Detection of horizontal sequence transfer in microorganisms in the genomic era

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Abstract:

Sequence transfer and genome remodeling are very frequent events in microorganisms, especially prokaryotes. This is due to the mosaic structure of the genomes, which calls into question the correct classification of genomes in terms of a single gene or a group of genes. We started here, as a first step, the inventory of bioinformatics tools applied to the detection of horizontal sequence transfers in microorganisms in the genomic era by applying bibliometric survey. Our bibliographic analysis allowed us to identify 17 main tools for the detection of chimeras. The first Bellerophon developed in 2004, followed by CCode and Pintail in 2005; Mallard in 2006; Mothur in 2009; Blackbox Chimera in 2010; Perseus, ChimeraSlayer, UCHIME 1 and UCHIME2, and ChimeraScan in 2011; Decipher in 2012; EBARDenovo and FunFrame in 2013; CATCh in 2015; Uchime 2 in 2016 and ChimeraMiner in April 2019. We then described each of these tools, highlighting their operating principles as well as the advantages and limitations of each (specificities, sensitivity, rapidity and frequent updates). The number of articles citing these tools has increased over the years especially for Mothur, Uchime, VSEARCH and ChimeraSlayer, thus demonstrating the interest of researchers in these tools and the need to decipher chimeras in genomic era.

52 **INTRODUCTION:**

53 Exchanges of genetic material between different species were very common during evolution
54 (Eisen 2000; Smith et al. 1992). Therefore, the detection of horizontal transfer was considered
55 crucial for the understanding of evolutionary processes and for the qualitative and quantitative
56 assessment of the exchange rate between microorganisms (Dutta & Pan 2002; Syvanen 1994).
57 The advent of new sequencing technologies has opened new avenues for evolutionary biology
58 research. The sequencing of new microbial clades and the depth of sequencing, provided by the
59 new sequencers, make it possible to study in depth the mechanisms of evolution of these
60 genomes. In particular, the mosaicity of genomes has been highlighted and the origin of
61 genomic content has been decrypted via the generation of "evolutionary bushes" or
62 rhizomes(Levasseur et al. 2017). This approach consists in integrating all the coding sequences
63 and determining their origin (HGT detection, horizontal gene transfer). Moreover, mosaic
64 structures within the sequences could also be highlighted (horizontal sequence transfer).
65 Beyond gene transfer, the transfer of sequences thus calls into question the current criteria of
66 taxonomy. Indeed, the use of individual molecular markers for taxonomy does not take into
67 account the mosaicity of exploited sequences (Haas, Gevers, Earl, Feldgarden, Ward,
68 Giannoukos, Ciulla, Tabbaa, Highlander, Sodergren, Methé, DeSantis, Petrosino, et al. 2011).
69 In addition, the use of several concatenated markers further biases the phylogenetic signal. To
70 do so, we carried out a state-of-the-art study of applied bioinformatics, the detection of
71 horizontal sequence transfers in microorganisms in the genomic era. Thus, we define in this
72 section the needs for sequence transfer analysis tools, from sequence quality control through
73 pre-processing to the analysis of genomic sequences by applying bibliometric survey. Then, we
74 describe the limitations and advantages for each tool. We list the tools and web resources
75 available to perform sequence transfer analysis. The global trend of research on chimera

76 detection tools is also introduced. Finally, their consequences in metagenomics, evolutionary
77 biology and taxonomy are discussed.

78 MATERIALS AND METHODS

79 Tool selection and data collection

80 Information from databases of scientific publications (such as NCBI PubMed, Web of Sciences
81 (WOS)) has been filtered to allow us to find recent tools, software or software packages that
82 aim to verify chimeras A keyword search for chimera check ("Chimeric Read" OR "Chimeric
83 data" OR "chimera detection" OR "detect chimeric sequences" OR "chimera finder" OR
84 "chimera check") was used. The search yielded from 1354 articles in PubMed and Medline, 52
85 articles from WOS on 05/07/2019. Subsequently, these articles were screened manually by
86 reading title and abstract. Only these 17 papers, the first papers describing new tools for chimera
87 control, have been selected and studied in detail. The total records of those publications,
88 including years of publications, titles, names of authors, affiliations, nationalities and names of
89 publishing journals were collected from each publication. Information on the tools and their
90 operating principles was collected from the original paper. The modeling of the operating
91 principle of the tools was carried out using the draw.io software (https://www.draw.io/). Excel
92 was used to create all the graphs in this article.

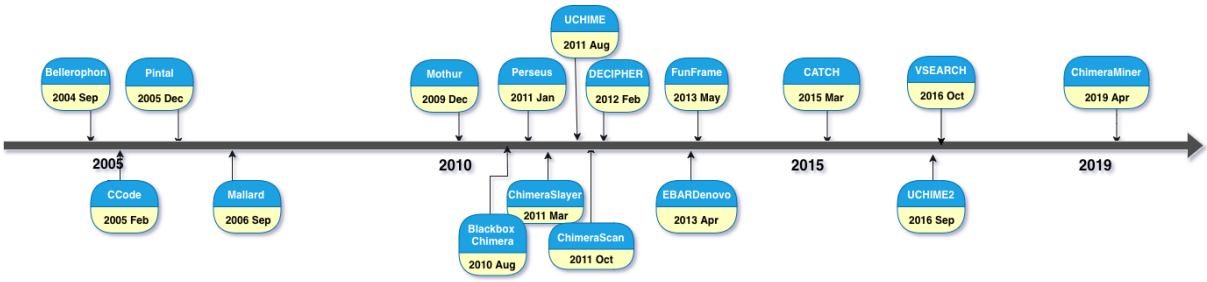
93 Bibliometric analysis and Visualization.

94 Using the WOS database, we collected the articles citing the original papers for different
95 chimera tools. Total citations counts were collected from the Web of Science database on
96 06/07/2019. The lists of papers citing the different tools were exported to Excel 2016. They
97 were subsequently merged, and the duplicates were removed. We used VOS viewer (Leiden

98 University, Leiden, Leiden, The Netherlands) for constructing and visualizing bibliometric
99 networks(Synnestvedt et al. 2005) (http://www.vosviewer.com).

100 **BIOINFORMATICS AND STATISTICAL TOOLS FOR CHIMERA DETECTION**

101 Our bibliographic analysis allowed us to identify 17 tools for the detection of chimeras. The
102 first Bellerophon developed in 2004, followed by CCode and Pintail in 2005; Mallard in 2006;
103 Mothur in 2009; Blackbox Chimera in 2010; Perseus, ChimeraSlayer, UCHIME 1 and
104 UCHIME2, and ChimeraScan in 2011; Decipher in 2012; EBARDenovo and FunFrame in
105 2013; CATCh in 2015; Uchime 2 in 2016 and ChimeraMiner in April 2019. Figure 1 illustrates
106 this dynamic in the development of these tools. We will describe each of these tools,
107 highlighting their operating principles and the advantages and limitations of each.



108

109 **Figure1:** Modeling Chimera detection tools

110 **1. Bellerophon**

111 Bellerophon (Huber et al. 2004a) is a tool that also allows the detection of chimeric sequences
112 specific to the 16S rRNA genes developed by Greengenes (DeSantis, Hugenholtz, Larsen, et
113 al. 2006a) through several sequence datasets. It is based on a partial tree approach (Wang &
114 Wang 1997), i.e. the phylogenetic tree that is derived from the independent regions is the result
115 of a multiple alignment whose branching patterns are compared to indicate chimeric sequences.

