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REPUBLICATION

(54) **DNA FRAGMENTS ARRAY FROM BIOMINING MICROORGANISMS AND METHOD FOR DETECTION OF THEM**

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(57) **ABSTRACT**

The present invention discloses an array of DNA fragments from biomining microorganisms and a method to identify readily and simultaneously said microorganisms in a sample. This method is a useful tool in biomining, in every circumstance where a global understanding of the present microbiological diversity is required, or simply to assess the presence of some microorganism with biomining relevance, either on the mineral, or in a bioleaching heap, in the biomining laboratory or in any other circumstance involving biomining microorganisms.

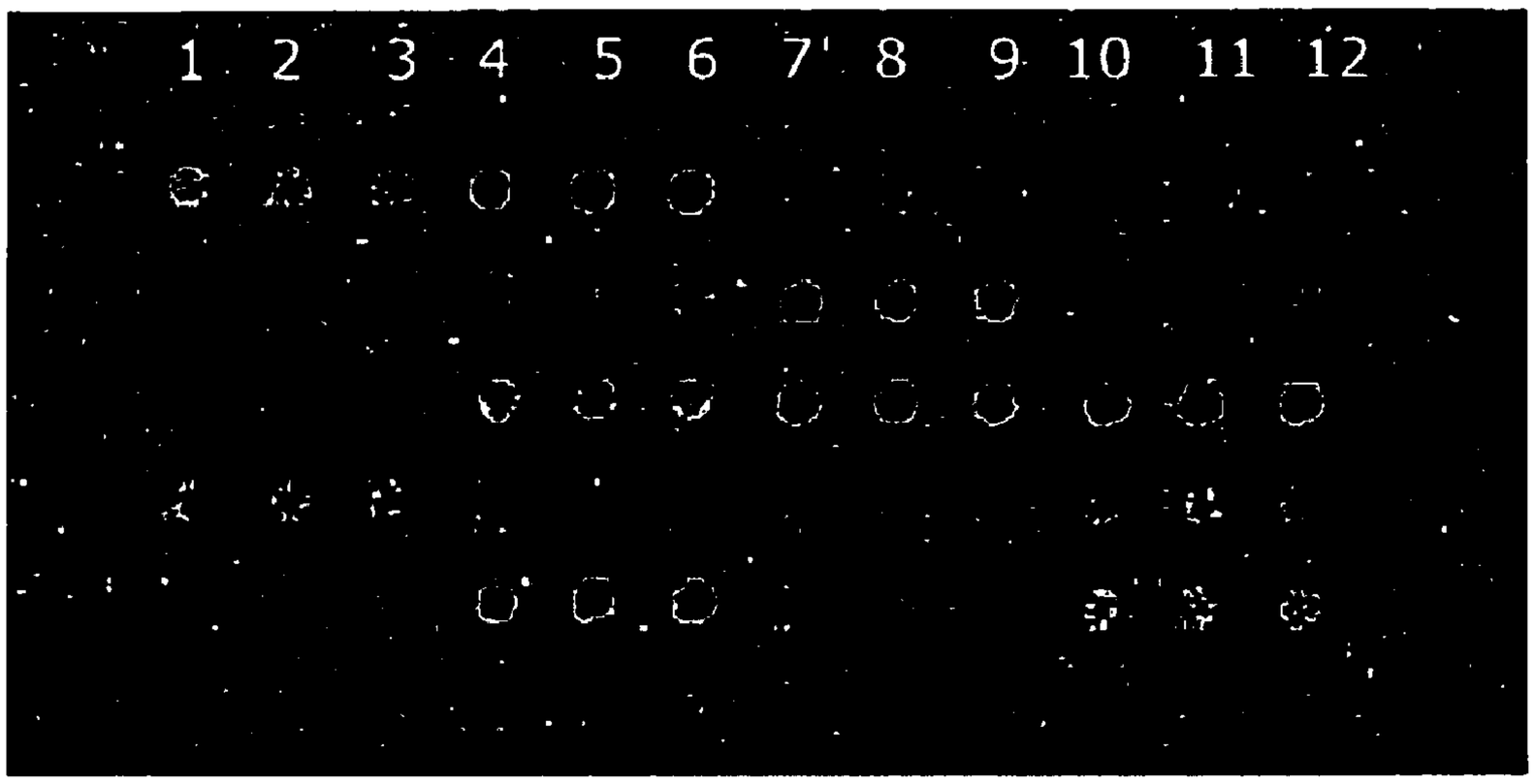


Figura 1. **M1**

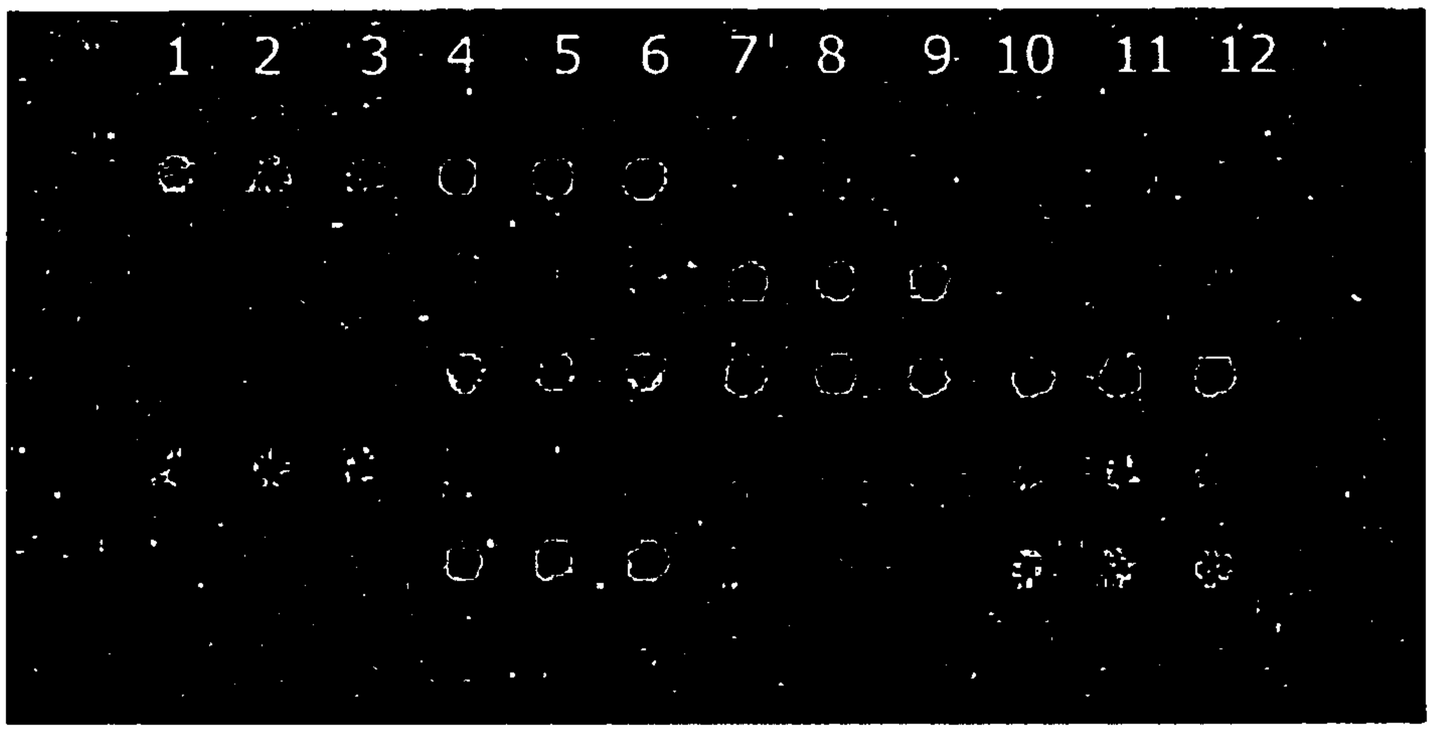
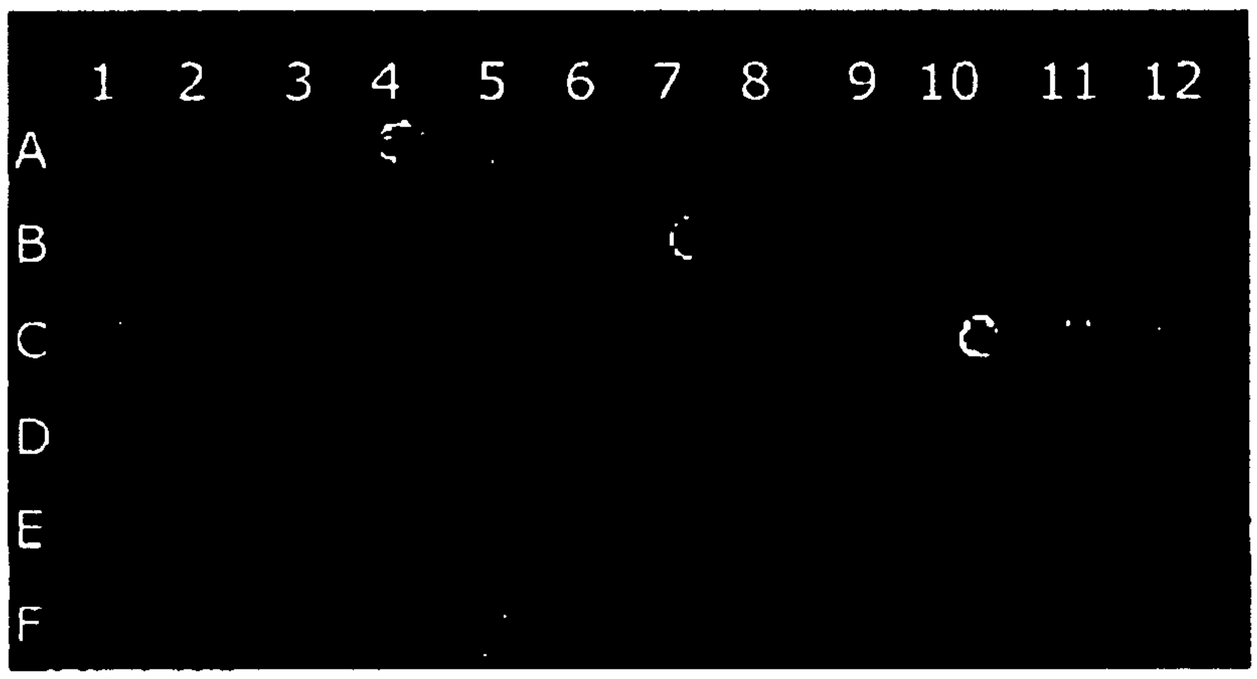


Figura 2. **M2**



DNA FRAGMENTS ARRAY FROM BIOMINING MICROORGANISMS AND METHOD FOR DETECTION OF THEM

FIELD OF THE INVENTION

[0001] The present invention discloses a biomining microorganism DNA fragment array and a method to identify said biomining microorganisms in a sample readily and simultaneously. This method is a useful tool in biomining, in every circumstance where a global understanding of the present microbiological diversity is required, or simply to assess the presence of some microorganism with biomining relevance, either on the mineral, or in a bioleaching heap, in the biomining laboratory or in any other circumstance involving biomining microorganisms.

