Bioprospecting Studies: Transforming the Natural Genetic Heritage into Biotechnological Richness

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Abstract The Brazilian microbiota has great potential richness for industrial use, given its mega-diversity. Despite the advances in international research that have provided access to such microbiota, via several approaches (metagenomics; second-generation DNA sequencing, in situ cultivation, and as a consequence high-throughput screening, etc.) a glimpse into the research output in Brazil demonstrates that such immense potential has been poorly explored. Even though the Brazilian scientific community has reached a degree of international excellence in research recognition, there is still strong centralization of knowledge and of biotechnology enterprises in the Southeast Region of the country, which greatly limits access to our multitude of biomes and ecosystems. Another problem is the lack of communication between the knowledge generation centers and practical efforts in the field, resulting in very little national intelligence reaching the consumer market. Consequently, the internal biotechnology market prioritizes imports, even though there are available domestic resources to generate competitiveness at a global level. Academic and industry integration initiatives through innovative agencies have demonstrated a path to bridge the gap between the "ownership" and the "usage capacity" of the country's rich microbial diversity.

Introduction

Brazil has a land mass of 8.5 million square kilometers, which makes it the largest country in South America. The country consists of 26 States and a Federal District, and contains five geographic regions. The great extent of the land reflects a variety

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of biomes that have enormous biodiversity. Thus, Brazil is a country that harbors mega-diversity, containing around 20% of the described species of the planet [1].

The diversity of the Brazilian biomes—Cerrado, Caatinga, Atlantic and Amazon forests, Pantanal, and Pampas [2]—allows the diversification of a variety of living forms. The considerable literature on the biodiversity of the country's plants and animals has outlined fundamental parameters that are followed for the management of conservation areas [3]. In contrast, studies considering microbial biodiversity are fewer and are still insufficient to understand the microbial biology and functional diversity of a particular environment [4]. Therefore, the great diversity of Brazilian microbial ecosystems represents a reservoir to be explored, and it may contain genes for new enzymes and products for biotechnological use.

The biomolecules produced by microorganisms may act as bioindicators of soil quality, and may be used for bioremediation. Also, biomolecules such as biopolymers have various uses, including their use as bioemulsifiers, while exopolysaccharides [5, 6] are used in medicine as drug carriers to deliver therapeutic molecules such as drugs and genes, and in chemotherapeutics as nanodrugs, and even for tissue engineering [7–9]. Brazil leads in studies of agricultural plant-bacterial interactions and in the selection of more efficient bacterial strains to fix nitrogen [10–12], mostly under harsh conditions. As a result, biological nitrogen fixation has been largely adopted in the country, promoting the replacement of conventional fertilization by inoculants, providing social, environmental, and economic benefits for the producer as well as for the consumer. For soybean the advantages of symbiotic nitrogen fixation are already well known, resulting in an annual revenue of US$7 billion for the country [13]. Soybean (*Glycine max*) and sugarcane (*Saccharum* spp.) are intensively cultivated throughout Brazil, thanks to the favorable soil and topological conditions, allowing the production of biofuels—biodiesel and bioethanol, respectively—both of which, with the great recent advances in production, use enzymes for their synthesis.

Biodiesel, when compared with fuels derived from oil, is less toxic and more easily degraded because it has fewer components, and it is considered a sustainable source of energy [14]. Oil/fat of animal or plant origin and a short-chain alcohol are used for its production, generating biodiesel and glycerol as by-products. The production of enzymes that use agroindustry by-products, with the aim of the further utilization of these enzymes for biofuel production is an alternative method to be considered, once the reduction of tailings and the economic feasibility of such procedures are considered [15].

In 2014, Raizen Energia S/A (Piracicaba, Brazil) and GranBio (São Miguel dos Campos, Brazil) started producing ethanol from sugarcane bagasse (second-generation ethanol) in Brazil, with the intention of producing it on a commercial scale. The enzymes involved were to be supplied by a Danish company (Novozymes, Bagsvard city, Denmark) that intends to build a plant in Brazil to better serve the needs of all three companies. While this might be considered as a breakthrough for biofuel production, it repeats a common scenario in Brazil: the export of primary commodities and the import of finished products, a situation that can be considered unfavorable to the economy of any country.

As well as its very large territory that is favorable for agriculture, Brazil has around 8000 km of marine coast. Microbial marine communities can be found from the surface of the water to deeper areas, such as the abyssal zone [16, 17], excluding the microorganisms associated with other marine species [18]. Important biotechnological products, such as cellulolytic enzymes from fungi in symbiosis with cnidarians, can be obtained from the marine environment [16]. Mangroves, which inhabit transition zones between the terrestrial and marine environments, seem to have unique features, and as a consequence they show autochthonous bacterial species adaptation, representing an important biotechnological resource [15].

Thus, although Brazil has great biodiversity and availability of fertile land for biomass production, as well as a number of different environments to be explored, at present only primary commodities are commercialized. So biotechnological development is compromised and the country faces serious challenges, because less than 1% of its scientific productivity reaches the consumer market [19]. One of the obstacles to be overcome is access to the country's immense biodiversity. However, despite its importance, knowledge of Brazilian microbial diversity is still sparse.

This chapter considers barriers to the search for new bioactive compounds for biotechnological and economic development in Brazil, as well as the search for promising methods for large-scale production of these compounds. For these purposes, we carried out a review of the legal procedures and an assessment of the potential rules governing microbiological research. In addition, we present an overview of microbiological research in the country, with special focus on the main biomes already mentioned above. A picture of some of the most commercialized products of microbial origin is drawn, stressing national participation in this scenario, contextualizing and emphasizing the main perspectives under development.

We also reflect on what needs to be improved so that national biotechnological efforts become competitive; academic and industrial research needs to be integrated with either public or private initiatives, leading to better use of the resources. The country still faces many problems that hinder advances in biotechnology, including a low level of investment in research and development, dependence on public financial support, poor intellectual property protection, the lack of public/ private sector arrangements, and other structural deficiencies. The distribution of scientific production in Brazil is still unequal, with biotech companies being concentrated in the Southeast Region, where the strongest academic centers can also be found; this unequal distribution is the main obstacle to potential access to biotechnology in the nation as a whole.

Use of Microorganisms and Legal Information on Biological Resources

The microbiota represents an immense reservoir of enzymes and bioactive metabolites with great potential for industrial utilization. Microbial resources, particularly enzymes, have been used by humans for at least 2000 years to fulfill human needs,

and in the past 50 years they have been used in many industrial processes (for domestic needs and in the production of food for humans and animals; for chemistry compounds from technical to high-quality (or A.C.S.) grades and in the pharmaceutical industry; in biofuel production; and in water treatment); this has increased their presence in the market in an exponential fashion. These enzymes are produced by submerged or solid-state fermentation, by either bacteria or fungi isolated from the environment or by recombinant bacteria [19–22].