116 **2. CCode**

117 To perform an analysis of suspected chimeric sequences, it is necessary to have all the closest
118 sequences in a database. A comparison will be made between the request sequences and the
119 reference sequences in order to confirm to confirm or refute the existence of a chimeric origin.

Note that a chimeric sequence is composed of at least two partial gene sequences and each fragment is examined according to size and distance. The precision on the chimeric or non-chimeric origin of a query sequence is based on a 95% confidence threshold value and an analysis of variance test (Sokal Rolph). CCode (Gonzalez et al. 2005) (Detection and evaluation of chimera and crossing) is written in programming language C available for free at (http://www.rtphc.csic.es/download.html and on http://www.irnase.csic.es/users/jmgrau/index .html). Note that the reference sequences used in this program were obtained using the blastn algorithm (Altschul et al. 1990). Using too long or too short, a sequence can cause a resignation of of the program sensitivity.

3. Pintial

Pintial(Ashelford et al. 2005a) is a utility for the detection and analysis of 16S rRNA chimeras and other abnormalities. The operating principle is very simple as it uses a graphical interface and is capable of running on various computer platforms. It compares the length evolution distance of the 16S rRNA gene that exists between the query sequence and the subject sequence by advancing a fixed number of bases at a time along the length of the gene. Using this method, 1399 sequences of 19 phyla, as defined by the RDP project, were sorted and found that 64.3 were chimeras, 14.3% were non-identifying sequencing errors and 21.4% were strongly degenerate. Although Pintial was originally a 16S anomaly detection tool, most of the anomalies it detects are chimeras.

4. Mallard

Mallard (Ashelford et al. 2006a) is a computer-based tool for the detection of chimeras and other artifacts written in Java 1.4 programming language based on 16S rRNA genes. Mallard develops the Pintial algorithm [15] which runs by making a pairwise comparison between the reference sequence and the query sequence based on an alignment to evaluate changes in distance that are scalable and uncorrected. The outlier values obtained are identified as those that are below the threshold set by the user for the sequence comparison. Mallard was used in

146 the analysis of the rRNA gene database with the same principle, i.e a multiple sequence
147 alignment with the reference sequence in order to detect putative abnormalities that are verified
148 with BLASTn. The comparison of Mallard with Bellerophon (Huber et al. 2004b) indicates
149 show that on average 73.1% of chimeras know were by Mallard counter to Bellerophon which
150 on average 59.8% of the chimeras were detected. Mallard more successfully detects putative
151 abnormalities in bacterial and archaic taxons.

152 **5. Mothur**

153 Mothur (Schloss et al. 2009a) both to be a software package that brings together all the software
154 intended for the microbial community, including the chimera detection tools (ChimeraSlayer
155 and Uchime) in the form of a single tool capable of analyzing all these data. Mothur is written
156 in C ++ with the help of object-oriented programming strategy. The first version was developed
157 in 2009 and implements a lot of analysis algorithms for the 16S rRNA gene. The design patterns
158 used in this program are enhanced for flexibility and software maintenance. This during mothur
159 has a limit on the speed of the RAM available on the user's computer.

160 **6. Blackbox Chimera check (B2C2)**

161 Blackbox Chimera check (Gontcharova et al. 2010). Chimera check is a chimera filtering tool
162 that is based on a graphical interface executable in the Windows NET platform to process a
163 batch of sequence of wide range. During the same process, several files initially selected can
164 be analyzed. The regions at the beginning and at the end of the contigues from the input
165 sequences are aligned through a BLAST database.

166 **7. Perseus**

167 The Perseus algorithm exploits the abundance of the sequences that are associated with the
168 pyrosequencing data by considering each sequence at a time by performing precise pairwise
169 alignments on all sequences of equal or greater abundance, on all possible parents (Quince et
170 al. 2011a). A query is marked as chimera if the distance calculated during the alignment is less

171 than 0.15 and less than or equal to the distance of the closest sequence between the best possible
172 parents. This hypothesis makes it possible to validate the existence of a chimera. Taking into
173 account that this chimeric model may have evolved, another factor called "chimera index" will
174 be calculated based on the query, the closest relative and the parent farthest away using ancestral
175 parsimony. Once the index is determined, there remains the supervised learning through a set
176 of data sets (V2 and V5) or for each sequence, a chimera matching criterion will be calculated,
177 then a classification will be performed to determine by comparison with the reference sequences
178 to form a logistic regression.

179 **8. Algorithm ChimeraSlayer CS**

180 The algorithm (Figure 2) runs by first passing through a NAST (DeSantis, Hugenholtz, Keller,
181 et al. 2006) format alignment using the NAST-iEr multiple alignment utility. Using this output
182 file, the end regions of the request sequence each corresponding to 30% of the request length
183 will be independently searched in the 16S reference database using Megablast. An identification
184 of the chimeric parent will be made, so that it is an in-silico chimera among several parent
185 reference 16S having a high alignment score. The best results for each search will be extracted
186 in NAST format through a dynamic alignment program CHECK-CHIMERA (Komatsoulis &
187 Waterman 1997). After identification, another NAST realignment will be done, but this time
188 based on the profile of the selected parents. Finally, the query sequences, which present a great
189 similarity with an in silico chimera formed between any two parental sequences, are marked in
190 an evolutionary framework, i.e. a bootstrap greater than 90% and a minimum divergence greater
191 than 1.007 (Haas, Gevers, Earl, Feldgarden, Ward, Giannoukos, Ciulla, Tabbaa, Highlander,
192 Sodergren, Methé, DeSantis, Consortium, et al. 2011a; Komatsoulis & Waterman 1997).

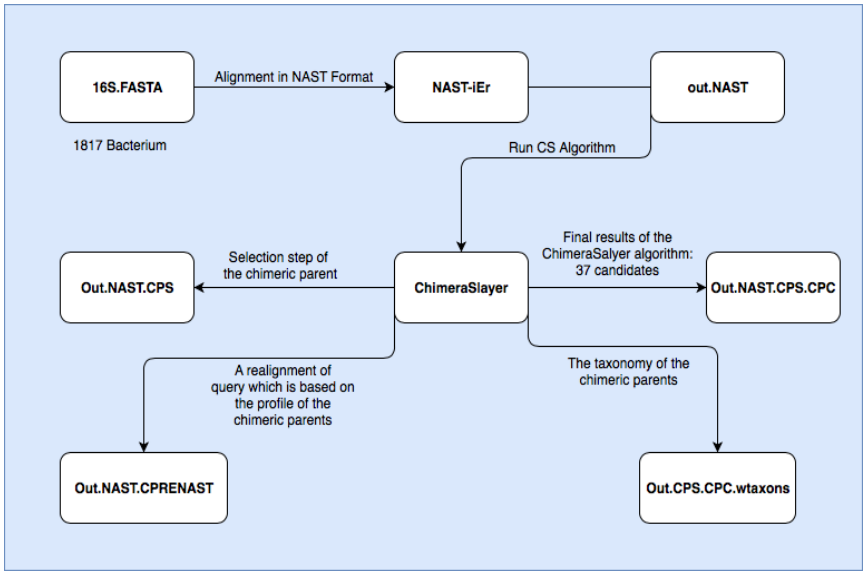


Figure 2: Modeling ChimeraSlayer Algorithm.

9. Uchime

Uchime (Figure 3) uses the query sequence and divides it into four pieces or segments, each portion is searched through a reference database, and the best matches of each search are noted. Among these correspondences, the two best candidates are identified, and a multiple alignment will be done (local, local-X and global-X) based on the profile of the two selected candidates. If the percentage sequence identity is ≥ 0.8%, an alignment score will be calculated and if the threshold is exceeded, the query is marked as chimera(Edgar et al. 2011a).