BACKGROUND OF THE INVENTION

[0002] Biomining is, in general terms, the use of microorganisms for the recovery of metals from minerals. Its most traditional expression is bioleaching, but we understand biomining as encompassing not only this process, but also the monitoring and intervention of the involved microorganisms, as these techniques are complex and subjected to permanent development; laboratory level research associated to the improvement of processes or the development of new methodologies are also included.

[0003] Bioleaching is defined as a method to solubilize metals from complex matrixes in an acid medium using direct or indirect microorganism action. Microorganisms that are useful in these processes belong both to Bacteria and Archaea domains and fulfill two basic conditions: they are acidophiles and chemolithotrophic.

Microorganisms Associated with Bioleaching Processes.

[0004] Many microorganisms have been described as being useful in bioleaching processes, among which we can identify genera *Acidiphilium* spp., *Leptospirillum* spp., *Sulfobacillus* spp., *Acidithiobacillus* spp. and species *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* belonging to Bacteria domain. From Archaea domain we can identify genera *Acidianus* spp., *Ferroplasma* spp., *Metallosphaera* spp., *Sulfolobus* spp. and *Thermoplasma* spp. (Rawlings D E. Annu Rev Microbiol. 2002; 56:65-91; Rawlings D E. Microb Cell Fact. 2005; 4(1):13).

[0005] Factors determining diversity and metabolic activity of the microbiological community associated to bioleaching processes.

[0006] The microorganisms belonging to each above mentioned genus and species produce compounds that increase the rate of different chemical reactions, which allows carrying out bioleaching processes in much shorter times. For this, microorganisms require in their turn a suitable environment to promote said reactions that, for instance, could be aerobic or anaerobic, or require some specific nutrient. Therefore, the environmental conditions under which the bioleaching process is carried out modify the activity and microbiological composition of the present community.

[0007] It has been proposed that microorganism participation in bioleaching processes could be direct or indirect (Rawlings D E. Microb Cell Fact. 2005; 4(1):13). It is direct when microorganisms act directly over the metal or over its counter-ion, in either case releasing one ion of the desired metal. On the other hand, the participation is indirect when the microorganism does not use the desired metal or its counter-ion as a substrate, but generates chemical conditions that accelerate or favor said metal solubilization, either by acidification of the medium (e.g., by generating sulfuric acid) or by generating an oxidizing agent that finally interacts with the salt (metal and counter-ion) to be solubilized. For instance, species belonging to genus *Acidithiobacillus* are able to produce elements that increase the oxidation rate of reduced sulfur compounds (such as sulfide, elemental sulfur, thionates, etc.) by using oxygen as electron acceptor. During this process they generate sulfuric acid as final product and reducing species such as sulfite and thiosulfate as intermediate products, which allows solubilizing sulfur associated metals in the mineral. In particular, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* contribute with biological components that favor iron (II) to iron (III) oxidation using oxygen as electron acceptor. Generated iron (III) is a major oxidizing agent that can oxidize present sulfides or any other compound to be oxidized.

[0008] The common mining practice in bioleaching processes is to leave a heap of mineral in an acid medium, generally sulfuric acid, and gradually remove the acid medium to recover the metal by electrolysis. Often efficient metal recovery yield heaps and "inefficient" heaps are obtained, these latter having a lower yield under the same operation conditions and characteristics of the leached substrate. Being bioleaching a microbiological process, differences of efficiency levels between heaps could be consequence of differences in abundance and type of species in the microbiological community composing them. In this way, this low yield problem could be solved, for example, by inoculating microorganisms that produce components favoring the desired reaction to be maintained during the process. Nevertheless, up to date there is no method allowing the fast, specific and simultaneous identification of bioleaching microorganisms present in a sample.

Microorganisms Detection Method.

[0009] According to the state of the art, if it would be desired to determine the major components of a microbiological population with biomining relevance that are present in a sample, it could be done using techniques such as: denaturing gradient gel electrophoresis (DGGE), fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), or selective cultures. These techniques are labor intensive, expensive and require highly qualified workers to perform them. For example, DGGE assay is slow, its completion taking about 3 weeks, and have low sensitivity (detection limit: 10^4 microorganisms/ml), which is inadequate for biomining communities, as normally there are relevant taxons at lower concentrations than said detection limit. PCR technique also has problems, requiring firstly an individual reaction for each of the species to be determined, which is slow and extremely laborious. In second place, when working with a metagenomic sample, there is the risk of primers having cross-reactivity with other of the species found in the sample, giving both false positive and false negative results. Finally, analysis of microbiological communities using the traditional identification method by culturing has the risk of some species that are present in lower proportion being lost in the process and not being detected. This risk is always present in the case of selective cultures, but is increased when dealing with biomin-

ing microorganisms, as the conditions that maintain the growth of these microorganisms are hard to achieve, and some of them are definitely not even able to be cultured in the laboratory. Other problem of the analysis by culture is the slow development of the process, which could take many days.

[0010] In consequence, in the state of the art there is no simultaneous detection method for many organisms that is simultaneously fast, specific and cheap.

[0011] By mean of the present invention said technical problem has been solved by creating a simultaneous identification method for biomining microorganisms using a DNA fragment array technique.

[0012] A good definition of DNA array is that proposed by Schena et al. (Trends Biotechnol. 16, 301-306): "a microscopic ordered nucleic acid array that allows simultaneous analysis of complex DNA samples" (Schena M., Heller, R. A., Theriault, P., Konrad, K., Lachenmeier, E. and Davis, R. W. (1998)). Depending on the diameter of the deposited DNA spots, there are 2 array types: macro-arrays (300 microns or more) and micro-arrays (less than 100 microns). The first can be manually manufactured in the laboratory and the spots can be observed without the help of special equipment. The second require an automated deposition process (normally a robotic deposition platform) and a specialized image acquisition and processing equipment.

[0013] In this particular case, DNA fragment arrays comprise an ordered series of spots deposited on a flat surface, such as a glass, silicon or nylon sheet, where every spot contains a large amount of copies of a known DNA fragment that is specific for a determined microorganism with biomining relevance.

[0014] The selection method using DNA fragment arrays comprise a simultaneous hybridization of the set of array "spots" with a labeled DNA extract of the studied sample. Normally, DNA from the sample, which has been labeled and fragmented as required, is subjected to a denaturation stage wherein the double stranded DNA is separated, e.g. by heating. When temperature is lowered, DNA will tend to hybridizes with its most complementary fragment according to its physicochemical characteristics. Being this DNA in contact with the array, if there is coincidence between sample DNA and the DNA fragment contained in a spot, labeled sample DNA copies will specifically attach to said spot with the largest possibility. This is due to the larger amount of complementary DNA copies contained in the array spot. In the acquisition and processing stage of the hybridized array image, this label will allow the detection of the microorganisms present in the studied sample.

[0015] DNA labeling can be done by any known labeling technique, being fluorescence and radioactive labeling the most common ones.

[0016] Arrays and their usage method are known, and we find examples of arrays in the state of the art used to detect the presence of microorganisms in a sample, but none of them is directed to microorganisms that are relevant in biomining.

[0017] At the present time, diverse published protocols exist for the manufacture of DNA fragment arrays, and there are also laboratories that offer manufacturing services for this type of arrays. Consequently, only the selection of genes and the design of used DNA fragments defines the specificity and utility of an array, as the manufacture can vary according to the matrix, the method used to bind DNA fragments to the matrix, the spatial distribution of the spots on the matrix, etc., depending on the manufacturing company or the protocol used to manufacture the array in the laboratory (Ye et al. Journal of Microbiological Methods 47 (2001): 257-272).

BRIEF DESCRIPTION OF THE INVENTION

[0018] The present invention discloses a DNA fragment array from biomining microorganisms and a method to readily and simultaneously identify said biomining microorganisms in a sample.

[0019] We have designed DNA fragments comprising 100 or less nitrogenous bases that allow the specific and unequivocal identification of the following taxons having biomining importance: bacteria *Acidiphilium* spp., *Leptospirillum* spp., *Sulfolobus* spp., *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*; and archaea *Acidianus* spp., *Ferroplasma* spp., *Metallosphaera* spp., *Sulfobacillus* spp. and *Thermoplasma* spp. Moreover, we have included sequences for the detection of strain Wenelen (DSM 16786), owned by Biosigma, which can be conveniently included in biomining processes.

[0020] By disposing at least one of these DNA fragments in an array, the presence of at least one biomining relevant microorganism can be detected and identified. Preferentially, arrays containing many of the designed DNA fragments are provided, which allows the simultaneous identification of many or all the biomining relevant microorganisms in a sample by using a single process.

[0021] Preferably, a sufficient number of different DNA fragments are included in the arrays to detect all the biomining relevant microorganisms in a sample.

