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Review Article

Microbiology Section

International Depository Authority and its Role in Microorganism's Deposition

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ABSTRACT

After the World Trade Organization's agreement on Trade Related Aspect of Intellectual Property Right, patents have come into major play. Patenting of work related to live organisms that have medical, agricultural and other uses is always a tedious, complicated and controversial job. In view of this an agreement called as Budapest Treaty was passed in 1977 for deposition of microorganisms in culture collection centers for patent purpose. To make a culture collection center an IDA the culture center has to follow rules and regulations made in Budapest Treaty. Today several culture collection centers are working as International Depository Authority (IDA) in India and abroad that are storing microorganisms for patent purpose. India has two IDA units; one is Microbial Culture Collection in National Centre for Cell Science Pune, India, and second one is Microbial Type Culture Collection and Gene Bank at Institute of Microbial Technology Chandigarh, India. IDA units are important for valuable sample depositions, supply of cultures, characterization of samples and conservation of biological materials.

Keywords: Budapest treaty, Culture collection, Patent

INTRODUCTION

Intellectual Property Right (IPR) is a lawful right given to inventors for their intellectual work. It includes Patents, Trade secrets, Copyrights, Trademarks, Plant varieties protection, Industrial designs and Geographical indicators. New invention is required for growing economy like India as it gives competitive edge form the rivals. Further as heavy investment is involved in new inventions, the inventor should have the right to get monitory benefits for his/her work. In this regard patent and other IP rights give necessary protection of original work from its misuse and at the same time provide monitory gain from the invention. In 1948 after World War II General Agreement on Tariffs and Trade (GATT) was made for International trade purposes [1]. GATT was eventually replaced by World Trade Organization (WTO) in 1995, which included Intellectual Property Right (IPR) for the first time in trade [2].

As per Article 27(3)(b), of Trade Related Aspects of Intellectual Property Right (TRIPS) agreement microorganisms, non-biological and microbiological processes can be patented [3]. India also follows the rules and regulation of TRIPS agreement and allows only that work to get patent that has commercial application, novelty and non-obvious in nature [4]. However, it is a very tedious job to get patent for live organisms. It is only given to those inventions that includes genetic modification and insets valuable characters in microorganisms that were initially not present in natural form of that organism such as attenuation in bacterial strain making it less infective which is required during vaccine preparation (Tuberculosis vaccine) [5], genetic modified oil eating bacteria [6] etc. In 1980, USA was the first country that had decided to grant the patent on live microorganisms, a genetically modified *Pseudomonas* bacteria was granted patent because of its ability to degrade harmful organic compound produce during oil spills [6]. One of the mandatory obligation for grant of patent for an invention is its repeatability by a person who has knowledge in that field, with work related to non-biological in nature, testing of invention is an easy process as most of the ingredient of work is stable in nature. But working with microorganisms is a very different phenomenon as bacteria always change their character in native environment due to selective pressure causing difficult to repeat the experiment that was previously performed/claimed by the inventor. To resolve those issues it was decided that inventor must deposit microorganisms in pure and viable form for its patent purpose in a depository house also known as bacterial depository bank or International Depository Authority (IDA) as per the rules and regulations set by International body in the form Budapest Treaty [7]. Further, single deposition of sample will be sufficient for its recognition for patent purpose by other countries that are part of this treaty.

1. Budapest Treaty

The proposal for International treaty on deposits of microorganisms was first put forward by United Kingdom. The treaty was adopted in 1977 at Budapest and came into existence in 1980. At that time 18 countries ratified this treaty such as France, USA, UK, Italy etc. The main objective of this treaty is to deposit live microorganisms for their patent purpose. To deposit live microorganisms in one place, member country has to develop IDA where depositors can safely deposit live microorganisms [7]. Today more than 80 countries are part of this treaty and there are 39 IDAs in 22 countries. To get the membership of Budapest treaty, each country has to ratify Paris convention which is related to protection of work having industrial application. Budapest treaty is not only restricted to microorganisms but plant and animal cells, hybridoma cell, cell lines, RNA, plasmids etc., can also be deposited in IDA. In 2001, India became part of Budapest treaty and currently there are two IDAs, Microbial Culture Collection (MCC) in National Centre for Cell Science (NCCS), Pune and Microbial Type Culture Collection and Gene Bank (MTCC) at Institute of Microbial Technology (IMTECH), Chandigarh respectively [8]. Along with two IDAs, India has thirteen more culture collection centers known as designated repositories, storing different kinds of biological resources and are recognized by National Biodiversity Authority (NBA) [9].