Because of the importance of such resources for the production of wealth, the biochemical and functional characterization of the microbiota has become essential to protect world biodiversity. Projects on a global scale such as "The Earth Microbiome Project" (http://www.earthmicrobiome.org), "International Census of Marine Microbes" (http://icomm.mbl.edu/index.html), and "Human Microbiome Project" (http://hmpdacc.org) all have some focus on such protection. Considering the strong impact of biotechnology on industry, the Economic Development and Cooperation Organization (EDCO) proposed the creation of the Global Biological Resource Centre Network (GBRCN), which consists of the biological collections of each country, and aims to promote access to high-quality biological material (http://www.gbrcn.org/).

Within Brazil, efforts are being made to evaluate the possibilities of the sustainable exploration of different ecosystems, with organizations such as the "Biota-FAPESP Project" and the "Brazilian Microbiome Project", which are standardizing the analytical profiles of Brazilian microbial communities using data from different DNA sequencing platforms [23].

In 2007, the "Rede Brasileira de Recursos Biológicos" (CRB-Br; the "Brazilian Network of Biological Resources") was established, created by Law Decree 6041, to maintain the diversity of macro- and microbiotas in Brazil. The CRB-Br covers all the major biotechnological sectors and consists of the Ministry of Science, Technology, and Innovation; the National Council of Metrology, Standardization, and Industrial Quality; the Technology Institute of Paraná; the Oswaldo Cruz Foundation (FIOCRUZ); the Brazilian Agricultural Research Corporation (EMBRAPA); the University of Campinas; the Rio de Janeiro Cell Bank; the Reference Centre on Environmental Information; the National Institute for National Property; and the Brazilian Society of Microbiology [24].

Once the guidelines for accessing biotechnological potential are established, one of the main questions to be considered is "how is this access going to be carried out?"; this is a subject that will be considered in the following section.

How to Access the Diversity of the Microbiota?

Despite all the potential represented by the microbiota, it is not always possible to access these microorganisms; this is because of the complexity required to faithfully represent the set of physicochemical factors that is necessary for their development [25]. So, it is estimated that only around 1% of these microorganisms can actually

be cultivated using traditional growth techniques and culture media plating on Petri dishes [21, 26–28], and because of these difficulties much of the microbiota's genetic diversity is not available to be retrieved.

In this context, with the development of research in the past few decades, some techniques have been developed to assess this vast diversity (microbial and molecular). The main techniques used are the metagenomic approach, second-generation large-scale DNA sequencing, in situ cultivation, and high-throughput screening. In the following sections, brief descriptions of each technique are presented, along with discussions on how research groups are currently using these techniques in institutions and universities.

Almost Two Decades of Metagenomics

Metagenomics is a technique that allows us to access and to study collective genomes, without the need for previous cultivation of donor organisms [29]. This technique makes it possible for scientists to better investigate the vast potential of different microbial sources, such as, for example, soils; microbial consortia; mangrove sediments; river, lake, and marine water samples; insect-associated microbiota; and the bovine rumen, etc.

The development of metagenomics has taken place in the context of advanced molecular biology technology and functional assays [29]. Studies that involve metagenomics include a DNA extraction phase; a second phase of cloning the DNA fragments using vectors that can be cosmids, fosmids, plasmids, or even vectors that can harbor large DNA inserts such as artificial bacterial or yeast chromosomes; a third phase with the growth of transformed clones harboring the cloned DNA fragments, using the host; for example, *Escherichia coli* competent cells. The obtained clones are then collected and kept as metagenomics libraries, which can be used for screening new natural products.

In recent years, function-driven searches for several genes and/or proteins of biotechnological importance have been made in these metagenomics libraries, with the aim being to conduct initial screening for the identification of a desired activity or expected expression, while sequence-driven searches have been used to identify DNA conserved target sequences [30].

Using function-driven metagenomic searches it was possible to identify a novel member of the GH16 family derived from sugarcane soil [31] and it was also possible to identify clones that showed excellent (>70%) hexadecane biodegradation in a metagenomics library from a Brazilian oil reservoir sample [32].

Still considering findings using a function-driven search of metagenomic samples, the group from the Plant and Microorganisms Biochemistry Laboratory (LBMP) of São Paulo State University at the Jaboticabal campus in Brazil has identified 30 clones with lipolytic activity in a metagenomic library derived from a microbial consortium able to degrade diesel oil.

Clones that showed great potential for tributyrin hydrolysis on Petri dishes assays were selected and, after DNA sequencing and annotation, three open reading frames

(ORFs), identified as coding for esterase/lipases, were cloned on an expression vector, with the aim being functional characterization and further biotechnological applications. The results showed three proteins, named ORF2, Est16, and EST3, which were expressed and purified as soluble and stable proteins. Functional characterization of Est16 [33] and EST3 [34] indicated that these esterases seemed to have strong potential for assays involving organic solvents and biofuel production, respectively. Sequence analysis and molecular modeling of ORF2 have shown that this protein is a new and undescribed member of the lipolytic bacterial family V [35], which, according to Arpigny and Jaeger [36], has members of mesophilic origin that are adapted to cold and heat.

In Brazil various other lipolytic enzymes of different origins are being investigated using metagenomics: in mangrove sediments [37] and fat-contaminated soil [38], in microorganisms such as *Lactobacillus plantarum* [39] and *Rhizomucor pusillus* [40], and also in plant material [41, 42].

Using the sequence-driven approach, the LBMP group has also been able to find two antibiotic gene groups (PKS I and II) in environmentally derived eucalyptus samples [43]. Also in Brazil, using the same approach, it was possible to identify, by metagenomic analysis, epoxide hydrolases and haloalkane dehalogenases originating from mangrove soil samples [44], laccases originating from sugarcane [45], and genes coding for proteins involved in biomass degradation, such as hydrolases and dehydrogenases. It was also possible to identify genes associated with bacterial efflux pumps or ABC-type transport systems from the metagenomic analysis of composting animal material samples [46]. In addition to these examples, the LBMP metagenomic libraries were mined, using both sequence- and function-driven mining approaches, to search for several genes of industrial interest, including genes for catalases, amylases, peptidases, cellulases, and laccases; genes for other antibiotic pathways; and genes for xyloseisomerases and phosphatases.

Various other metagenomic projects have been or are still being developed in Brazil by other research teams, such as those from EMBRAPA, FIOCRUZ, “Universidade de São Paulo” (USP, São Paulo State University) and the “Centro de Energia Nuclear na Agricultura” (CENA/USP, Center for Nuclear Energy in Agriculture) among others [23].