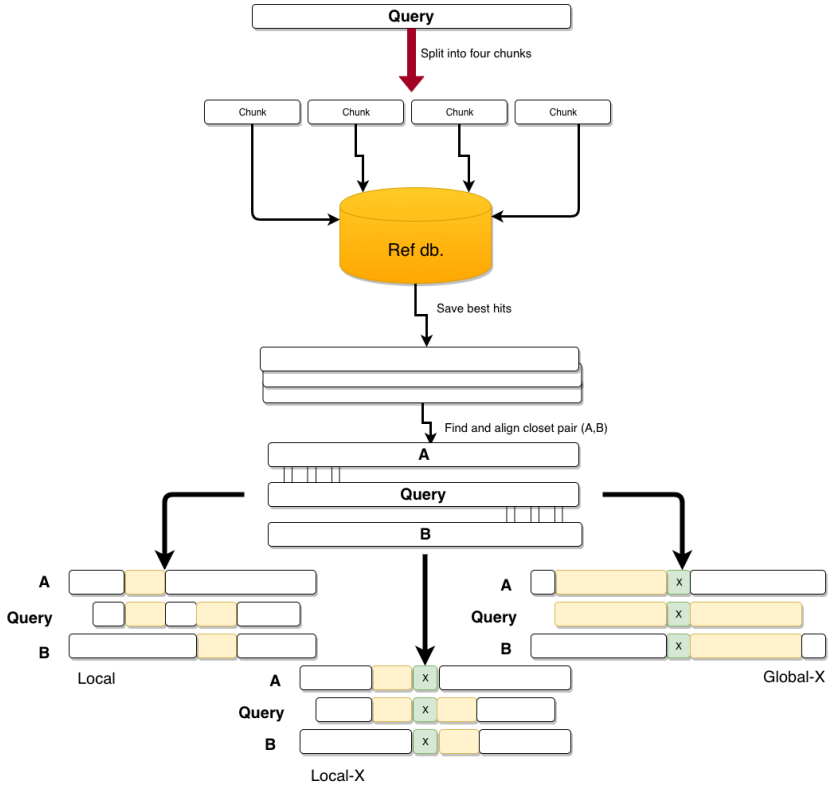


Figure 3: Modeling Uchime Algorithm: The query sequence is divided into 4 pieces; each piece is searched in the reference database and the best ones are selected after calculating the percentage of identity. Three types of alignment are made (Local, local-X and Global-X) between the query (Q) and both parents (A, B)(Edgar et al. 2011a).

10. ChimeraScan

ChimeraScan is an open source chimera detection software package that includes features such as: processing long matched reads greater than 75bp, handling ambiguous mapping reads, detecting readings covering a merge junction, integrating with very popular aligner of nodes (Langmead et al. 2009). The comparison of ChimeraScan with other tools, such as deFuse (McPherson et al. 2011) , shortFuse (Kinsella et al. 2011) and MapSplice (Wang et al. 2010) shows that 90% of the positives among 78 chimera predictions were detected by ChimeraScan.

11. Decipher

DECIPHER(Wright et al. 2012) is a new method for detecting chimeric 16S rRNA sequences using a chimeric sequence search approach. It is based on the detection of short fragments that

217 are rare in a phylogenetic group where a query sequence is classified but found in another
218 phylogenetic group. Calibration of the algorithm was carried out for complete sequences
219 (fs_DECIPHER) and short sequences (ss_DECIPHER) and was then compared to other
220 algorithms namely WigeoN (Pintail) (Ashelford et al. 2005a), ChimeraSlayer (Haas, Gevers,
221 Earl, Feldgarden, Ward, Giannoukos, Ciulla, Tabbaa, Highlander, Sodergren, Methé, DeSantis,
222 Consortium, et al. 2011b) and Uchime (Edgar et al. 2011a). In fact ss_DECIPHER and Uchime
223 provided a better detection of chimera on short sequences of around 100 to 600 nucleotides in
224 length, ie 79% and 81%, but if we move on to longer sequences, Uchime's performance begins
225 to deteriorate while those of ss_DECIPHER increases with a detection rate around 89%. Based
226 on either tool, positive rates were estimated to be approximately 1.3% and 1.6%. Indeed, the
227 rate of false positive of fs_DECIPHER was very low compared to other programs, i.e. a rate of
228 less than 0.20%. DECIPHER was only surpassed by ChimeraSlayer and Uchime when the
229 chimeras are formed from close relatives, a difference of less than 10%. With fs_DECIPHER,
230 the detection of chimeras in the RDP (Cole et al. 2007), SILVA(Pruesse et al. 2007) and
231 Greengenes(DeSantis, Hugenholtz, Larsen, et al. 2006b) were outperformed, equivalent to 1%
232 and 2% more chimeras.

233 **12. EBARDenovo**

234 EBARDenovo (Chu et al. 2013) runs in three main stages: extension, bridge and chimera
235 detection. Before performing these steps, the algorithm points to the first base pairs for each
236 read (default 15pb) as the index key, then a sort will be applied based on read abundance. As
237 part of the detection of sequence repeat detection (chimeras), predictable readings are used to
238 identify repetitive sequences. EBARDEnovo is implemented in C programming language and
239 can be run on Windows, Linux and Windows operating systems. 64-bit Mac OS. It is imperative
240 to install the Mono program for execution (http://www.mono-project.com/).

241 **13. Funframe**

242 FunFrame is a pyrosequencing error analysis pipeline using HMM-FRAME (Zhang & Sun
243 2011) ; UCHIME (Edgar et al. 2011a) for chimera detection and ESPRIT-Tree (Cai & Sun
244 2011) for clustering using the OTU array. FunFrame is coded in R and Python programming
245 language and the execution is performed by a bash script and can be done individually.

246 **14. CATCH**

247 CATCH (Mysara et al. 2015), which groups the classifiers (CATCH reference and CATCH de
248 novo) is a machine learning algorithm for the detection of chimeric and non-chimeric
249 sequences from a set of input data. Using CATCH, the input data are scores from the various
250 chimera detection tools such as mothur (Schloss et al. 2009b) that implement the algorithms
251 (ChimeraSlayer UCHIME and Pintial) that use the Gold Reference Database (on the Broad
252 Microbiome Utilities website, version micorbiomeutil-r20110519) and DECIPHER that uses
253 the RDP. This database during each tool is run separately and the output scores are processed
254 by a classifier to finally predict whether the reading is chimeric or not. This automatic learning
255 is done in three steps: (i) firstly, the identification of the output values of the different tools for
256 detecting chimeras, (ii) in a second step, the classifier is trained in a supervised learning it learns
257 to establish a correct prediction according to the given function from the tools and, at the end,
258 (iii) the learning is validated the classifier is currently being able to predict chimeric sequences
259 with other data different from those used as examples. For the reference-based CATCH
260 classifier, the features are (i) the score and final decision that are calculated for UCHIME and
261 ChimeraSlayer (ii) and the standard deviation and final decision that are calculated for Pintial
262 and (iii) the final decision that remains for DECIPHER. While for the de novo CATCH
263 classifier, the score characteristics and final decision are chosen as input parameters for
264 UCHIME, ChimeraSlayer and Perseus.

265 **15. Uchime2**

UCHIME2 (Edgar 2016) is an optimized update of the UCHIME (Edgar et al. 2011b) chimera detection algorithm that operates by building a model and performing multiple alignment of several models and the most important results of the most similar sequence. If the fractional divergence is large, then one has a model and the query is more likely to be marked as a chimera and if the divergence is small, the model is more likely to be a false.