1.1 Key features of Budapest treaty:

1) IDA formation and recognition of single deposition of microorganisms for patent purpose: Budapest treaty has given clear rules and regulations of creating and maintenance of IDA, daily working protocol of IDA and guidelines to depositor. It was decided that each member country will make IDA for microorganisms

deposition with full infrastructure facility, also the IDA will accept microorganisms from depositors of native country as well as outside from country. One of the burning issue that was solved in this treaty was to stop multiple deposition of sample in more than one IDA for patent purpose. The treaty says that single deposition of sample for patent purpose in IDA will be enough to give recognition by other member countries of Budapest treaty [10].

2) Broad definition of microorganisms: Budapest treaty has not defined the definition of microorganisms; because of this situation today not only microorganisms but plasmid, cell lines, fungi, yeast, RNA, plant and animal cells etc., can also be deposited.

3) Safe deposition of microorganisms: It is the duty of IDA to safely deposit microorganisms without affecting its viability and purity.

4) Deposition and furnishing of samples: A brief rule and regulation is given for deposition of samples. Further, sample can be stored up to 5 to 30 years in IDA. Also, IDA has to furnish samples to depositor or concern authority as per the requirement.

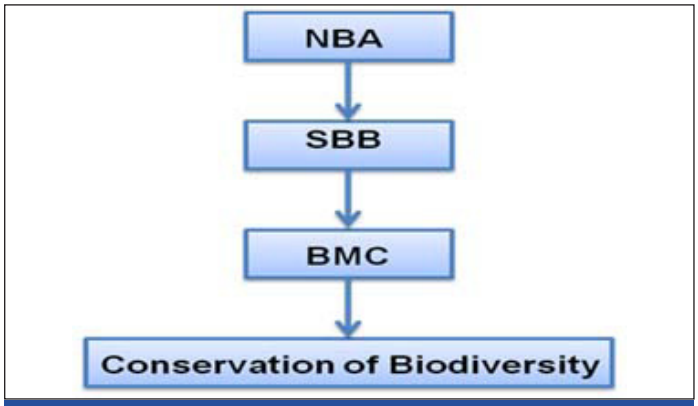
2. What is an IDA?

Any culture collection center can become IDA but it has to give assurance to members of Budapest treaty and World Intellectual Property Organization (WIPO) that it will deposit microorganism from any depositors, it will accept and store microorganism in safe and proper manner and furnish the sample to any authorized person as per the requirement in strict manner, obeying all the rules and regulations of Budapest treaty [11].

2.1 Main functions of IDA:

1) Storage of microorganisms for patent purpose;
2) Long term storage of microorganisms in safe manner;
3) Furnishing of samples as per the requirement;
4) Creates an environment of good microbiological practices;
5) Identification and characterization of samples;
6) Gives accession number to samples;
7) Maintain secrecy of deposited microorganisms;
8) Provides human resource work such as training program related to microbial techniques;
9) Protection of environment;
10) Proper transport of sample.