BRIEF DESCRIPTION OF THE FIGURES

[0022] FIG. **1** shows the result of hybridizing a biomining sample 1 with an array of the invention. Fragments of the invention correspond to sub-fragments of fragments of 16S rDNA and tRNA-synthases genes described in Table 2. Each fragment was deposited by triplicate. In Table 1, the content of each position in the microarray is detailed.

[0023] Conclusion: The results indicate the presence of *A. ferrooxidans, Leptospirillum* spp., and *Ferroplasma* spp. in sample 1 (M1). Data indicate that correlation always existed between spots with 16S rDNA fragments and spots with tRNA-synthases. All positive controls showed a hybridization signal and negative controls remained unmarked.

[0024] FIG. **2** shows the result of hybridizing a second biomining sample with a microarray of the invention. The used microarray is the same used for sample 1, the content of which is described in Table 1. The fragments used in the example are contained in the sequences for 16S rDNA and tRNA-synthases described in Table 2. Each fragment was deposited by triplicate.

[0025] Conclusion: The results indicate the presence of *A. ferrooxidans, A. thiooxidans, Acidianus* spp., *Leptospirillum* spp., *Ferroplasma* spp., *Metallosphaera* spp. and *Sulfolobus* spp. in sample 2 (M2). Data indicate that correlation always existed between spots with 16S rDNA fragments and spots

with tRNA-synthases. All positive controls showed a hybridization signal and negative controls remained unmarked.

DETAILED DESCRIPTION OF THE INVENTION

[0026] A method that allows fast and simultaneous detection and identification of biomining microorganisms would have applications in diverse industrial areas. As an example, the identification of microorganisms present in a bioleaching heap could become a tool to appropriately control the bioleaching process, as it could be established whether it is necessary to inoculate some particular microorganism into the heap or not, thus maximizing the amount of mineral recovered in the process.

[0027] With our resources put in the former objective, we have designed DNA fragments useful to detect and identify the different microorganisms relevant in biomining by disposing them in a DNA array. These microorganisms relevant in biomining are bacteria *Acidiphilium* spp., *Leptospirillum* spp., *Sulfolobus* spp., *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*; archaea *Acidianus* spp., *Ferroplasma* spp., *Metallosphaera* spp., *Sulfobacillus* spp. and *Thermoplasma* spp.; and strain Wenelen (DSM 16786).

[0028] The strain Wenelen (DSM 16786), owned by Biosigma, has an increased oxidizing activity, especially in the case of chalcopyrite, in comparison with other known microorganisms. This feature makes its incorporation in biomining processes convenient and the arrays of the present invention would allow monitoring its presence in the media where it has been inoculated.

[0029] All DNA arrays base their detection capacity on the DNA fragments designed to be synthesized and bound onto a solid substrate. These DNA fragments, short nucleotide sequences having 300 or less nitrogenous bases in our case, are designed from specific genomic sequences of the microorganisms to be detected. Therefore, the first step is choosing the genes, regions or sequences of the genomes of the desired microorganisms from which the deposited fragments will be selected.

[0030] For a greater convenience in the design of DNA fragments and eventually in the use of the array, a limited genomic region can be selected, which has to be conserved in all the relevant genomes, i.e. have to be present in all the relevant microorganisms. Furthermore, said region must be variable enough (in its nucleotide sequence) to allow distinguishing between different species and even between different strains in case it should be required.

[0031] An advantage of using a genomic region is given by the fact that the array can be used in conjunction with the product of a PCR that universally amplifies the selected region, which eventually could increase the sensitivity of the method.

[0032] Advantageously, in a microorganism identification array many different spots that identify the same microorganism are included, which assures that the user could be more trustful on obtained data. In this way, each point set identifying a particular microorganism acts as an internal control by itself for "false positives" or "false negatives" generated by interferences during the course of the method.

[0033] We have opted to include both strategies in our arrays. In one hand, we work with the same genomic region for every microorganism to be identified, taking into account that arrays can be eventually provided only with this set of spots. On the other hand, we have also included many genomic regions of the same microorganism, and in this way the whole array, which contains all the DNA fragments designed by us, will contain a set of spots for the identification of each biomining microorganism.

[0034] The first gene selected by us is the gene codifying for the 16S ribosomal RNA molecule, herein referred as 16S rDNA, traditionally used for identification. The other selected characteristic genomic regions are genes codifying for different tRNA-synthases, which are represented in substantially all microorganisms due to their relevance. These genes have the required conservation and variability features that are adequate to identify microorganisms, as set forth above.

[0035] Once these regions were selected, DNA fragments having 100 nitrogenous bases were designed, each one specific for each of the microorganisms to be identified in the process, i.e. for bacteria *Acidiphilium* spp., *Leptospirillum* spp., *Sulfobacillus* spp., *Acidithiobacillus* spp., *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*; archaea *Acidianus* spp., *Ferroplasma* spp., *Metallosphaera* spp., *Sulfolobus* spp. and *Thermoplasma* spp.; and strain Wenelen (DSM 16786).

[0036] The designed fragments can be deposited on the array either entire, or comprised in a larger fragment that contains them, or in partial form, i.e. as any of the sub-fragments comprised in the fragment, or as the reverse complementary sequences of any of the former options. Advantageously, sub-fragments having 50 or 70 nucleotides were deposited.

[0037] DNA fragment design was carried out using a proprietary method protected by patent application CL 2102-05, filed by Biosigma. Said method is applied in the oligonucleotide design software "Massive Primer Designer". For more clarity, we will briefly explain the method.

[0038] A database was selected, SHIFTSIXTEEN®, which contains all sequences from the selected genomic regions for all taxons or strains to be determined. In this first stage, we will refer to nucleotide sequences as words having defined length in the alphabet {A,C,T,G}. Each sequence is computationally scanned from 5' to 3' to obtain all existing words that have the defined length for desired DNA fragments to be designed, 100 in this case. This oligonucleotide candidate pass through the following tests in the same described order, wherein a rejection in one of the tests means the total rejection of the candidate.

[0039] 1. GC composition level: This is a filter that allows discarding a priori candidates that have very high or very low hybridization temperature values, by performing very cheap calculations in terms of time. The candidate is rejected if its GC composition falls off the limits imposed at the moment of executing the calculation.

[0040] 2. Hybridization temperature: Given a sequence and environmental conditions (salt concentration, nucleotide concentration, etc.), the hybridization temperature between said sequence and its complementary one is calculated. When executing the calculation, an oligonucleotide is rejected if its hybridization temperature falls off the established limits to be used during the hybridization assay.

[0041] 3. Secondary structure: for a reference temperature, for each candidate sequence is examined to know whether a stable secondary structure (three-dimensional fold of the oligonucleotide by itself) is formed or not. If a secondary structure is formed, the oligonucleotide is replaced by its reverse complementary sequence; if this reverse complementary also forms a secondary structure, then the oligonucleotide is rejected. If only the original form of the oligonucleotide forms a secondary structure, but not its reverse complementary sequence, then this latter is selected as candidate oligonucleotide for the following tests.

[0042] 4. Specificity: using one or more sequence alignment algorithms, each candidate is tested for relevant similarity with sequences pertaining to other microorganisms that can probably be present in a biomining sample.

[0043] If all tests were approved, the candidate is selected as possible oligonucleotide to be used as deposited DNA fragment in an array.

[0044] In this method some other optimizations are included, such as the initial selection of sequences that are relatively specific for each microorganism, in order to limit the oligonucleotide search in selected regions.

[0045] Using this method, a plurality of oligonucleotides was obtained that fulfill all the described requirements and can be used in an array for the detection and identification of biomining relevant microorganisms.

[0046] We have designed a total of 70 DNA fragments for the identification of different biomining relevant taxons, each one having 100 nucleotides. The sequences of all designed 70 DNA fragments were included in the listing of sequences.

[0047] From all the 70 designed sequences, sequence No 1 is specific for *Acidiphilium* spp., corresponding to a 16S rDNA gene fragment.

[0048] There are 9 specific sequences for *Leptospirillum* spp., sequences No 2 to 10, from which sequence No 2 corresponds to a 16S rDNA sequence and the others (sequences 3 to 10) are fragments of genes codifying for different tRNA-synthases.

[0049] One sequence is specific for *Sulfobacillus* spp., sequence No 11, corresponding to 16S rDNA.

[0050] Two sequences are specific for *Acidithiobacillus* spp., namely sequences No 12 and 13. Sequence No 12, corresponding to 16S rDNA, specifically recognizes *Acidithiobacillus* spp. different from species *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. On the other hand, sequence No 13, also corresponding to 16S rDNA, is specific for the entire genus.

[0051] Sequences No 14 to 26 recognize the species *Acidithiobacillus ferrooxidans*, where sequences 14 to 17 correspond to 16S rDNA and sequences 18 to 26 correspond to tRNA-synthases.

[0052] Sequence No 27 is specific for *Acidithiobacillus thiooxidans* and corresponds to 16S rDNA.

[0053] One of the sequences is specific for *Acidianus* spp., sequence No 28, corresponding to 16S rDNA.

[0054] There are 11 sequences that are specific for *Ferroplasma* spp., namely sequences No 29 to 39, from which sequence No 29 corresponds to 16S rDNA and sequences No 30 to 39 correspond to tRNA-synthases.

[0055] Sequence No 40 is specific for *Metallosphaera* spp. and corresponds to 16S rDNA.

[0056] There are 10 sequences that are specific for *Sulfolobus* spp., namely sequences No 41 to 50, from which sequence No 41 corresponds to 16S rDNA and sequences No 42 to 50 correspond to tRNA-synthases.

[0057] Sequences No 51 to 59 are specific for *Thermoplasma* spp., from which sequence No 51 corresponds to 16S rDNA and sequences No 52 to 59 correspond to tRNA-synthases.

[0058] Finally, there are 11 sequences that are specific for strain Wenelen (DSM 16786), namely sequences No 60 to 70, where sequence No 70 is a tRNA-synthase and sequences No 60 to 69 correspond to specific sequences for the strain.

