the factors determining survival tend to be strain specific.[35] Factors affecting survival are becoming better understood at the subcellular level, but many strains from a wide range of species remain difficult to preserve.

Cryopreservation techniques

When lyophilization is not amenable for suitable cell preservation, cryopreservation techniques can be considered valuable. Cryopreservation, usually, involves storage at temperatures of around -70°C in a mechanical freezer and, more advisable, at −196°C in liquid or circa -150°C in vapor phase of nitrogen[28] (Figure 2).

Generally, vapor-phase storage is more convenient and safer for regular access to stored material than liquid-phase storage. Furthermore, some manufacturers (*e.g.*, CBS distributed by PhiTech Intl., Milton Keynes, UK) are now producing vapor-phase storage systems in which liquid nitrogen is retained in the vessel walls, which improves safety and appears to provide a temperature profile superior to standard vapor-phase systems.[36]

Figure 1. Freeze drying equipment [photo by kind permission of CABI Microbial Service (Egham, Surrey, UK)].

Figure 2. Cryo-storage in liquid nitrogen tanks [photo by kind permission of CABI Microbial Service (Egham, Surrey, UK)].

The effectiveness of microbial cryopreservation can be affected by several factors, for example, species, strain, growth phase and rate, growth medium composition, pH, osmolarity and aeration, cell water content, lipid content and composition of the cells, density at freezing, composition of the freezing medium, cooling rate, temperature and duration of storage, warming rate and recovery medium.[37] Furthermore, the freezing process has adverse effects on cells, since the removal of water during intracellular and extracellular freezing causes mechanical injury to the cell and changes the physical and chemical nature of the solutions within and around the cells.[38,39] For this reason the rate of cooling is extremely significant and it should be controlled. In general, the best results for preservation of bacteria have been achieved with slow cooling at 1°C min^{-1} over the critical phase.[27] This step can be controlled using a freezer which has an adjustable cooling rate. If a programmable control is not available, slow cooling can be obtained by placing the filled glass or plastic vials in a stainless steel pan at the bottom of a mechanical freezer at -60°C for 1 h in order to achieve the rate of cooling of approximately -1.5°C min^{-1}. Then, if the frozen vials have to be stored in liquid or vapor phase of nitrogen, they can be plunged into a liquid-nitrogen bath for 5 min before transferring in liquid-nitrogen tank.[28]

Good results for microbial preservation can also be achieved using a commercially available container, *Mr. Frosty* System (Nalgene, Rochester, NY, USA). The container is filled with isopropyl alcohol at room temperature; this provides a slow rate of cooling of cells, close to the ideal of -1°C min^{-1}, when placed at -80°C.[40]

It has been reported that the best recovery of the cryopreserved cultures is obtained by rapid thawing since slow warming may cause damage owing to the recrystallization of ice. A rapid rate of warming can be achieved by quickly immersing the frozen vials in a 37°C water bath with moderate agitation until all the ice melts.[27,28]

Several researchers have attempted to optimize cryopreservation processes through approaches that circumvent damage caused by water crystallization. Most improvements have been obtained using cryoprotective agents. Generally, glycerol and DMSO, which can penetrate the cells, contrary to other cryoprotectants, have proven to be the most effective for a wide range of bacteria, since they are able to provide both intracellular and extracellular protection against freezing.[28]

However, they should be used carefully because of their harmful toxicity at high concentrations.[39] DMSO and glycerol are routinely used at final concentration of 5% (v/v) and 10% (v/v), respectively in the proper suspending medium.[28] Nevertheless, higher concentrations of glycerol (25% v/v) have been reported to be successful for AAB cryopreservation.[41,42] Recently, the stability of *A. pasteurianus* strain AB0220 over 9 years of preservation has been investigated. The phenotypic and genotypic data on the strain subcultures preserved for long time, at −80°C with glycerol, have confirmed the suitability of the method for the *authentic strain* maintenance.[43] Nevertheless studies performed on the preservation of *Ga. xylinus* have revealed the influence of cryoprotective agents on cellulose production. In particular the use of glycerol or skimmed milk was not recommended as both altered the structure of cellulose produced by *Ga. xylinus* and influenced the bacterial metabolism. On the contrary, freezing in suspension with DSMO guaranteed high survival rates and no determinable influence on cellulose structure and production.[44]