2.2 Convention on Biological Diversity (CBD) and IDA: CBD was signed by more than 200 countries during Earth summit at Rio de Janeiro, Brazil in 1992 [12]. The agreement gives the right to nations to conserve their biodiversity. The aim includes: conservation of biological resources, sustainable use of biological diversity and equal sharing of benefit among the people who are using the genetic resources of biodiversity. IDA which is used for safe and long term deposition of microorganisms can work as preservation house for microbial population. In view of above situation two organizations World Federation of Culture Collections (WFCC) and World Data Centre for Microorganisms (WDCM) were established. The WFCC is an institution that helps in establishment of culture collections centers. It gives different guidelines for establishment, authentication and maintenance of cultures in culture centers. Also, creates an online networking between culture centers for better communications. WFCC has created WDCM whose main role is to maintain statistical data of culture collection centers, for example how many people are working, what kind of cultures are maintained in each culture centers, different facilities provided by culture centers etc. In 2003 India has established, National Biodiversity Authority (NBA). NBA is an autonomous body of government of India and it's headquarter is in Chennai. The main function of NBA is to implement CBD agreement at regional level in India. For CBD implementation more efficiently, NBA has created 29 State Biodiversity Boards (SBBs) and 37,769 Local Level Biodiversity Management committees (BMCs) [13], [Table/Fig-1]. SBB takes suggestion from NBA regarding conservation of biodiversity at state level while BMC is involved at ground level in promoting conservation, sustainable use and documentation of biological diversity. In situ conservation protects the natural habitat of the species, while ex situ includes captive breeding programme for animals, zoo, botanical garden,



[Table/Fig-1]: NBA and its role in biodiversity conservation. NBA-National Biodiversity Authority, SBB- State Biodiversity Board, BMC- Biodiversity

in vitro production of plants, bacterial and other biological material storage and propagation in culture collection centers (IDA) etc.

Management Committee

2.3 Process involved in microorganisms deposition to IDA: There are several rules and regulation that have been included in this treaty for both depositors and culture collection center (IDA) for microorganism's deposition in appropriate manner [14]. A brief process of microorganism's deposition in IDA is as follows [Table/Fig-2]:

2.3.1 Depositor's obligation [Table/Fig-3]: Rule 6.1(a): Depositor submits an IDA application form called BP1 (Budapest) mentioning his/her name, signature and address, full description of microorganism, chemicals, media and protocol for its proper storage in IDA. The form mentions information whether the said material is hazardous in nature or not or any other information in this regard. Further depositor has to mention in the form whether the culture is in pure or in mixed form and conditions for its long term storage.

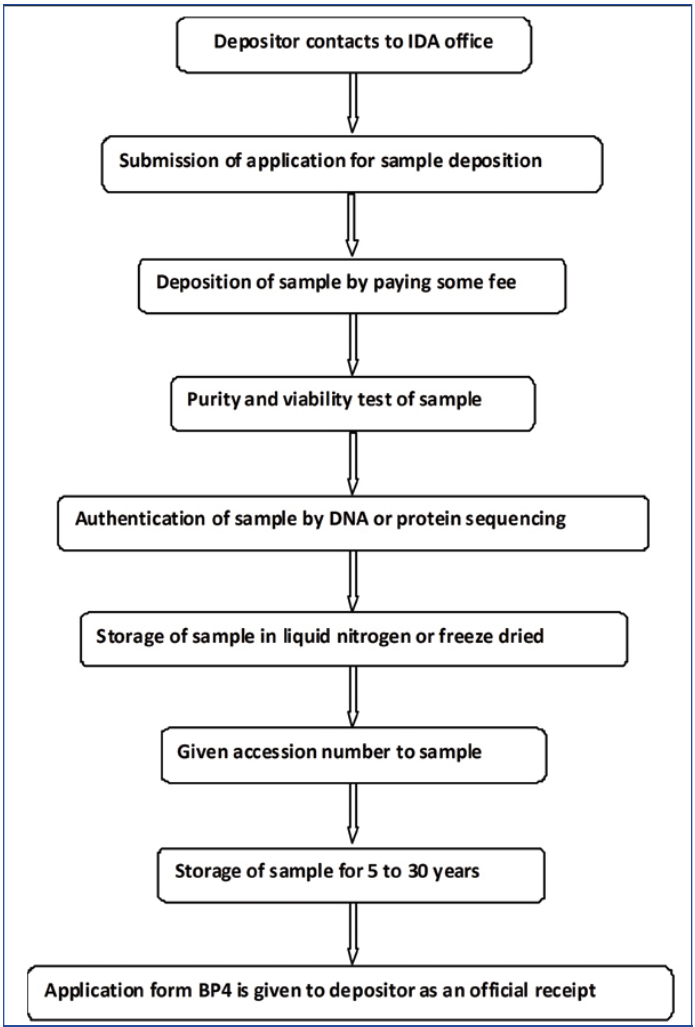
New deposition of original sample: Rule 4 says a new deposition of original sample can be performed in following situations:

1) If IDA is not able to furnish the sample for some reasons such as the stored microorganism is no more viable; 2) Sample is not given to foreign country where depositor wants to use it due to export or import restriction or if the status of IDA of the culture institute is cancelled.

In all these situations depositor has the right to make new deposition of the original sample by giving signed statement that the new deposited sample is equivalent to original sample. Form BP2 is used if the deposition is made in same IDA, while form BP3 in case of new IDA.

Rule 5.1: This rule comes when it is necessary to transfer samples from one IDA to another IDA. There could be many regions when this situation can arise such as when culture centre having IDA status fails to work under rules and regulations of Budapest treaty and its IDA status has withdrawn. In that case instead of new deposition of original samples, samples can be transferred to old IDA to new IDA. The new IDA has to inform depositors by giving following information in International form BP6:

1) Name and address of new IDA where his or her sample has been deposited;
2) Name and address of depositor;
3) Date of transfer of sample from previous IDA to substituted IDA;



[Table/Fig-2]: Process of microorganism's deposition in IDA.

Rules	Type of Functions	Different Forms use in IDA
Rule 3	Kinds of microorganisms accepted by IDA	Form BP2 & 3
Rule 4	New deposition of original sample	Form BP6
Rule 5.1	Transfer of samples from one IDA to another IDA	Form BP1
Rule 6.1(a)	Process of original sample deposition by depositor	
Rule 6.3(a)	Sample deposition in agar slant /liquid suspension/ lyophilized form	
Rule 6.4(a)	Refusal of taking microorganisms for deposition in IDA	
Rule 6.4(b),(c) and (d)	Deals with acceptance of original deposit in IDA	Form BP4,5 &6
Rule 7.1	After receiving and accepting the sample from depositor, IDA provides a hand written official receipt to depositor	
Rule 9.1	Deals with storage of microorganisms in IDA	Form BP9
Rule 10.1 and 10.2	Viability testing of sample	Form BP10
Rule 11	Furnishing of sample by IDA	

[Table/Fig-3]: Rules and regulations for sample deposition and holding in IDA.

4) Identification reference given by the depositor for that microorganism;
5) Accession number given by IDA;
6) Accession number given by the previous IDA;
7) Name and address of previous IDA;
8) Scientific description of the microorganism.

Rule 6.3(a): This rule deals with, in which form the sample will be deposited to IDA. IDA may ask depositors to provide sample in agar slant/liquid suspension/lyophilized form, sample may be required in specific number of replicates etc.

2.3.2 IDA obligation [Table/Fig-3]

Language: In most country English is used as communication language. However, country wise language can also be used such as Japanese language is being used in IDA of Japan for sample deposition.

Rule 3: This rule says any culture collection center which acquires the IDA status should mention what kinds of microorganisms will be accepted for deposition under Budapest treaty, fees for storage and furnishing of samples, extension of list of kinds of microorganism accepted, official language of the said institution etc.

Rule 6.4(a): It deals with refusal of taking microorganisms for deposition in IDA in specific situations. There are situations when IDA does not accept microorganisms such as if the microorganism type is different from mandate of IDA, due to specific property of bacteria that makes it very difficult to be cultivated by the IDA personal despite that microorganism comes under IDA mandate for deposition or in case if microorganism is missing from the sample or breakage of sample during its shipment.