16. VSEARCH

The VSEARCH algorithm (Rognes et al. 2016) uses a fast heuristic based on databases shared by the query and target sequences to identify similarities between them. This strategy is also used by USEARCH (https://www.drive5.com/ usearch /). With VSEARCH, dynamic programming will perform optimal global alignment of the query on potential target sequences and pairwise alignments are calculated through vectorization and multiple threads. It is a multithreaded 64-bit tool that is free and open source and intended for metagenomic data analysis.

17. ChimeraMiner

ChimeraMiner (Lu et al. 2019a) is an improved chimera detection tool for the analysis of MDA (Multiple Displacement Amplification) sequencing data. To compare the efficiency of the algorithm with the previous tools, two sets of MDA data sets were used (MDA1 and MDA2). In terms of treatment time, it is estimated at 43.4% and that 83.60% of the chimeras detected by the previous tools were found by ChimeraMiner, plus 6,736,168 chimeras not detected by the previous methods.

GLOBAL TREND OF CHIMERAS TOOLS RESEARCH: A BIBLIOMETRIC ANALYSIS

Bibliometrics applies mathematical and statistical techniques to the analysis of books, articles and other documents (Otlet 1934; Pritchard 1969), thus highlighting the current level of knowledge in a scientific field through the compilation of data obtained from bibliographic

291 databases and their quantitative and qualitative analysis (Ekinci et al. 2015). Bibliometrics can
292 be used to inform policy decisions (Russell & Rousseau 2010), allocate research funding
293 (Cronin & Sugimoto 2014), help libraries prioritize acquisitions(Engler 2014) and evaluate
294 scholarly activities (Rousseau et al. 2018). The objectives of this section were to provide a
295 brief bibliometric analysis of the articles that cited the original articles from each of these
296 tools.

297 **Bibliographic analysis of chimera's detection tools original papers**

298 The authors who contributed to the development of these tools came from 13 different
299 countries. The USA contributed to the development of these 10 tools (Uchime et Uchime2,
300 FunFrame, DECIPHER, ChimeraScan, Chimera Slayer, Blackbox Chimera check (B2C2),
301 Perseus, Bellerophon et Mothur) ; 06 from United Kingdom (Perseus, Mallard, Uchime,
302 Mothur, VSEARCH and Pintail) ; 02 from Canada (Mothur and EBARDenovo) ; 02 from
303 Norway (VSEARCH and Perseus). The authors from Taiwan, Spain, Belgium, China,
304 Australia, Germany, France, Slovenia and Austria contributed respectively for EBARDenovo,
305 CCode, CATCh, ChimeraMiner, Bellerophon, VSEARCH, VSEARCH, Mothur and Mothur
306 (Table 1). Bioinformatics is the journal in which most of these papers (06 publications) were
307 published, followed by Applied and Environmental Microbiology (05 publications) and one in
308 each of the bioRxiv, BMC Bioinformatics, Genome Research, International Journal of
309 Molecular Sciences, PEERJ and Open Microbiology Journal (Table 1). Among the features of
310 chimera detection tools, the most commonly used programming language is C++, the reference
311 interface is often command line based and stability is checked for most tools (Table 2).

312 **Citation analysis of papers citing chimera's detection tools**

313 Of the 17 tools, only the 15 indexed in the WOS database were used for further analysis. These
314 are: Mothur, UCHIME, Chimera Slayer, Bellerophon, Perseus, VSEARCH, Pintail, Mallard,
315 DECIPHER, ChimeraScan, CCode, EBARDenovo, CATCh, FunFrame et ChimeraMiner.
316 They were cited respectively 8424; 5121; 1335; 1246; 857; 590; 575; 552; 345; 141; 60; 15;
317 12; 8 and 0 times. And an average per year respectively of 765,82; 569; 148,33; 77,88; 95,22;
318 147,5; 38,33; 39,43; 43,13; 15,67; 4; 2,14; 2,4; 1,14 et 0 (Table 3). The total number of papers
319 citing the chimera detection tools was 14876. The number of papers citing these tools has grown
320 over the years for Mothur, Uchime, VSEARCH et Chimera Slayer which demonstrates the
321 interest of researchers for these tools (Figure 4). Their specificity, precision, speed and frequent
322 updates can be an explanation. There has been a loss of interest in tools such as Perseus, Pintail,
323 Mallard, Decipher, ChimeraScan, CCode, EBARDenovo with the progressive decrease in the
324 number of citations in recent years (Figure 4 and Figure 5).

325 **Table 3: Number of citations of publications on chimera detection tools**

326

Tools	Publication date	Total Citations	Average per Year
Mothur	Dec 2009	8424	765,82
UCHIME	Aug 2011	5121	569
VSEARCH	Oct 2016	590	147,5
Chimera Slayer	Mar 2011	1335	148,33
Perseus	Jan 2011	857	95,22
DECIPHER	Feb 2012	345	43,13
Bellerophon	Sep 2004	1246	77,88
Pintail	Dec 2005	575	38,33
ChimeraScan	Oct 2011	141	15,67
Mallard	Sep 2006	552	39,43
CATCh	Mar 2015	12	2,4
CCode	Feb 2005	60	4
EBARDenovo	Apr 2013	15	2,14
FunFrame	May 2013	8	1,14
ChimeraMiner	Apr 2019	0	0

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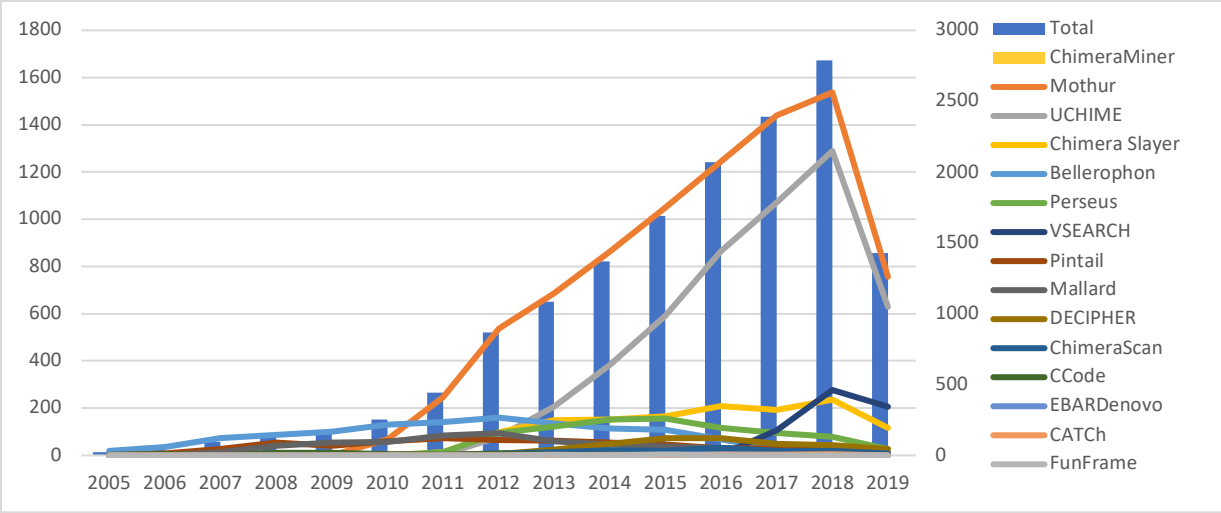

