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(54) **METHOD OF PREDICTING AND THEN PRODUCING A MIX OF MICROBIOTA SAMPLES**

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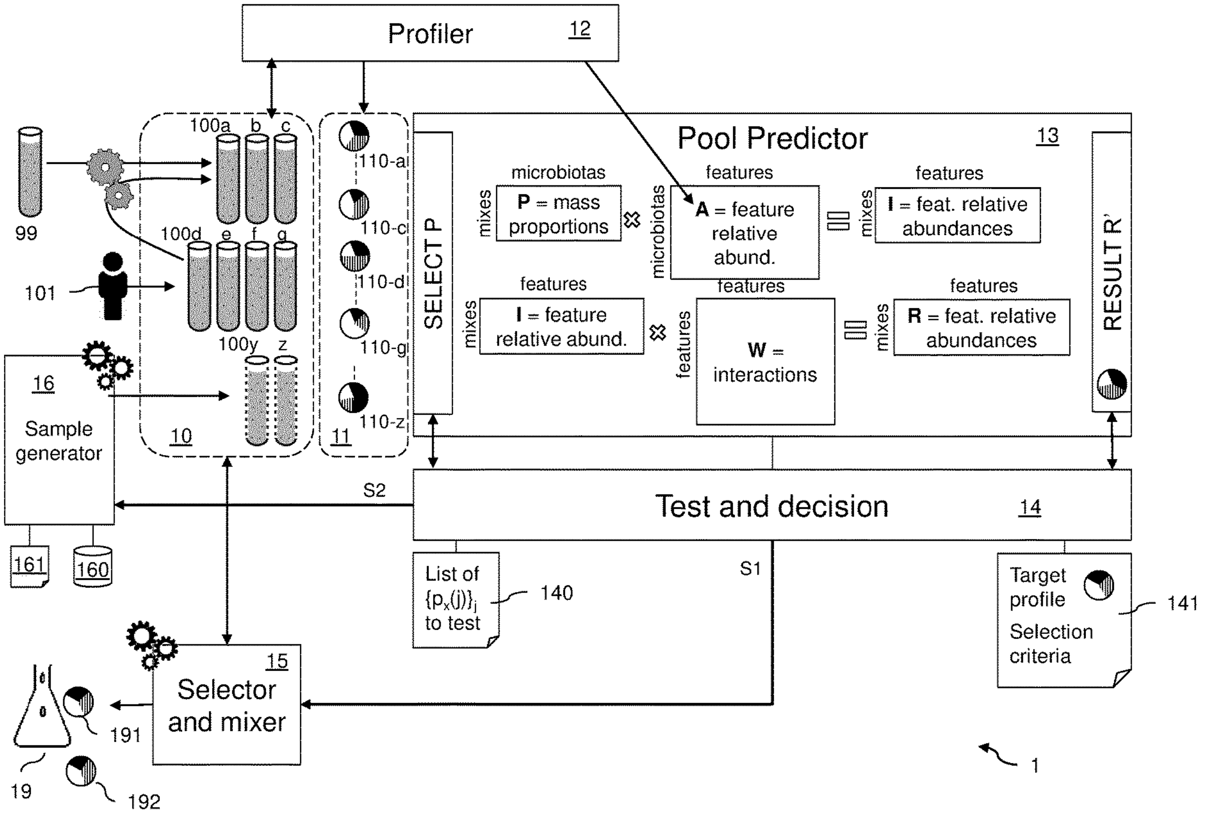
(57) **ABSTRACT**

Prediction of a mix of complex communities of microorganisms includes a linear prediction, e.g. matrix-based, that is corrected using an interaction model, e.g. a matrix, learnt from reference true mix profiles and corresponding reference linear-predicted profiles. Reverse prediction makes it possible to determine a mix of samples to be made given a target mix profile.



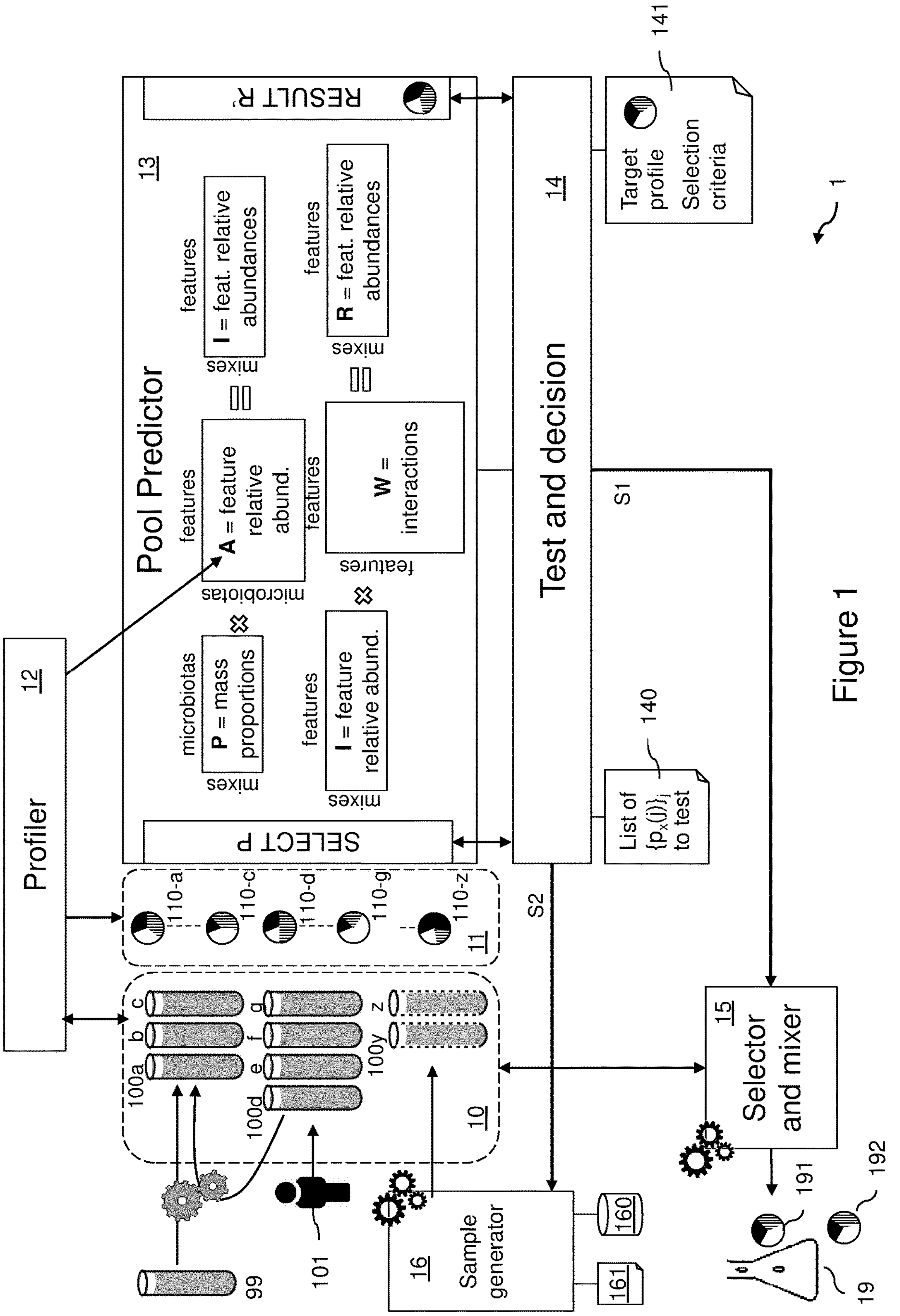

Profiler
12
Pool Predictor
13
microbiotas
P = mass proportions
mixes
features
A = feature relative abund.
microbiotas
features
I = feat. relative abundances
mixes
features
I = feature relative abund.
mixes
features
W = interactions
features
features
R = feat. relative abundances
mixes
SELECT P
RESULT R'
Test and decision
14
S1
S2
List of {p_x(j)}_j to test
140
Target profile
Selection criteria
141
1
100a
b
c
100d
e
f
g
100y
z
10
110-a
110-c
110-d
110-g
110-z
11
99
101
16
Sample generator
160
161
15
Selector and mixer
191
192
19

Figure 1

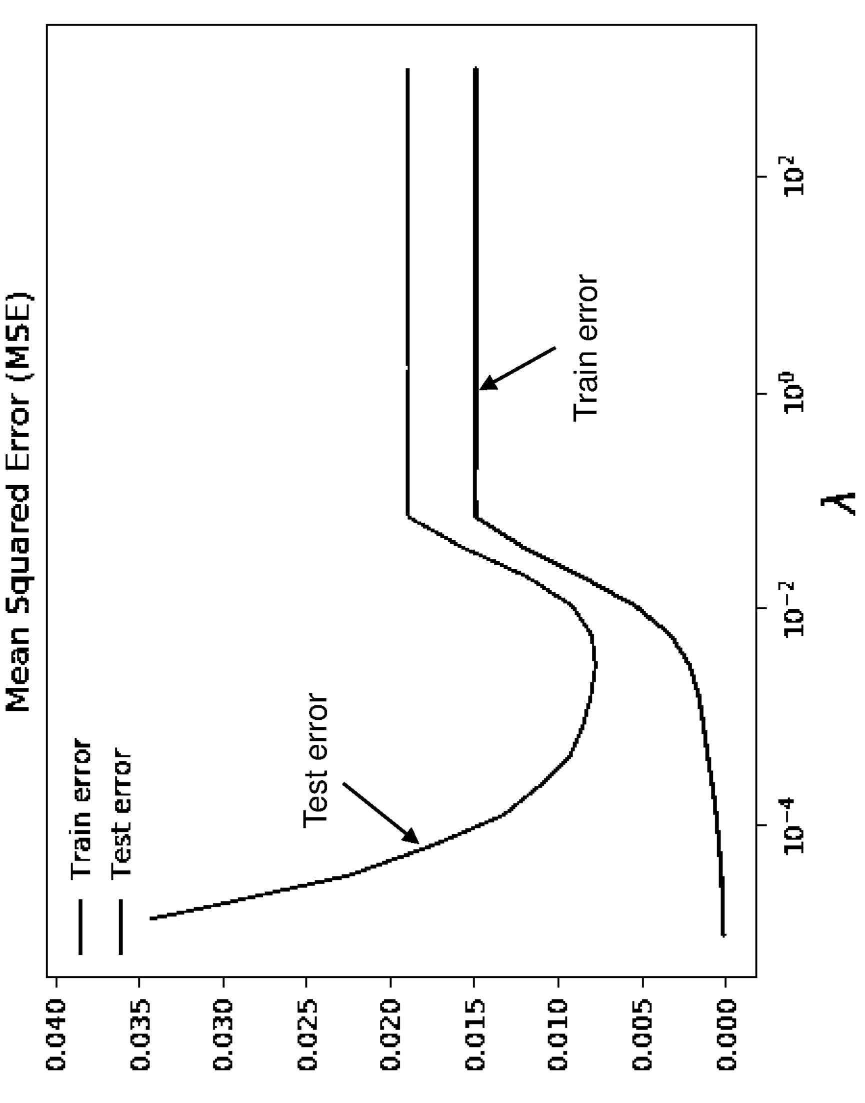

Mean Squared Error (MSE)
Train error
Test error
Test error
Train error
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
10^{-4}
10^{-2}
10^{0}
10^{2}
λ

Figure 1a

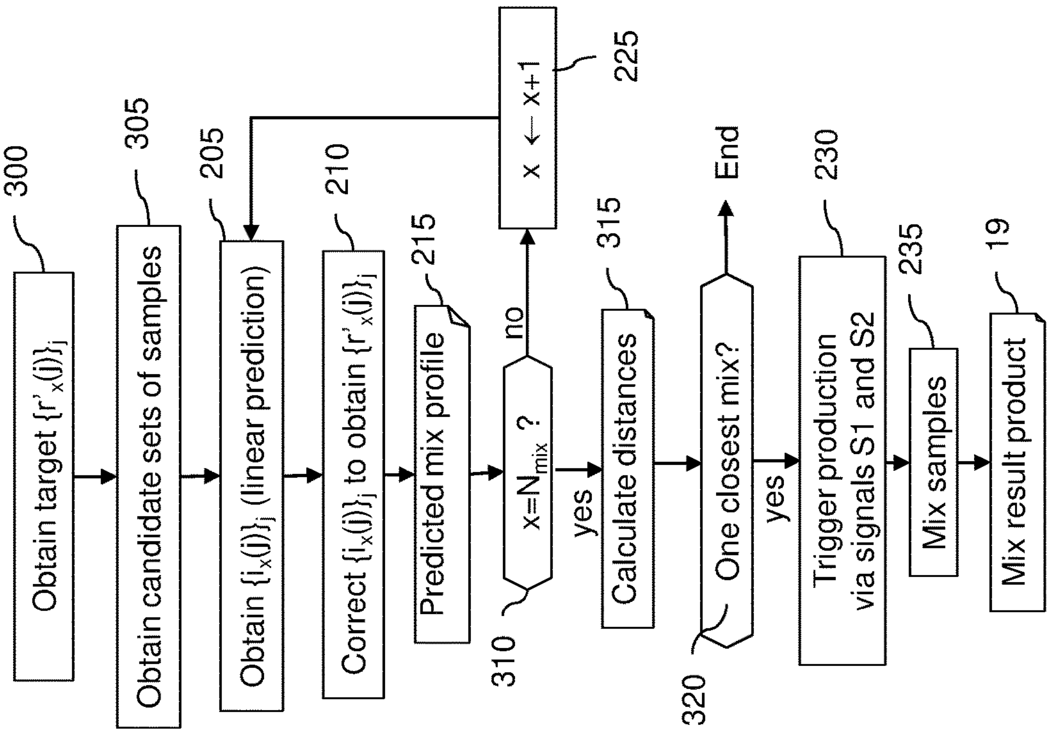

300
Obtain target $\{r'_x(j)\}_j$
305
Obtain candidate sets of samples
205
Obtain $\{i_x(j)\}_j$ (linear prediction)
210
Correct $\{i_x(j)\}_j$ to obtain $\{r'_x(j)\}_j$
215
Predicted mix profile
310
$x=N_{mix}$?
no
yes
$x \leftarrow x+1$
225
315
Calculate distances
320
One closest mix?
yes
End
230
Trigger production via signals S1 and S2
235
Mix samples
19
Mix result product