Rule 6.4(b), (c) and (d): Rule 6.4(b) and (c) deals with acceptance of original deposit in IDA. The depositor has to give all the required information as per the rule 6.1(a) and rule 6.3(b) in order to get the sample deposition in IDA. Rule 6.4(d) is used when a deposition is made in non-IDA culture collection institute, but if the culture collection is converted to IDA in that case the depositor has to follow all the requirements as mentioned in rule 6.1(a) and 6.3(b) to deposit sample in IDA.

Rule 7.1: After receiving and accepting the sample from the depositor, IDA provides a hand written official receipt also known as international form BP4 to depositor mentioning following information:

1) Name and address of the IDA;
2) Name and address of the depositor;
3) Date of deposition of the sample;
4) Any identification reference given by the depositor;
5) Any taxonomic or scientific information which is mentioned by the depositor, obeying the rule 6.1(a);
6) Accession number given by the IDA to that deposit.

Form BP/4 is very important document which certifies that the sample is deposited in IDA by the depositor; obeying all rules and regulations of Budapest treaty.

Rule 9.1: This rule deals with storage of microorganisms in IDA. IDA has the responsibility to store the sample in safe and viable manner for its further use. The sample can be stored up to five to thirty years in IDA as per the requirement. In no case, secrecy of deposited sample is disclosed by IDA and Rule 9.2 has given specification in this regard. IDA does not give any information regarding microorganism that has been deposited for patent purpose, only in exceptional cases information can be given such as if the information is required by Industrial Property Office (IPO) for patent purpose or concern authority of IDA.

Rule 10.1 and 10.2: Viability testing of sample by IDA is required for proper storage of sample. Each sample that is deposited to IDA is tested for their viability at regular interval. The test depends on kind of microorganism that has been deposited and condition of storage. IDA not only perform viability test but also gives written report to depositor or any concern authority about the condition of sample if the concern authority ask for that. As per rule 10.2 following information should be present in viability test statement:

1) Name and address of IDA issuing it;
2) Name and address of depositor;

3) Result of viability test (sample is viable or not);
4) Accession number of sample given by IDA;
5) Date of viability test;
6) Information about the condition at which viability test was performed.

Rule 11: This rule talks about furnishing of sample to depositor or organization or person authorized to IDA. There are three rules in this regard Rule 11.1, Rule 11.2 and Rule 11.3. Rule 11.1 are used to give microorganism from IDA to IPO of contracting country in following situations:

IPO will request the IDA to give microorganism for patent procedure of a depositor who has filed an application for patent of that microorganism in said office; further sample will only be used by IPO for patent procedure. Rule 11.2 says IDA has to furnish the sample on request of depositor. Rule 11.3 says that sample can be provided to any person or organization by IDA only when it is certified by IPO, mentioning that the said microorganism is filed for patent purpose in that office.

Rule 12: Each IDA takes some fee in response to storage of microorganism. Fee can also be charged during viability testing and furnishing of samples.

For example MCC, Pune charges INR 2000 to 3000 for supply of culture. Charges on sample deposition depend on nature of deposition, no fee is taken for general deposition and INR 10,000 is taken for safe deposition. In case of patent, deposition the fee can be up to INR 20,000 per sample.

3. Microbial Resources Centers (MIRCEN)- Developed countries have money as well as good infrastructure facilities that helps in formation of IDA but poor countries and third world nations do not have capacity to invest on IDA. A proper awareness on IPR, monetary help, and creation of good infrastructure is required in those countries, as most of them have economy based on agriculture. In a partnership with the United Nations Environment Programme, and the United Nations Development Programme (UNDP), 34 Microbial Resources Centers (MIRCENS) have been established worldwide. MIRCEN was formed to give developing countries help in preserving and utilization of useful bacteria [15]. Although it was made for developing countries, developed countries such as United State has American Type Culture Collection (ATCC) which works as both IDA and MIERCEN centers. It has following objectives:

1) Provide infrastructure help in developing countries to develop culture collection centers;
2) Main emphasis has given to Rhizobacteria for its preservation, utilization and making in biofertilizer;
3) Give proper training to human resource for suitable working of bacterial deposition centers;
4) Establish International collaboration between developing countries for knowledge sharing of useful bacteria.