Next-Generation DNA Sequencing (NGS)

Next-generation DNA sequencing (NGS) techniques, and progress in data analysis methods and platforms, have allowed the exploration of microbial diversity in microbiota that are still not cultivable (including non-abundant microbiota) and the search for genes with high technological value; the new techniques even allow the detection of differences within a set of genomes (like the human genome). These new DNA sequencing techniques could cause a revolution in genetics, because the high-throughput systems used can generate thousands or even millions of nucleotide sequences in one single run, allowing us to answer questions at an unprecedented speed [47].

Various technologies are used for NGS, such as the commercial platforms Illumina Miseq and HiSeq2000 (Illumina, San Diego, CA, USA), Ion PGM and Ion Proton (Life Technologies, Carlsbad, CA, USA), and Pacbio (Pacific Biosciences, Menlo Park, CA, USA). These DNA sequencers differ based on their adopted chemistry or amplification method, the resultant Mb amount per run, run duration, amplified fragment length, and Mb cost. These platforms are currently used for quite different purposes; one example being for studying the microbial ecology of fermented or unfermented food [48, 49]. By using NGS it has become possible to identify new microbial species and to correlate them with particular food and production steps, knowledge that is very important for promoting quality control and food production security [48].

Against this background, the Brazilian Microbiome Project (BMP), which aims to build a Brazilian metagenomic database, was developed [23]. The BMP intends to link their information to systems of functional genetic diversity, and compare these systems with other microbiome projects throughout the world. The BMP also intends to describe microorganisms that can be used for the production of new products, with the purpose of improving the use of Brazilian biodiversity that favors biotechnology.

High-Throughput Screening (HTS)

High-throughput screening (HTS) is a system that is able to identify chemical probes on libraries containing a large number of compounds, using sophisticated assays and detection platforms [50]. As this technology can be used to evaluate a great number of chemical substances, it can be adopted for key targets of biomedical research, which could lead to the screening of new drugs [51]. HTS is reported to be the most productive technique for use on different targets [51].

Drug screening is just one of the many uses or strategic options that can be implemented for the control of diseases that, to date, are not amenable to cure. In a recently published paper [52], a research group reported on developing and improving a drug-screening assay for human African trypanosomiasis (HAT) and identifying potential candidates for the development of new drugs against HAT. After screening a library of 4000 putative kinase inhibitors, they found 13 scaffolds that indicated activity against *Trypanosoma brucei*. Their SYBR Green-based HTS is an effective way of detecting *T. brucei* when compared with resazurin (standard assay), as it is faster and more sensitive and reliable.

Cultivating the Uncultivable: Ichip

The isolation chip (Ichip) is a new method for the in situ cultivation of environmental microbial communities, the aim being to access the great hitherto inaccessible microbial diversity [53]. With this method the Ichip, which consists of

hundreds of micro-chambers, is used to allow the separation of each single cell of a cellular mixture into individual micro-chambers, after the environmental sample has been distributed over the specimen slide. The assembled Ichip is returned to the environment for incubation, because the development of colonies is based on diffusion by natural sources; after the incubation period, the material is recovered for further analysis, such as, for example, microscopy, DNA sequencing, etc. [53].

As recently reported, a new antibiotic, named teixobactin, was isolated from a soil sample using the Ichip as a tool [21]; besides allowing the discovery of this natural product, the Ichip also enabled the isolation of the relevant microorganism, tentatively named *Eleftheria terrae*, which, up to that time, had not been cultivable. With a high-throughput method, the Ichip promoted the recovery of 50% of the inoculated microorganisms in the micro-chambers; in other words, its use has made possible the in situ cultivation of observed colonies [53].

Teixobactin has shown high activity against gram-positive pathogens and drug-resistant strains, and interestingly, this antibiotic action was found to be related to the main biosynthetic pathway of *Staphylococcus aureus*, indicating that, in view of its interaction with peptidoglycan precursors, this antibiotic might be a new inhibitor of peptidoglycan synthesis. The efficient ligation of teixobactin to bacterial wall teichoic acid, a precursor of undecaprenyl-PP-GlnNAc (lipid III), liberates autolysis, producing cell wall lysis, consequently resulting in the pathogen´s death [21]. Even if other new drugs in the class are not developed, teixobactin is the first member of a new antibiotic class that targets lipid III [21, 54].

Brazil Compared with Other Countries

The choice of a technique or a set of techniques is a key point to be taken into account when an experimental design is to be considered for a scientific project. In this phase, it is essential to raise the questions to be answered by the project´s aims and/or to state the study's hypotheses, while bearing in mind the infrastructure available for the correct development of the experiments, the necessary equipment and reagents, the time required for the experiments to be carried out, and the data analysis.

We analyzed how Brazilian procedures compare with those of other countries in terms of the different techniques and methods used for investigating biomolecules. Does Brazil implement the currently available scientific and technological methods in its laboratories and research groups? If the answer is yes, which of the techniques listed above (metagenomic approach, NGS, HTS, and Ichip), are currently more accepted by the Brazilian scientific community?

To answer these two questions, a search was done of published scientific papers, using the databank from the Web of Science (http://wokinfo.com/), which is based on one of the most important world sources of information, the Thomson Reuters BiologyBrowser. A search for published scientific papers and not for other types of

information was performed because this is a standard scientific procedure worldwide, and with such a search it is possible to obtain information on the methods used and results, as well as the names of the research groups who carried out the experiments and the names of the relevant institutions and countries.

For this particular analysis two search mechanisms were used: the first one looked for works that had used the above-mentioned techniques and so the keywords "metagenome", "next-generation sequencing", "high-throughput screening", or "Ichip" were inserted in the search field. For the second analysis, as well as using the topic search field, we searched for the location at which the experiments were carried out, so for this option a second search field was added, in which data from countries such as Brazil, the United States, India, China, and Japan were individually analyzed. The data were collected in mid-August 2015 and Excel software generated the graphics.

The data clearly showed that, of the four above-mentioned techniques, HTS was the most commonly used worldwide by the scientific community, with 42,667 published articles in the period 2010–2015, almost double the number of articles using NGS (24,412). The number articles using the metagenomic approach was significantly lower (1883), followed by those using the Ichip (14 articles).

Alhough the HTS technique was the one most commonly used by the laboratories involved with this type of research in scientific institutions and/or private companies abroad, within Brazil this technique was listed as the second option, after NGS, in terms of the number of published articles, at 152 and 181, respectively.

It was interesting to observe that, in Brazil, although the NGS technique was used in the greatest number of published studies, metagenomics was, by far, the most prominent technique in comparison with other countries. The number of articles from Brazilian groups using the metagenomic approach corresponded to 2% of all the published articles worldwide (Fig. 1a), followed by NGS with 1% of all the published articles (Fig. 1b), and then HTS (Fig. 1c) and Ichip (Fig. 1d). These findings emphasize that, to date, and according to the data obtained in this analysis, there have been no publications by Brazilian research groups or institutions using the Ichip technique, in contrast with results in the United States, with five articles involving this option.