Figure 3

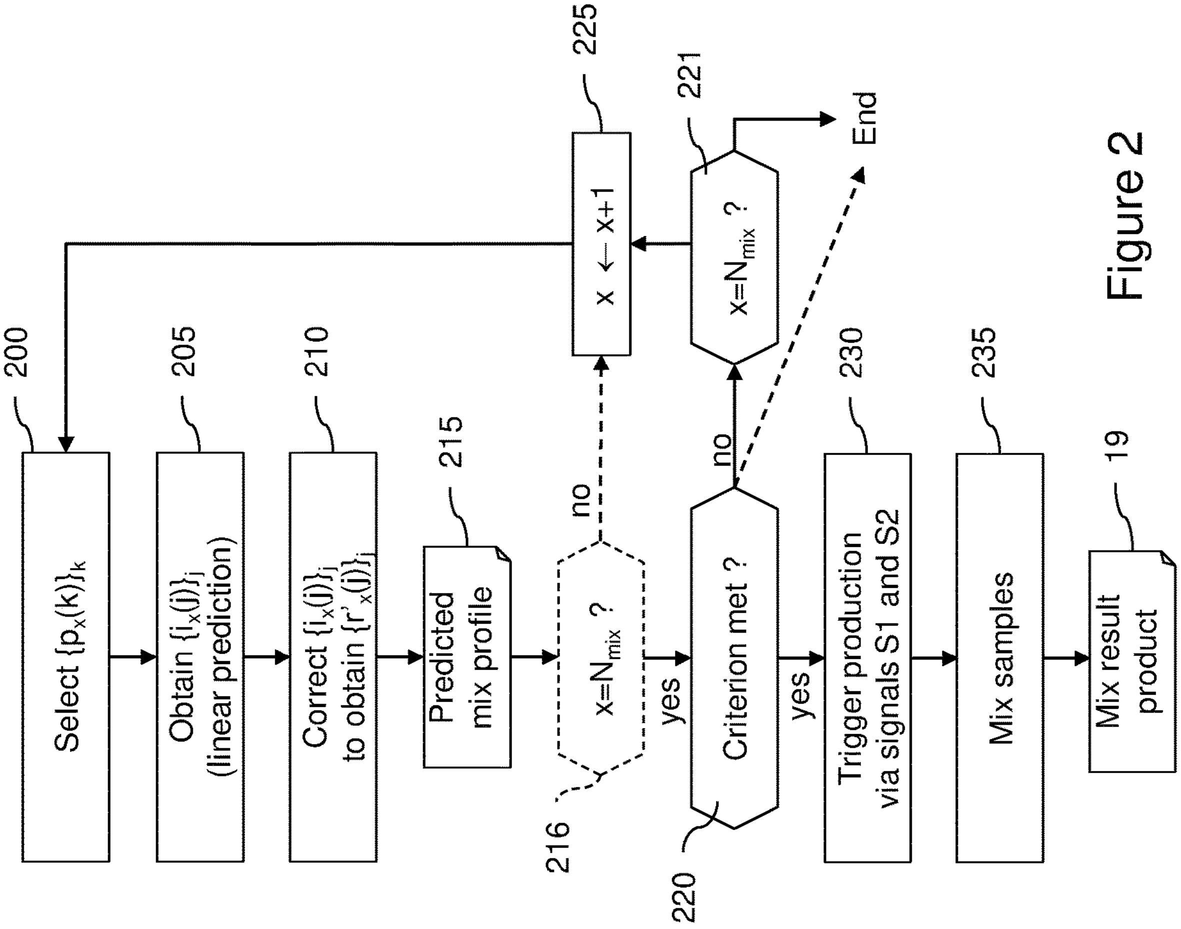


Figure 2

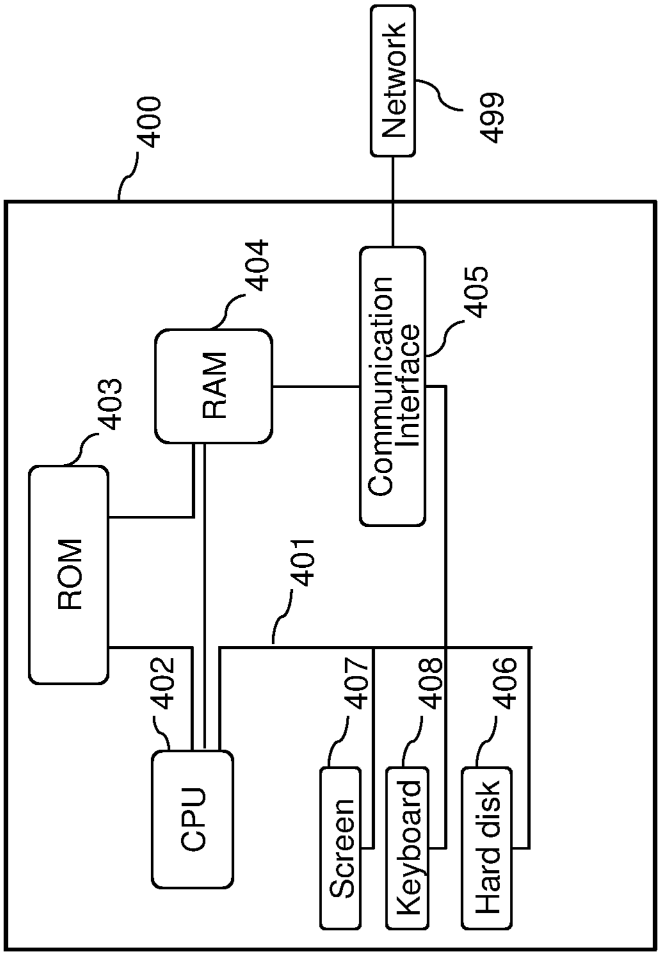

400
ROM
403
RAM
404
Communication Interface
405
Network
499
401
CPU
402
Screen
407
Keyboard
408
Hard disk
406

Fig. 4

Figure 5a

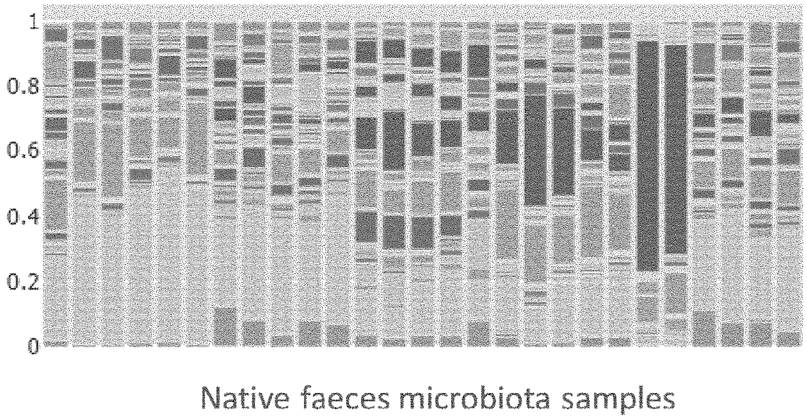


Figure 5b

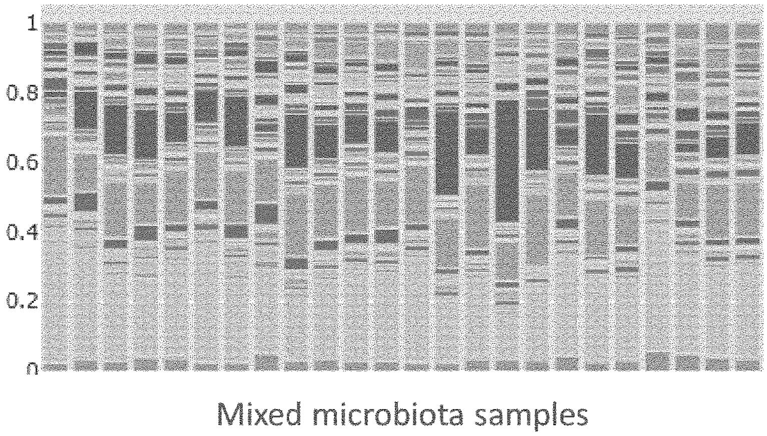


Figure 5c

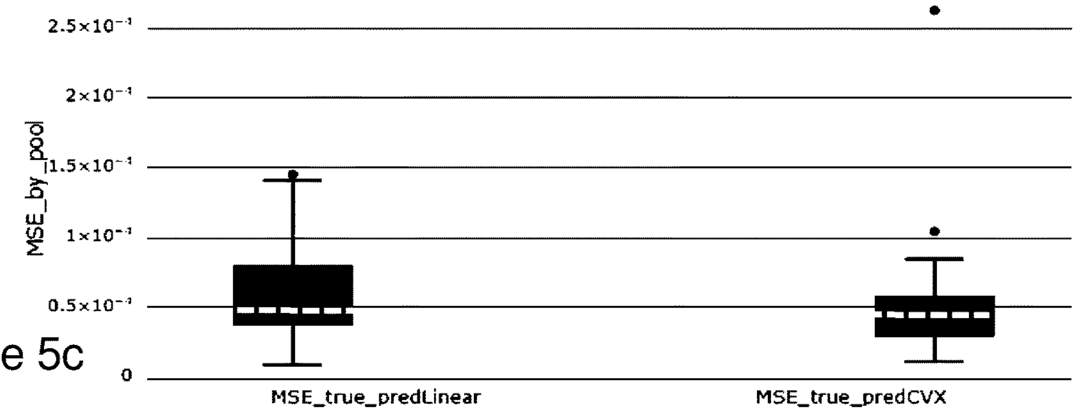


Figure 6a

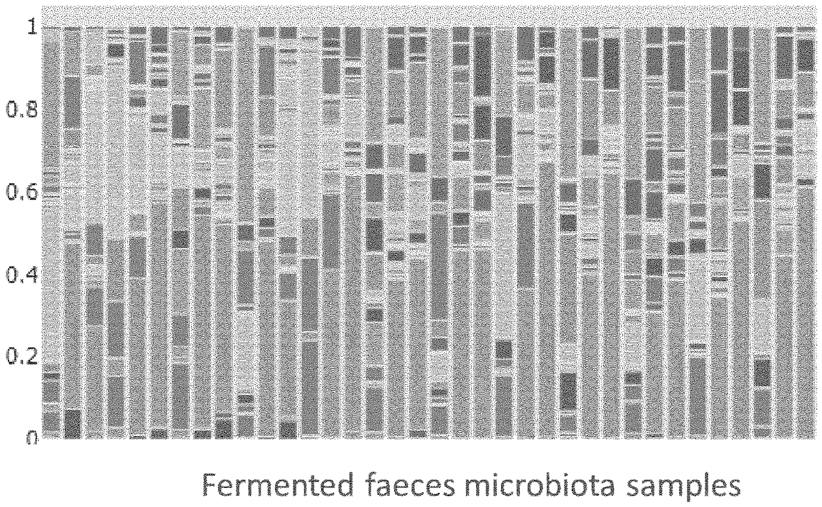


Figure 6b

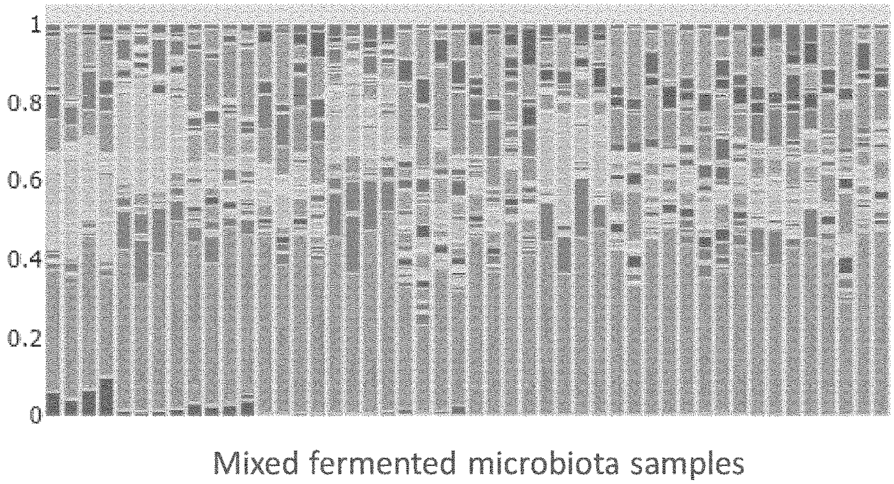
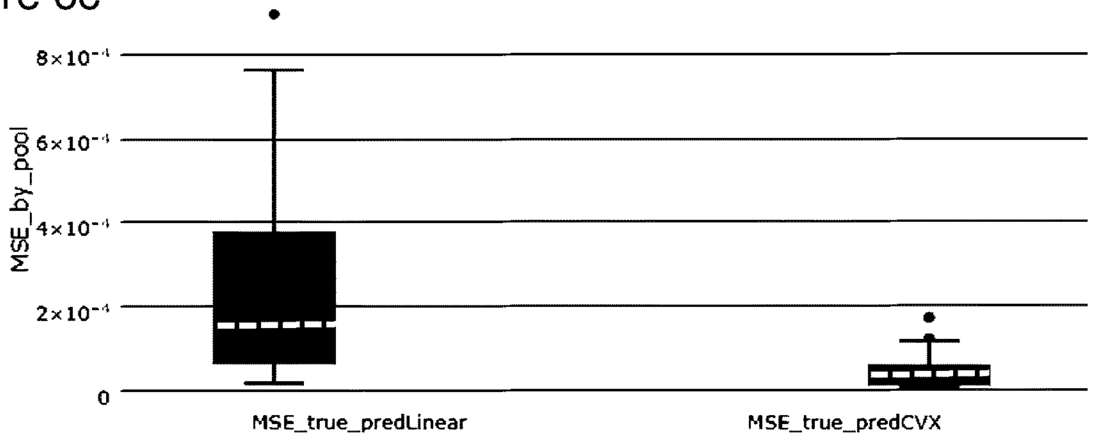


Figure 6c



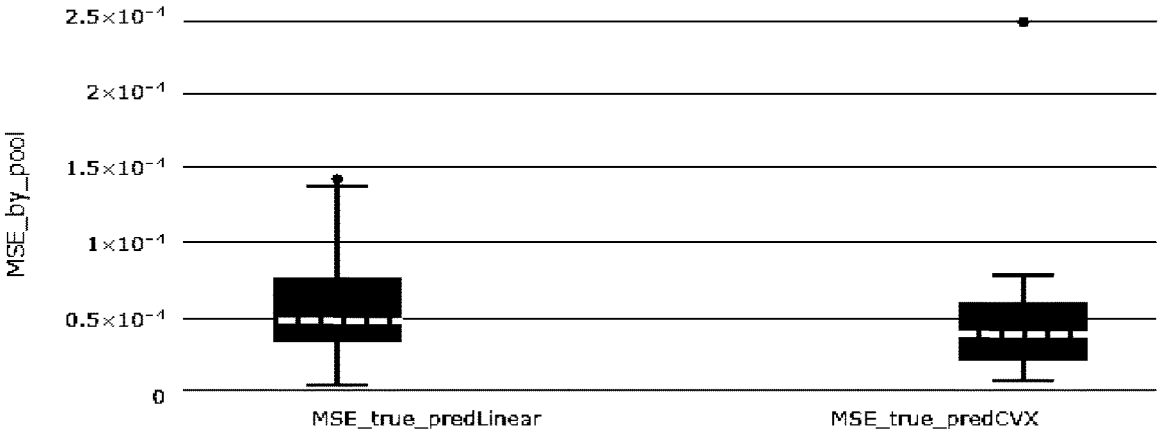


Figure 7a

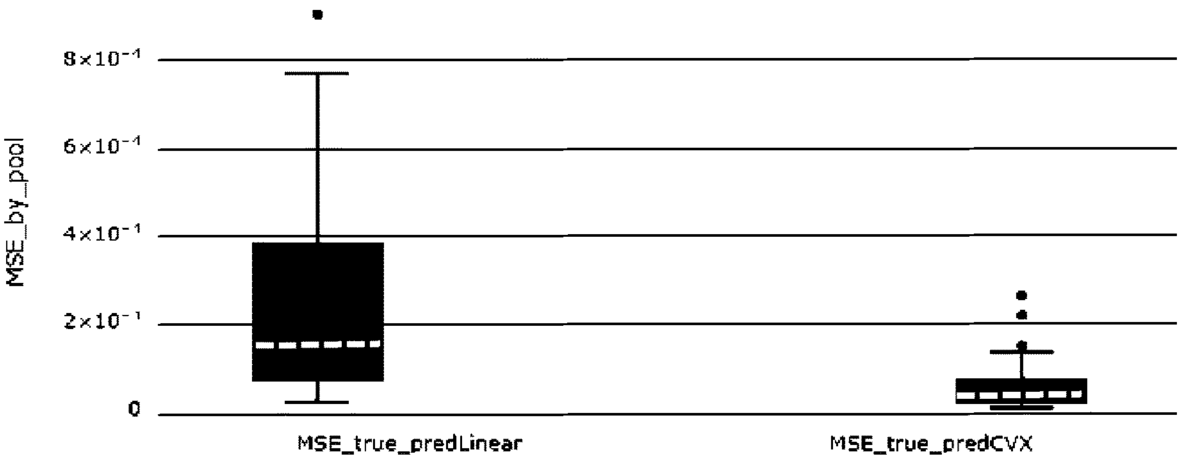


Figure 7b

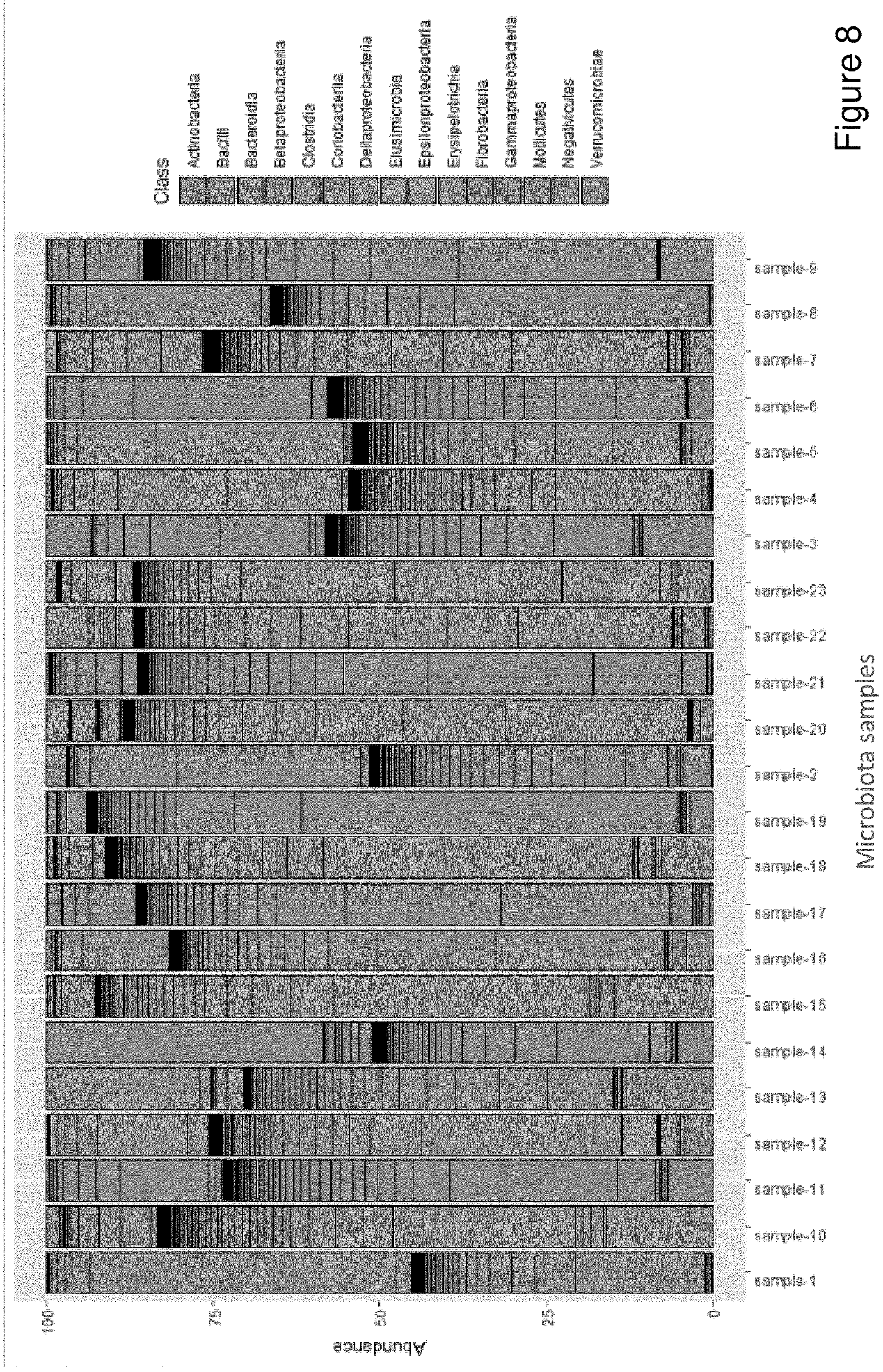

Class
Actinobacteria
Bacilli
Bacteroidia
Betaproteobacteria
Clostridia
Coriobacteriia
Deltaproteobacteria
Elusimicrobia
Epsilonproteobacteria
Erysipelotrichia
Fibrobacteria
Gammaproteobacteria
Mollicutes
Negativicutes
Verrucomicrobiae
sample-9
sample-8
sample-7
sample-6
sample-5
sample-4
sample-3
sample-23
sample-22
sample-21
sample-20
sample-2
sample-19
sample-18
sample-17
sample-16
sample-15
sample-14
sample-13
sample-12
sample-11
sample-10
sample-1
100
75
50
25
0
Abundance
Microbiota samples