4. Indian Scenario on Culture Collection

According to World Data Center for Microorganisms (WDCM) [16], India is among the top five countries that store or hold maximum numbers of microbial cultures [Table/Fig-4]. Today, India has 30 culture collection centers that store approximately 194174 cultures as per WDCM record [Table/Fig-5]. While all over world there are 713 culture collection centers in 73 countries [Table/Fig-6]. Out of 2556283 numbers of microbial culture collections, 1045361 is contributed by bacteria while 783450, 37916 and 31833 comes from fungi, virus and cell lines respectively.

Rank	Country	Total number of hold of Cultures
1	USA	261637
2	Japan	254830
3	India	194174
4	China	187794
5	South Korea	167090

[Table/Fig-4]: Top five countries in world that hold maximum numbers of cultures.

Name of the Institute	Type of Collection	WDCM Registration number
DMSRDE Culture Collection, DRDO, New Delhi	Lichens, Fungi	166
National Collection of Dairy Cultures, Karnal, Haryana	Bacteria, Fungi, Yeasts	775
Culture Collection, Department of Microbiology, Bose Institute, Kolkata	Bacteria, Fungi, Yeasts	119
National Bureau of Agriculturally Important Microorganisms, Mau	Agriculturally Important Microbes	1060
Institute of Microbial Technology, Chandigarh	All kinds of Microbes	773
Indian Type Culture Collection, IARI, New Delhi	Bacteria, Fungi	430
NII Microbial Culture Collection, Kerala	Bacteria, Fungi, Yeasts	961
Microbial Culture collection, Pune	Microbes	930

[Table/Fig-5]: List of important culture collection centers in India.

Name of the Institute	Type of Collection	Total number of deposits	WDCM regis-tration number
American Type Culture Collection(ATCC), USA	B, F, Y, A, V, Protozoa, Cell lines, Plant tissue, Seed	72000	1
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Germany	B, F, Y, P, Cell Lines, V, Archaea	23000	274
CAB International (CABI), UK	B, F, Y, Plant parasitic Nematodes	28000	214
NBRC Culture Collection, Japan	B, Y, V, Microalgae, Human cDNA Clones, Archaea	126000	825
National Center for Agricultural Utilization Research (NCAUR), USA	A,B,F,Y	94000	97
Centraalbureau voor Schimmelculture (CBS), Netherlands	F,Y	72000	133
Universiteit gent-laboratorium voor microbiologie, Belgium	B	25000	296
B- Bacteria, F- Fungi, Y- Yeast, A- Algae, V- Virus			

[Table/Fig-6]: List of some important culture collection centers in world.

4.1 The Microbial Type Culture Collection and Gene Bank (MTCC): MTCC culture centre was established in 1986 at IMTECH, Chandigarh and is jointly funded by DBT and CSIR [17]. In 2002, WIPO has given the centre the status of IDA (WDCM registration number 773) and it was first centre in India that got this status. Currently, MTCC has stored almost 20000 cultures from *Actinomycetes*, bacteria, fungi, yeasts and plasmids of hazard group 1 (not dangerous to humans) and 2 (may cause human disease but rarely spread in human community and treatment is available). It performs following functions.

1) Sample deposition: There are three kinds of deposit that MTCC takes: general deposits, safe deposits and patent deposits [18].

a) General deposits: Here, researcher can deposit the valuable culture of identified strain for safe storage in MTCC. Depositor has to give culture data sheet, mentioning the relevant information of strain and provide the strain in freeze-dried form to the center. MTCC does not charge any fee for this deposition and store samples up to five years.

b) Safe deposits: Cultures are stored in secure place and only depositor has access to it. MTCC charge an annual fee from depositor and an agreement is signed between depositor and

MTCC. In most cases safe deposits are made for five years.

c) Patent deposits: Microorganisms are deposited for patent purpose in a confidential manner in MTCC for upto 30 years. A onetime fee is also given by depositor to MTCC in this regard. Cultures can be deposited for International and national patent filing as MTCC is recognized as IDA by WIPO.