Figure 4: Evolution of the number of citations of chimera detection tools

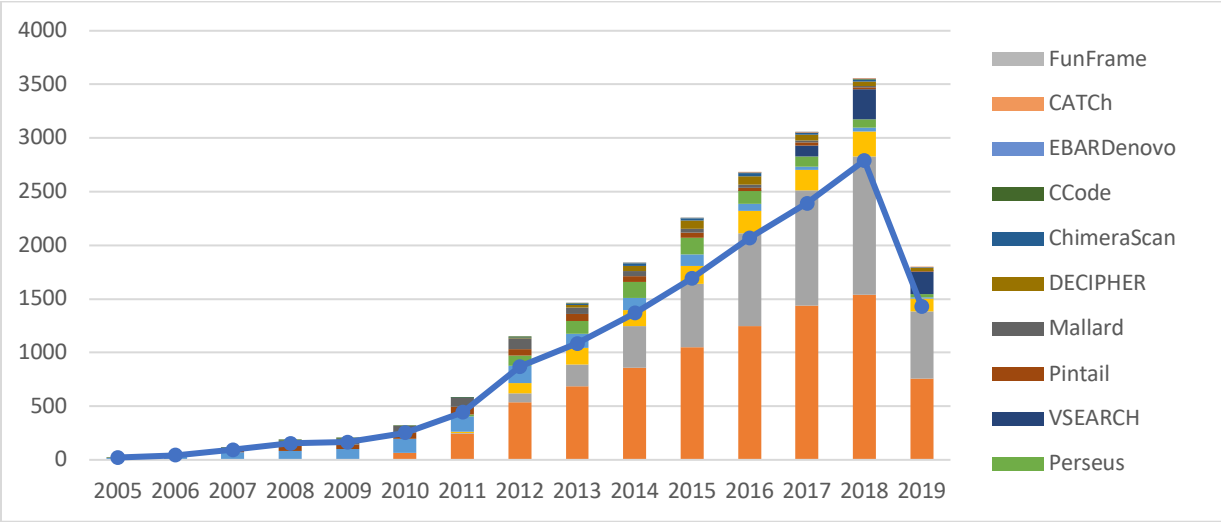

Figure 5: Evolutions curved of the top 20 most productive organizations

Distribution of published journals on papers citing chimera's detection tools

The citing document was published in 1525 journals, with 788 who published more than 2 citing documents. Plos One is the most used (1054 documents), followed by Frontiers in Microbiology (1013 documents) and Scientific Reports (608 documents) (Figure 6). After the year 2015, we can see in Figure 7 the decrease of the most used source (Plos One, Applied and Environmental Microbiology; FEMS Microbiology Ecology and Environmental microbiology) and the increase of some sources such as Frontiers in Microbiology and new sources like

Scientific Reports, Science of the Total Environment, Microbiome, and Peerj. These most interesting sources are interested by microbial ecology in human and environment.

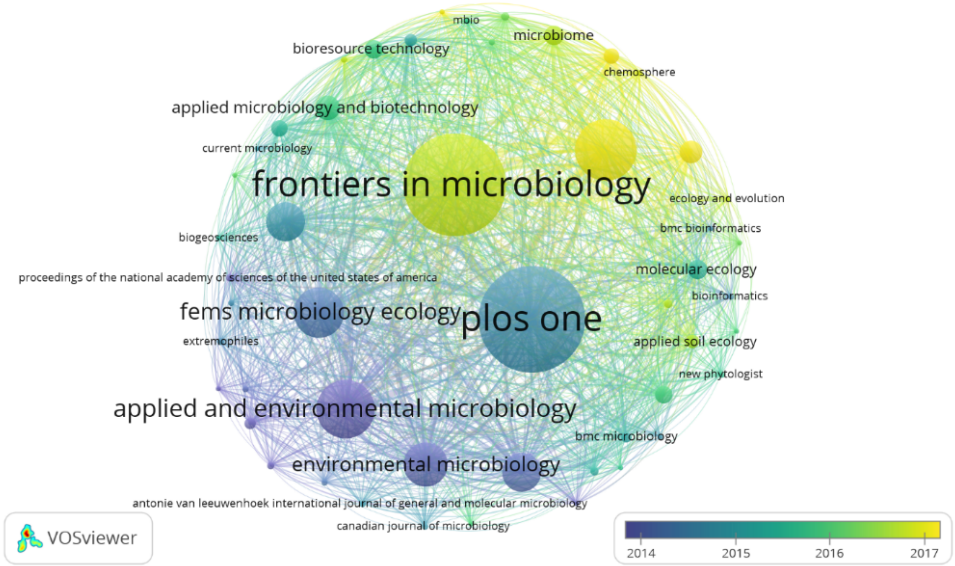

Figure 6: Combined mapping and clustering of top 50 most active journals according to the number of publications

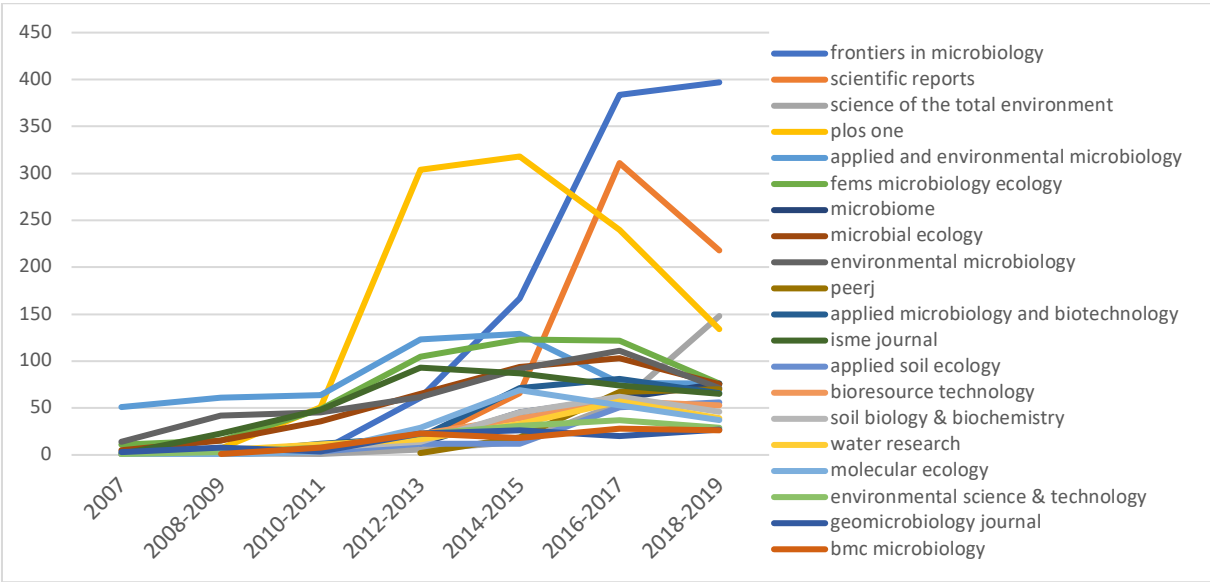

Figure 7: Evolutions curved of the top 20 most active journals according to the number of publications

Countries contributing to global publications on papers citing chimera's detection tools

More than 58,760 authors from various parts of the world have contributed to studies that have cited these chimera detection tools. In general, they were affiliated with institutions in 149 countries, the top ten of which was the USA first (5,146 items), followed by China (3,468 items), Germany (1,384 articles), Canada (967 articles), France (902 articles), Australia (811 items), England (804 items), Spain (741 items), Japan (598 items) and South Korea (555 items) (Figure 8). American documents were the most cited with 202,396 citations, followed by German documents with 44,309 citations and China with 42,219 citations. After 2017, China has become the country with the highest number of publications citing Chimera tools (Figure 9). Furthermore, they developed the last chimera tools to our knowledge, ChimeraMiner (Lu et al. 2019b), released early this year. France, a pioneer in this field, has also increased its position and is now one of the top 4 countries interested in studies related to the Chimera (Figure 9).