[0059] Apart from these DNA fragments that are specific for each biomining relevant microorganism, it is convenient to include negative and positive controls in each array. Negative controls must be nucleotide sequences that should never be found in a biomining context. Positive controls must be nucleotide sequences that are always present in a test sample.

[0060] It should be noted that the arrays disclosed in the present invention are those comprising at least one of the DNA fragments included in sequences No 1 to 70, either entirely, or in a larger region comprising them, such as a PCR product, or partially, i.e. as one of the sub-fragments contained in each of the fragments herein disclosed, or as the reverse complementary sequence of any of the former options. This is vitally relevant, as the specificity of a nucleotide sequence is the same specificity of its reverse complementary sequence, and it is this feature, i.e. specificity, the more difficult goal to achieve in the design of DNA fragments. It could be possible that the stability of the reverse complementary sequence will not be suitable for the sequence to be used in an array, but nevertheless the skilled person will distinguish between thermodynamically stable and unstable oligonucleotides by means of diverse tools existing in the art. All reverse complementary sequences of fragments No 1 to 70 of the present invention, either entirely, or in a larger region comprising them, such as a PCR product, or partially, i.e. as one of the sub-fragments contained in each of the fragments herein disclosed, are to be considered within the scope of the present invention.

[0061] Preferably, the array will contain at least one fragment or sub-fragment for each biomining relevant species. It is also possible to manufacture an array containing all disclosed DNA fragments or sub-fragments. All these options, together with all the intermediate possible combinations, are comprised within the scope of the present invention.

[0062] The efficiency of the arrays of the invention is given by the specificity and stability of the fragments to be deposited. These characteristics are retained by each sub-fragment contained within the designed fragments. This means that specificity is retained if nucleotides 1 to 100, or 42 to 92, or 15 to 65, or any other possible selection is used. All selections are sub-fragments and are comprised within the scope of the present invention.

[0063] It is also possible to have DNA fragments that contain fragments or sub-fragments of the invention flanked by

other oligonucleotides, either by synthesis or as PCR products. These larger fragments that contain the fragments of the present disclosure, the specificity of said fragments given by the fragments or sub-fragments designed by us, are also to be considered within the scope of the present invention.

[0064] Each selected fragment or sub-fragment have to be synthesized in many hundreds of copies and deposited as a homogeneous point on a suitable array support, such as glass, silicone, nylon or other support in the art.

[0065] As we mentioned before when discussing the background of the invention, synthesis techniques for DNA fragments and array manufacture are known, and any of them could be used to manufacture the arrays of the present invention.

Use of the Array

[0066] For the detection and identification of biomining microorganisms by using the arrays of the present invention, a sample DNA to be evaluated must be firstly isolated. It is also possible to work with cDNA, the only difference being that RNA is firstly isolated from the sample in this case. Many DNA and RNA extraction methods from mineral or soil samples are known in the art and any of them can be used, considering in each case the particular nature of the sample.

[0067] In a second stage, the entire sample DNA or RNA must be converted to short labeled fragments, which are suitable for hybridization with the fragments that are deposited in the array spots. In case that sample DNA was isolated, this DNA has to be fragmented and labeled. In case that the study is being carried out using sample RNA, fragmentation is not required and only labeling must be performed to obtain labeled cDNA. A technique that enables to fragment and label DNA at the same time is labeling by using random 6-nucleotide DNA primers. Labeling can be done using labeled nucleotides or the sample can be labeled using any of the other techniques in the art, such as radioactivity, biotin, fluorescence labeling or other. Preferably, if a macroarray is to be used, labeling will be done by using radioactivity, ^{32}P, and if a microarray is to be used, fluorescence should be preferred, e.g. using Cy5 or Cy3.

[0068] Alternatively, if the selected array comprises DNA fragments belonging to a single genomic region, such as e.g. 16S rDNA or methionyl-tRNA-synthase, a PCR amplification stage can be performed using universal primers that contain the selected regions, and directly labeling the PCR product using labeled nucleotides or nucleotides that are able to be labeled.

[0069] The methods described for the preparation of DNA or cDNA for the array do not limit the present invention, and any existent method can be used for the preparation of DNA or cDNA without causing the use of the array to be out of the scope of the present invention.

[0070] Once the DNA is prepared, it is subjected to a DNA denaturation stage, and subsequently said denatured DNA is incubated on the array by putting an aliquot of the DNA mix on the array. The array is left to hybridize at a suitable temperature for at least one hour, and preferably overnight.

[0071] After the hybridization stage, the array must be carefully washed, usually using buffer solutions at moderate temperatures between 35-50° C., preferably between 40-45° C.

[0072] Once washed, the array is preferably dried, advantageously by centrifugation, for example in a Falcon tube, for a short time at moderate velocity.

[0073] Finally, labeled spots should be visualized, and the position of each labeled point indicates the presence of the biomining microorganism from which the corresponding DNA fragment was designed.

[0074] Furthermore, the negative control spots are controlled for absence of signal, as the presence of hybridization with said DNA fragments would indicate an unspecific reaction and therefore the obtained results should be discarded due to the presence of false positives.

[0075] Likewise, positive control spots must be marked, as the absence of hybridization with said DNA fragments would indicate interferences in the reaction and therefore those spots without signal could be false negatives.

[0076] Consequently, the determination of the composition of a biomining microbiological community would be reduced to the reading of labeled spots in an array of the present invention.

EXAMPLES

Example 1

Microarray to Detect and Identify the Presence of Biomining Relevant Microorganisms

[0077] A microarray was manufactured having twenty different DNA fragments that specifically identify the 10 most relevant taxons in biomining: *Acidiphilium* spp., *Leptospirillum* spp., *Sulfobacillus* spp., *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Acidianus* spp., *Ferroplasma* spp., *Metallosphaera* spp., *Sulfolobus* spp. and *Thermoplasma* spp.

[0078] In all cases, a DNA fragment from the 16S rDNA genomic region was included, and for some taxons two fragments corresponding to different tRNA-synthases were included, in order to have an internal control in these cases. One positive control and three negative controls were also included in the microarray. In the following Table 1, the content of each position in the microarray is detailed.

TABLE 1

	Position in the microarray	
Microorganism	16S rDNA	tRNA-synthase
A. ferrooxidans	A4-A6	B7-B9; C7-C9
A. thiooxidans	A7-A9	—
Acidiphilium spp.	E7-E9	—
Acidianus spp.	C1-C3	—
Leptospirillum spp.	C4-C6	C10-C12; E4-E6
Sulfobacillus spp.	D4-D6	—
Ferroplasma spp.	D1-D3	E10-E12; D10-D12
Metallosphaera spp.	B4-B6	—
Sulfolobus spp.	D7-D9	F4-F6; A10-A12
Thermoplasma spp.	E1-E3	F1-F3; F10-F12
Positive control	A1-A3	
Negative control	F7-F9; B10-B12; B1-B3	

[0079] All deposited fragments were 60 nucleotides long. Selected DNA fragments of the invention are 60-nucleotide-long sub-fragments of the fragments detailed in Table 2, which are defined in the list of sequences.

TABLE 2

Microorganism	tRNA-synthase Amino-acyl	tRNA-synthase Sequence N°	16S rDNA Sequence N°
A. ferrooxidans	Alanyl	19	14
	Leucyl	26	—
A. thiooxidans	—	—	27
Acidiphilium spp.	—	—	1
Acidianus spp.	—	—	28
Leptospirillum spp.	Isoleucyl	3	2
	Alanyl	6	—
Sulfobacillus spp.	—	—	11
Ferroplasma spp.	Threonyl	37	29
	Valyl	39	—
Metallosphaera spp.	—	—	40
Sulfolobus spp.	Glutamyl	41	41
	Alanyl	43	—
Thermoplasma spp.	Isoleucyl	54	51
	Histidyl	56	—

[0080] Each fragment was deposited by triplicate. The manufacture of the microarray was carried out by a specialized company in the field.

Example 2

Use of the Microarray to Detect and Identify Biomining Relevant Microorganisms

[0081] The microarray obtained in Example 1 was used to determine the composition of the microbiological community of two bioleaching heap effluent samples, sample 1 (M1) and sample 2 (M2).

[0082] Total DNA was extracted from M1 and M2 using traditional DNA extraction methods.

[0083] 2 μl were taken from the DNA samples and put in Eppendorf tubes. In each case, the following method was carried out:

[0084] 36 μl of ddH_2O and 3.3 ml of 6-nucleotide random primers were added. The mix was boiled for 5 minutes and then the work was continued on ice.

[0085] 2 μl of a nucleotide mix were added, where dUTP was labeled with a Cy fluorophore. Cy3 was used for M1, with green fluorescence, while Cy5 was used for M2, with red fluorescence. Subsequently, 4 μl of a polymerase and 5 μl of buffer solution were added, and the mix was incubated for 4 hours at 37° C.

[0086] The reaction was stopped with 5 μl 0.5 M EDTA, pH 8. Labeled DNA was recovered using an ion exchange column. The DNA containing eluate was dried under vacuum.

[0087] DNA was resuspended by adding 100 μl of a buffer solution and was brought to 100° C. for one and a half minute, to denature DNA. Hybridization was carried out on the array at 55° C. overnight.

[0088] The following morning, each microarray was washed twice with 2×SSC, 0.1% SDS, at 45° C.; once with 0.2×SSC, 0.1% SDS, at 42° C., and once with 0.2×SSC, at 42° C.

[0089] Each microarray was put in a case with MilliQ water for 15 minutes and subsequently dried by centrifugation in a Falcon tube for 1 minute at 1100 rpm.

[0090] Finally, the results obtained for each microarray could be observed, which are shown in FIG. **1** for M1 and in FIG. **2** for M2.

[0091] In Table 3, the microarray positions of each different fragment are indicated and hybridization results with DNA from M1, which are shown in FIG. **1**, are summarized. It can be observed that, in every case, the families of spots (replicates of the same DNA fragment) had the same result and there always was total correlation between results obtained with 16S rDNA fragments and tRNA-synthase fragments. All positive controls showed hybridization and negative controls remained unmarked.