2) Supply of cultures: MTCC also supplies the cultures for research purposes. It supplies the cultures in freeze dried form while cultures of fungi and yeast in active form after the request from the researcher. It takes some fee in this regard.

3) Characterization of Samples

a) Phenotypic characterization: It includes morphological, physiological, biochemical and antimicrobial susceptibility test. The test includes, Gram staining, morphology study, colonial pigmentation, motility, oxidase, H_2S, indole, urease and catalase production, growth at different temperatures (4-37°C), salt concentrations and different pH are examined, citrate utilization, nitrate reduction etc.

b) 16S rRNA analysis: This method is used for identifying and classifying of bacteria. 1500 bp of gene for 16S rRNA gene is amplified by universal primers and send for sequencing. The obtained DNA sequence is matched with other bacterial DNA sequence database to check whether the bacteria are novel or already known.

c) Fatty acid methyl esters test: They are present in phospholipid bilayer with proteins on bacterial membranes. The presence of diverse molecules on membrane of bacteria in different combination gives every bacterial species unique identification property. Fatty acid profiles are usually determined by using Gas Chromatography (GC) method.

d) MALDI-TOF analysis: Like DNA fingerprinting, Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) method known as protein fingerprinting. Bacterial protein is taken, converted to volatile form and fragmented to different sizes of polypeptide chains, which moves according to mass by charge ratio (m/z) from negative charged plate to positive charged plate, heavy polypeptides move slowly while lighter ones move quickly (time of flight is calculated) and then send for sequencing. Identified sequences are compiled together and searched for its similarity or dissimilarity with other sequences present in protein database of bacteria for identification purpose.

4) Other services: MTCC also performs some other services such as bacterial DNA isolation for genomic DNA sequencing purpose, G+C (Guanine+Cytosine) content percentage and ratio count of sample DNA which helps in identification of microorganism and freeze drying of microbial culture for their long term storage. Using advance methods such as Biolog (a biochemical test) and Vitek (growth-based method) are being used for bacterial identification. It also provides training programmes and workshops on microbial culture techniques for human resource development.

4.2 Microbial Culture Collection (MCC): MCC culture collection center is situated in NCCS, Pune and is funded by Department of Biotechnology (DBT). In 2011, it was recognized by WIPO as IDA and is registered in WDCM with registration number 930. At present MCC accepts bacteria, yeasts, fungi and plasmids in a host and/or as isolated DNA preparations belonging to hazards group 1 and 2 as per classification in India [19]. MCC works in same manner as MTCC by obeying the all rules and regulations of Budapest Treaty. However, few services such as phenotypic characterization, Biolog test and Vitek system are still to be included at the institute.

CONCLUSION

IDA plays an important role in conservation of microbial population. A culture collection center not only stores valuable microorganisms but also stores other useful biological materials which can be used in research, agriculture, industry, and pharma sector etc. Everyday new microorganisms are being discovered; IDA provides a platform for storage of these bacterial strains in pure form thus preventing loss of biodiversity. The stored bacterial strains can further be utilized for the research and potential application. To convert a culture collection centre into an IDA requires huge financial support, infrastructure and manpower which itself is a big challenge. Budapest treaty gives provision for interstate deposition of microorganisms in IDA. Valuable bacterial strains found in countries where there is no IDA can be deposited in IDA of other countries.

It is important to understand that the misuse of IDA should also be avoided. IDA stores both pathogenic and non-pathogenic bacteria; pathogenic bacteria could be used in making biological weapons. In upcoming years, more and more countries will ratify the Budapest treaty and give emphasis on storage of valuable microorganisms at culture collection centers for their better utilization.

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