With scientific and technological advances, new methodologies are being proposed for the search of biomolecules, such as, for example, ultrahigh-throughput screening using drop-based microfluidics [55]. According to our analysis, within the past 5 years three articles in which this particular technique was used were published by Brazilian research groups in partnership with United States institutions, while none has been published in Brazil alone.

Thus, our data analysis has revealed that, although Brazilian research groups are implementing different bioprospecting methods for biomolecules, this is being done at a much lower speed than that in developed countries such as Japan and the United States. When we compare Brazil with India, whose human development index (HDI) for 2014 was lower than the Brazilian HDI (http://hdr.undp.org/en/countries), at 0.586 and 0.744, respectively, it can be seen that Brazil has less significant

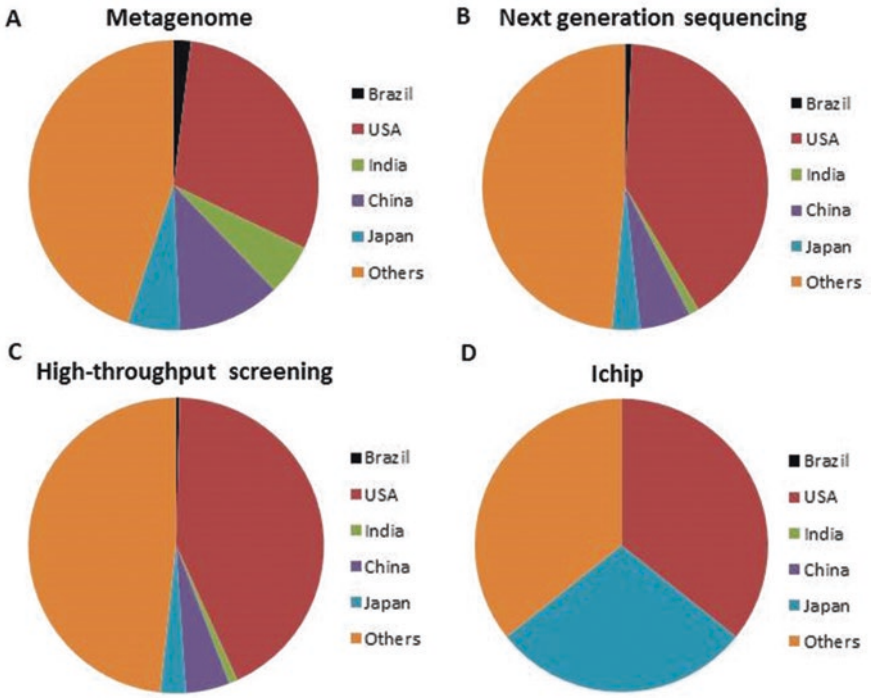

Fig. 1 Comparative analysis of bioprospecting in Brazil and other countries. The search for scientific articles that adopted metagenomic (**a**), next-generation sequencing (**b**), high-throughput screening (**c**), or Ichip (**d**) techniques was carried out using the Web of Science databank. The data were collected in August 2015 and the graphics were generated using Excel software

participation in bioprospecting than India (Fig. 1a, b, and c). This situation prompts us to think about the reasons that could be slowing the development of Brazilian science, particularly in regard to bioprospecting assays, which can lead to a better understanding of our own biodiversity.

Research Frontiers: Where the Microbial Metabolic Wealth is Mostly Explored

Brazil has the world's sixth largest microbial culture collection, holding 109,626 microbial isolates shared by 75 collections throughout the country (World Data Center for Microorganisms, 2015; accessed on August 23, 2015).

Surprisingly, this great biochemical and genetic microbial diversity has rarely been explored, especially when one takes into account the diversity of biomes in Brazil; opposed to this diversity is the extremely unequal centralization of the human and financial resources that are commonly destined for scientific research.

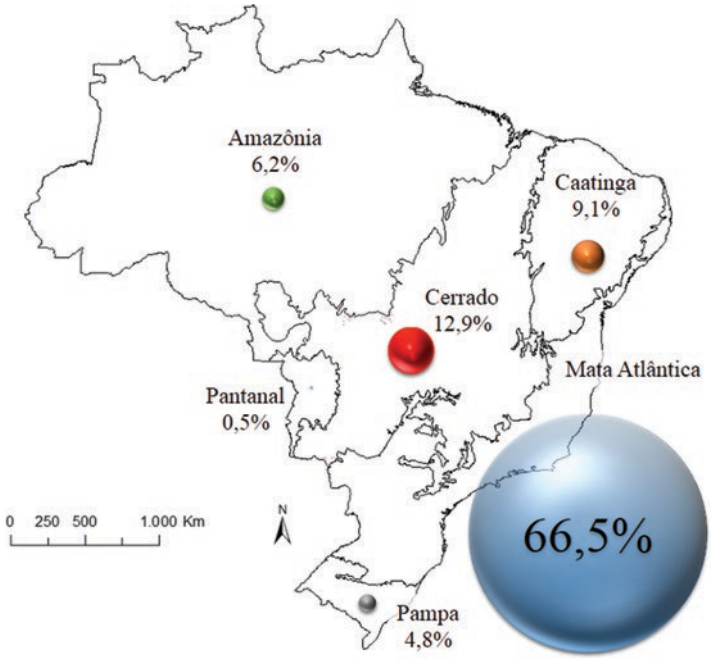

Fig. 2 Impact of studies involving microorganisms or their products isolated in Brazilian territory in articles published within the past 5 years in Brazil. The articles were searched for using the Web of Science tool [56] and the following keywords: "Microorganism", "Biomolecules", "Enzymes", and "Brazil". The data were collected in August 2015

Using the Web of Science tool (accessed between August 17 and 23, 2015), we carried out a bibliographic review of articles involving microorganisms or their products isolated in Brazilian territory published in the past 5 years in indexed periodicals. Most of the articles related to prospective studies of microbial communities and their metabolites (66.5%), with results from research developed at São Paulo State University and most referred to the Atlantic forest biome (Fig. 2). The second most commonly studied biome was the Cerrado (12.9%), followed by the Caatinga (9.1%). Despite their importance, the other biomes were mostly not studied, and biodiversity research and microbial prospecting for biotechnological purposes in these biomes were almost completely absent.

Economic Importance of Microorganisms: Global and National Impacts

Enzymes and antibiotics, mainly those of microbial origin, are among the biotechnological resources of high economic importance [19–22, 57].

The world market for enzymes is constantly expanding. Around 50% of this world market is destined for technical applications (bioenergy, textiles, etc.), while 37% of the market is used for food production and 12% is directly used for nourishment [58].