TABLE 3

Sample 1 (M1)

Microorganism	Position in the microarray: 16S rDNA	R	tRNA-synthase 1	R	tRNA-synthase 2	R
A. ferrooxidans	A4-A6	+	B7-B9	+	C7-C9	+
A. thiooxidans	A7-A9	–	/	/	/	/
Acidiphilium spp.	E7-E9	–	/	/	/	/
Acidianus spp.	C1-C3	–	/	/	/	/
Leptospirillum spp.	C4-C6	+	C10-C12	+	E4-E6	+
Sulfobacillus spp.	D4-D6	–	/	/	/	/
Ferroplasma spp.	D1-D3	+	D10-D12	+	E10-E12	+
Metallosphaera spp.	B4-B6	–	/	/	/	/
Sulfolobus spp.	D7-D9	–	A10-A12	–	F4-F6	–
Thermoplasma spp.	E1-E3	–	F1-F3	–	F10-F12	–
Positive control	A1-A3	+	/	/	/	/
Negative control	F7-F9	–	B1-B3	–	B10-B12	–

Legend:
(R): result;
(+): positive;
(–): negative;
(/): does not exist.

[0092] The results show the presence of *A. ferrooxidans, Leptospirillum* spp., and *Ferroplasma* spp. in M1.

[0093] In Table 4, once again the microarray position of each different fragment is indicated and hybridization results with DNA obtained from M2, which are shown in FIG. **2**, are summarized. As for M1, it is possible to observe that the new families of spots had always the same results, and correlation was kept between results obtained with 16S rDNA fragments and tRNA-synthase fragments. All positive controls showed hybridization and negative controls remained unmarked.

TABLE 4

Sample 2 (M2)

Microorganism	Position in the microarray and result: 16S rDNA	R	tRNA-synthase	R	tRNA-synthase	R
A. ferrooxidans	A4-A6	+	B7-B9	+	C7-C9	+
A. thiooxidans	A7-A9	+	/	/	/	/
Acidiphilium spp.	E7-E9	–	/	/	/	/
Acidianus spp.	C1-C3	+	/	/	/	/
Leptospirillum spp.	C4-C6	+	C10-C12	+	E4-E6	+
Sulfobacillus spp.	D4-D6	–	/	/	/	/
Ferroplasma spp.	D1-D3	+	D10-D12	+	E10-E12	+
Metallosphaera spp.	B4-B6	+	/	/	/	/
Sulfolobus spp.	D7-D9	+	A10-A12	+	F4-F6	+
Thermoplasma spp.	E1-E3	–	F1-F3	–	F10-F12	–

TABLE 4-continued

	Sample 2 (M2)					
	Position in the microarray and result					
Microorganism	16S rDNA	R	tRNA-synthase	R	tRNA-synthase	R
Positive control	A1-A3	+	/	/	/	/
Negative control	F7-F9	−	B1-B3	−	B10-B12	−

Legend:
(R): result;
(+): positive;
(−): negative;
(/): does not exist.

[0094] The results show the presence of *A. ferrooxidans, A. thiooxidans, Acidianus* spp., *Leptospirillum* spp., *Ferroplasma* spp., *Metallosphaera* spp. and *Sulfolobus* spp. in sample 2 (M2).

List of Sequences.

Sequence No.: 1

Length: 100

Type: DNA

Microorganism: *Acidiphilium* spp.

Category: 16S rDNA

[0095] Sequence:

ACA CAC GTG CTA CAA TGG CGG TGA CAG TGG GAA GCC
AAG CAG CGA TGC TGA GCT GAT CCT GAA AAG CCG TCT
CAG TTC GGA TTG CAC TCT GCA ACT CGGG

Sequence No.: 2

Length: 100

Type: DNA

Microorganism: *Leptospirillum* spp.

Category: 16S rDNA

[0096] Sequence:

ACG CAG CAA CGC CGC GTG TGG GAA GAA GGC CTT CGG
GTC GTA AAC CAC TTT TAC TCG GGA CGA AAA AGG GAT
ATC AAA TAA ATA TCC CCG ATG ACG GTAC

Sequence No.: 3

Length: 100

Type: DNA

Microorganism: *Leptospirillum* spp.

Category: Isoleucyl-tRNA-synthase

[0097] Sequence:

TAT GGT GTC CAA ACT GCG AAA CAG CAC TCG CCC GAT
GCG GAA GTC GAG TAT GAG CCC CAT TCT TCG ACA TCA
GCT ACT GTC CTC TTT CCT GAA GTC CCGG

Sequence No.: 4

Length: 100

Type: DNA

Microorganism: *Leptospirillum* spp.

Category: Threonyl-tRNA-synthase

[0098] Sequence:

CCT TTT CAT ATC CTG ATT TTT CGG GAA AGC GTC CAG
AGC TAC CGG GAC CTT CCC ATC CGG TTG TCG GAA CTG
GGA ACC GTC TAC CGG TAT GAA CGT TCGG

Sequence No.: 5

Length: 100

Type: DNA

Microorganism: *Leptospirillum* spp.

Category: Glutamyl- and glutaminyl-tRNA-synthase

[0099] Sequence:

TGA GGC GAT CGC CGC TAT CCT CGA CGG AAT GCG CTG
GCT CGA TTT GAG TTG GGA TGA GGG TCC CAT CTA CCA
GAC CAG TCG TCT GGA ACG CTA CCG GGAG

Sequence No.: 6

Length: 100

Type: DNA

Microorganism: *Leptospirillum* spp.

Category: Alanyl-tRNA-synthase

[0100] Sequence:

GAC GAA GCG GCC TCC CTT TGG CAG AGC GTT GCG GGT
GTC GAT CCC TCC CGA ATC GTT CGA CTC GGA GAA AAG
GAC AAT TTC TGG CAG ATG GGA AAC ACCG

Sequence No.: 7

Length: 100

Type: DNA

Microorganism: *Leptospirillum* spp.

Category: Histidyl-tRNA-synthase

[0101] Sequence:

AGA AGG CCT TCG TCT CGG CCG AAA GAG AAG GCG CCC GCT ACA TCG GAC TCG CCG GTG AAA GCG AAC GCT CAG ACG GAA CCT TGA CCA TCA AGG ATC TGAA

Sequence No.: 8

Length: 100

Type: DNA

Microorganism: *Leptospirillum* spp.

Category: Methionyl-tRNA-synthase

[0102] Sequence:

CCG AGG CGG TGC GCC CCG AGT CCC GCT ACA ACG AGG TCA TGG GTT TCC TCA GGA AGC CTC TGG GGG ACC TCT GCA TCT CCC GCC CCA AGA GCC GGG TTC C

Sequence No.: 9

Length: 100

Type: DNA

Microorganism: *Leptospirillum* spp.

Category: Aspartyl-tRNA-synthase

[0103] Sequence:

GGT GGG GAC ATC GGA GGA GGT CTC GGA GAG CCT TCG ACT GAC TTA TCG CTA CCT CGA CAT GCG TTC CCC CCG GCT TCT GGA GGC ACT GAG GTT CCG CTCG

Sequence No.: 10

Length: 100

Type: DNA

Microorganism: *Leptospirillum* spp.

Category: Phenylalanyl-tRNA-synthase

[0104] Sequence:

GGA TTC CAG GAG ATT CTC TCG AAT ATC CTG ACA TCG ATC GAA AAG GAC ACG ACA GAC CTC GGA CGT CCT TCG GAC ACG ACG GTC GAA ATC GAC AAC CCCG

Sequence No.: 11

Length: 100

Type: DNA

Microorganism: *Sulfobacillus* spp.

Category: 16S rDNA

[0105] Sequence:

GGG TGC GTA GGC GGT GTT GTG GGT CTG AGG TGA AAG GTC GGG GCT CAA CCC TGA GAA TGC CTT GGA AAC TGC AAG ACT TGA GTG CTG GAG AGG CAA GGGG

Sequence No.: 12

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus* spp. no (ferrooxidans, thiooxidans)

Category: 16S rDNA

[0106] Sequence:

CGC GTG GAT GAA GAA GGC CTT CGG GTT GTA AAG TCC TTT CGT GGG GGA CGA AAA GGT GGT TCC TAA TAC GAG CTC CTG TTG ACG TGA ACC CAA GAA GAAG

Sequence No.: 13

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus* spp.

Category: 16S rDNA

[0107] Sequence:

ACG AAA AGG CGG GTC CTA ATA CGA TCT GCT GTT GAC GTG AAC CCA AGA AGA AGC ACC GGC TAA CTC CGT GCC AGC AGC CGC GGT AAT ACG GGG GGT GCAA

Sequence No.: 14

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: 16S rDNA

[0108] Sequence:

GTA ATG CGT AGG AAT CTG TCT TTT AGT GGG GGA CAA CCC AGG GAA ACT TGG GCT AAT ACC GCA TGA GCC CTG AGG GGG AAA GCG GGG GAT CTT CGG ACCT

Sequence No.: 15

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: 16S rDNA

[0109] Sequence:

CCG CAT GAG CCC TGA GGG GGA AAG CGG GGG ATC TTC
GGA CCT CGC GCT AAG AGA GGA GCC TAC GTC CGA TTA
GCT AGT TGG CGG GGT AAA GGC CCA CCAA

Sequence No.: 16

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: 16S rDNA

[0110] Sequence:

CCG CAT GAG CCC TGA GGG GGA AAG CGG GGG ATC TTC
GGA CCT CGC GCT AAG AGA GGA GCC TAC GTC CGA TTA
GCT AGT TGG CGG GGT AAA GGC CCA CCAA

Sequence No.: 17

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: 16S rDNA

[0111] Sequence:

ACT AGA TGT TTG GTG CCT AGC GTA CTG AGG TCG TAG
CTA ACG CGA TAA GTA TTC CGC CTG GGA AGT ACG GCC
GCA AGG TTA AAA CTC AAA GGA ATT GACG