Figure 8

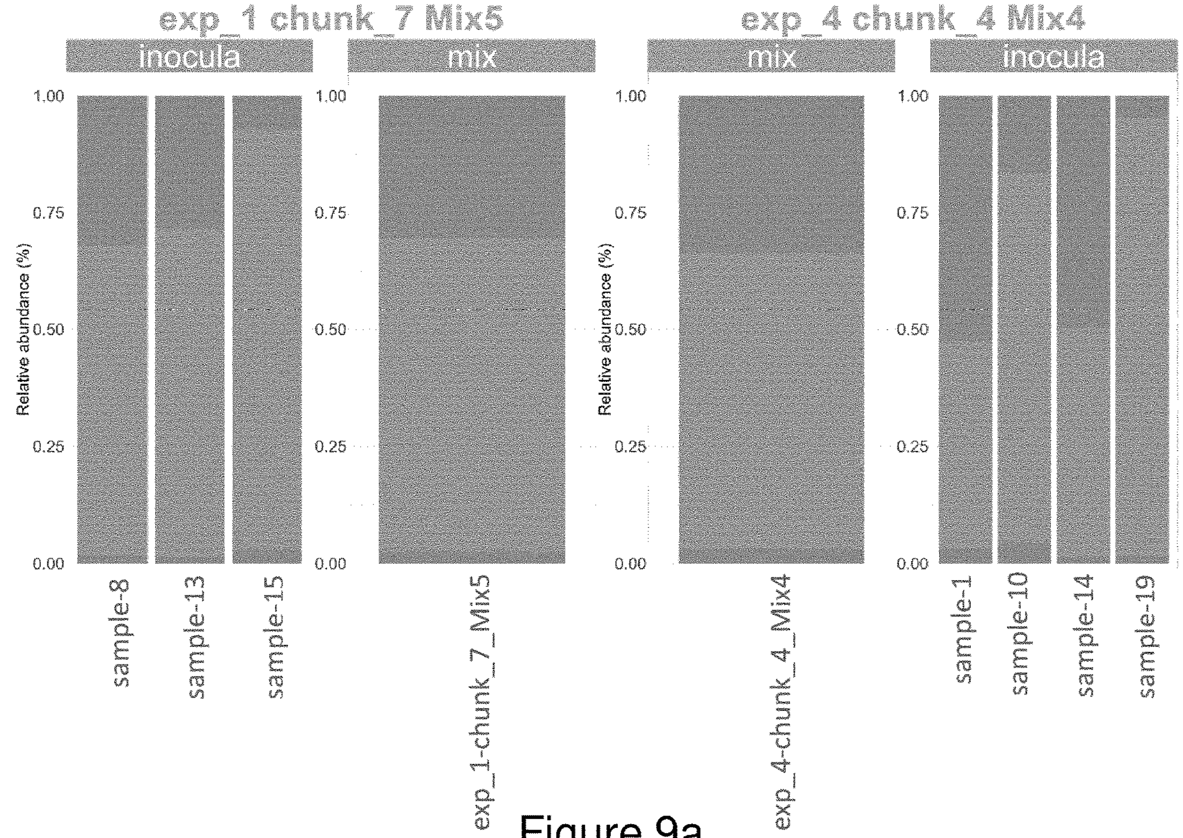


Figure 9a

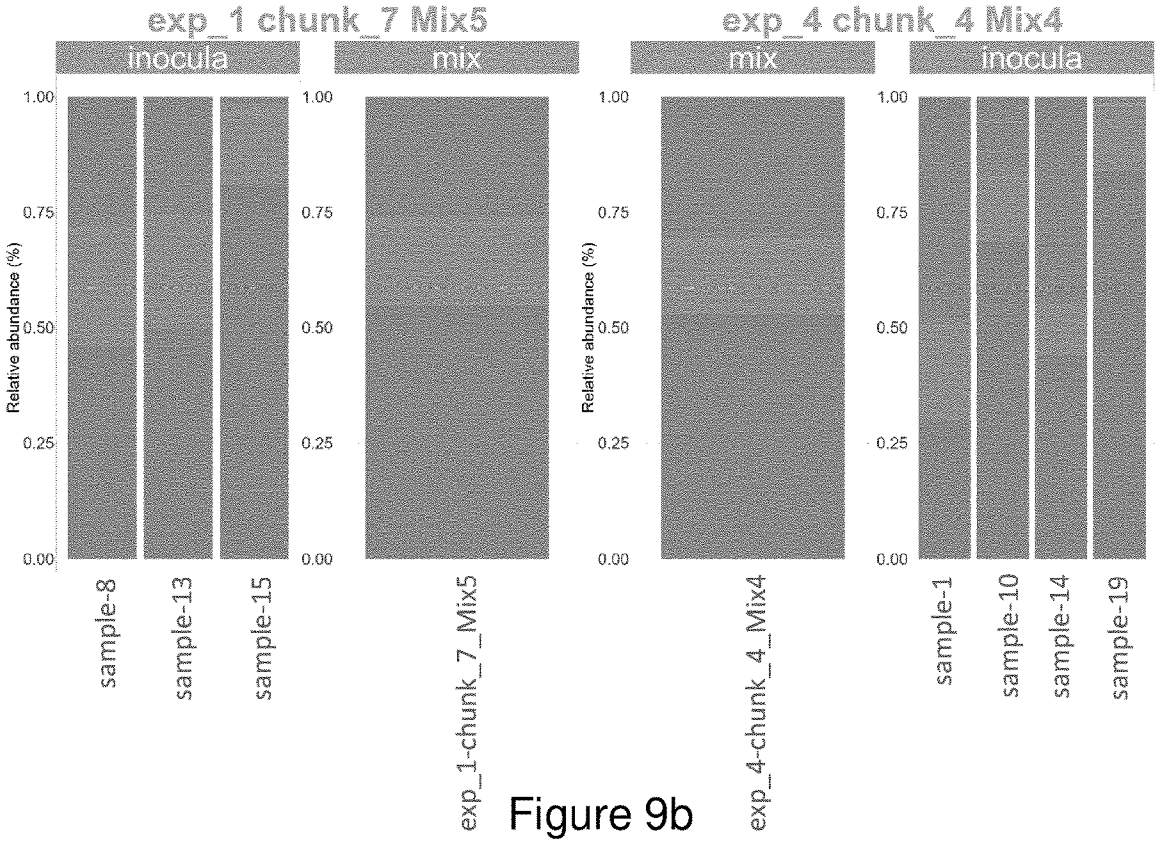


Figure 9b

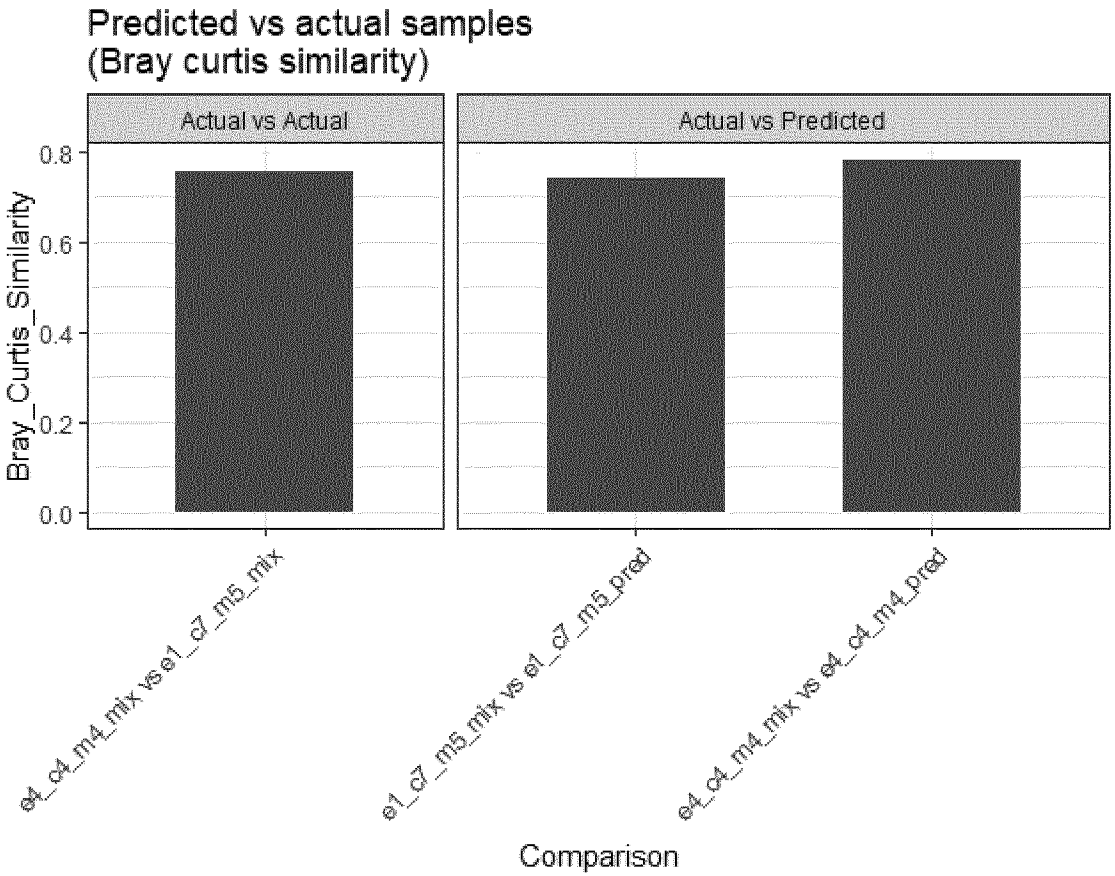

Predicted vs actual samples
(Bray curtis similarity)
Actual vs Actual
Actual vs Predicted
Bray_Curtis_Similarity
0.8
0.6
0.4
0.2
0.0
e4_c4_m4_mix vs e1_c7_m5_mix
e1_c7_m5_mix vs e1_c7_m5_pred
e4_c4_m4_mix vs e4_c4_m4_pred
Comparison

Figure 10

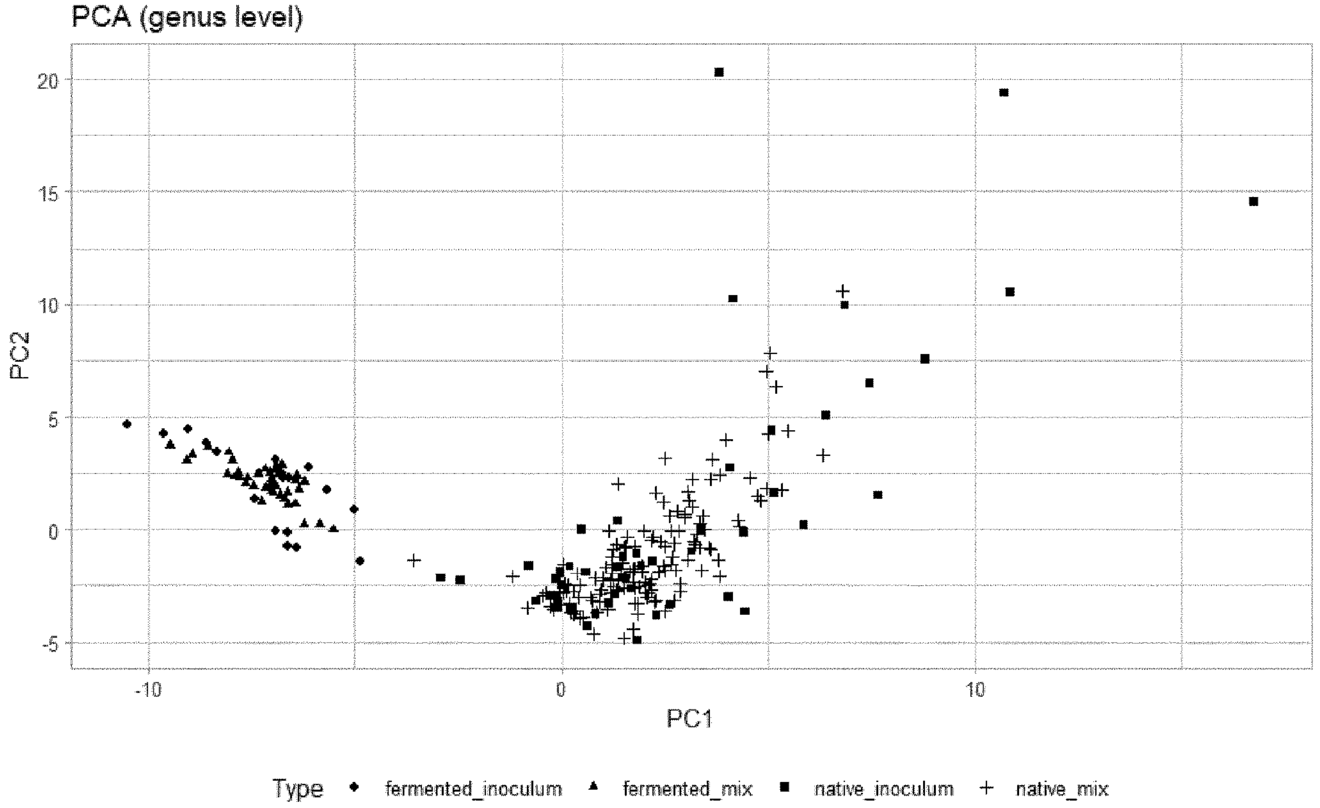


Figure 11

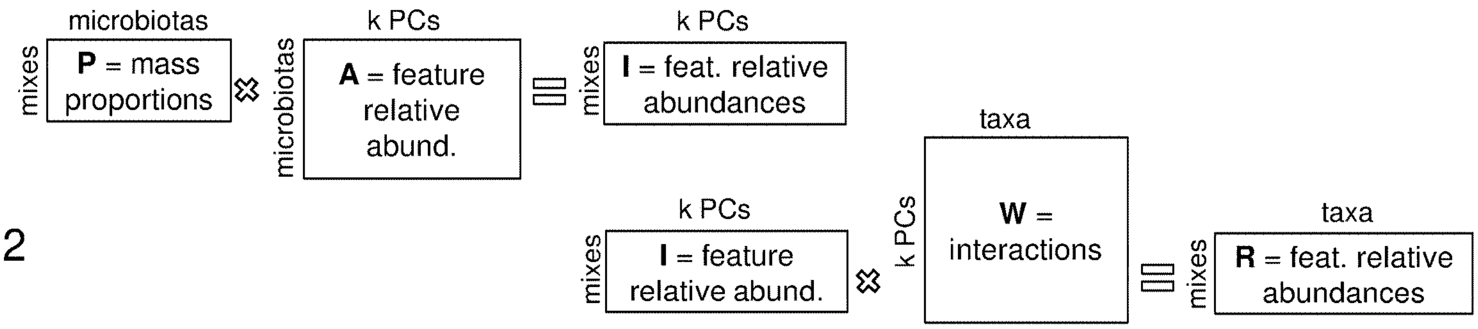

microbiotas
mixes
P = mass proportions
⊗
k PCs
microbiotas
A = feature relative abund.
=
k PCs
mixes
I = feat. relative abundances
k PCs
mixes
I = feature relative abund.
⊗
taxa
k PCs
W = interactions
=
taxa
mixes
R = feat. relative abundances

Figure 12

METHOD OF PREDICTING AND THEN PRODUCING A MIX OF MICROBIOTA SAMPLES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a National Stage of International Application No. PCT/EP2022/062226 having an International Filing Date of 5 May 2022, which designated the United States of America, and which International Application was published under PCT Article 21(2) as WO Publication No. 2022/234053, which claims priority from and the benefit of European Patent Application No. 21172578.3, filed on 6 May 2021, the disclosures of which are incorporated herein by reference in their entireties.

BACKGROUND

Field

[0002] The present disclosure concerns the mixing or "pooling" of complex communities of microorganisms, or microbiotas, and more particularly methods and devices using a learnt model, for example a matrix-based predictor, linking the individual profiles or compositions of initial microbiota samples with the profiles of resulting mixes thereof.

Brief Description of Related Developments

[0003] Complex communities of microorganisms, also known as microbiotas, play a key role in health and diseases. In particular, it has been discovered that the administration or transplantation of a complex community of microorganisms, for instance via Fecal Microbiota Transplantation (FMT), may treat infections and diseases.

[0004] In case of administration or transplantation of a complex community of microorganisms, it is important for the administrated or transplanted sample to have an appropriate profile in terms of viability and diversity of microorganisms such as bacteria, archaea, viruses, phage, protozoa and/or fungi.

[0005] Some administration and transplantation methods are often empirical and take no particular precaution to ensure the diversity of the microorganisms present in the used samples, or to best preserve the viability of the microorganisms.

[0006] Furthermore, samples collected from donors may not offer satisfactory profiles of complex communities of microorganisms for an efficient treatment.