Validation of preservation techniques and quality control requirements

In compliance with the Organization for Economic Co-operation and Development (OECD) best-practice guidelines (http://www.oecd.org/dataoecd/7/13/38777417.pdf), microorganisms should be preserved preferably by two different methods and as master cell banks and as stocks for distribution. Where two distinct methods are not applicable to the biological material, cryopreserved stocks should be maintained in separate locations. To avoid the risk of losing significant strains, their deposit in a different collection is strongly advised, especially strains cited in publications, or, more generally, used in research so that they will be available to the international scientific community in both academic and industrial institutions. Moreover BRCs, among several services, can offer safe deposit of strains where their distribution is restricted at the discretion of the depositor. Furthermore culture collections recognized as *International Depository Authority* (IDA) can hold strains associated with patents under the Budapest Treaty. Culture collections can often apply specific protocols for cultures preservation, which are generally described in their own databases or reported in publications. However, validation of preservation techniques between collections is still not common and, for this reason, recent projects have taken this important aspect into consideration.[45] Quality control of the preserved cultures is fundamental to validate the methodology used. BRCs are required to carry out quality controls on the received material, at least three occasions: upon receipt of the original material, after preservation of the first batch of samples, and after each subsequent new batch preservation.[6] Specifically, quality control is performed in order to assess: viability of the culture on the medium/media recommended, purity, authenticity (conforms to the description provided by the depositor) and properties confirmed by phenotypic, metabolic and molecular characterization.[6] Usually 16S ribosomal RNA gene sequencing is helpful for bacteria species identity, however, for some AAB, which show high 16S rRNA gene sequence similarities, other methods as multilocus sequence analysis can be more adequate for species differentiation in the family *Acetobacteraceae*.[46] Moreover, several DNA *fingerprinting* methods, which may produce strain-specific banding patterns are more sensitive and suitable to detect any genetic drift, changes or deterioration during preservation. Examples for these methods are: amplified fragment-length polymorphism PCR (AFLP), pulsed field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD) analysis, REP-PCR (repetitive extragenic palindromic-PCR), ERIC-PCR (enterobacterial repetitive intergenic consensus sequences-PCR), BOX-PCR (derived from the *box*A element), $(GTG)_5$-PCR and ribotyping.[47] A major disadvantage of some of these fingerprint-based methods is that the results are often very difficult to compare when they have been obtained in different laboratories due to the lack of standardization. A key requirement of the techniques chosen is that they are reproducible.[47,48] All the tests performed need to be made according to documented procedures and the results need to be recorded and retained for future reference.

Databases and Biological Resources Centers networking

Nowadays almost all BRCs have open catalogue databases available online with advanced search functionality. The actual level of detail contained within catalogues depends on the governance of the individual culture collection. Some catalogues only state minimal information on the strains *e.g.* strain number, species name and basic information about the depositor, whereas other BRCs offer detailed information on the origin of the culture, its history, its isolation, references to scientific literature and sequence information.[49]

In order to standardize and integrate the information among different culture collection databases, several initiatives have been designed from various BRCs. Among these, the Common Access to Biological Resources and Information (CABRI; http://www.cabri.org/guidelines.html) offers world-wide access to catalogue databases of different types of organism, genetic materials and other biological in Europe and allows to simultaneous check on the availability of specific organisms or genetic resources. As a result, strains of organisms are supplied from member collections with traceability, conforming to national and international regulatory requirements. Another important initiative recently realized is the platform StrainInfo.net

(http://www.straininfo.net). This provides essential support for the exchange of microbial information and meta-information by using a Microbial Common Language (MCL).[49] Accordingly, strain designations, historic information on their deposit in BRCs, growth characteristics, genomics and other data, and relevant publications are shown to the user in an integrated way. This system has the potential to evolve into a global microbiological data-network of which the data can be directly used in scientific research, for example in combination with genomics for comparative studies.[50]

Two main culture collection organizations, the World Federation for Culture Collections (WFCC) and the European Culture Collection Organization (ECCO), act as connection among collections and users, to coordinate activities as well as to exchange information and technologies.[1]