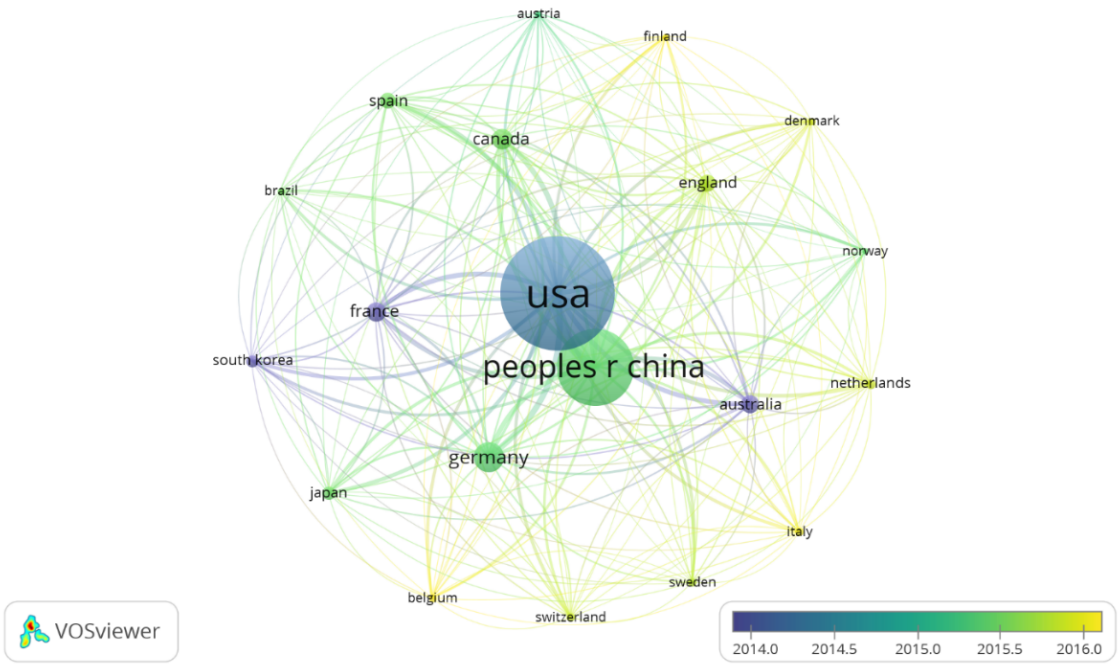

Figure 8: Networked visualization of collaborations between countries among the 20 most productive countries of publications citing chimera detection tools. Links represent the strength of collaboration

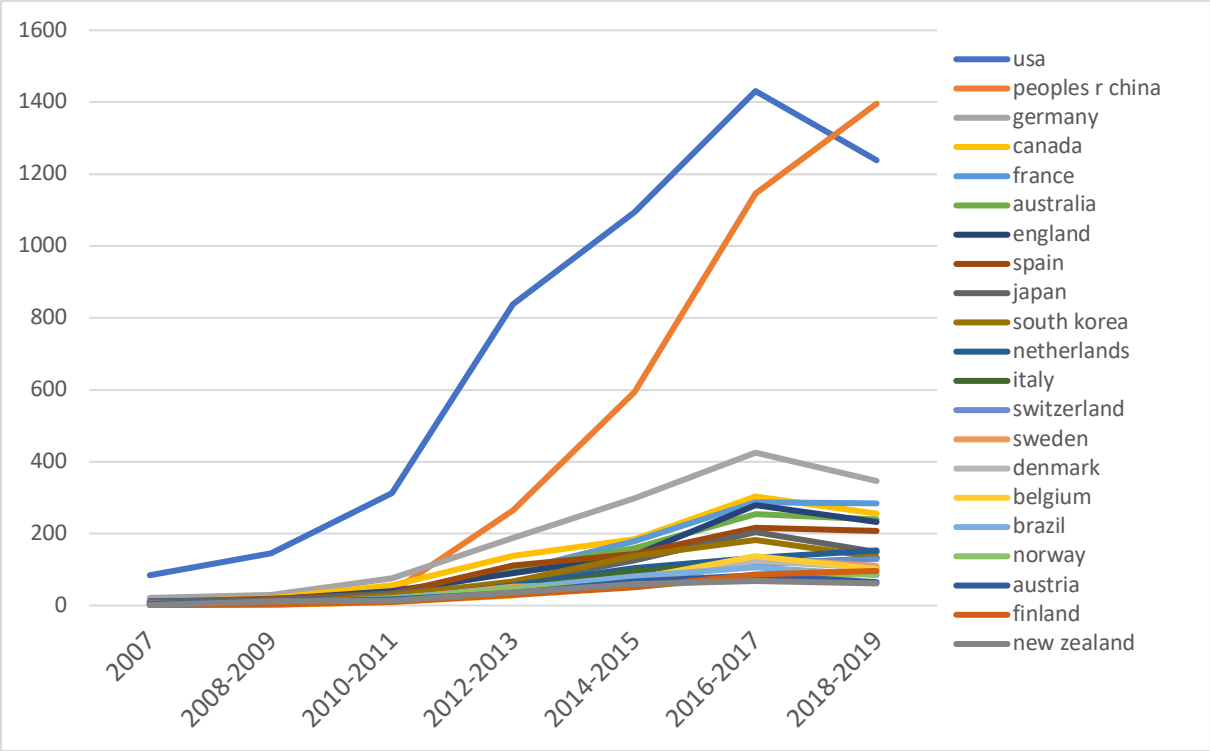

Figure 9: Curved evolution of the 20 most productive countries

Distribution of institutes paying attention to chimera detection tools

The authors contributed in the document citing these chimera stools are affiliated with 9000 organizations, and 1466 organizations have at least 1466 documents. Among the top 20 organizations frequently citing these stools, Chinese organizations are in first and second place (respectively Chinese Acad Sci and Univ Chinese Acad Sci), followed by American organizations (Univ Michigan) (Figure 10; Figure 11). The French institutions, INRA and CNRS are respectively in 17th and 18th place, in terms of contribution cited (figure 10).