[0007] Mixes of complex microorganism community samples collected from several donors have thus been considered to increase the diversity of the samples that can be used as inocula for administration or transplantation.

[0008] To test various mixes, the mixing of samples is actually performed randomly, and resulting products are then sequenced in order to obtain final mix profiles, from which curative and treatment properties are inferred. This test-based approach has some drawbacks. In particular, it consumes rare material given the harsh difficulties in obtaining samples from donors and takes several weeks to be completed due to sequencing analysis time.

[0009] Prediction of the mix composition, i.e. of the profile of the mix product, has thus been contemplated.

[0010] A simple way to predict the mix composition from the individual profiles of the complex microbial communities used as starting material consists in applying a linear prediction for each profiling feature: for instance, by summing the relative abundances of said profiling feature in the individual profiles after weighting them by the ratio of the corresponding complex communities in the mix.

[0011] However, some shifts or drifts between such linear-predicted profiles and the true profiles (obtained by profiling the mix result products) were observed. Hence, the linear prediction of profiles is thus considered as being a naive approach.

[0012] An assumption of the inventors is that the shifts may result from some important and quick adjustments of the microorganisms due to their interactions in the shared environment, for instance an adaptation to new conditions of the shared environment or a competition between microorganisms.

[0013] There is thus a need to perform accurate predictions to conduct the pooling in a way that guaranties the delivery of a precise complex community product composition e.g. with expected treatment efficacy.

SUMMARY

[0014] The present disclosure seeks to overcome some of the foregoing concerns by computer-aided designing these shifts when predicting mix compositions, with a view of driving, controlling or directing an actual mixing of microbiota samples for use thereof in, inter alia, administration or transplantation methods.

[0015] In this respect, the present disclosure proposes a computer-aided method of predicting a mix composition resulting from the mixing of complex microorganism community samples belonging to an initial sample collection, the method comprising:

- **[0016]** predicting, using a linear approach, an intermediary mix profile for a mix of selected complex microorganism community samples, and
- **[0017]** correcting the intermediary mix profile into a predicted mix profile, using an interaction model learnt from reference linear-predicted mix profiles and corresponding reference true mix profiles.

[0018] In particular, the predicted mix profile may be used to control actual picking and mixing of complex microorganism community samples from the initial sample collection to obtain a mix result product. Picking samples may simply mean taking or retrieving appropriate and sufficient quantities of the samples from the initial collection. The picking may be made either manually by an operator or automatically by controlled robots.

[0019] It turns out that the present disclosure also provides a method of producing a complex microorganism community product, comprising:

- **[0020]** selecting complex microorganism community samples from an initial sample collection,
- **[0021]** using the above prediction method to predict a mix profile resulting from the mixing of the selected samples,
- **[0022]** comparing the predicted mix profile to a selection criterion, for example the sufficient presence of taxa of interest or any target mix profile, and
- **[0023]** depending on the outcome of the comparing, actually picking and mixing the selected samples to

obtain a mix result product. The selected samples are preferably mixed using the relative abundancies used for the prediction.

[0024] Of course, should the outcome of the comparing be deceiving, no actual mixing may be performed, but another selection of samples may be made to predict another mix profile with the teachings of the disclosure. Thus, a plurality of sets of selected samples may be successively considered. Next, for each set, the using and comparing steps are performed and then the actual picking and mixing are performed depending on the outcome of their comparing.

[0025] Conversely, the present disclosure also proposes a reverse approach with a computer-aided method of determining a target set of complex microorganism community samples in an initial sample collection given a target mix profile representing a target mix result product, the method comprising:

[0026] selecting candidate sets of complex microorganism community samples from the initial sample collection,

[0027] for each candidate set selected, using the above prediction method to predict a mix profile resulting from the mixing of the samples of the selected candidate set,

[0028] comparing the predicted mix profiles to the target mix profile to choose one candidate set as the target set.

[0029] The target mix profile may be general, i.e. quantifying each profiling feature considered, or be specific to one or some profiling features, e.g. defining some feature specifications such as the presence or absence of one (or more) profiling feature(s) and/or its relative abundance or quantity or ranges of quantities, or defining a minimum level of diversity for instance in terms of a number of profiling features with minimum relative abundancies. The target mix profile may thus be a set of profiles scanning various possible values for a given feature specification.

[0030] The target set of samples may then be used to control actual picking and mixing of complex microorganism community samples from the initial sample collection to obtain a mix result product function of the target mix profile (it may have the target mix profile or be close to it given approximations).

[0031] It turns out that the present disclosure also provides a method of producing a complex microorganism community product having a target mix profile representing a target mix result product, comprising:

[0032] selecting, using the above determining method, a target set of complex microorganism community samples belonging to an initial sample collection given the target mix profile, and

[0033] actually picking and mixing the samples of the selected target set to obtain a mix result product.

[0034] The present disclosure advantageously makes it possible to instantaneously simulate various mix compositions at low cost, in particular without consuming any actual material (samples of the initial sample collection).

[0035] It further allows efficient sets of complex microorganism community samples to be found with a view of obtaining a mix result product that meets mix criteria, for instance a target community profile or composition adapted to cure a disease.

[0036] Hence a pooling strategy can be defined ahead of a production routine, depending on the needs of the intended use (e.g. therapeutic, prophylactic, environmental, . . .).

[0037] The mix result product so obtained can then be administrated or transplanted into a human or animal body or to plants as a fertilizer or even to environment media, including water, soil and subsurface material, e.g., for treating contamination via bioremediation.

[0038] Preferably, Microbiome Ecosystem Therapy products can be produced using the above methods.

[0039] Correlatively, the disclosure also provides a computer device comprising at least one microprocessor configured for carrying out the steps of any of the above methods. The computer device may thus be configured to emit a signal to control a mixing device to actually pick and mix complex microorganism community samples from the initial sample collection to obtain a mix result product.

[0040] Optional features of aspects of the disclosure are defined in the appended claims. Some of these features are explained here below with reference to a method, while they can be transposed into device features.

[0041] In some aspects, predicting the intermediary mix profile includes computing a matrix product between a first matrix defining the mix in terms of proportions of the complex microorganism community samples of the initial sample collection and a second matrix defining the individual profiles of the complex microorganism community samples. The second matrix, denoted A below, is defined by the initial sample collection available.

[0042] In some aspects, correcting the intermediary mix profile includes computing a matrix product between a matrix representing the intermediary mix profile and a square interaction matrix of the learnt interaction model. Here, the interaction model may be the square interaction matrix learnt from the reference linear-predicted mix profiles and the corresponding reference true mix profiles.

[0043] Using matrices to perform the prediction of sample mixes advantageously allows a large number of profiling features to be taken into account and quick computations to obtain one or more predicted mix profiles for mix result product or products.

[0044] In some aspects, the predicting method further comprises clipping each negative value in the predicted mix profile, i.e. the negative values are set to 0. This is to correct theoretical predictions (for instance relative abundance becomes negative) to nature reality.

[0045] In some aspects, the predicting method further comprises normalizing to 1 a sum of relative abundancies of profiling features defining the predicted mix profile. Again, this aims at normalizing theoretical predictions to nature reality. This is to have true relative abundancies, the sum of which represents an entire composition.

[0046] It is also expected that a profiling feature not present in the selected samples (that are mixes) should not be present in the predicted mix profile. Hence, non-zero abundances in the predicted mix profile for profiling features initially not present in the selected samples are set to zero.

[0047] In some aspects regarding the reverse approach, determining the set of samples may include determining a relative abundance of each sample within the set. In other words, the reverse prediction aims at obtaining the relative proportions of the complex microorganism community samples to be mixed together.

[0048] In other aspects regarding the reverse approach, comparing the predicted mix profiles to the target mix profile includes computing a distance between each predicted mix profile and the target mix profile and selecting, as target set, the candidate set having the lowest distance.

[0049] In some aspects, a profile of a complex community of microorganisms (sample or mix) includes relative abundancies of profiling features in the complex community of microorganisms.

[0050] In specific aspects, the relative abundancies are representative of mass or volume proportions of the profiling features in the complex community of microorganisms.

[0051] In some aspects, profiling features forming a profile of a complex community of microorganisms include one or more features from taxa, genes, antibiotic resistance genes, functions, metabolite traits, and metabolite and protein production, preferably include taxa.

[0052] In some aspects, an individual profile of a complex microorganism community sample is obtained using a profiling technology such as 16S rRNA gene amplicon sequencing, NGS shotgun sequencing, amplicon sequencing other than 16S rRNA gene-based, NGS amplicon-based targeted sequencing, phylochip-based profiling, whole metagenome sequencing (WMS), Polymerase Chain Reaction (PCR) identification, a mass spectrometry (e.g. of LC/MS type or GC/MS type), near-infrared (NIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, preferably using the 16S rRNA gene amplicon sequencing or NGS.

[0053] In some aspects, a profile of a complex community of microorganisms defines profiling features with respect to one or more microorganisms present in the complex community of microorganisms from bacteria, archaea, viruses, phage, protozoa and fungi, preferably with respect to bacteria and/or archaea.

[0054] In some aspects, a profile of a complex community of microorganisms defines profiling features that specify relative abundances of microorganisms considered at one or more taxonomic levels from strains, species, genus, families and orders, preferably one or more taxonomic levels from genus, families and orders.

[0055] In some aspects, a profile of a complex community of microorganisms includes relative abundancies, in the complex community of microorganisms, of bacteria and/or archaea taxa considered at a taxonomic level of genus, families and orders.

[0056] In some aspects, a profile of a complex community of microorganisms includes relative abundancies, in the complex community of microorganisms, of bacteria and/or archaea taxa defined by the presence/absence or expression of certain genes and/or functions (e.g., production of butyrate, antibiotic resistance genes, production of enzymes such as organophosphate hydrolases, phosphodiesterases, superoxide dismutases, etc., production of anti-microbial peptides, organophosphate hydrolyases or other enzyme useful in bioremediation processes, . . .).

[0057] In some aspects, the initial sample collection comprises samples selected from the group consisting of raw complex microorganism community samples, engineered/processed complex microorganism community samples, artificial complex microorganism community samples (e.g., bacterial consortia obtained by mixing isolated strains) and virtual complex microorganism community samples.

[0058] In some aspects, the initial sample collection includes one or more of faecal, skin, buccal, vaginal, nasal, tumoral, human, animal, plant, water, soil samples. For instance, it may include one or more faecal samples coming from at least one donor, preferably coming from at least two donors.

[0059] In some aspects, the interaction model (e.g. the square interaction matrix) is obtained using machine learning that minimizes a formula function of a difference between reference predicted mix profiles obtained from the reference linear-predicted mix profiles and the interaction model (preferably, a matrix product is performed with the square interaction matrix), and the corresponding reference true mix profiles.

[0060] The reference data (here profiles) are known as training data for the machine learning process. It is searched to minimize the error between matrix-based predicted profiles and corresponding true profiles, possibly given a regularization term.

[0061] In this respect, the formula may add a regularization term, preferably a Ridge-based regularization term, to said difference.

[0062] In particular aspects, the regularization term includes a difference between a square interaction matrix of the interaction model and the identity matrix.

[0063] The regularization tends to penalize model solutions too far from the identity. Indeed, it is expected that the interactions between the microorganisms within the mixes are not too substantial, so that model solutions far from the identity are far from biological reality. The regularization term thus avoids such accidental solutions (theoretical solutions due to the particular set of training data) to be obtained.

[0064] In some aspects, negative values of relative abundancies of profiling features in the reference predicted mix profiles are clipped before minimizing the formula.

[0065] In some aspects, the method further comprises normalizing to 1 a sum of relative abundancies of profiling features defining one of the reference predicted mix profiles before minimizing the formula. Preferably, several or all the reference predicted mix profiles are individually normalized to 1 if necessary.

[0066] In some aspects, a reference linear-predicted mix profile is predicted, using a linear approach, from individual profiles of complex microorganism community samples mixed together to produce a reference mix product and the corresponding reference true mix profile is obtained from profiling (e.g. sequencing or 16S rRNA gene amplicon sequencing) the reference mix product.

[0067] In some aspects relating to the producing method, the selection criterion includes one or more from a diversity criterion representative of an increase in profiling feature diversity, a minimum or maximum relative abundance of one or more profiling features, a non-zero relative abundance for one or more specific profiling features or for a minimum number of profiling features, a relative ratio between at least two profiling features, a closeness (or similarity such as minimal distance) to a target mix profile.

[0068] In some aspects, one selected complex microorganism community sample is a virtual sample and the method further comprises actually producing a complex microorganism community sample corresponding to the selected virtual sample from isolated strains and/or complex microorganism community samples. This advantageously allows defining a pooling strategy ahead, without consuming material nor having yet the samples. A bacteria consortium identified as being useful to produce a desired mix result product can then be produced by a mere mixing of isolated strains. Similarly, a sample identified as being useful to produce a desired mix result product can be produced by mixing one or several of isolated strains with one or several samples, resulting in an engineered sample enriched in the desired strains.

[0069] Another aspect of the disclosure relates to a non-transitory computer-readable medium storing a program which, when executed by a microprocessor or computer system in a device, causes the device to perform any method as defined above.

[0070] At least parts of the methods according to the disclosure may be computer implemented. Accordingly, the present disclosure may take the form of an entirely hardware embodiment, an entirely software embodiment (including firmware, resident software, micro-code, etc.) or an embodiment combining software and hardware aspects that may all generally be referred to herein as a "circuit", "module" or "system". Furthermore, the present disclosure may take the form of a computer program product embodied in any tangible medium of expression having computer usable program code embodied in the medium.

[0071] Since the present disclosure can be implemented in software, the present disclosure can be embodied as computer readable code for provision to a programmable apparatus on any suitable carrier medium. A tangible carrier medium may comprise a storage medium such as a hard disk drive, a magnetic tape device or a solid-state memory device and the like. A transient carrier medium may include a signal such as an electrical signal, an electronic signal, an optical signal, an acoustic signal, a magnetic signal or an electromagnetic signal, e.g. a microwave or RF signal.

BRIEF DESCRIPTION OF THE FIGURES

[0072] FIG. **1** illustrates a complex microorganism community mixing platform implementing aspects of the present disclosure;

[0073] FIG. **1***a* illustrates the behavior of an error measurement depending on an hyper-parameter of a regularization term when modelizing;

[0074] FIG. **2** illustrates, using a flowchart, general steps of producing a mix result product, including predicting mix profiles, according to aspects of the disclosure;

[0075] FIG. **3** illustrates, using a flowchart, general steps of determining and then producing a mix result product given a target mix profile, according to aspects of the disclosure;

[0076] FIG. **4** shows a schematic representation a computer device in accordance with aspects of the present disclosure;

[0077] FIGS. **5***a*, **5***b* and **5***c* illustrate results of a first experiment of the present disclosure, based on mixing native complex community samples of microorganisms;

[0078] FIGS. **6***a*, **6***b* and **6***c* illustrate other experimental results of the present disclosure, based on mixing fermented complex community samples of microorganisms;

[0079] FIGS. **7***a* and **7***b* illustrate yet other experimental results of the present disclosure, mixing native and fermented samples;

[0080] FIG. **8** illustrate a collection of sample profiles used in a second experiment of the present disclosure;

[0081] FIGS. **9***a* and **9***b* illustrate results of the second experiment seeking to find a mix composition to obtain a mix product close enough to a target mix product;

[0082] FIG. **10** illustrates the similarities between actual mixes or actual and predicted mixes when comparing the target mix product and the best predicted mix product shown in FIGS. **9***a* and **9***b;*

[0083] FIG. **11** illustrates the PCA based on genus relative abundances obtained from NGS shotgun sequencing of samples in Experiment 3; and

[0084] FIG. **12** illustrates the PCA-based approach used in Experiment 3.

DETAILED DESCRIPTION

[0085] The present disclosure concerns the mixing or "pooling" of complex communities of microorganisms, or "microbiotas" or "microbiota samples". It is more particularly directed to methods and devices using a learnt predictor model linking the individual profiles or compositions of initial complex microorganism community samples with the profiles of resulting mixes thereof.

[0086] As used herein, the expressions "microbiota", "microbiota composition" and "complex community of microorganisms" can be used interchangeably to refer to any population of microorganisms comprising a high number of microorganisms of different species which live together and potentially in interaction. Microorganisms possibly present in a complex community of microorganisms include yeasts, bacteria, archae, virus, fungi, algae, phages, and any protozoa of different origins such as soil, water, vegetal, animal, or human origins.

[0087] Microbiotas according to the present text include naturally occurring complex communities of microorganisms (such as, for example, gut microbiota, i.e., the population of microorganisms living in the intestine of an animal), as well as "engineered complex communities of microorganisms", i.e., complex communities resulting from transformation steps such as addition of isolated beneficial strains, treatments to remove potential deleterious microorganisms (e.g., by using rare-cutting endonucleases targeting genes specific for pathobionts), expansion by culture in specific conditions (e.g., fermentation in appropriate medium), etc. "Isolated beneficial strains" herein designate natural strains known to have a beneficial effect in certain conditions (e.g., Akkermansia muciniphila), as well as genetically modified strains, including strains in which a potential deleterious gene has been knocked out (for example using a rare-cutting endonuclease such as Cas9) and strains in which a transgene has been introduced (e.g., by the use of a bacteriophage, or the CRISPR system).

[0088] Complex communities of microorganisms and microbiotas according to the present text include "raw" or "native" complex communities or microbiotas, i.e., directly obtained from a source, a donor or donors without being treated by post-processing and "processed complex communities of microorganisms", including engineered complex communities or microbiotas and any complex microorganism communities resulting from a treatment on or post-processing of or transformation of one or more natural raw complex microorganism communities (e.g., a complex community or microbiota which been filtered, frozen, thawed and/or lyophilized, and/or which has been extracted, isolated or separated from its initial matrix by techniques well-known for the skilled person such as, for example, those described in WO 2016/170285 and WO 2017/103550).