Currently, a small group of well-established European collections are working together in a European Union (EU)-sponsored project, the European Consortium of Microbial Resource Centers (EMbaRC; http://www.embarc.eu), aiming to improve, coordinate and validate collection delivery to European and international researchers.[51] The goals are to ensure harmonization of the quality of microbial collections, taking the current OECD best-practice guidelines and emerging national quality standards for microbial resource centers to the international level. The consortium agrees that coordinated action is needed to store all relevant strains resulting from European research and that they need to work together to provide the capacity and expertise.[51] Furthermore the Microbial Resources Research Infrastructure (MIRRI; http://www.mirri.org) has been recently established with the purpose to provide services facilitating access to high quality microorganisms, their derivatives and associated data for research, development and application. It connects resource holders with researchers and policy makers to deliver the resources and services more effectively and efficiently to meet the needs of innovation in biotechnology.

The linkage between acetic acid collection and stakeholders

The increased knowledge on AAB physiological, biochemical and genetic properties has encouraged advancement in biotechnology and innovative research methods. For instance, food technology has been largely advantaged by using AAB as functional starter cultures in acidification process. Culture collections are fundamental tools for selecting the ideal culture for any particular food application as they can provide a large pool of candidates to find the best one. In this way they can support biotechnology companies to develop new starters, with required performance parameters, which are superior to the ones found in nature, by using mutant selection and genetic engineering. The optimization of AAB starter cultures for vinegar production has been extensively investigated.[52-54] Despite the several types of vinegar produced worldwide, selection criteria must always take in account the characteristics of the raw material, desired AAB metabolic activities, applied technology and desired characteristics of the final product.[14,55] In addition to vinegars, AAB are also involved in the production of other fermented beverages and food products (e.g. nata, kombucha and cocoa). Generally, the advances in enzyme technology help to a greater extent in the search for a biocatalyst with the desired specificity for industrial needs. Therefore, the characterization of microorganisms which are able to thrive in peculiar or in extreme environments has received great attention as a valuable source for novel enzymes.[56] AAB, being acidophilic microorganisms that grow in very specialized habitat might be of potential interest. Their extracellular enzymes might be highly stable at low pH and therefore could be particularly useful in acidic degradations and in the food industry.[57] Other interesting biotechnological applications of some AAB strains regard to their ability to produce exopolysaccharides (EPS), which may have beneficial effects on human health. Bacterial EPS generally have unique rheological properties caused by their high purity and regular structure and, therefore, they can be used as thickening, gelling, or stabilizing agents in the food industry.[58] Furthermore, bacterial cellulose has been extensively investigated for new materials useful for paper additives, diaphragm for headphones and artificial skin.[59,60] Among AAB, strains belonging to *Ga hansenii*, *Ga. intermedius* and *Ga. kombuchae* have been reported to be capable of producing bacterial cellulose, however, *Ga xylinus* is certainly the most efficient producer.[61-64]

Concerning *G. oxydans,* it is mainly used by biopharmaceutical industry for the production of high-value organic chemicals *e.g.*, precursors of vitamin C, precursors of miglitol, precursors of D-tagatose, dihydroxyacetone, D-gluconic acid, shikimate, L-ribulose and others.[20,65,66] In the last decades, nitrogen-fixing AAB strains belonging to *Gluconacetobacter, Acetobacter* and *Swaminathania* genera have been considered valuable for agriculture as a tool for improving crop performance and environmental conditions, since they promote plant growth as well potentially reducing or avoiding the use of chemical fertilizers.[67-69] Additionally, biosensor technology using AAB enzymes or whole-cells has been recently developed for testing and diagnosis in clinical, food, as well as environmental and agricultural fields.[57]

Conclusions

Acetic acid bacteria culture collection management requires dedication and expertise of the curators in different areas of microbial biology. The integration of sequence data with transcriptional and functional studies supplied from culture collections is essential to better define complex traits and optimize AAB biotechnological applications. Furthermore culture collections need to use high-level research and scientific services to meet the expectations of their customers and stakeholders in solving a variety of problems as well as exploiting the potential of AAB diversity. The essential role of culture collections and their significant contribution in AAB characterization, preservation as well as biotechnological application should be generally recognized and financially supported.

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