Sequence No.: 18

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: Glutamyl- and glutaminyl-tRNA-synthase

[0112] Sequence:

ACC GGA TCG GGG GGG CGA TCA CGG CCT CTA CCG GCA
GAT GCA GCG CAT GGC CGT CTA TCG GGA AGT GCT GTG
CGC ATT TCC AGT CCA GTG GAA AGG CCTA

Sequence No.: 19

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: Alanyl-tRNA-synthase

[0113] Sequence:

GGA GTA TTT TGT GGA ACA GGG GCA TCA GAT CGT GCC
CTC CAG CCC CCT GAT CCC TCG TAA CGA TCC GAC CCT
GCT GTT TAC CAA TGC CGG TAT GGT GCCC

Sequence No.: 20

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: Isoleucyl-tRNA-synthase

[0114] Sequence:

TCC GAC ACC ATC CTC CAG CAA CTC GGC GAC AGC TAC
CGG CGC ATC CGT AAT ACG GCC CGC TAT ATG CTG GGC
AAC ACC CAC GAC TTT AAT CCA GCC ACGG

Sequence No.: 21

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: Phenylalanyl-tRNA-synthase

[0115] Sequence:

TGC CCA TGA CGC GGG GAC CGC AGG CAG CCA CTT TAC
GCA GTG TTT TGC AGG CGC GTG ATT ATC ATG AGG TGA
TCA CCT ACA GTT TTA TCT CCC GTC AGGC

Sequence No.: 22

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: Histidyl-tRNA-synthase

[0116] Sequence:

GGC ATT ACC CCG CCG TTC GTG CCG CCC TGG ATG ACC
TGC AGC GCG TAT GGT CGG TAT TAC GGG CAC GTT ATC
CAG ACC TGG CGA TTC AAT GCG ATC TCTC

Sequence No.: 23

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: Methionyl-tRNA-synthase

[0117] Sequence:

AGC ACT GGT GCG CGG CCC ACC GCC GCA ATT TGG CCG ATT ACT GGG GCC CGG ATT CCG CTG CCG AGA TCT ACC ATT TTA TCG GCA AGG ACA TCA TTT ATTT

Sequence No.: 24

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: Aspartyl-tRNA-synthase

[0118] Sequence:

CTT CGG CGT CGA TCG CCC CGA TCT ACG CAA TCC GCT GGA ACT GAC AGA GCT CAC CGA TCT CAT GCG CGC GGT GGA CTT CAA AGT GTT CCG CGA AGC CGCC

Sequence No.: 25

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: Threonyl-tRNA-synthase

[0119] Sequence:

TAT GAG GGC AAG TTT CCA GTC TGG CTC GCT CCG GTA CAG GCC GTG GTG CTG CCT ATC AGC GAG CAT TAC TCG GAA TAC GCC GAG TCG GTA AGT GAC GTAT

Sequence No.: 26

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus ferrooxidans*

Category: Leucyl-tRNA-synthase

[0120] Sequence:

GAC AAC ATC GCC CAC ATG CGC GGG CAA TTG CAG CGG CTG AGG CCT GTC TTA CGA CTG GTC GCG GGA GTT CGC TAC CTG CAC GCC GGC ATT ATT ATC GCTG

Sequence No.: 27

Length: 100

Type: DNA

Microorganism: *Acidithiobacillus thiooxidans*

Category: 16S rDNA

[0121] Sequence:

TAC CTG GGC TTG ACA TGG ATC GGG AAT CCT GCA GAG ATG CGG GAG TGC CTT TCG GGG AGT CGG ATC ACA GGT GCT GCA TGG CTG TCG TCA GCT TCG TGTC

Sequence No.: 28

Length: 100

Type: DNA

Microorganism: *Acidianus* spp.

Category: 16S rDNA

[0122] Sequence:

ACG TGG TCA ACC TAA CCT CGG GAC TTG GAT ACC TCC GGG AAA CTG GAG CTA ATC CAA GAT AGG CAA AGG AAT CTG GAA CGA TCC TTT GCT TAA AGG CCTC

Sequence No.: 29

Length: 100

Type: DNA

Microorganism: *Ferroplasma* spp.

Category: 16S rDNA

[0123] Sequence:

AAG GGA TAA CTT CGG GAA ACT GAA GGT AAT ACC TTA TAA TTG CTT AAA ACT CGA ATG TTT TTG CAA TAA AAG TTA CGA CGC TCA AGC ATC AGT CTC CGAC

Sequence No.: 30

Length: 100

Type: DNA

Microorganism: *Ferroplasma* spp.

Category: Glutamyl- and glutaminyl-tRNA-synthase

[0124] Sequence:

TTC AGT TAA CCT TGA AAA ATT CGA TAA GAT GAT TTC AGG CCA TTA CTC CGA GGG TGA AGC AGC ACT GGT AAT GAA AAC GGA CAT AAA TCA CCC GAA TCCC

Sequence No.: 31

Length: 100

Type: DNA

Microorganism: *Ferroplasma* spp.

Category: Alanyl-tRNA-synthase

[0125] Sequence:

AGA GGG CAC TAA GTA TTC CAT GAT GCC GCT AAA AAT TGT TGA TAC AGG TTA CGG ATT GGA AAG GCT TGT GTG GCT TTC TAC AGG CAC GCC CAC AGT TTAC

Sequence No.: 32

Length: 100

Type: DNA

Microorganism: *Ferroplasma* spp.

Category: Isoleucyl-tRNA-synthase

[0126] Sequence:

ATA ATA GTG TTC CAT GGA AAG ATA AAT TTG TCA TGG ATG TTA ATC CCG ATA TAA TCG GAT ACT TAA AGG AAA ATA ACC TTC TAT TTA AAA GCC AGA AAAT

Sequence No.: 33

Length: 100

Type: DNA

Microorganism: *Ferroplasma* spp.

Category: Phenylalanyl-tRNA-synthase

[0127] Sequence:

TGA CAA AAT GGT GCC TGT AAT ACT TGA CAG CAA GGA AGA TGT AAT GTC AAT GCC CCC CAT AAT AAA TGG GCT GAA ATC TAA AAT TGG AAA TAA TAC ATCA

Sequence No.: 34

Length: 100

Type: DNA

Microorganism: *Ferroplasma* spp.

Category: Histidyl-tRNA-synthase

[0128] Sequence:

CTG ATG CAG AAA TTG TTG GAT TGG CTT CAA GCA TTC TTG ATT ACC TTG GTT TAT CCG GGG TAT ATG AAA TTA ACA TTA ATG ACA GAT TTT TGA TGG AATA

Sequence No.: 35

Length: 100

Type: DNA

Microorganism: *Ferroplasma* spp.

Category: Methionyl-tRNA-synthase

[0129] Sequence:

AAA GGA TGT AGA TGA ATT TTT TAT TAA CCT GCT GGA AAA AGG ATA CCT TGT AAA ACG CTA TAT GAT ATC ACC ATT CTG CGT TGA AAT CAA TAA ATT CATG

Sequence No.: 36

Length: 100

Type: DNA

Microorganism: *Ferroplasma* spp.

Category: Aspartyl-tRNA-synthase

[0130] Sequence:

CAG TCA CCC CAG CTC TAT AAG GAA ATT CTT ATA TCA TCA GGG TTT GAT AAA GTA TTC GAA GTA GGG CCT GCA TTT CGG GCT GAA AAG GAA AAC ACT GTAA

Sequence No.: 37

Length: 100

Type: DNA

Microorganism: *Ferroplasma* spp.

Category: Threonyl-tRNA-synthase

[0131] Sequence:

TAT GAA AGA ATT GAA TGA AAA AAG CGG GTG GGA AGA GGT ATG GAC CGC ACA TGC TTT CAA AGA CAT TAT ATG GAA ACA ATC TGG ACA TTA TTA TAA GTAT

Sequence No.: 38

Length: 100

Type: DNA

Microorganism: *Ferroplasma* spp.

Category: Leucyl-tRNA-synthase

[0132] Sequence:

AAA AAT ATG GCT GTA ATT TTT TAT GAA ACA ACA AGG AAA GCT GTT ACA AGG TCT GGC TCC AGG GTT ATA GTG GCA GTA TTA AAA GAC CAG TGG TTC ATTG

Sequence No.: 39

Length: 100

Type: DNA

Microorganism: *Ferroplasma* spp.

Category: Valyl-tRNA-synthase

[0133] Sequence:

GGA ATC TGA CAT AAA AGC CAG ATG AAA CTG TCA ATG GCA GCA CCA CTG GAC ATT TAC GTA AAA TTG CAC GGA GCC ATG CTA AAT ATT ATA TCT CCT GAGC

Sequence No.: 40

Length: 100

Type: DNA

Microorganism: *Metallosphaera* spp.

Category: 16S rDNA

[0134] Sequence:

TGC GGG AAA CCG TGA GGG CGT TAC CCC TAG TGC CCT CGC AAG AGG GCT TTT CTC CAC TCC AGA AAG GTG GAG GAA TAA GCG GGG GGC AAG ACT GGT GTCA

Sequence No.: 41

Length: 100

Type: DNA

Microorganism: *Sulfolobus* spp.

Category: 16S rDNA

[0135] Sequence:

GGA GGG AGA TAA CCC CGG GAA ACT GGG GAT AAT CTC CCA TAG GCG AGG AGT CCT GGA ACG GTT CCT CGC TGA AAG GCT CAT GGG CTA TTC CCC GCT CATG

Sequence No.: 42

Length: 100

Type: DNA

Microorganism: *Sulfolobus* spp.

Category: Glutamyl- and glutaminyl-tRNA-synthase

[0136] Sequence:

AGA TCC AAT AGC TAA GAG ACT AAT GTT TGT AAA AGA TCC AAA GGA GTT TAT CAT CGA GCT ACC AGA ACC AAT AAA GGC TAA AAT ACC ATA TAA TCC CTCT

Sequence No.: 43

Length: 100

Type: DNA

Microorganism: *Sulfolobus* spp.