[0089] The expression "samples", "complex microorganism community samples" and "microbiota samples" can be used interchangeably and refer to initial complex communities or microbiotas in the meaning of the disclosure, i.e. that are available for mixing.

[0090] The term "Microbiome Ecosystem Therapy product" herein refers to any composition comprising a complex community of microorganisms (either naturally occurring or engineered, native or processed), provided it is in a form suitable for administration to an individual in need thereof. A Microbiome Ecosystem Therapy aims at modifying an individual's microbiota to obtain a health benefit (e.g., preventing or alleviating the symptoms of a disease, increasing the chances that the individual responds to a treatment, etc.). Typically, a Microbiome Ecosystem Therapy is done by replacing at least part of a dysfunctional and/or damaged ecosystem by a different complex community of microorganisms in a subject in need thereof. Microbiome Ecosystem Therapies include Fecal Microbiota Transplantation (FMT). In the present text, unless specified otherwise, the term "FMT" is broadly used to refer to any kind of Microbiome Ecosystem Therapy.

[0091] As shown in FIG. **1** illustrating a complex microorganism community mixing platform **1** implementing aspects of the present disclosure, samples **100** are available through an initial sample bank or collection **10**. Although a single collection or bank is shown, the samples may be stored in a plurality of sub-banks that altogether form collection or bank **10**.

[0092] The samples of the present disclosure may comprise or may consist of microorganisms coming from one or more sources and/or from one or more donors **101**.

[0093] The samples of the present disclosure may come from:

[0094] a single source,

[0095] at least two sources,

[0096] a single donor,

[0097] at least two donors,

[0098] a single source and a single donor,

[0099] a single source and at least two donors,

[0100] at least two sources and a single donor, or

[0101] at least two sources and at least two donors,

[0102] As used herein, the term "source" refers to any environment from where the sample comes from such as a soil, water, parts of a vegetal, parts of animal body or fluids or parts of human body or fluids. In case of a human or an animal, the source may refer to any part of the body (skin, nasal mucosa, . . .) or to body fluids such as the content of the intestine (e.g., a stool sample).

[0103] As used herein, the term "donor" refers to a vegetal, a physical location (for sources such as soil or water), an animal or a human, preferably a human.

[0104] The donors may be pre-selected according to the method and criteria described in the prior art, such as for example in WO2019/171012 A1.

[0105] In the example shown, some samples, referenced **100***d*, **100***e*, **100***f*, **100***g*, are raw complex communities of microorganisms or microbiotas, i.e., directly obtained from a donor or donors without being treated by post-processing.

[0106] Other samples, referenced **100***a*, **100***b*, **100***c*, are "processed samples", i.e., engineered complex microorganism communities resulting from a treatment on or post-processing of or transformation of one or more natural raw complex communities. As mentioned above the treatment may include filtration, centrifugation, fermentation, freezing, freeze-drying the initial complex community, and even mixing of initial complex communities, but also treatments aimed at isolating spores and spore forming bacteria such as the use of ethanol, chloroform or heat.

[0107] As shown, an initial complex community may be one sample **100***d*, **100***e*, **100***f*, **100***g* belonging to the initial sample collection **10** or be an external sample **99**.

[0108] The initial sample collection **10** may include one or more samples from any source (faecal, skin, nasal, buccal, vaginal, tumoral . . .) of any origin (human, animal, plant, soil, . . .), preferably one or more faecal samples coming from at least one donor, preferably coming from at least two donors.

[0109] According to a particular embodiment, the samples of the collection **10** include faeces samples.

[0110] Faeces samples collected from donors may be controlled according to the method and qualitative criteria described in the prior art, such as for example in WO2019/171012 A1. For example, the qualitative criteria of the sample may comprise sample consistency between 1 and 6 on the Bristol scale; absence of blood and urine in the sample; and/or absence of specific bacteria, parasites and/or virus, as described in WO2019/171012 A1.

[0111] Faeces samples may be collected according to any method described the prior art, such as for example in WO2016/170285 A1, WO2017/103550 A1 and/or WO2019/171012 A1.

[0112] Preferably, the samples may be collected and then placed in anaerobic conditions. For example, as described in WO2016/170285 A1, WO2017/103550 A1 and/or WO2019/171012 A1, within 5 minutes following taking of the sample, the samples may be placed in an oxygen-tight collecting device.

[0113] The samples may be prepared according to the methods described in the prior art, such as for example in WO2016/170285 A1, WO2017/103550 A1 and/or WO2019/171012 A1.

[0114] All samples **100***a*-**100***g* shown in the Figure are actual samples stored in at least one bank.

[0115] Samples **100***y*-**100***z* represented with dotted lines are theoretical samples that are not actually collected from a donor or produced, hence not actually stored in the storage bank or banks **10**. As explained below, these "virtual" samples **100***y*-**100***z* are depicted to illustrate theoretical complex community profiles **110***z* imagined by an entity, for instance a computer, an operator, a researcher, and so on.

[0116] The initial sample collection **10** may comprise only native samples **100***d*-**100***g*, or may comprise only processed samples **100***a*-**100***c*, or may comprise only virtual samples **100***y*-**100***z*, or any combination thereof.

[0117] A first object of the present disclosure relates to the prediction of a mix composition resulting from the mixing of samples **100***a*-**100***z* belonging to the initial sample collection **10**. The prediction is two-fold:

[0118] predicting, using a linear approach, an intermediary mix profile for a mix of selected complex microorganism community samples, and

[0119] correcting the intermediary mix profile into a predicted mix profile, using an interaction model learnt from reference linear-predicted mix profiles and corresponding reference true mix profiles. The interaction model is preferably a squared interaction matrix learnt from the reference linear-predicted mix profiles and the corresponding reference true mix profiles.

[0120] The present inventors have surprisingly found that a learnt interaction model, and more particularly the matrix-based method, provides accurate prediction results once the interaction model or matrix is learnt, hence giving relevant hints to a final product without consuming any material of the initial sample collection.

[0121] As the prediction can be computer-implemented, the predicted mix profiles can be quickly obtained despite a large number of mixes to predict, a large number of samples available in the initial sample collection **10**, and a large number of features profiling the complex communities of microorganisms (samples and mixes).

[0122] A second object of the present disclosure relates to a reverse operation where a target set of samples is determined from the initial sample collection **10** given a target mix profile representing a target mix result product. The target mix result product may for instance represent a desired complex community of microorganisms having curative properties with respect to a disease or infection.

[0123] The reverse operation includes:

- **[0124]** successively selecting candidate sets of complex microorganism community samples from the initial sample collection,
- **[0125]** for each candidate set successively selected, using the above prediction method to predict a mix profile resulting from the mixing of the samples of the selected candidate set,
- **[0126]** comparing the predicted mix profiles to the target mix profile to choose one candidate set as the target set.

[0127] Both prediction operation and reverse operation can be used to actually produce a mix result product.

[0128] With respect to the prediction operation, the predicted mix profile may then be compared to a selection criterion, for instance the sufficient presence of taxa of interest. Then, depending on the outcome of the comparing, the selected samples are retrieved and actually mixed to obtain the mix result product. The predicted mix profile can thus be used to control actual picking and mixing of samples from the initial sample collection to obtain the mix result product.

[0129] The selection criterion may be set function of desired properties for the mix result product.

[0130] Such an approach, including the interaction-model-based or matrix-based prediction, is illustrated in FIG. **2**, described with more details below.

[0131] With respect to the reverse operation, a target set of samples belonging to the initial sample collection is selected using the reverse operation given a target mix profile, corresponding for instance to a mix result product having desired curative properties. Next, the samples of the target set are picked and actually mixed to obtain the desired mix result product. The target set of samples determined by the reverse predicting method can thus be used to control actual picking and mixing of samples from the initial sample collection to obtain a mix result product function of the target mix profile.

[0132] Aspects of such reverse approach using the target mix profile are illustrated below with reference to FIG. **3**.

[0133] By "mix" it is meant any actual mixing of samples that results in a new complex community of microorganisms or new microbiota composition. The result is also referred to as mix result product as it may be used for administration or transplantation as described above. The mix result product may for instance be used as an FMT inoculum.

[0134] By "profile" it is meant a description of the composition of the complex community of microorganisms or microbiota composition concerned (be it a sample or a mix). A profile for instance specifies the relative abundancies of profiling features in the complex community or microbiota composition. "relative" means that the sum of the abundancies equals to 1. The relative abundancies may be expressed in mass (or weight) or volume proportions of the profiling features in the complex community of microorganisms.

[0135] Depending on the application concerned (for instance, in the therapeutic field, depending on the disease targeted and, in the bioremediation field, depending on the pollutants to eliminate), the profiling features may be of different types. Usually, they are selected from a group including taxa, genes, antibiotic resistance genes, functions, and metabolite traits, and metabolite and protein production. A profile may mix profiling features of different types, for instance taxa and antibiotic resistance genes. A particular embodiment considers only taxa to profile a complex community of microorganisms.

[0136] Functions describe the known action of a protein or a protein family (as phylogenetically defined, e.g. databases of KEGG KOs or NCBI COGs or Enzyme Commission Number), or they can define a metabolic context (e.g. database of BiGG models at the reaction level, or KEGG pathways at the metabolic pathway level), some databases can be specialized as for example the CaZy database which is a catalogue of Carbohydrate-active enzymes. Any of those function categories (or a combination thereof) can be used as features in the matrix model.

[0137] KEGG stands for "Kyoto Encyclopedia of Genes and Genomes", while KO stands for "KEGG Orthology". NCBI stands for "National Center for Biotechnology Information", COG stands for "Cluster of Orthologous Groups" and BiGG stands for "Biochemical Genetic and Genomic".

[0138] Various profiling techniques are known to obtain complex community profiles, including 16S rRNA gene amplicon (i.e. metagenome) sequencing, NGS shotgun sequencing, amplicon sequencing other than 16S rRNA gene-based, NGS amplicon-based targeted sequencing. 18S/ITS gene sequencing, metagenomic sequencing, a phylochip-based profiling, a Polymerase Chain Reaction (PCR) identification, a mass spectrometry (e.g. of LC/MS type or GC/MS type), near-infrared (NIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy.

[0139] As shown in FIG. **1**, a profiler (or sequencer) **12** is preferably used to provide a profile, e.g. a 16S sequencing, of the actual samples **100***a*-**100***g*. The corresponding individual profiles so obtained are referenced **110***a*-**110***g* and form an initial profile collection or bank **11**. Of course, the 16S rRNA sequencing is not mandatory and other methods can be used as defined above, alone or in combination, to provide the profiles **110**.

[0140] The individual profiles, whatever the sequencing technique used, are converted in the same format and stored in a memory (not shown) of a computer as matrices or vectors a_x. The coefficient $a_x(j)$ of individual profile 'x' indicates the relative abundance of the profiling feature 'j' in the sample considered.

[0141] As mentioned previously, some individual profiles **110***z* may be artificially built by an operator, e.g. by defining coefficients $a_x(i)$ representing the relative abundances of the profiling features 'j' in a theoretical sample.

[0142] The initial profile collection **11** may thus comprise only individual profiles **110***d*-**110***g* corresponding to native samples **100***d*-**100***g*, or may comprise only individual profiles **110***a*-**110***c* corresponding to processed samples **100***a*-**100***c*, or may comprise only virtual profiles **110***y*-**110***z* corresponding to virtual samples **100***y*-**100***z*, or any combination thereof.

[0143] Any other profile handled thereafter (e.g. so-called intermediary profiles or mix profiles) follows the same profile format, for instance a vector made of the same profiling features 'j' in the same order.

[0144] Preferably, bacterial abundance profiles are obtained, meaning that the profiles specify relative abundancies of profiling features concerning bacteria. More generally, a profile of a complex community of microorganisms may define profiling features with respect to one or more microorganisms present in the complex community (bacteria, archaea, viruses, phage, protozoa and fungi), preferably with respect to bacteria and/or archaea. Of course, profiling features within the same profile may concern different microorganisms as previously listed.

[0145] Preferably, genus-based bacterial abundance profiles are obtained, meaning the profiling features describe the relative abundancies of bacteria at genus level in the complex community of microorganisms. More generally, a profile of a complex community of microorganisms may define profiling features that specify relative abundances of microorganisms considered at one or more taxonomic levels from strains, species, genus, families and orders, preferably one or more taxonomic levels from genus, families and orders.

[0146] The prediction operation and the reverse operation are conducted by the pool predictor module **13** under the control of the module **14**. Module **14**, referred to as "test and decision module" or "decision module", drives platform **1** with a view of predicting mix profiles and/or determining a set of samples given a target mix profile and/or producing at least one mix result product.

[0147] Modules **13** and **14** are preferably implemented through a computer, having an input/output interface (e.g. keyboard, mouse, screen) to allow an operator to interact with platform **1**.

[0148] As shown in the Figure, pool predictor **13** is matrix-based and comprises two steps for predicting a result mix profile from initial profiles of samples mixed together.

[0149] Matrix A defines the individual profiles of all the samples available in the collection **10**. It may be formed by profiler or sequencer **12** or at least by the individual profiles obtained from the profiler. Additionally any virtual individual profile is also added to the matrix.

$$\text{Preferably } A = \begin{bmatrix} \{a_1(j)\}_j \\ \{a_n(j)\}_j \end{bmatrix}$$

[0150] where j=1 . . . m, with m the number of profiling features considered and n the number of individual profiles **110** in the initial profile collection **11**, hence of samples **100** (including the virtual ones) in the initial sample collection **10**.

[0151] Square matrix W is the interaction matrix defined above modelling the interaction between the microorganisms. A description of the modelling matrix W, including how it is learnt, is provided below with more details. The interaction matrix aims at representing the non-linear interactions between the various profiling features of samples when the latter are mixed together.

[0152] The prediction operation comprises a first matrix-based step of predicting, using matrix A, an intermediary mix profile, formed by matrix I, for at least one mix of selected samples: I=P*A, where P is a matrix representing the at least one mix of selected samples from collection **10**.

[0153] Matrix P may define each mix in terms of mass or volume proportions of the samples of the initial sample collection.

[0154] For instance,

$$P = \begin{bmatrix} \{p_1(k)\}_k \\ \{p_t(k)\}_k \end{bmatrix}$$

[0155] where $\{p_x(j)\}$; defines a mix 'x' with $p_x(k)$ the proportions of sample k (k from 1 to the number Nsamp of samples in collections **10/11**). The sum of the proportions equals 1: $\Sigma_{k=1\ .\ .\ .\ Nsamp}(p_x(k))=1$. Where a sample r is not used in the mix x, $p_x(r)=0$.

[0156] The matrix-based approach advantageously allows a varying number of mixes to be predicted together: each line of P defines a mix to predict (hence 't' mixes are defined in the above example), which number 't' can vary from one prediction to the other.

[0157] The mixes to test, i.e. a list of $\{p_x(k)\}$: may be defined in advance in list **140** of test and decision module **14**. Upon starting a new test procedure, module **14** reads list **140** and forms matrix P defined above using one or some or all the mix definitions of the list. Of course, in a variant or in combination, an operator may define at least one new mix on the fly by selecting, on a screen connected to module **14**, samples **100** from the collection **10** and by specifying their relative proportions. Hence a mix $\{p_x(k)\}$ can be created on the fly.

[0158] The prediction operation I=P*A is for instance computer-implemented.

[0159] Matrix

$$I = \begin{bmatrix} \{i_1(j)\}_j \\ \{i_t(j)\}_j \end{bmatrix}$$

with j=1 . . . m, is obtained defining the linear-predicted or "intermediary" mix profiles for the tested mixes P. Those mix profiles are "naïve" predictions because they do not take into account the interactions between the microorganisms when the mix is actually performed.

[0160] That is why, according to the disclosure, the prediction operation comprises a second step of correcting, using the interaction model, in particular interaction matrix W, the intermediary mix profiles, i.e. matrix I, into predicted mix profiles, represented by matrix

$$R = \begin{bmatrix} \{r_1(j)\}_j \\ \{r_t(j)\}_j \end{bmatrix};$$

R=I*W, with j=1 . . . m.

[0161] Predicted mix profiles can thus be obtained quickly for a varying number of mixes, without consuming any material of collection **10**.

[0162] It is expected that the relative abundancies r_x(j) are not negative and form together an entire composition (i.e. their sum equals 1 for a given mix 'x'). However, this may not be the case with a matrix product. Aspects of the disclosure thus include post-processing result matrix R into R' in order to meet biological constraints.

[0163] For instance, each negative value in R is clipped, meaning the negative abundancies are set to 0. Thereafter, the relative abundancies r_x(j) are normalized, i.e. adjusted (using a linear interpolation for instance) into r'_x(j) so that their sum equals 1: $\Sigma_{j=1\ ...\ m}(r'_x(j))=1$. The final mix result matrix is the following one

$$R' = \begin{bmatrix} \{r'_1(j)\}_j \\ \{r'_k(j)\}_j \end{bmatrix}$$

where $\{r'_x(j)\}$; is a vector representing the predicted mix profile for tested mix x (defined by $\{p_x(k)\}$). Optionally, before normalizing, non-zero relative abundancies (non-zero value in R) for profiling features that are absent in the initial samples mixed together (i.e. a_x(j) is zero for all samples x mixed together) are set to zero.

[0164] The efficiency of the present method comes from the modelling of the real positive and negative interactions between the microorganisms of mixed samples into a matrix, so-called interaction matrix W. Then a two-step matrix-based process is efficiently used to predict real mix profiles.