Of note, antibiotics and other bioactive entities originating from microbes raise billions of dollars in the world economy, because there is a constant need for new molecules for the control of human and animal diseases [19, 21, 43, 57].

According to available data for the year 2012 in the Observatory of Economic Complexity data bank (available at http://atlas.media.mit.edu/en/), world export transactions involving enzymes reached US$4.7 billion during this period. The countries that had the highest export transactions were Denmark (US$1.08 billion), the United States (US$487 million), and Finland (US$383 million), while Brazil (US$45.5 million) was ranked in the 16th position and the 5th position in the Americas. For antibiotics, the most relevant export transactions involved China (US$3.19 billion), Switzerland (US$1.92 billion), and the United States (US$1.7 billion), with Brazil in the 22nd position, with exports of US$69.68 million. More details for the world percentage distributions are shown in Fig. 3.

According to BCC Research (Fig. 4a), the best growth projections for enzymes related to biofuels are for the cellulase class, with a prospective doubling of the

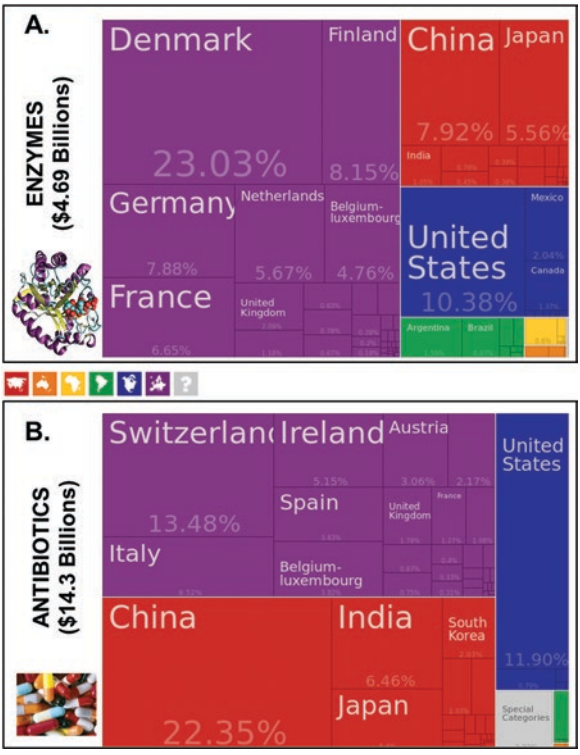

Fig. 3 World enzyme (**a**) and antibiotic (**b**) markets in 2012. Source: Observatory of Economic Complexity, accessed in August 2015

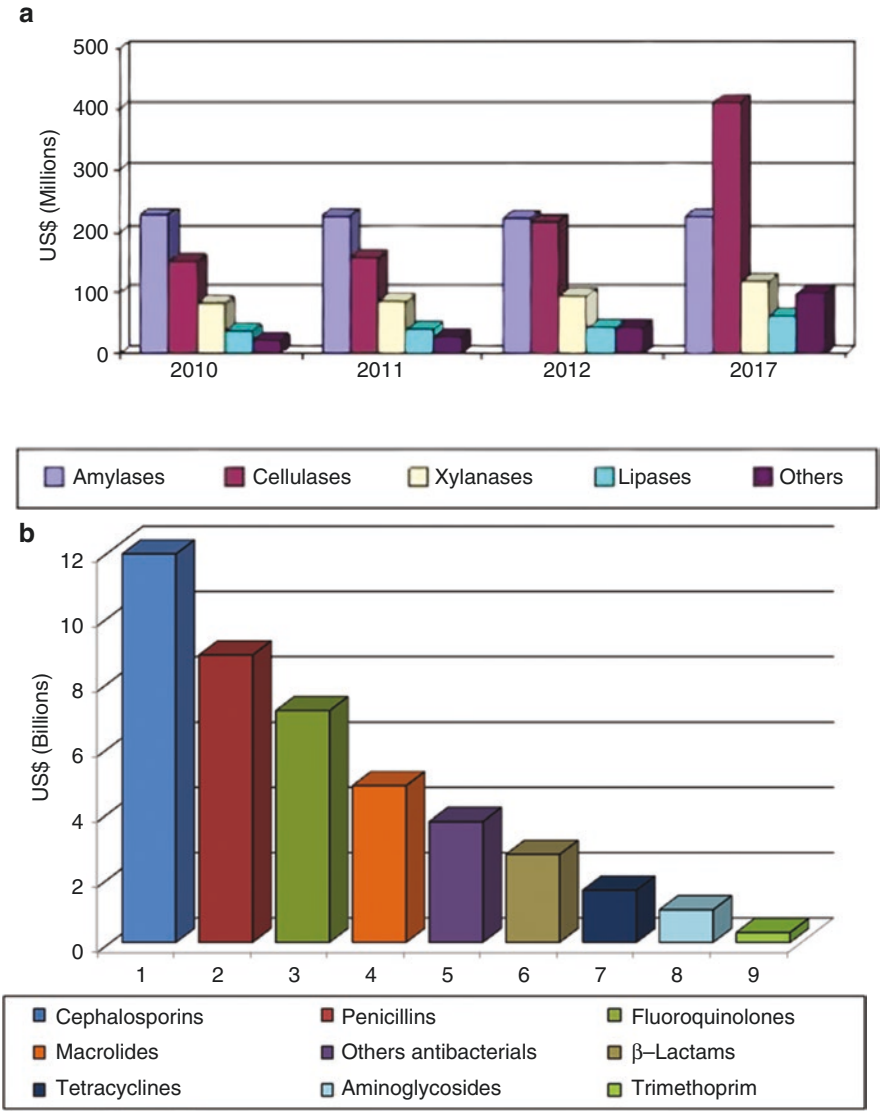

Fig. 4 Economic importance of microorganisms. (**a**) Projections of global enzyme markets for biofuels. Source: BCC Research (2013) [20]; (**b**) main commercial molecules worldwide in 2009 (adapted from Hamad, 2010 [57], with permission)

revenue by the year 2017, reaching a value of US$400 million on the world´s enzyme market. The main use of cellulases is related to the expansion of new technologies for the production of second-generation biofuels based on industrial residues, such as, for example, second-generation ethanol [59–62].

Figure 4b illustrates the worldwide revenues for commercial pharmaceuticals in the year 2009, with emphasis on the polypeptide class and cephalosporins, whose market share was US$11.9 billion [57].

Brazil is the world's second greatest ethanol producer, with vast experience in production scaling; thus, the country has an important reference role for the development of this biofuel. Annual Brazilian ethanol production has already reached 28 billion liters [63]. Of the by-products of ethanol production in the country, sugarcane bagasse, with an annual amount of around 160 million tons, should be emphasized. This lignocellulosic biomass (at present used for the cogeneration of power in production plants) has great potential to increase the production of biofuels, via second-generation ethanol, and its use could raise overall ethanol production by 40% compared with that obtained by first generation process [64]. However, there is still a need for better coordination among the various biotechnology research centers in Brazil so that the country can also be a leading second-generation ethanol producer [59, 65].