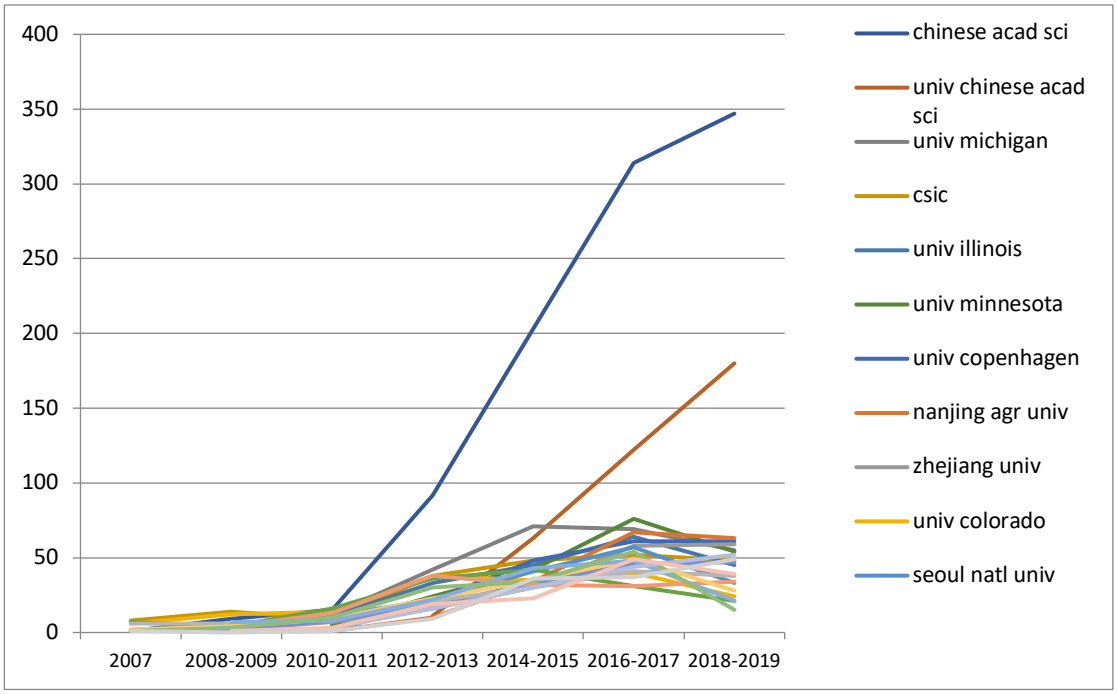

Figure 10: Curved evolution of the top 20 most productive organizations

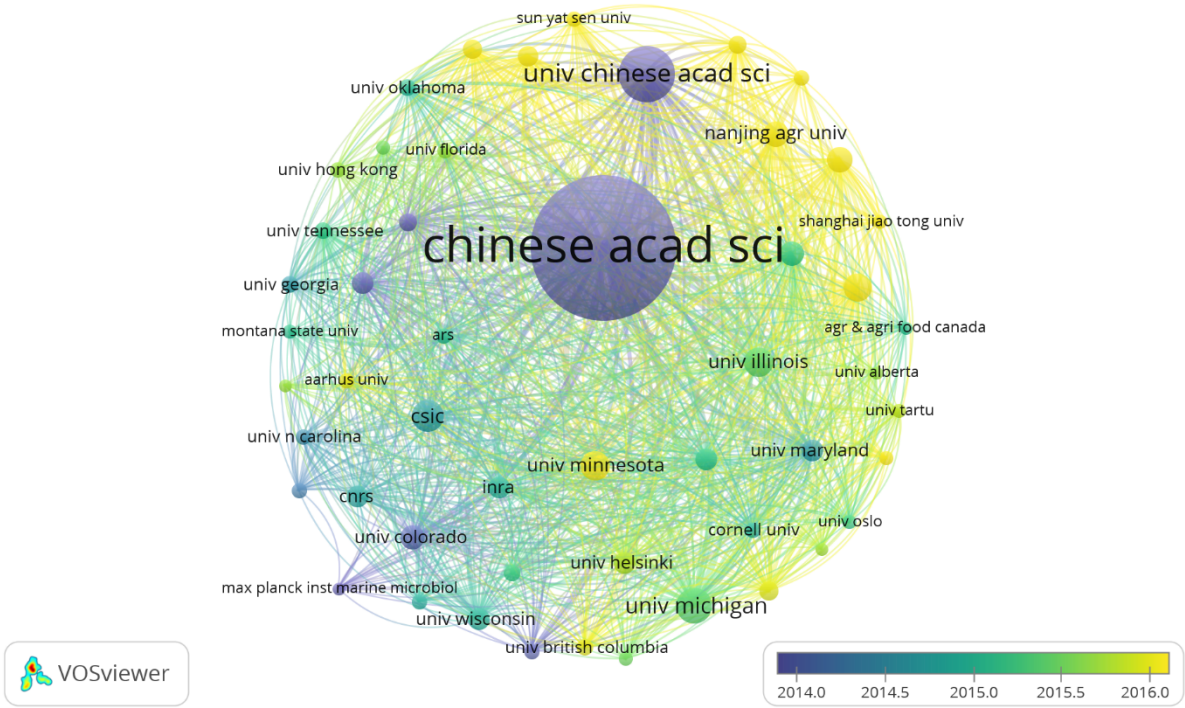

Figure 11: Networked visualization of inter-organizational collaborations among the 50 most productive organizations in publications citing chimera detection tools. Links represent the strength of collaboration

381 **Hotspots of studies citing chimera's detection tools**

382 We apply bibliometrics to the keywords of the analyzed articles. The keywords represent the
383 author's opinion (or about) the most important words of his articles. Second, keyword analysis
384 can potentially detect trends in research topics, both currently and in the past
385 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6480778/). The authors of the citing
386 document used 23670 keywords and 3646 were used more than 5 times. The 100 most frequent
387 keywords used by the authors in the reference document were classified into 5 groups (Figure
388 12). The clusters are made up of the keywords related to microbiology techniques and microbial
389 diversity. The group 1 color red, composed of 30 elements, is specifically constituted by the
390 keywords related to wastewater treatment (activated sludge, wastewater, wastewater treatment
391 and bioremediation) and biological processes induced by microorganisms (stable isotope
392 probing, anammox, ammonia-oxidizing archaea, biodegradation, biofilm, denitrification and
393 nitrification). Group 2, green in color, consisted of 29 items and the items specific to it are:
394 bacteria (actinobacteria, bacteria and cyanobacteria), fungi (arbuscular mycorrhizal fungi,
395 fungal community and fungi) and environment (Antarctica, bacterioplankton, rhizosphere,
396 biogeography, arctic and soil). The Cluster 3, yellow in color and composed of 28 elements, is
397 specifically made up of keywords related to human studies on probiotics, prebiotics, treatments
398 (probiotics, lactobacilli and lactic bacteria, diet and antibiotics) and health status.(obesity,
399 inflammation and dysbiosis). The group 4, of blue color and composed of 8 elements, is
400 specifically constituted by the keyword relative to methanogenic archaea (anaerobic digestion,
401 archaea, methane, methanogen, methanogenesis and methanogens). The last cluster 5, pink in
402 color and composed of 6 elements, is specifically constituted by keywords relative to phylogeny
403 and taxonomy (Figure 12, Figure 13). This analysis highlights the main research areas related
404 to chimera stools. We can cite microbial diversity of human and environment; phylogeny and

taxonomy; archaea and methanogen; human probiotics and dysbiosis; waste treatment and biological process induced by microorganisms.