[0165] Interaction matrix W is learnt for a given set of m profiling features. Should the profiling features be reordered in the profiles, the coefficients of interaction matrix W should be reordered accordingly.

[0166] The m profiling features may also evolve over time, for instance because new features are discovered, some features become less meaningful hence they are deleted, and/or some features can be split into more features to be more precise. Evolution in the profiling features may also result from the enhancement of the profiling/sequencing methods and profilers/sequencers **12** that provide new profiling data, as well as the improvements of the bioinformatics method that combines algorithm and reference databases of features.

[0167] Different sets of profiling features may also be considered, for instance with respect to different diseases or treatments that are targeted.

[0168] The profiling features themselves but also the number of features in the sets can evolve or change.

[0169] Hence, each time a new set of profiling features is considered, interaction matrix W can be computed anew, as well as matrix A describing the initial profile collection **11**. The computed interaction matrices W may be stored in memory of pool predictor **13** so that they may be reused, should the corresponding set of profiling features be used anew.

[0170] Interaction matrix is preferably obtained using machine learning. The machine learning is made using a set of training data. The training data are built from reference mix products 'ref' resulting from a plurality of mixes $\{p_{ref}(k)\}$ of samples k.

[0171] An actual reference mix of samples is homogenised during a period of 10 minutes to 3 hours, preferably between 30 min and 1.5 hour. The homogenisation is made at a temperature between 0° C. and 10° C., preferably between 2° C. and 6° C., more preferably at about 4° C.

[0172] The mix is then considered as stable for a couple of hours, at least up to 16 hours from the mixing, preferably up to 24 hours therefrom.

[0173] It means that the interaction matrix is representative of the interactions that should occurred between the microorganisms for a stabilized mix at 4° C.

[0174] Other interaction matrices may be produced that are representative of other mixing conditions.

[0175] The individual profiles $\{a_x(j)\}_j$ with j=1 . . . m of the samples x are known or obtained from a sequencer profiling the samples x. Hence, reference linear-predicted mix profiles $\{i_{ref}(j)\}_j$ are also known by using the above linear formula I=P*A.

[0176] The mix profiles of the reference mix products 'ref', referred as to reference true mix profiles $\{r_{true}(j)\}_j$, are also known or obtained from a sequencer profiling the reference mix products 'ref'.

[0177] Reference predicted mix profiles $\{r_{pred}(j)\}_j$ correspond to a matrix product between the reference linear-predicted mix profiles $\{i_{ref}(j)\}$; and the square interaction matrix W (in the process of being learnt): $R_{pred}=I_{ref}*W$ or $\{r_{pred}(j)\}_j=\{i_{ref}(j)\}_j*W$ for a single reference mix product 'ref'.

[0178] The machine learning seeks to minimize an error in the prediction of the reference mix profiles. In other words, it seeks to minimize a formula that is based on a difference between the reference true mix profiles

$$R_{true} = \begin{bmatrix} \{r_{true\text{-}1}(j)\}_j \\ \{r_{true\text{-}N}(j)\}_j \end{bmatrix}$$

and the corresponding reference linear predicted mix profiles

$$R_{pred} = \begin{bmatrix} \{r_{pred\text{-}1}(j)\}_j \\ \{r_{pred\text{-}N}(j)\}_j \end{bmatrix} = I_{ref} * W.$$

'pred-i' and 'true-i' reference the predicted and true reference mix profiles corresponding to the same reference mix product 'i', respectively. 'N' represents the number of reference mix products considered.

[0179] The training data for the machine learning are I_{ref} and R_{true}.

[0180] In some aspects, the formula to minimize is the residual vectors

$$\{r_{true-i}(j)\}_j - \{r_{pred-i}(k)\}_k = \{r_{true-i}(j)\}_j - \{i_{ref}(k)\}_k * W,$$

[0181] or the residual matrix $R_{true}-R_{pred}=R_{true}-I_{ref}*W$.

[0182] Any norm may be used: L1, L2, Lp and so on. Preferably, the Sum of Squared Difference (SSD) or its derived Mean-Squared Error (MSE) may be used. Also the minimum Chi-squared method may be used alternatively.

[0183] The machine learning may then seek to solve the following convex optimization problem:

$$\min\left(\frac{1}{N}*\left\|I_{ref}*W-R_{true}\right\|^2\right)$$

where $\|\cdot\|^2$ is the MSE and N is the number of mix products considered in R_{true} and R_{pred}.

[0184] In aspects avoiding overfitting W, the formula adds a regularization term, preferably a Ridge, L2,-based regularization term, to said difference. In a variant a Lasso, L1,-based regularization term can be used. The Ridge approach advantageously helps having a higher number of non-zero coefficients in W, hence modelling more precisely the interactions between the profiling features.

[0185] Hence, the machine learning seeks to solve the following convex optimization problem:

$$\min\left(\frac{1}{N}*\|I_{ref}*W-R_{true}\|^2+1*\|W-ID\|_2\right)$$

where $\|\cdot\|^2$ is the regularization term (preferably Ridge), ID is the identity matrix, and A is an hyper-parameter for the regularization weighting.

[0186] In addition, constraints may be set during the machine learning so that R_{pred} has no negative relative abundancies and the sum of the relative abundancies of each reference predicted mix profile is 1. In other words, a modified matrix R'_{pred} is preferably used corresponding to clipping the negative relative abundancies in I_{ref}*W and then to normalizing to 1 the sum of the relative abundancies for each reference predicted mix profile, i.e. for each line in I_{ref}*W. Modified I_{ref}*W is noted $\widetilde{I_{ref}*W}$. Therefore, in aspects, the machine learning seeks to solve the following convex optimization problem:

$$\min\left(\frac{1}{N}*\|\widetilde{I_{ref}*W}-R_{true}\|^2+1*\|W-ID\|_2\right).$$

[0187] The set of training data, let say N reference mix results, is split into two subsets, one for the optimization of the hyper-parameter λ and the other for the optimization of W.

[0188] Various methods to optimize λ are known, including inter alia a minimizing information criteria approach (for instance minimizing Akaike or Bayesian Information Criterion) or a minimizing cross-validated residual approach, that use the first subset of training data. For this optimization, W may be set by default different from ID.

[0189] For example, the MSE of the above formula with λ varying between 10^{-5} and 10^{3} is computed for a training dataset and a test dataset (splitting the subset for optimization of hyper-parameter λ). The resulting MSE is as shown in FIG. **1***a*.

[0190] As shown, when A is small, the train dataset MSE is close to 0 while the test dataset MSE is very high. In this situation, the model is overfitted.

[0191] On the other hand, when A is high, the model is underfitted.

[0192] λ may therefore be chosen to minimize the MSE for the test dataset.

[0193] Once λ is known, the second subset of training data is used to learn W by minimizing cross-validated residuals: a k-fold procedure is performed.

[0194] The subset of training data (i.e. $\{r_{true-i}(j)\}_j$ and $\{i_{ref-i}(j)\}_j$) is split into k subsets, preferably k is selected from integers 3 to 20, preferably from 4 to 10, more preferably is equal to 5.

[0195] Each of the k subsets is successively selected in a round-robin fashion (circular order) to define a test subset, while the k−1 remaining subsets define a training subset.

[0196] For each of the k rounds, the model is trained using the training subset, i.e.

$$\min\left(\frac{1}{N}*\|\widetilde{I_{ref}*W}-R_{true}\|^2+1*\|W-ID\|_2\right)$$

is solved to find W. Advantageously, all the linear-predicted mix profiles of the training subset are fed into a single matrix I_{ref} (and the true mix profiles in R_{true}) so as to learn W in a single pass.

[0197] The learnt interaction matrix W is then checked with the test subset; the test subset is applied to the matrix-based model $R_{true}=I_{ref}$*W. A score based on any norm, for instance on the

$$MSE\frac{1}{N}*\|\widetilde{I_{ref}*W}-R_{true}\|^2$$

is obtained.

[0198] As this operation is repeated for each k test subset, k scores are obtained.

[0199] The learnt interaction matrix W corresponding to the best score (i.e. the lowest one) can then be selected to configure pool predictor **13**.

[0200] Of course, other methodologies for machine learning can be used, provided a learnt interaction matrix W is obtained.

[0201] In some aspects, the profiling features of samples **100** (i.e. used to form matrix A) are the same as the profiling features of the final mix result (i.e. used to form matrix R). As mentioned above, they may be taxa (as in the Experiments 1 and 2 below), genes, antibiotic resistance genes, functions, and metabolite traits, and metabolite and protein production.

[0202] In other aspects, the profiling features of samples **100** (i.e. used to form matrix A) are different (in part or in whole) from the profiling features of the final mix result (i.e. used to form matrix R). Any of the above profiling features (taxa, genes, functions, and so on) may be used.

[0203] As an example, where a profiling technique such as NGS shotgun sequencing is used, a higher number of profiling features is obtained per sample **100** compared to 16S sequencing. Samples **100** can therefore be profiled using NGS shotgun sequencing (hence matrix A is formed with the NGS-shotgun profiling features) while the final mix result may be kept with a reduced number of profiling features, e.g. those obtained using 16S sequencing (hence matrix R is formed with 16S profiling features). In that case, matrix I is formed with the NGS-shotgun profiling features and interaction matrix W is not a square matrix and still models the interactions between the microorganisms, but as relationships between the NGS-shotgun profiling features and the 16S profiling features in the example.

[0204] In specific implementation seeking to reduce the high number of NGS-shotgun profiling features, a Principal Component Analysis (PCA) is performed, projecting this high number of features onto k principal components (k PCs). In one embodiment, the PCA is performed on the features profiling the samples, i.e. when building matrix A. In another embodiment, matrix I is generated with the high number of profiling features, and the PCA is performed on matrix I.

[0205] As mentioned above, pool predictor **13** outputs a final mix result matrix

$$R' = \begin{bmatrix} \{r_1'(j)\}_j \\ \{r_k'(j)\}_j \end{bmatrix}$$

when mixes

$$P = \begin{bmatrix} \{p_1(k)\}_k \\ \{p_k(k)\}_k \end{bmatrix}$$

are provided as inputs.

[0206] Mix result matrix R', i.e. the predicted mix profiles $\{r'_x(j)\}_j$, obtained by test and decision module **14** can then be used to control a process of actual producing a mix result product **19**. For instance, it may be used to control, through signalling **S1** and optionally **S2**, an actual picking and mixing of samples from the initial sample collection **10** to obtain the mix result product.

[0207] One of the predicted mix profiles in R' may be selected by decision module **14** to trigger the production of the mix result product **19**.

[0208] One or more selection criteria may be used to select one of the predicted mix profiles.

[0209] The selection criteria may be stored in a file **141** in memory. Criteria may be input in the system (list **141**) by an operator and mirror requirements of the mix result product to have for instance curative or treatment properties.

[0210] The criteria relate to the profiling features of the profiles. Hence, it is synonymous to a target mix profile, the constraints on profiling features being more or less loosened depending on the aspects.

[0211] The criteria may include a diversity criterion, for instance a bacterial diversity criterion.

[0212] By "diversity" or "bacterial diversity" it is meant the diversity or variability of the complex community of microorganisms (mix or sample), e.g. measured at the level of the genus, species, genes, functions or metabolites. The diversity can be expressed with alpha-diversity parameters to describe the complex community such as richness (number of species or genera or genes observed), Shannon index, Simpson index and Inverse Simpson index; and with beta-diversity parameters to compare complex communities such as Bray-Curtis index, UniFrac index and Jaccard index.

[0213] A diversity criterion may thus represent a requirement in terms of presence (i.e. non-zero corresponding relative abundancies) of a minimum number of profiling features (e.g. bacteria genera) or of one or more predefined profiling features. The minimum number of profiling features may be considered with respect to all the m profiling features or in a variant with respect to a predefined sublist of the m profiling features. This allows the selection process to be focused on specific features for the desired mix result product **19**.

[0214] A predicted mix profile of R' that satisfies the presence of the minimum number of profiling features may for instance be selected.

[0215] A diversity criterion may represent a minimum or maximum relative abundance of one or more specific profiling features. For instance, a given bacteria genus may be desired in the mix result product within at least 5% in proportion (mass) compared to the other bacteria (specified in other profiling features). The diversity criterion may also define a range to which the relative abundance of one or more specific profiling features should belong. Of course, various diversity criteria may be mixed: a minimum or maximum relative abundance for one profiling feature with a range for another feature and/or with a maximum relative abundance for a third feature. And so on.

[0216] Similarly, a relative ratio (possibly minimum and/or maximum ratio) between at least two profiling features may be used as a diversity criterion.

[0217] A predicted mix profile of R' that satisfies the minimum or maximum relative abundancies of the specific profiling features may for instance be selected.

[0218] A diversity criterion may also represent an increase in profiling feature diversity.

[0219] A diversity criterion may define a closeness or similarity to a specific target mix profile. For instance, a target mix profile may be defined when a mix result product exactly matching the target mix profile is desired. Usually, the target mix profile is provided together with a maximal value corresponding to a distance (measurement) evaluated between profiles. A mix profile is said to be close to the target mix profile when the distance between both profiles (given the measurement) is below the maximal value. The measurement may be any norm, L1, L2, . . . , Lp, the SSD, the MSE, Beta-diversity indexes or any other known distance measurement between the profiling features (e.g. Bray-Curtis, Jaccard, unifrac distances or similarity measures).

[0220] The predicted mix profile of R' with the minimal distance to the target mix profile may for instance be selected.

[0221] All or part of the above-defined criteria may be combined.

[0222] The one or more selection criteria to be used to select one of the predicted mix profiles (i.e. more generally the target mix profile) are retrieved by decision module **14** and applied to R'.

[0223] The predicted mix profiles within R' may be considered successively in order.

[0224] The first predicted mix profile meeting the selection criterion or criteria may be selected for the production of the mix result product **19**.

[0225] In a variant, all the predicted mix profiles of R' are evaluated with regard to the selection criterion or criteria, and the one having the best score (e.g. meeting some criteria and/or being the closest to some others) is selected.

[0226] More generally, a reverse prediction may be contemplated where the selection criterion/target mix profile is defined, for instance that corresponds to target mix products having curative properties. File **140** may define candidate sets of samples to be tested from which it is searched the “best” set given the target mix profile. The process may be iterative meaning that a first group of disparate candidate sets (i.e. with disparate mixes in terms of which samples are mixed together and with which respective proportions) can be first tested through file **140** to find the “best” one, and then another group of candidate sets in the vicinity of the “best” set (e.q. with modification of the proportions $p_x(k)$ of the samples within the “best” set and/or with the addition or deletion of only 1 or at most 2 samples to/from the set) may then be tested. Of course one or more additional iterations may be contemplated to progressively refine the “best” result set of samples given the target mix profile.

[0227] At each iteration, the predicted mix profile of R' with the minimal distance (Bray-Curtis, Jaccard, unifrac distances and so on.) to the target mix profile may for instance be selected as the “best” one. In a variant, multiple (e.g. matrix-based) predictions may be made, and the “best” candidate set is selected from the multiple (e.g. various matrices R') predicted mix profiles so obtained. A comparison between the predicted mix profiles and the target mix profile is thus conducted to choose one candidate set as the “best” one, i.e. as a target set.

[0228] Once a predicted mix profile is selected, hence the corresponding target set of samples from the initial collection is known, the process to produce the mix result product **19** starts.

[0229] Decision module **14** first retrieves the mix composition $\{p_x(k)\}_k$ corresponding to the selected predicted mix profile, i.e. retrieves the proportions $p_x(k)$ for each sample k of the target set of samples in the initial collection **10**. It then signals, using S**1**, a selector and mixer **15** with these proportions $\{p_x(k)\}_k$. In a variant, the signal S**1** may be a display to an operator: for instance the proportions $\{p_x(k)\}_k$ are displayed, on a screen, to the operator for him or her to manually perform the actual picking and mixing of samples.

[0230] Selector and mixer **15** may be a machine having mechanical access (for instance through a controlled articulated arm) to the collection **10** of samples and including a bioreactor where performing the mixing of samples.

[0231] In response to signal S**1**, selector and mixer **15** picks, i.e. retrieves or takes, the samples having non-zero proportions $p_x(k)$ from bank **10**, takes an amount of each sample given the corresponding proportion $p_x(k)$ and a total volume or mass targeted for the mix result product **19**. The taken amounts of all the samples are poured in the bioreactor where they are actually mixed.

[0232] Preferably they are homogenised during a period of 10 minutes to 3 hours, preferably between 30 min and 1.5 hour. The homogenisation is made at a temperature between 0° C. and 10° C., preferably between 2° C. and 8° C., more preferably at about 4° C. The mix result product is then considered as stable for a couple of hours, at least up to 16 hours from the mixing, preferably up to 24 hours therefrom.

[0233] The true mix profile **191** $\{r_{true}(j)\}_j$ of the resulting mix result product **19** may be obtained using profiler/sequencer **12**. As it may slightly differ from the selected predicted mix profile **192** $\{r'_x(j)\}_j$. it may be used (together with the corresponding intermediary mix profile $\{i_x(j)\}_j$) as further training data in order to improve W. A new round as described above may be implemented with this sole new item of training data (or when a couple of items is obtained) where □ may be kept unchanged and W is initially set to its current value. This iterative learning of W as the platform **1** is used, advantageously refines W, hence provides better future mix profile predictions.

[0234] As mentioned above, some samples **100*y*-100*z*** may be virtual. In case such a virtual sample is selected by decision module **14** (i.e. its corresponding relative abundance $p_x(k)$ in the selected predicted mix profile is not zero), there is a need to actually produce said sample from its virtual definition (i.e. the corresponding individual profile).