In addition to its strategic internal market for enzymes, Brazil has great potential for the production of enzymes that are used in different industrial sectors, especially considering the use of cheap raw material obtained from the country's own industrial waste. According to a recent study [58], the national capacity to produce concentrated amylases, cellulases, and lipases could reach annual levels of 31, 32, and 310 million tons, respectively; with respect to xylanases the value might hit 2.9 million tons.

Based on a rough idea of how these values are actually expressed, within the past 15 years Brazil has imported 14,401 tons of amylases and exported only 3517 tons (data obtained from the web server AliceWeb of the Brazilian Ministry of Development, Industry, and Foreign Trade, available at http://aliceweb.desenvolvimento.gov.br/; Fig. 5). Together, these values are equivalent to 0.05% of the estimated amylase production during a 1-year period, based exclusively on our own net resources (31 million tons) [58].

In 2012, the total revenues generated by the export of amylases and cellulases in Brazil were, respectively, US$7.5 and US$1.6 million, while the expenditures for these items were, respectively, US$12.3 and US$1.2 million . The world transactions for the same period for each of these enzymes were above US$200 million (Fig. 4).

Brazil's share of the total world market for enzymes is still narrow, despite its great biodiversity, as can be seen by the national data in comparison with those of the United States (Fig. 6).

Brazil occupied a strategic position in the portfolio of United States imports (Fig. 7), mainly during the period from 2011 to 2014 (fifth position). According to the Intelligence Base for US Imports and Exports (Zepol web server, available at http://www.datamyne.com/zepol-archived-trade-reports/), Brazil was the world's second largest enzyme importer. In June 2015, a total of US$6.7 million was expended, with the export revenue being around US$65 million for the same period.

The main destinations for enzymes exported from Brazil were Venezuela, Argentina, Japan, and Denmark (Fig. 8).

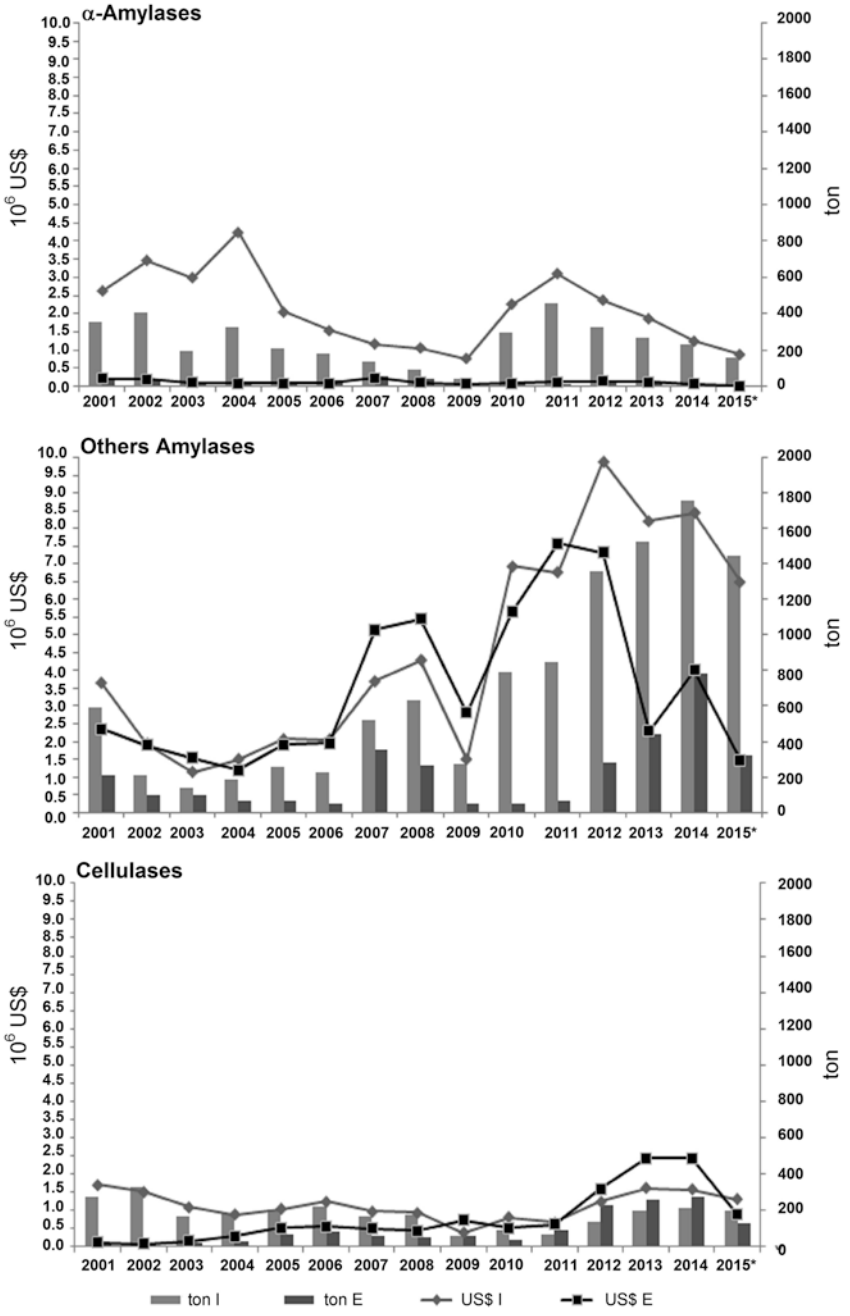

Fig. 5 Data for Brazilian enzyme import (*tonI*; US$ I) and export (*tonE*; US$ E) markets. The data were recovered from the webserver AliceWeb, using the terms "NCM 35079011" (α-amylases from *Aspergillus oryzae*); "NCM 35079019" (other amylases), and "NCM 35079041" (cellulases), in August 2015. The graphics were generated by Excel software