Category: Alanyl-tRNA-synthase

[0137] Sequence:

AAA ACC CAT AGA TGA ACA TTT AGC AAA GCA ATT AGG ACT TGA TTT GAA GTA TGT AGA TGA AGA ACT AAC AAG AGC TGC TAG AGT ATT TCA GGT ATT TGAT

Sequence No.: 44

Length: 100

Type: DNA

Microorganism: *Sulfolobus* spp.

Category: Isoleucyl-tRNA-synthase

[0138] Sequence:

ATT GGT GTT CTA GAT GTG AGA CCA CAT TAG CGG ACT ATG AGG TTT CCG AGT ATA GAG ACT TAG AAG ATC CAT CCA TAT ATG TTA AAT TCA GGG TAA AAGG

Sequence No.: 45

Length: 100

Type: DNA

Microorganism: *Sulfolobus* spp.

Category: Phenylalanyl-tRNA-synthase

[0139] Sequence:

TTT ATT TTG ATT AAA AAT GAA AAG ATA CTT GAT AGT GAG TAT GTG AAA ATA CTT AAC CCA ATT ACA GTG GAA TAT AAT GCA GTA AGA AAT TCA OTA ATAC

Sequence No.: 46

Length: 100

Type: DNA

Microorganism: *Sulfolobus* spp.

Category: Histidyl-tRNA-synthase

[0140] Sequence:

TAA GAA AAA CTA TGT ATG TAT TTA AAG ATA AGG CAG ATA GAG AAG TCG CTT TAA GAC CTG AGA TTA CAC CTA GTA TAG TTA GAG TTT ATC TAA ACT CGTT

Sequence No.: 47

Length: 100

Type: DNA

Microorganism: *Sulfolobus* spp.

Category: Methionyl-tRNA-synthase

[0141] Sequence:

TTT TTT GAT TTA TCA GAA TTT AAT GAT AAA ATA AGG GAT TGG ATT AGC AGT TCT AAT ACA ATG CCT GAT AAT GTT AAG TCT GTC GCA TTA AGC TGG GTTA

Sequence No.: 48

Length: 100

Type: DNA

Microorganism: *Sulfolobus* spp.

Category: Aspartyl-tRNA-synthase

[0142] Sequence:

GAT ACA CCG TTT CAT TTA GCA GAA TTT ATT AGT ATG GAT GTA GAA ATG GCG TTT GCT GAT TAT AAC GAT GTA ATG CAA CTT TTA GAG AAA ATA TTA CATA

Sequence No.: 49

Length: 100

Type: DNA

Microorganism: *Sulfolobus* spp.

Category: Leucyl-tRNA-synthase

[0143] Sequence:

AGA ATA CTG CGA AAA AAA TAG GTT TGG GTA TAG ATT GGA GAA GAG AAT TTA CTA CAA TTG ACC CAA TTT TTG AGA AAT TTG TAC AGT GGC AGT TTA GTAA

Sequence No.: 50

Length: 100

Type: DNA

Microorganism: *Sulfolobus* spp.

Category: Valyl-tRNA-synthase

[0144] Sequence:

GCT AGA AGA TCT ATA AAA AGT ATG AAA TTC ATT CCT CCT AGA ATG AAG TAT TAC TTT GAG GAT TGG ATA AAT AGT TTA GAA TGG GAA TGG AAT ATG ATTA

Sequence No.: 51

Length: 100

Type: DNA

Microorganism: *Thermoplasma* spp.

Category: 16S rDNA

[0145] Sequence:

ATC TCC TGG GCT ACA CGC GCG CTA CAA AGG GCG GGA CAA TGG GCT CCG ACA CCC AAA GGT GAA GGT AAT CTC GAA ACC CGT CCG TAG TTC GGA TTG AGGG

Sequence No.: 52

Length: 100

Type: DNA

Microorganism: *Thermoplasma* spp.

Category: Glutamyl- and glutaminyl-tRNA-synthase

[0146] Sequence:

CGG CCA TAT GTA TGT CTG CAC CTG CCC CAG GGA GGA GTT CAA GAA GAG GAA GCT AGA ATC AAT ACC ATG CAA AGA TCG CGA TAA CCC TCC TGA AAC CAAT

Sequence No.: 53

Length: 100

Type: DNA

Microorganism: *Thermoplasma* spp.

Category: Alanyl-tRNA-synthase

[0147] Sequence:

AAC TCA TCA GTT TcT GAT ATA GAC GAA GAA TTT CTG TCA GAG GTT GTA AAG GCC TCG GTT ATG AAA GAG CCA TAC GAA GAA TCA TTC GTC ATT TCG CAAC

Sequence No.: 54

Length: 100

Type: DNA

Microorganism: *Thermoplasma* spp.

Category: Isoleucyl-tRNA-synthase

[0148] Sequence:

TGA TGG ACG AGA AGC TAG AAA GTG AGA TGG ATC GTG CAT ATT CGG TCA TCG AAA CTG TGC GCA GAC TCA GGC AGG AGA ACT CCA TAA AGG GCA GGC AGCC

Sequence No.: 55

Length: 100

Type: DNA

Microorganism: *Thermoplasma* spp.

Category: Phenylalanyl-tRNA-synthase

[0149] Sequence:

TGG AGA CAG CAT ACA TTA TAC GAC AGT GAG CAG GAA
CCA AAG GAT GCA AAC CTA CGA TGG AAT GGA AGG AAC
CGT CGA TTG GAT CAT CAA AAA CCA TGAA

Sequence No.: 56

Length: 100

Type: DNA

Microorganism: *Thermoplasma* spp.

Category: Histidyl-tRNA-synthase

[0150] Sequence:

GGA TGT TGA GAA GTT CAT ATT CAA AAC GGC AGA GGA
GGC TGC AGA GGC ATT CGG TTT CAG GCG AAT AGA CTT
TCC AAG TCT TGA GTA TCT TGA TCT TTAC

Sequence No.: 57

Length: 100

Type: DNA

Microorganism: *Thermoplasma* spp.

Category: Methionyl-tRNA-synthase

[0151] Sequence:

CAA TGC TGA TGG GCT ATG GTG GTT TCA ACC TGC CAT
ACG ACA TTC CTG CTA ACG AAT ACC TGA CGT TCA AAG
GCC AGC AAT TCT CCA AGA GCA GAG GGAT

Sequence No.: 58

Length: 100

Type: DNA

Microorganism: *Thermoplasma* spp.

Category: Threonyl-tRNA-synthase

[0152] Sequence:

GTT ATA TAA AAG CAT TCA AGC TTC TGA ACA TTG CCA
GCG CGG TTT ACA AGC ACG ATG AGA GCA AGA CCC TCG
TAA GGA TAT ACG GAA CCG CCT TTC CTGA

Sequence No.: 59

Length: 100

Type: DNA

Microorganism: *Thermoplasma* spp.

Category: Valyl-tRNA-synthase

[0153] Sequence:

GAT GAC AAT GCG GTA GGT GAG GAC GAC ATC AAG GAC
GGG GAT ACG GAC AAG GTC ACC ATA GAG GAG TAT ACC
GCC ATA TTC TTC CGT GGA AAG TCC TTCG

Sequence No.: 60

Length: 100

Type: DNA

Microorganism: Wenelen (DSM 16786)

Category: Specific region for Wenelen

[0154] Sequence:

CCA ATA CTG GAC GTC ACC TAT ATT ATT CGC CAG CGA
CAG CCG TAA AGG TTT TCC GGG CAT CCT ACA GGC CTG
GCC CAT CGC TTC CCG CGA CAA GTG CGGC

Sequence No.: 61

Length: 100

Type: DNA

Microorganism: Wenelen (DSM 16786)

Category: Specific region for Wenelen

[0155] Sequence:

CCC CAC CAA GCC CCC AAT CGG GTC CGA CTG GCG AGG
AGA CCA CCG ATG AAT AAG CAA CCT TGG ATC TCT TAG
GAG GGC CAC AAC TCA TGA AAA AAT CGCG

Sequence No.: 62

Length: 100

Type: DNA

Microorganism: Wenelen (DSM 16786)

Category: Specific region for Wenelen

[0156] Sequence:

CCG ACA CCC TGA GCT GCG TGA ACC GTC GCA CGG GGA
ACG TCA TTT CGG CGC CAA TTC ACG CCT ATG TCG TGA
GTA GCG GCG GGC ATG GCG GTG TTC CCGG

Sequence No.: 63

Length: 100

Type: DNA

Microorganism: Wenelen (DSM 16786)

Category: Specific region for Wenelen

[0157] Sequence:

CAC CTG TAC GTA TGA AAA CCC CTA CGC CCC CAA TCG
CGG AGA TTG GCT GGC GGC CTA CTG TAA TAA CGG CTG
CGG GTA TAC GGG TTG TAT GGG TCA AGAG

Sequence No.: 64

Length: 100

Type: DNA

Microorganism: Wenelen (DSM 16786)

Category: Specific region for Wenelen

[0158] Sequence:

CAA CGC CGG TCC CGC GCC GAT TAT CCA GGT CAT CAA
CAC GGC CCA TCG GGA ACT GAA TAT TGG CGT CTA TTA
CCT GGA TGA CCG CAA GGT GCT TCG CGCC

Sequence No.: 65

Length: 100

Type: DNA

Microorganism: Wenelen (DSM 16786)

Category: Specific region for Wenelen

[0159] Sequence:

GAA GCC GAG ATG CTA TCC CAG GCC CTG CGC CGG GTT
GTC ATT CGC GGA CAC CTG AAT GAT GTA TAT GAT CCG
ATA ACG AAG TTT GAT TAT CAA GGG GTTG

Sequence No.: 66

Length: 100

Type: DNA

Microorganism: Wenelen (DSM 16786)

Category: Specific region for Wenelen

[0160] Sequence:

TCC ACC TAG CCA GGG CAT CGC GTC TCT GAT GAT TGC
CGG TAT CAT GGA GCG TTT GGG GCT AGG CGA TGT TGA
CCC CCT TAG TGC CGA ATT GAT ACA CGGC