[0235] When decision module **14** detects such non-zero relative abundance for a virtual sample **100*y*-100*z*** corresponding to a bacterial consortium, it signals, using S**2**, a sample generator **16** with the need of producing said artificial sample. S**2** may identify the sample concerned and indicate the amount of material needed (i.e. the corresponding proportion $p_x(k)$ multiplied by the target total volume or mass for the mix result product **19**).

[0236] Sample generator **16** may be a machine having mechanical access (for instance through a controlled articulated arm) to a bank of isolated strains **160** and having storage access to a file **161** defining the composition of samples in terms of mix of individual strains. Sample generator **16** also includes a bioreactor where performing the mixing of the strains.

[0237] In response to signal S**2**, sample generator **16** retrieves the definition of artificial samples (bacterial consortia) in terms of strains and takes the appropriate amount of each required strain from the strain bank **16** given the signalled amount of material needed. The taken amounts of all required strains are poured in the bioreactor where they are actually mixed, for instance during 30 minutes at 4° C.

[0238] In aspects, sample generator **16** may have access to bank **10** and/or even to a bank of external samples **99**. When decision module **14** detects a non-zero relative abundance for a virtual sample corresponding to an engineered or processed complex community (i.e. a mix involving a sample), it signals, using S**2**, sample generator **16** with the need of producing said engineered or processed sample. S**2** may identify each strain and/or each sample in bank **10** and/or each external sample concerned by the mix and indicate the amount of material needed (i.e. the corresponding proportion $p_x(k)$ multiplied by the target total volume or mass for the mix result product **19**).

[0239] In response to signal S**2**, sample generator **16** retrieves or picks the materials, pours them in the bioreactor where they are actually mixed.

[0240] Once the mix is done and stabilized, the sample has been generated, hence it is stored in the initial sample collection or bank **10** where the selector and mixer **15** can take it to actually produce mix result product **19**.

[0241] Although signals S**1** and S**2** are described above as control signal to drive the selector and mixer **15** and the sample generator **16**, one or both of them can be mere signals displayed to an operator for him or her to actually and manually perform the mixing.

[0242] FIG. **2** illustrates, using a flowchart, general steps of producing such mix result product **19**, including predicting mix profiles. These steps are performed by platform **1**.

[0243] At step **200**, test and decision module **14** selects a set of samples from those available in the initial sample collection **11**. This step may merely consist in providing or selecting one mix definition $\{p_x(k)\}_k$ from list **140**.

[0244] The definition of mix ‘x’ is provided to pool predictor **13** for prediction.

[0245] 'x' is initially set to 1 when the mix definitions in list **140** are indexed from 1 to N_{mix}.

[0246] At step **205**, pool predictor **13** first performs a linear prediction of the mix profile to obtain an intermediary mix profile: $\{i_x(j)\}_j=\{p_x(k)\}_k*A$. This is a matrix product.

[0247] At step **210**, pool predictor **13** then performs the correction of the intermediary mix profile using interaction matrix W: $\{r_x(j)\}_j=\{i_x(j)\}_j*W$. This is a matrix product.

[0248] Clipping and normalization of $\{r_x(j)\}_j$ are also performed if needed to obtain the predicted mix profile **215** for mix 'x', $\{r'_x(j)\}_j$.

[0249] At step **220**, it is checked whether $\{r'_x(j)\}_j$ satisfies a selection-criterion-based condition. The condition may be defined by a single selection criterion to satisfy or by a multiplicity thereof. The condition may mirror criteria defining a complex community of microorganisms or a microbiota composition with desired curative or treatment properties.

[0250] If $\{r'_x(j)\}_j$ does not satisfy the condition, another mix is considered through step **225** (incrementing the mix index) before looping back to step **200**. Of course, if all the mixes have yet been considered (test **221**), the process ends without having any mix satisfying the condition. An alert message may then be issued to an operator.

[0251] If $\{r'_x(j)\}_j$ does satisfy the condition, it is selected and the process goes to step **230**.

[0252] At step **230**, the corresponding mix definition $\{p_{select}(k)\}_k$ is retrieved from list **140** by decision module **14** and sent to selector and mixer **15** or displayed to the operator (signal S**1**).

[0253] Optionally, where a virtual sample 'k' has a corresponding non-zero proportion $p_{select}(k)$, decision module **14** triggers its actual production by sample generator **16** or displays the information to the operator, through the sending of signal S**2**.

[0254] At step **235**, the samples with corresponding non-zero $p_{select}(k)$ are retrieved by selector and mixer **15** from bank **10** and then mixed together in a bioreactor.

[0255] It results the desired mix result product **19**.

[0256] Thanks to the above prediction operation, an accurate profile of a mix result product may be obtained (at least estimated) quickly without consuming material (samples).

[0257] However, the samples may disappear over time (to actually produce some products or because they deteriorate over time) while new samples may be collected from new donors. It turns out that the collection **10** may evolve over time (thus A evolves), after a mix definition is determined to produce a target mix result product. Thanks to the disclosure, pool predictor **13** may be configured anew with the evolved collection (A is redefined and W is learnt) and another mix definition corresponding to the evolved collection can also be determined (using the prediction of the disclosure) that allows a similar mix result product to be generated.

[0258] The above sequence of steps **200-235** selects the first mix in list **140** that satisfies the condition.

[0259] In a variant, a predicted mix profile may be estimated by pool predictor **13** for all the mixes defined in list **140** before checking the condition at step **220** to find the "best" mix, i.e. set of samples.

[0260] This approach, as illustrated in FIG. **3**, seeks to find a mix definition $\{p_x(k)\}_k$ (i.e. a set of samples of collection **10**) from a target mix profile. Such determination of a target mix definition (i.e. a target set of samples) may take place in a production process as described now. Indeed, once the target mix definition $\{p_x(k)\}_k$ for the target mix profile has been obtained by test and decision module **14**, it may be used to control a process of actual producing a mix result product **19** as described above: decision module **14** can send signal S**1**, and optionally signal S**2**, to control modules **15** and **16** in the production process or inform the operator of the operations to conduct.

[0261] FIG. **3** illustrates, using a flowchart, general steps of producing such mix result product **19** given the target mix profile. These steps are performed by platform **1**.

[0262] At step **300**, the target mix profile $\{r'_x(j)\}_j$ corresponding to a desired mix result product is set in file **141**.

[0263] The target mix profile may be a profile with well-defined values for the profiling features, but also a profile defining more loosened values, for instance a minimum relative abundance for one or more profiling features, a maximum relative abundance for one or more profiling features, a range for the relative abundance of one or more profiling features, a minimum number of non-zero relative abundances in the profile or in a predefined subset of profiling features, predefined profiling features with non-zero relative abundancies, a defined ratio between relative abundancies of two or more profiling features, and so on.

[0264] The target mix profile may be defined in file **141** in such a way test and decision module **14** is able to feed pool predictor **13** with this target mix profile as $\{r'(j)\}_j$ (output of the model).

[0265] At step **305**, a group of candidate sets of samples from collection **10** are obtained. They may be predefined.

[0266] Random selection of samples from the collection can be performed as well as random selection of respective mix proportions $p_x(k)$.

[0267] The number of samples to mix may be selected within a range of authorized numbers, e.g. 2 to 1000 samples, preferably 3 to 100. In practice, 3 to 10 is easily handled. Of course, computer-implemented method according to the disclosure makes it possible at low cost to perform predictions for a higher number of samples mixed together.

[0268] The mix proportions may be selected from a group of predefined proportions (given the number of samples mixed together as the total of proportions must be 100%).

[0269] An initial group of candidate sets may be randomly formed, usually resulting into very disparate sets of samples. Another group of candidate sets may be formed given one or more know sets (e.g. determined as "best" sets in a previous iteration of the process of FIG. **3**). The other group of candidate sets may for instance include other candidate sets that depart from the know set or sets by only different mix proportions $p_x(k)$ and/or by a limited number of different samples (e.g. only 1 or 2 different samples).

[0270] The candidate sets are defined in file **140** in such a way test and decision module **14** is able to feed pool predictor **13** with these candidate mixes (lists of $\{p_x(k)\}_k$).

[0271] Next, steps **205**, **210**, **215** described above are performed in order to predict one or more mix profiles $\{r_x(j)\}_j$ for the candidate sets/mixes. Thanks to the matrix-based approach, the mix profiles of multiple candidate sets (possibly all) may be simultaneously predicted, where P thus includes multiple or all $\{p_x(k)\}$: from list **140**.

[0272] Step **310** checks whether all the candidate sets/mixes have been processed (test **216**). In the negative, the next mix definition is considered through step **225**.

[0273] Next, at step **315**, the predicted mix profiles are compared to the target mix profile (selection criterion) with a view to choose one candidate set as the target set.

[0274] For instance, a distance, e.g. a Bray-Curtis distance or a Jaccard distance or an unifrac distance or a combination thereof, is calculated for each predicted mix profile (hence for each candidate set).

[0275] Step **320** determines the closest predicted mix profile or profiles to the target mix profile given the considered distance. Preferably, the closest one is determined.

[0276] A distance margin may be implemented to guarantee the closest predicted mix profile is close enough to the target mix profile. In this situation, the closest predicted mix profile must satisfy the margin, meaning its calculated distance must be less that this margin. If no predicted mix profile satisfies the test, the process ends and an alert message may be issued to an operator.

[0277] Otherwise predicted mix profile $\{r'_{select}(j)\}_j$ **325** corresponding to target set/mix composition 'select' has been determined when entering step **230** described above.

[0278] In some aspects (not shown in the Figure), this target set/mix composition may be used to define a new group of candidate sets as explained above in order to perform another (even more) round of the process and refine the target set/mix composition to be used at step **230**.

[0279] At step **230**, the mix definition 'select' is sent to selector and mixer **15** or to the operator (signal S**1**).

[0280] Optionally, where a virtual sample 'k' has a corresponding non-zero proportion $p_{select}(k)$, decision module **14** triggers its actual production by signalling sample generator **16** or informing the operator, through the sending of signal S**2**.

[0281] At step **235**, the samples with corresponding non-zero $p_{select}(k)$ are retrieved or picked, e.g. by selector and mixer **15** from bank **10**, and then mixed together in a bioreactor.

[0282] It results the desired mix result product **19**.

[0283] A Fecal Microbiota Transfer (FMT) product and more generally a Microbiome Ecosystem Therapy product can be built from several samples. The mixing strategy as defined by the present disclosure allows the diversity of the final FMT product to be efficiently improved compared to a mono sample strategy, furthermore without wasting material.

[0284] Platform **1** described above with reference to FIG. **1** comprises several modules that are under the control of a central computer. For instance pool predictor **13** and test and decision module **14** are implemented in the central computer while sequencer **12**, selector and mixer **15**, sample generator **16** and bank **10** are separate machines connected to the central computer.

[0285] The description above mainly uses a matrix-based prediction model, in particular a square interaction matrix. Alternatives to the latter include deep learning models, such as neural networks made of multiple layers of parameterized differentiable nonlinear modules that can be trained or learnt by backpropagation.

[0286] FIG. **4** schematically illustrates a computer device **400** managing the production platform **1**. Computer device **400** may for instance implement pool predictor **13** and test and decision module **14** and may control sequencer **12**, selector and mixer **15** and sample generator **16** via adapted signalling (S**1** and S**2**).

[0287] The computer device **400** is configured to implement at least one embodiment of the present disclosure. The computer device **400** may preferably be a device such as a micro-computer, a workstation or a light portable device. The computer device **400** comprises a communication bus **401** to which there are preferably connected:

[0288] a central processing unit **402**, such as a microprocessor, denoted CPU;

[0289] a read only memory **403**, denoted ROM, for storing computer programs for implementing the disclosure;

[0290] a random-access memory **404**, denoted RAM, for storing the executable code of methods according to aspects of the disclosure as well as the registers adapted to record variables and parameters necessary for implementing methods according to aspects of the disclosure;

[0291] a communication interface **405** connected to a network **499** in order to communication with a user or operator device and/or with other devices of platform **1**, for instance sequencer **12**, selector and mixer **15** and sample generator **16**; and

[0292] a data storage means **406** such as a hard disk or a flash memory, for storing computer programs for implementing methods according to one or more aspects of the disclosure as well as any data necessary for aspects of the disclosure, including inter alia individual sample profiles (i.e. collection **11**), lists **140** and **141**.

[0293] Optionally, the computer device **400** may also include a screen **407** serving as a graphical interface with an operator, for instance to configure the platform by means of a keyboard **408** or any other pointing means (e.g. defining lists **140**, **141** as well as collection **11** and virtual samples **110***y-z*) and/or to display the results of the prediction process or of the reverse operation, for instance to display the target mix definition $\{p_{select}(j)\}_j$.

[0294] The computer device **400** may be optionally connected to various peripherals useless for the present disclosure, the sequencer **12**, each being connected to an input/output card (not shown).

[0295] Preferably the communication bus provides communication and interoperability between the various elements included in the computer device **400** or connected to it. The representation of the bus is not limitative and in particular the central processing unit is operable to communicate instructions to any element of the computer device **400** directly or by means of another element of the computer device **400**.

[0296] The executable code may optionally be stored either in read only memory **403**, on the hard disk **406** or on a removable digital medium (not shown). According to an optional variant, the executable code of the programs can be received by means of the communication network **499**, via the interface **405**, in order to be stored in one of the storage means of the computer device **400**, such as the hard disk **406**, before being executed.

[0297] The central processing unit **402** is preferably adapted to control and direct the execution of the instructions or portions of software code of the program or programs according to the disclosure, which instructions are stored in one of the aforementioned storage means. On powering up, the program or programs that are stored in a non-volatile memory, for example on the hard disk **406** or in the read only memory **403**, are transferred into the random-access memory **404**, which then contains the executable code of the program or programs, as well as registers for storing the variables and parameters necessary for implementing the disclosure.

Experimental Results

Scope of the Experiments

[0298] The purpose of the experiments was to investigate the efficiency of the interaction matrix W, including its machine learning procedure as proposed, to predict the mix profile of a mix of microbiota samples (Experiment 1) and to determine a mix composition given a target mix profile (Experiment 2).

Experiment 1—Protocol

[0299] Initial sample collection **10** was considered. Corresponding initial profile collection **11** was obtained by sequencing, using a 16S based microbiota taxa profiling, each of the microbiota samples. Hence, 131 taxa (at genus level) were evaluated as profiling features.

[0300] Next, mixing of the samples were realized. Each mix product was a combination of three to six samples with respective ratios. The mixing was performed at 4° C. and the mix homogenized during 30 min to 1 h30 after mixing. The mix products were sequenced, using the same 16S based microbiota taxa profiling, during their stable state (i.e. during the hours following the homogenization, less than 16 h from the mixing).

[0301] A k-fold cross-validation strategy was employed with k=5 to configure pool predictor **13**, i.e. to learn λ and interaction matrix W. The k-fold strategy ensured that none of the observations was used as training data and as test set during the a same evaluation.

[0302] The modelization method as described in the material and methods section was tested and applied at three different taxonomic ranks: species, genus, family and order. However, the species level datasets were very sparse, so it was excluded from the testing procedure. Starting at the genus level, the assignation tables were rich enough to allow the analyses, so the less resolved levels (family, order) were deduced from the genus tables, only for visualization purposes when needed, but not used as is in the modelization procedure. The main reason is that it is not possible to deduce from a taxa level used in the training, the composition of a higher resolved level, and having the genera information is important in our application perspective.

[0303] We have trained the models for native samples only (FIG. **5**) and for fermented samples (FIG. **6**) separately as well as both combined (FIG. **7**). The MSE was used to quantify the quality of the modelization when applied on the data. The MSE were systematically compared between the machine learning model and the linear model (the one providing the naïve predictions).

Experiment 1—Results

[0304] FIG. **5***a* illustrates the initial profile collection **11** corresponding to an initial sample collection **10** comprising only native faeces microbiota samples. 27 microbiota samples were considered. Their individual profiles are depicted in the Figure.

[0305] FIG. **5***b* illustrates the mix profiles of 24 mix products mixing three to six microbiota samples from amongst the 27 microbiota samples of FIG. **5***a*, with respective ratios or proportions. The mix definitions $\{p_x(k)\}_k$ are saved.

[0306] FIG. **5***c* illustrates, on the left side, the error resulting from a linear prediction of the mix profiles given the mix definitions $\{p_x(k)\}_k$ and the individual sample profiles $\{a_x(j)\}_j$. The linear prediction corresponds to the sole step **205**: I=A*P.

[0307] The Figure also illustrates, on the right side, the error resulting from the prediction according to the disclosure (steps **205** and **210**), i.e. involving the interaction matrix W. W was machine-learned using only the sample and mix profiles of FIGS. **5***a* and **5***b* (native samples) with the k-fold cross-validation strategy.

[0308] The model-based method of the disclosure returns a better performance than the linear method for the native dataset.

[0309] FIG. **6***a* illustrates the initial profile collection **11** corresponding to an initial sample collection **10** comprising only fermented faeces microbiota samples. 36 microbiota samples were considered. Their individual profiles are depicted in the Figure.

[0310] FIG. **6***b* illustrates the mix profiles of 48 mix products mixing three to six microbiota samples from amongst the 36 microbiota samples of FIG. **6***a*, with respective ratios or proportions. The mix definitions $\{p_x(k)\}$ are saved.

[0311] FIG. **6***c* illustrates, on the left side, the error resulting from a linear prediction of the mix profiles given the mix definitions $\{p_x(k)\}_k$ and the individual sample profiles $\{a_x(j)\}_j$. The linear prediction corresponds to the sole step **205**: I=A*P.

[0312] The Figure also illustrates, on the right side, the error resulting from the prediction according to the disclosure (steps **205** and **210**), i.e. involving the interaction matrix W. W was machine-learned using only the sample and mix profiles of FIGS. **6***a* and **6***b* (fermented samples) with the k-fold cross-validation strategy.