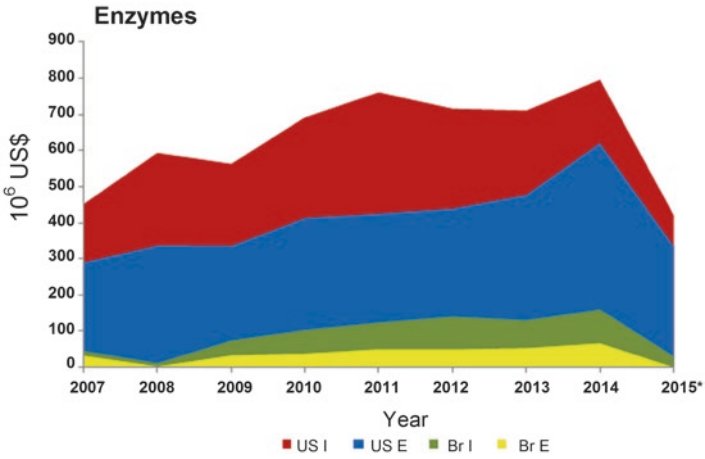

Fig. 6 Import/export data for enzymes in the United States (*USI*, *USE*) and Brazil (*BrI*, *BrE*). The data were recovered by the web servers AliceWeb (Brazil) and Zepol (United States). The data were obtained in August 2015 and the graphics were generated by Excel

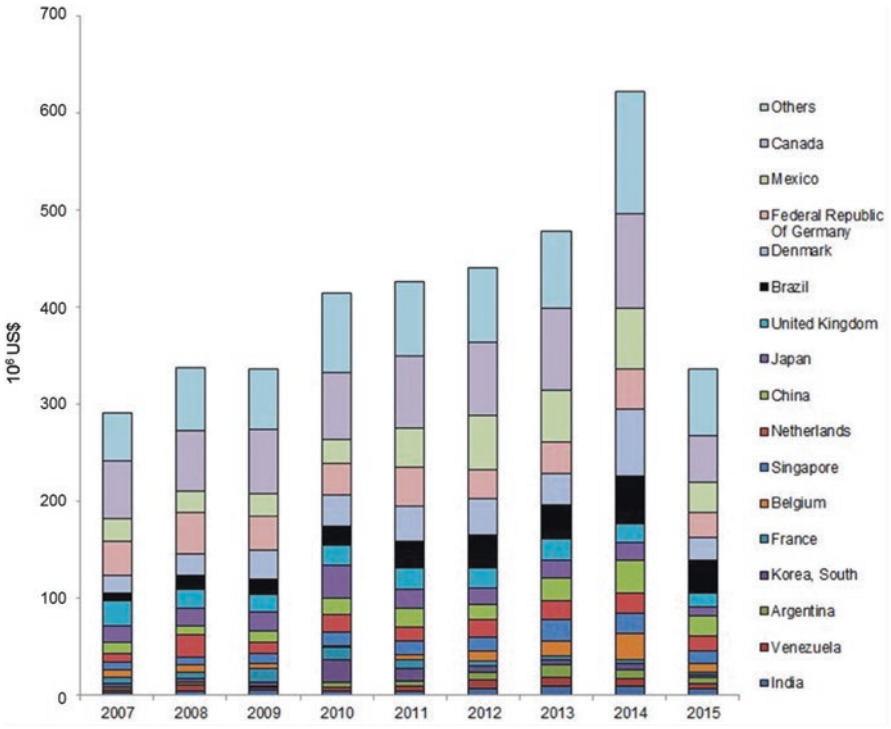

Fig. 7 Main destinations of enzymes exported from the United States. The data were recovered by the web server Zepol, using the keyword "NCM 3507" (Enzymes; Prepared Enzymes). The data were obtained in August 2015 and Excel generated the graphics

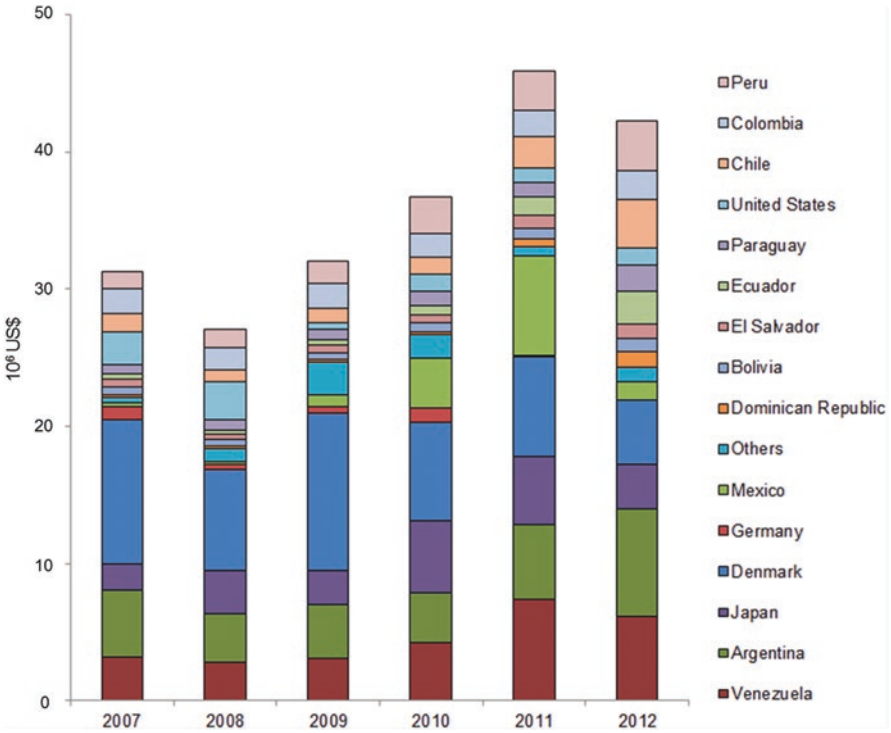

Fig. 8 Main destinations of enzymes exported from Brazil. The data were recovered by the web server Observatory of Economic Complexity, using the keyword "NCM 3507" (Enzymes; Prepared Enzymes). The data were obtained in August 2015 and Excel generated the graphics

Although Brazilian biotechnology has achieved several successful breakthrough initiatives for the development of plant cultivars, such initiatives have not taken place in the pharmaceutical industry, which is developing slowly because of the importation of the active principles of drugs [19]. Based on data from the Observatory of Economic Complexity, in 2012 Brazil exported less than 0.5% of the total world demand for commercial antibiotics, as opposed to China, which exported 22.3% of the total world demand (Fig. 3).

Despite its low export flow of antibiotics, Brazil has a strong internal market for these agents, both for human medical treatment and for agricultural use. The import and export volumes of chloramphenicol are low, in contrast to the import volumes of penicillin and tetracycline, both of which are high (Fig. 9).