Sequence No.: 67

Length: 100

Type: DNA

Microorganism: Wenelen (DSM 16786)

Category: Specific region for Wenelen

[0161] Sequence:

ACC TCG CGA AGA GCG TGA CGG TGG AGT AAT GGG GCG
GTT CGG TGT GCC GGG TGT GTT CGC AGG TAA TAA AGT
TTG CTC ATG GGT AAT AAA GTT TGG ACAT

Sequence No.: 68

Length: 100

Type: DNA

Microorganism: Wenelen (DSM 16786)

Category: Specific region for Wenelen

[0162] Sequence:

TGC CGG GCC TTG GGC TAT TGC AGC TGA CGT CGC CCG
GCA TCT GGG CGT TGT GAA GGT TAA GGG CAC CGT TTA
CCG CTG ACG CGA ACA CAA AGG GTC TCCC

Sequence No.: 69

Length: 100

Type: DNA

Microorganism: Wenelen (DSM 16786)

Category: Specific region for Wenelen

[0163] Sequence:

GTT TGA GAC CCA AGA GGG CTT TGA GAT AGC GAA ACG
CAT GGT TCT GGG TGA CCA ACA TGA TGA CGG CCC ACT
CCA CTA ACC CGG ATT GGC TCT GCC CCGC

Sequence No.: 70

Length: 100

Type: DNA

Microorganism: Wenelen (DSM 16786)

Category: Valyl-tRNA-synthase

[0164] Sequence:

GGT GGC GCG AAT GAT CAT GAT GGG TCT GCG CTT CAT
GGA CGA TGT GCC ATT CCA TGA AGT CTA TGT CCA CGG
TCT GGT GCG CGA TGC CGA GGG CCA GAAG

What is claimed is:

1. Array for the detection and identification of biomining microorganisms wherein said array comprises one representative of either one, many or all of the following DNA fragments bound to its surface:

a. at least one DNA fragment that specifically identifies *Acidiphilium* spp.,

b. at least one DNA fragment that specifically identifies *Leptospirillum* spp.,

c. at least one DNA fragment that specifically identifies *Sulfobacillus* spp.,

d. at least one DNA fragment that specifically identifies *Acidithiobacillus* spp.,

e. at least one DNA fragment that specifically identifies *Acidithiobacillus ferrooxidans,*

f. at least one DNA fragment that specifically identifies *Acidithiobacillus thiooxidans,*

g. at least one DNA fragment that specifically identifies *Acidianus* spp.,

h. at least one DNA fragment that specifically identifies *Ferroplasma* spp.,

i. at least one DNA fragment that specifically identifies *Metallosphaera* spp.,

j. at least one DNA fragment that specifically identifies *Sulfolobus* spp.,

k. at least one DNA fragment that specifically identifies *Thermoplasma* spp.,

l. at least one DNA fragment that specifically identifies strain Wenelen (DSM 16786),

wherein each DNA fragment is present in hundreds of copies that form spots that have a homogeneous composition spatially distributed on the surface support.

2. Array according to claim 1, wherein said DNA fragment that allows the identification of *Acidiphilium* spp. is selected from the DNA fragments defined by sequence No 1 and its reverse complementary sequence.

3. Array according to claim 2, wherein said DNA fragment is either in its complete form, or contained in a larger sequence, the specificity of which given by said fragment, or is any of the sub-fragments contained in said DNA fragment.

4. Array according to claim 3, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

5. Array according to claim 1, wherein said DNA fragment that allows the identification of *Leptospirillum* spp. is selected from the DNA fragments defined by sequences No 2-10 and their respective reverse complementary sequences.

6. Array according to claim 5, wherein said DNA fragments are either in their complete form, or contained in a larger sequence, the specificity of which is given by said fragments, or are any of the sub-fragments contained in said DNA fragments.

7. Array according to claim 6, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

8. Array according to claim 1, wherein said DNA fragment that allows the identification of *Sulfobacillus* spp. is selected from the DNA fragments defined by sequence No 11 and its reverse complementary sequence.

9. Array according to claim 8, wherein said DNA fragment is either in its complete form, or contained in a larger sequence, the specificity of which is given by said fragment, or is any of the sub-fragments contained in said DNA fragment.

10. Array according to claim 9, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

11. Array according to claim 1, wherein said DNA fragment that allows the identification of *Acidithiobacillus* spp. is selected from the DNA fragments defined by sequences No 12 and 13 and their respective reverse complementary sequences.

12. Array according to claim 11, wherein said DNA fragments are either in their complete form, or contained in a larger sequence, the specificity of which is given by said fragments, or are any of the sub-fragments contained in said DNA fragments.

13. Array according to claim 12, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

14. Array according to claim 1, wherein said DNA fragment that allows the identification of *Acidithiobacillus ferrooxidans* is selected from the DNA fragments defined by sequences No 14 to 26 and their respective reverse complementary sequences.

15. Array according to claim 14, wherein said DNA fragments are either in their complete form, or contained in a larger sequence, the specificity of which is given by said fragments, or are any of the sub-fragments contained in said DNA fragments.

16. Array according to claim 15, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

17. Array according to claim 1, wherein said DNA fragment that allows the identification of *Acidithiobacillus thiooxidans* is selected from the DNA fragments defined by sequence No 27 and its reverse complementary sequence.

18. Array according to claim 17, wherein said DNA fragments are either in their complete form, or contained in a larger sequence, the specificity of which is given by said fragments, or are any of the sub-fragments contained in said DNA fragments.

19. Array according to claim 18, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

20. Array according to claim 1, wherein said DNA fragment that allows the identification of *Acidianus* spp. is selected from the DNA fragments defined by sequence No 28 and its reverse complementary sequence.

21. Array according to claim 20, wherein said DNA fragments are either in their complete form, or contained in a larger sequence, the specificity of which is given by said fragments, or are any of the sub-fragments contained in said DNA fragments.

22. Array according to claim 21, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

23. Array according to claim 1, wherein said DNA fragment that allows the identification of *Ferroplasma* spp. is selected from the DNA fragments defined by sequences No 29-39 and their respective reverse complementary sequences.

24. Array according to claim 23, wherein said DNA fragments are either in their complete form, or contained in a larger sequence, the specificity of which is given by said fragments, or are any of the sub-fragments contained in said DNA fragments.

25. Array according to claim 24, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

26. Array according to claim 1, wherein said DNA fragment that allows the identification of *Metallosphaera* spp. is selected from the DNA fragments defined by sequence No 40 and its reverse complementary sequence.

27. Array according to claim 26, wherein said DNA fragment is either in its complete form, or contained in a larger sequence, the specificity of which is given by said fragment, or is any of the sub-fragments contained in said DNA fragment.

28. Array according to claim 27, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

29. Array according to claim 1, wherein said DNA fragment that allows the identification of *Sulfolobus* spp. is selected from the DNA fragments defined by sequences No 41-50 and their respective reverse complementary sequences.

30. Array according to claim 29, wherein said DNA fragments are either in their complete form, or contained in a larger sequence, the specificity of which is given by said fragments, or are any of the sub-fragments contained in said DNA fragments.

31. Array according to claim 30, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

32. Array according to claim 1, wherein said DNA fragment that allows the identification of *Thermoplasma* spp. is selected from the DNA fragments defined by sequences No 51-59 and their respective reverse complementary sequences.

33. Array according to claim 32, wherein said DNA fragment is either in its complete form, or contained in a larger sequence, the specificity of which is given by said fragment, or is any of the sub-fragments contained in said DNA fragment.

34. Array according to claim 33, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

35. Array according to claim 1, wherein said DNA fragment that allows the identification of strain Wenelen (DSM 16786) is selected from the DNA fragments defined by sequences No 60-70 and their respective reverse complementary sequences.

36. Array according to claim 35, wherein said DNA fragments are either in their complete form, or contained in a larger sequence, the specificity of which is given by said fragments, or are any of the sub-fragments contained in said DNA fragments.

37. Array according to claim 36, wherein said sub-fragments comprise preferably 50 to 70 nucleotides.

38. Array according to claim 1, wherein said array optionally comprises a DNA fragment that does not correspond to any biomining relevant microorganism as a negative control.

39. Array according to claim 1, wherein said array comprises an oligonucleotide from a region that is highly conserved in microorganisms as a positive control.

40. Method for the identification of biomining microorganisms, wherein said method comprises:

a. incubating a labeled DNA sample on an array as defined in claim 1,

b. after the incubation, washing the array to remove non-hybridized labeled DNA fragments,

c. visualizing the array spots that have a mark,

d. establishing which microorganisms are present in the sample.

41. Method for the identification of biomining microorganisms according to claim 40, wherein said method comprises, when required, the following steps:

a. extracting DNA or cDNA from a biomining sample; and/or

b. fragmenting and labeling a DNA sample using labeled nucleotides or nucleotides that are able to be labeled,

before incubating a labeled DNA sample on an array as comprising

c. at least one DNA fragment that specifically identifies *Acidiphilium* spp.,

d. at least one DNA fragment that specifically identifies *Leptospirillum* spp.,

e. at least one DNA fragment that specifically identifies *Sulfobacillus* spp.,

f. at least one DNA fragment that specifically identifies *Acidithiobacillus* spp.,

g. at least one DNA fragment that specifically identifies *Acidithiobacillus ferrooxidans,*

h. at least one DNA fragment that specifically identifies *Acidithiobacillus thiooxidans,*

i. at least one DNA fragment that specifically identifies *Acidianus* spp.,

j. at least one DNA fragment that specifically identifies *Ferroplasma* spp.,

k. at least one DNA fragment that specifically identifies *Metallosphaera* spp.,

l. at least one DNA fragment that specifically identifies *Sulfolobus* spp.,

m. at least one DNA fragment that specifically identifies *Thermoplasma* spp.,

n. at least one DNA fragment that specifically identifies strain Wenelen (DSM 16786),

wherein each DNA fragment is present in hundreds of copies that form spots that have a homogeneous composition spatially distributed on the surface support.

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