[0313] The model-based method of the disclosure returns a dramatically better performance than the linear method for the fermented dataset (median MSE is 5× lower with the ML model predictions).

[0314] For FIGS. **7***a* and **7***b*, interaction matrix W was machine-learned using both sample and mix profiles of FIGS. **5***a*, **5***b*, **6***a* and **6***b* (i.e. native and fermented samples) as training data. Again, the k-fold cross-validation strategy was used.

[0315] FIG. **7***a* shows the result when the dataset of FIGS. **5***a*, **5***b* (i.e. native samples and mixes thereof) are applied to pool predictor **13** so configured.

[0316] The left side of the Figure depicts the error resulting from a linear prediction of the mix profiles given the mix definitions $\{p_x(k)\}_k$ and the individual sample profiles $\{a_x(j)\}_j$.

[0317] The right side depicts the error resulting from the prediction according to the disclosure (steps **205** and **210**), i.e. involving the interaction matrix W so learnt.

[0318] As for the single dataset model, the combined datasets model improves slightly the estimation when applied to the native dataset.

[0319] FIG. **7***b* shows the result when the dataset of FIGS. **6***a*, **6***b* (i.e. fermenter samples and mixes thereof) are applied to pool predictor **13** so configured.

[0320] The left side of the Figure depicts the error resulting from a linear prediction of the mix profiles given the mix definitions $\{p_x(k)\}_k$ and the individual sample profiles $\{a_x(j)\}_j$.

[0321] The right side depicts the error resulting from the prediction according to the disclosure (steps **205** and **210**), i.e. involving the interaction matrix W so learnt.

[0322] As for the single dataset model, the combined datasets model improves dramatically the estimation when applied to the fermented dataset (median MSE is 4× lower with the ML model predictions).

Experiment 1—Discussion and Conclusion

[0323] In all cases, the model-based prediction improves the naïve (linear) methods estimation. It is especially important for the fermented dataset where the naïve approach does not perform well, especially for some groups of taxa. The model-based correction approach was more efficient, probably as there were more room for improvement. If more data are added to train the model, one can assume that the overall performances and the robustness will improve. The training method, also part of this disclosure, allows such a model evolution.

Experiment 2—Protocol

[0324] In this experiment, the interaction matrix W learned using native and fermented samples (i.e. W of FIGS. **7***a* and **7***b*) is used.

[0325] Another collection of samples was considered for this experiment. It is made of 23 samples. Corresponding collection of profiles was obtained by the same sequencing of each of the 23 microbiota samples: using a 16S based microbiota taxa profiling where 131 taxa (at genus level) were considered as profiling features. FIG. **8** illustrates the profile collection (at class level).

[0326] Pool predictor **13** was used to generate **160** mixes with different input microbiota samples (mixing 2 to 4 samples out of 23) with different mixing conditions. Four rounds (exp_1 to exp_4) of predictions were made, in which eight different sets of samples (chunk_1 to chunk_8) were considered with five different sets of proportions (Mix1 to Mix5).

[0327] Each generated predicted mix profile can then be identified by a triplet (i, j, k) where i=1 . . . 4 (exp), j=1 . . . 8 (chunk) and k=1 . . . 5 (Mix), and a corresponding name "exp_i-chunk_j-Mixk"

[0328] The different sets of proportions (in %) were predefined as follows (depending on the number of samples in the mix).

TABLE 1

sample proportions in mixes of 4 samples

	Sample 1	Sample 2	Sample 3	Sample 4
Mix1	10	10	20	60
Mix2	20	20	20	40
Mix3	10	20	30	40
Mix4	20	30	20	30
Mix5	25	25	25	25

TABLE 2

sample proportions in mixes of 3 samples

	Sample 1	Sample 2	Sample 3
Mix1	10	20	70
Mix2	20	20	60
Mix3	10	50	40
Mix4	10	10	80
Mix5	33	33	34

TABLE 3

sample proportions in mixes of 2 samples

	Sample 1	Sample 2
Mix1	10	90
Mix2	20	80
Mix3	30	70
Mix4	40	60
Mix5	50	50

[0329] The different candidate sets of samples were defined as follows.

TABLE 4

compositions of candidate mixes

		Sample 1	Sample 2	Sample 3	Sample 4
exp_1	chunk_1	sample-7	sample-14	sample-17	sample-20
exp_1	chunk_2	sample-2	sample-4	sample-11	sample-22
exp_1	chunk_3	sample-5	sample-6	sample-23	
exp_1	chunk_4	sample-1	sample-3		
exp_1	chunk_5	sample-9	sample-21		
exp_1	chunk_6	sample-16	sample-19		
exp_1	chunk_7	sample-8	sample-13	sample-15	
exp_1	chunk_8	sample-10	sample-12	sample-18	
exp_2	chunk_1	sample-9	sample-12	sample-15	
exp_2	chunk_2	sample-3	sample-5	sample-16	
exp_2	chunk_3	sample-6	sample-8		
exp_2	chunk_4	sample-7	sample-18	sample-23	
exp_2	chunk_5	sample-13	sample-19	sample-22	
exp_2	chunk_6	sample-1	sample-2	sample-21	
exp_2	chunk_7	sample-4	sample-10	sample-14	
exp_2	chunk_8	sample-11	sample-17	sample-20	
exp_3	chunk_1	sample-6	sample-10	sample-14	sample-16
exp_3	chunk_2	sample-1	sample-5	sample-11	
exp_3	chunk_3	sample-3	sample-4	sample-7	
exp_3	chunk_4	sample-9	sample-19	sample-22	
exp_3	chunk_5	sample-2	sample-12		
exp_3	chunk_6	sample-15	sample-20		
exp_3	chunk_7	sample-13	sample-21		
exp_3	chunk_8	sample-8	sample-17	sample-18	sample-23
exp_4	chunk_1	sample-6	sample-9	sample-15	
exp_4	chunk_2	sample-2	sample-18		
exp_4	chunk_3	sample-3	sample-5	sample-12	
exp_4	chunk_4	sample-1	sample-10	sample-14	sample-19
exp_4	chunk_5	sample-16	sample-21	sample-22	sample-23
exp_4	chunk_6	sample-4	sample-8		
exp_4	chunk_7	sample-13	sample-17	sample-20	
exp_4	chunk_8	sample-7	sample-11		

[0330] For the present experiment, mix "exp_1-chunk_7-Mix5", i.e. a mix made of 33% of sample-**8**, 33% of sample-**13** and 34% of sample-**15**, was considered as a target mix. Its predicted mix profile was used as a target mix profile.

[0331] The similarity between all the proposed mixes "exp_i-chunk_j-Mixk" and the target mix "exp_1-chunk_7-Mix5" was evaluated using the Bray-Curtis index at the genus level (FIG. **9**).

[0332] The Bray-Curtis index is forced to 0 when at least two samples of the mix considered come from the same donor. This was to avoid identifying mixes having the same samples.

[0333] Mix "exp_1-chunk_7-Mix5" and the most similar mixes for both metrics were actually mixed, and the resulting products were sequenced using the same sequencing technique with a view of comparing their composition at the phylum and family levels.

Experiment 2—Results

[0334] Table 5 below shows the 15 highest Bray-Curtis similarities (based on the genera abundances) calculated from the predicted mix profiles. The Bray-Curtis similarity equals 1−Bray-Curtis dissimilarity measure.

TABLE 5

Bray-Curtis similarities to exp_1-chunk_7-Mix5	
exp_4-chunk_4-Mix4	0.861
exp_4-chunk_4-Mix5	0.852
exp_4-chunk_4-Mix2	0.848
exp_4-chunk_4-Mix3	0.835
exp_4-chunk_4-Mix1	0.805
exp_4-chunk_2-Mix4	0.772
exp_4-chunk_2-Mix3	0.763
exp_1-chunk_8-Mix5	0.762
exp_4-chunk_2-Mix5	0.749
exp_4-chunk_2-Mix2	0.747
exp_1-chunk_8-Mix2	0.745
exp_3-chunk_1-Mix4	0.743
exp_3-chunk_1-Mix5	0.743
exp_1-chunk_8-Mix3	0.742
exp_2-chunk_7-Mix5	0.734

[0335] FIG. **9***a* shows the true profiles of the samples composing exp_1-chunk_7-Mix5 and the closest mix, exp_4-chunk_4-Mix4 (made of 20% of sample-**1**, 30% of sample-**10**, 20% of sample-**14** and 30% of sample **19**), as well as their true mix profiles, at phylum level.

[0336] FIG. **9***b* illustrates the same comparison at family level.

[0337] Although the initial samples have very different profiles between exp_1-chunk_7-Mix5 and exp_4-chunk_4-Mix4, the final products have very similar mix profiles at both phylum and family levels.

[0338] Table 6 and FIG. **10** show the Bray-Curtis similarity (at genus level) results of the comparisons between exp_1-chunk_7-Mix5 and the closest mix, exp_4-chunk_4-Mix4, as well as their true mix profiles.

TABLE 6

Bray-Curtis similarity (genus level) between predicted and actual mixes

Sample_1	Sample_2	Bray-Curtis similarity	Type
exp_4-chunk_4-Mix4 real mix	exp_1-chunk_7-Mix5 real mix	0.753	Actual vs Actual
exp_4-chunk_4-Mix4 real mix	exp_4-chunk_4-Mix4 predicted	0.783	Actual vs Predicted

TABLE 6-continued

Bray-Curtis similarity (genus level) between predicted and actual mixes

Sample_1	Sample_2	Bray-Curtis similarity	Type
exp_1-chunk_7-Mix5 real mix	exp_1-chunk_7-Mix5 predicted	0.739	Actual vs Predicted

[0339] Although the initial samples of the two mixes have very different profiles, the final products have very similar mix profiles at genus level according to the Bray-Curtis distance metric. Table 6 and FIG. **10** shows a similarity gap between an actual mix and its prediction. This however does not impact substantially the similarity between the actual mixes, which similarity remains at an acceptable level.

Experiment 2—Discussion and Conclusion

[0340] The experiment 2 shows that the prediction tool can be used iteratively to predict mixes very close to a target sample (Bray-Curtis similarity at the genus level greater than 0.86), and to select corresponding samples and mix proportions for the in vitro experience phase.

[0341] It also illustrates that two mixes produced according to the prediction recipe are actually very close together (Bray-Curtis similarity at the genus level greater than 0.75). This demonstrates the performances of the prediction tool, and its applicability in a real life context.

Experiment 3—Protocol

[0342] In this experiment, NGS shotgun sequencing has been used to profile samples **100**. Metagenomic sequencing data were obtained for 76 pools and 69 samples from donors, or individual fermentors.

[0343] Due to the high number of NGS shotgun profiling features (compared to 16S sequencing especially when looking at the species level instead of the genus level, or for certain functions), PCA has been used in order to reduce the dimensions of each sample profile, to k PCs.

[0344] FIG. **11** depicts the PCA based on genus relative abundances obtained from NGS shotgun sequencing of native samples (native, inoculum or mix), fermentation samples (fermented, inoculum or mix). Fermentation samples tend to cluster together, as well as native samples.

[0345] This PCA-based strategy is summarized in FIG. **12** where it is clear that instead of learning a "Taxa×Taxa" interaction matrix W (as in Experiments 1 and 2), a "top k principal components×Taxa" interaction matrix W is learnt in Experiment 3.

[0346] The methodology to learn this interaction matrix W is the same as for 16S analyses of Experiments 1 and 2.

Experiment 3—Results

[0347]

TABLE 7

comparison of prediction results between linear prediction, taxa without PCA prediction and taxa with PCA prediction

		MSE on		Bray Curtis on	
Data	Type of model	native samples	fermented samples	native samples	fermented samples
Genus	Linear prediction	36.1e−6	222.6e−6	0.87	0.78
	Taxa (loss MSE when learning W)	29.7e−6	40.1e−6	0.88	0.89
	Taxa PCA (loss MSE)	28.4e−6	25.6e−6	0.88	0.91
Species	Linear prediction	11.4e−6	25.6e−6	0.80	0.79
	Taxa (loss MSE)	9.0e−6	7.27e−6	0.82	0.85
	Taxa PCA (loss MSE)	8.21e−6	5.05e−6	0.82	0.88

[0348] The interaction matrix W has been learnt using MSE. Also the comparisons between predicted mix results (using W) and true mix results have been made based on MSE or on the Bray Curtis distance.

[0349] Both modeling approaches (with or without PCA) improve the taxonomic profile predictions (according to the MSE or BC metrics) at the genus and the species levels. The correction according to the disclosure (based on matrix W) has a stronger impact in the prediction of mixes from fermented samples, compared to native samples.

[0350] The reduction of profiling features using PCA seems to improve notably the prediction accuracy from fermented samples, while slightly improving it for predictions from native samples.

[0351] Although the present disclosure has been described herein above with reference to specific aspects, the present disclosure is not limited to the specific aspects, and modifications will be apparent to a skilled person in the art which lie within the scope of the present disclosure.

[0352] Many further modifications and variations will suggest themselves to those versed in the art upon referring to the foregoing illustrative aspects, which are given by way of example only and which are not intended to limit the scope of the disclosure, that being determined solely by the appended claims. In particular, the different features from different aspects may be interchanged, where appropriate.

[0353] In the claims, the word "comprising" does not exclude other elements or steps, and the indefinite article "a" or "an" does not exclude a plurality. The mere fact that different features are recited in mutually different dependent claims does not indicate that a combination of these features cannot be advantageously used.

What is claimed is:

1. A computer-aided method of predicting a mix composition resulting from the mixing of complex microorganism community samples belonging to an initial sample collection, the method comprising:

predicting, using a linear approach, an intermediary mix profile for a mix of selected complex microorganism community samples, and

correcting the intermediary mix profile into a predicted mix profile, using an interaction model learnt from reference linear-predicted mix profiles and corresponding reference true mix profiles.

2. The method of claim **1**, wherein the predicted mix profile is used to control actual picking and mixing of complex microorganism community samples from the initial sample collection to obtain a mix result product.

3. The method of claim **1**, wherein predicting the intermediary mix profile includes computing a matrix product between a first matrix defining the mix in terms of proportions of the complex microorganism community samples of the initial sample collection and a second matrix defining the individual profiles of the complex microorganism community samples.

4. The method of claim **1**, wherein correcting the intermediary mix profile includes computing a matrix product between a matrix representing the intermediary mix profile and a square interaction matrix of the learnt interaction model.

5. A computer-aided method of determining a set of complex microorganism community samples in an initial sample collection given a target mix profile representing a target mix result product, the method comprising:

selecting candidate sets of complex microorganism community samples from the initial sample collection,

for each candidate set selected, using the prediction method of claim **1** to predict a mix profile resulting from the mixing of the samples of the selected candidate set,

comparing the predicted mix profiles to the target mix profile to choose one candidate set as the target set.

6. The method of claim **5**, wherein the target set of samples is used to control actual picking and mixing of complex microorganism community samples from the initial sample collection to obtain a mix result product function of the target mix profile.

7. The method of claim **5**, wherein comparing the predicted mix profiles to the target mix profile includes computing a distance between each predicted mix profile and the target mix profile and selecting, as target set, the candidate set having the lowest distance.

8. The method of any of claim **1**, wherein a profile of a complex community of microorganisms includes relative abundancies of profiling features in the complex community of microorganisms.

9. The method of any of claim **1**, wherein profiling features forming a profile of a complex community of microorganisms include one or more features selected from the group consisting of taxa, genes, antibiotic resistance genes, functions, metabolite traits, and metabolite and protein production, preferably include taxa.

10. The method of claim **1**, wherein a profile of a complex community of microorganisms defines profiling features with respect to one or more microorganisms present in the complex community of microorganisms from bacteria, archaea, viruses, phage, protozoa and fungi, preferably with respect to bacteria and/or archaea, and/or.

defines profiling features that specify relative abundances of microorganisms considered at one or more taxonomic levels from strains, species, genus, families and orders, preferably one or more taxonomic levels from genus, families and orders, and/or

includes relative abundancies, in the complex community of microorganisms, of bacteria and/or archaea taxa considered at a taxonomic level of genus, families and orders.

11. The method of any of claim **1**, wherein the interaction model is obtained using machine learning that minimizes a formula function of a difference between

reference predicted mix profiles obtained from the reference linear-predicted mix profiles and the interaction model, and

the corresponding reference true mix profiles.

12. A method of producing a complex microorganism community product, comprising:

selecting complex microorganism community samples from an initial sample collection,

using the prediction method of claim **1** to predict a mix profile resulting from the mixing of the selected samples,

comparing the predicted mix profile to a selection criterion, and

depending on the outcome of the comparing, actually picking and mixing the selected samples to obtain a mix result product.

13. The method of claim **12**, wherein the selection criterion includes one or more from a diversity criterion representative of an increase in profiling feature diversity, a minimum or maximum relative abundance of one or more profiling features, a non-zero relative abundance for one or more specific profiling features or for a minimum number of profiling features, a relative ratio between at least two profiling features, a closeness to a target mix profile.

14. A method of producing a complex microorganism community product having a target mix profile representing a target mix result product, comprising:

selecting, using the determining method of claim **5**, a target set of complex microorganism community samples belonging to an initial sample collection given the target mix profile, and

actually picking and mixing the microorganism community samples of the selected target set to obtain a mix result product.

15. The method of claim **14**, wherein one selected complex microorganism community sample is a virtual sample and the method further comprises actually producing an artificial complex microorganism community sample corresponding to the selected virtual sample from isolated strains.

16. A computer device comprising at least one microprocessor configured for carrying out the method of claim **1**.

17. A non-transitory computer-readable medium storing a program which, when executed by a microprocessor or computer system in a device, causes the device to perform the method of claim **1**.

* * * * *