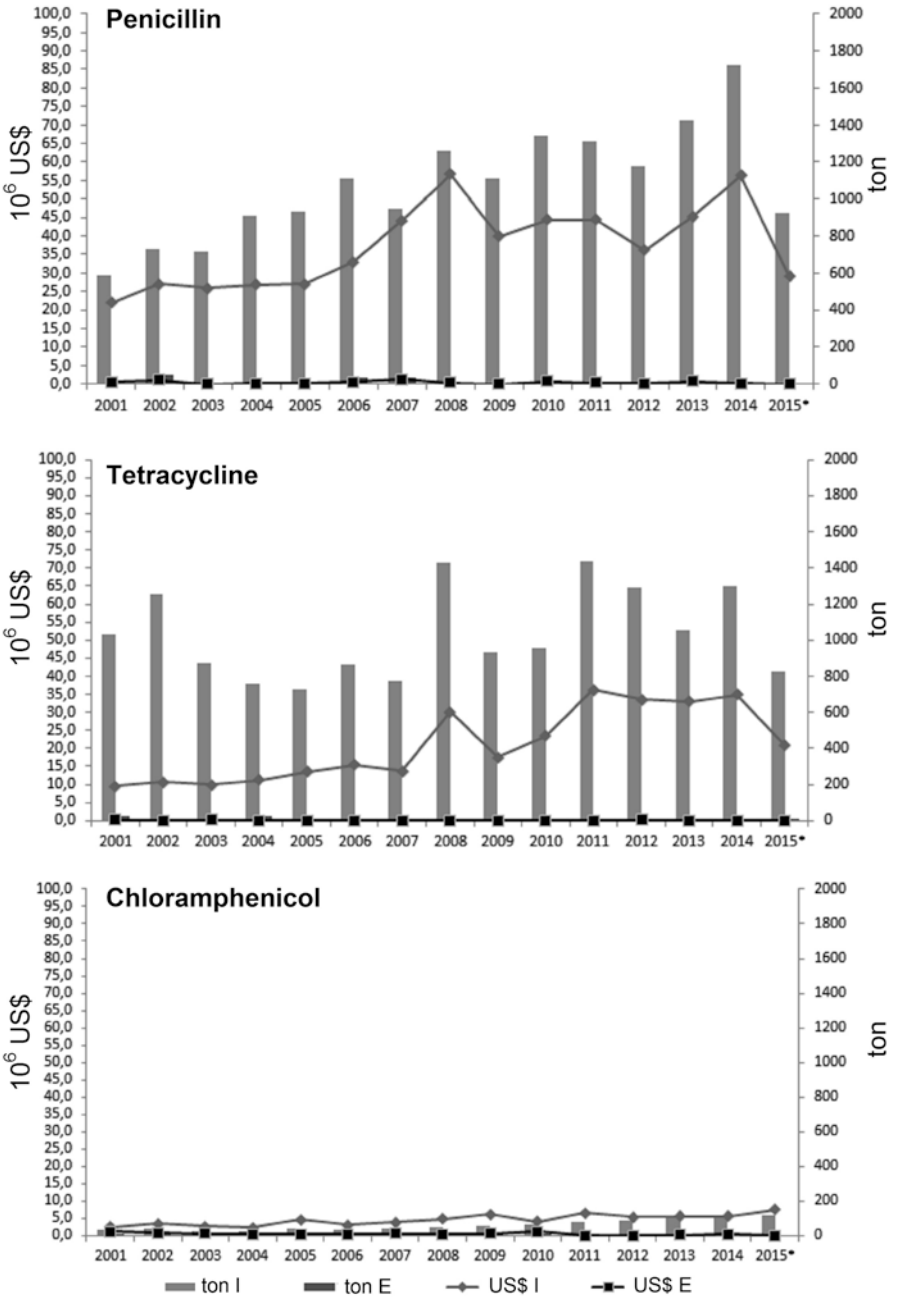

Fig. 9 Antibiotic import data (*ton I*, *US$ I*) and export data (*tonE US$ E*) for the Brazilian market. The data were recovered by the web server AliceWeb, using the key words "NCM 294110" (penicillin), "NCM 294130" (tetracycline), and "NCM 294140" (chloramphenicol). The data were obtained in August 2015 and Excel generated the graphics

The Gap Between the University and Industry: Challenges for Biotechnological Advances

Over several years of efforts and dedication, Brazilian science has gained the recognition and respect of the international scientific community [59, 66]. In fact, born in the birthplace of a developing nation, Brazilian academia seems to have been permeated by an increasing desire to find itself in the world context.

One of the first great Brazilian endeavors that have raised the country's scientific research status in international competition has been the *Xylella fastidiosa* DNA sequencing project [67]. The maturity acquired by the group in this project has generated involvement in other research approaches, such as that of cancer genome sequencing and the sugarcane expressed sequence tag project [68]. The Allelix (an anagram of *Xylella*) company is one example of this successful scientific research [66].

However, in the search for recognition, the national research goals seem to have become an entity turned to the academic world itself, centering efforts on university science and innovation, where there is still an unwise aspect regarding biotechnological potential. Academic research has progressed with its own expertise, hoping that this aspect would attract the eyes of industry [19, 69].

Despite sharing common interests, the academic and private biotechnological sectors are mostly not integrated, a situation that jeopardizes our ability to extend the Brazilian biotechnological network to reach world visibility [19]. There are 237 private biotechnology companies in Brazil, with almost 80% being in the Southeast Region; 63% of the companies have been active for only 15 years and most companies can be considered as micro or small companies (85%). Also, most companies are involved with human (39.75%) and animal (14.3%) health; while only 9.7% are related to agriculture, with 14.8% using environmental and bioenergy biotechnological solutions. Around 25% of the companies export some products, while 85% import mainly equipment and reagents [70].

The Brazilian academic sector is considered to be significant and well structured, as mentioned previously, although it is greatly centralized in certain regions of the country [59, 66]. About 1000 PhDs in science are awarded each year in Brazil, but only a small proportion of this human resource finds employment in the private sector. Consequently, the university-generated knowledge is underutilized, with less than 1% of this knowledge reaching the commercial market. This situation is rather different in other countries, with the United States accounting for 70% of the global biotechnology market [19]; this explains the results shown in Figs. 6, 7, and 8.

Biotechnology companies in Brazil do not attract Brazilian partners, as there is a lack of incentives, such as poor venture capital conditions and poor patent policies, which are considered unsuitable for large investors. Government involvement is also not appropriate, often being seen as only promises [19].

The absence of dialogue between the academic and industrial sectors is a fact. We need a bridge to unite university interests (frequently based on the number of publications) and those of the community, such as knowledge transference to industry, with rare interfaces aiming to measure this process [19, 58].

Some initiatives to improve knowledge transfer have emerged inside the universities themselves, through the "Agência Unesp de Inovação" (AUIN, São Paulo State University the Innovation Agency) and through "Agência USP de Inovação" (INOVA, University of São Paulo the Innovation Agency), both of which are specific entities that support researchers in the development and recognition of their patents. Together with these initiatives are projects to evaluate the possibility of sustainable exploration of the country's different ecosystems, such as the Biota-FAPESP Project and the Brazilian Microbiome Project [23], discussed at the beginning of this chapter.

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