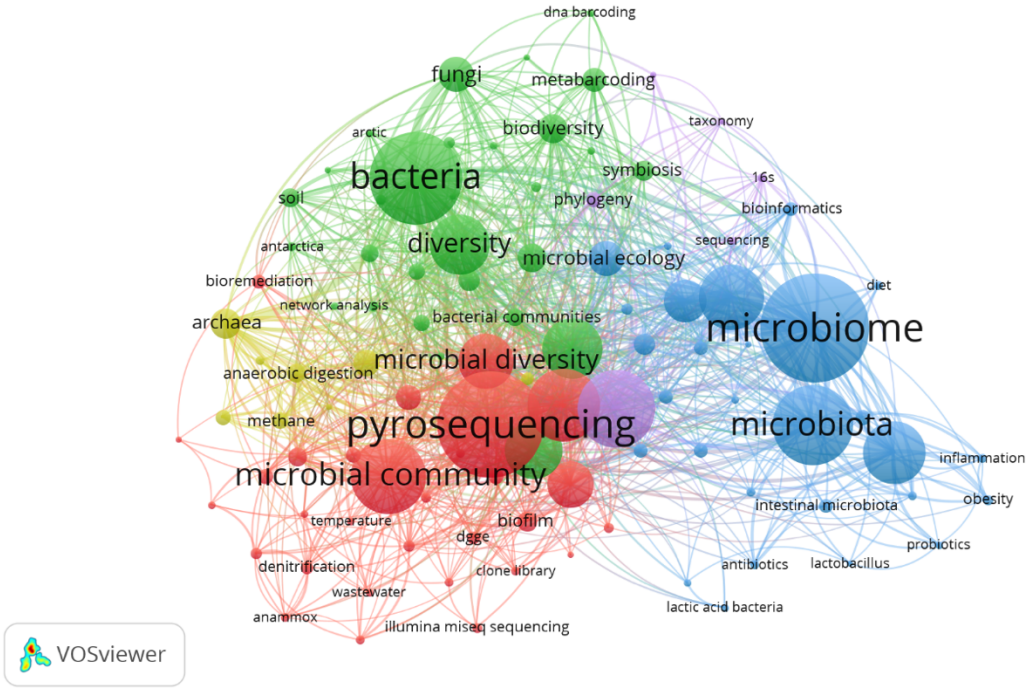

Figure 12: Network visualization of top 100 most used keywords of publications citing chimera's detection tools. The keywords were divided into four clusters according to different colors generated using default paramaters: wastewater treatment (down in red), microorganisms and environment (up in green), archaea methanogen related study (right in blue), probiotics prebiotics and treatment related study (left in yellow) and phylogeny and taxonomy (up in pink). A large circle size represents the keyword that appears at a high frequency.

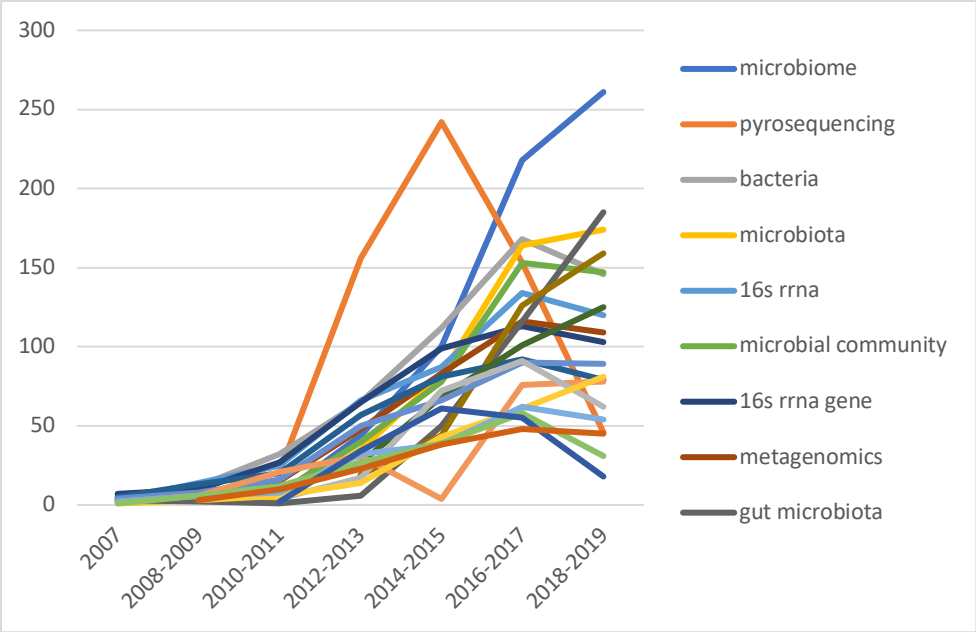

Figure 13: Evolutions curved of the top 20 most frequent used keywords

THE CONSEQUENCES IN TERMS OF METAGENOMIC STUDIES AND EVOLUTIONARY BIOLOGY AND TAXONOMY:

Advances in Next Generation Sequencing (NGS) have made a significant contribution to advanced microbial ecology studies, hence the implementation of metagenomics. This is defined as a process of analysis of the DNA without the need to perform a culture. With the implementation of databases and tools, bioinformatics and statistical analysis have made it possible to better exploit metagenomic data (Oulas et al. 2015). Together with marker genes, the use of metagenomics is an efficient approach to obtain a taxonomic profile of a given community using PCR amplification and sequencing of conserved marker genes such as 16S rRNA (Tringe & Hugenholtz 2008). The latter was considered a fundamental fold for the characterization of a given microbial community, but as a result, this approach is overshadowed by metagenomics, which provides the overall functioning of a community rather than an inventory of these inhabitants (Tringe & Hugenholtz 2008).

The essential function of the 16S gene, as well as its highly conserved sequence and structure, has made it the molecule of choice for microbial evolution and ecological studies [57,58]. The

numerous highly conserved regions covering the length of the gene allow the amplification of sequences from a wide range of species. However, these same highly conserved regions contribute to cross hybridization and amplification priming errors that create chimeric sequences. The correct identification of chimeric 16S sequences is a difficult computational problem and they are more likely to be classified as novel organisms if not correctly identified as outliers.

CONCLUSION AND DISCUSSION:

The evaluation of the accuracy of sequence transfer detection for all the tools testifies to the great variety of algorithms. For example, BellerophonGG is able to identify chimeras mainly restricted to the most divergent sequence pairs, while Pintail is highly sensitive to the detection of chimeras in full-length sequences, not chimeras in shorter sequences.

However, the perfect detection of chimeras is still an unresolved problem. More complex chimeras and sequence anomalies may escape detection. This underlines the importance of obtaining and validating the sequences that represent the new bacterial diversity and of continuing to develop the reference database and additional analytical tools. The problem of identifying rare species that correlate with diseases or other important functions of the microbial ecosystem remains difficult. This method is the only one currently able to detect sensitive chimeras in short and long readings of 16S sequences. Indeed, perfect detection of chimeras is still an unresolved problem due to divergence and length, as detection accuracy begins to degrade as divergence from reference sequences increases.

457

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Table 1: Bibliographic Informations of chimeras detection tools

Tools	Title	Authors	Source Title	Countries of contibuted authors	DOI	AVAILABILITY
Bellerophon (Huber et al. 2004a)	Bellerophon: a program to detect chimeric sequences in multiple sequence alignments	Huber, T et al, 2004 SEP	Bioinformatics	Australia, USA.	10.1093/bioinformatics/bth226	http://foo.maths.uq.edu.au/~huber/bellerophon.pl
CCode (Gonzalez et al. 2005)	Evaluating putative chimeric sequences from PCR-amplified products	Gonzalez, JM et al, 2005 FEB	Bioinformatics	Spain	10.1093/bioinformatics/bti008	http://www.irnase.csic.es/users/jmgrau/index.html and http://www.rtphc.csic.es/download.html.
Pintail (Ashelford et al. 2005b)	At least 1 in 20 16S rRNA sequence records currently held in public repositories is estimated to contain substantial anomalies	Ashelford, KE et al, 2005 DEC	Applied And Environmental Microbiology	United Kingdom	10.1128/AEM.71.12.7724-7736.2005	http://www.cardiff.ac.uk/biosi/research/biosoft/
Mallard (Ashelford et al. 2006b)	New screening software shows that most recent large 16S rRNA gene clone libraries contain chimeras	Ashelford, Kevin E. et al, 2006 SEP	Applied And Environmental Microbiology	United Kingdom	10.1128/AEM.00556-06	http://www.cardiff.ac.uk/biosi/research/biosoft/
Mothur(Schloss et al. 2009a)	Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities	Schloss, Patrick D et al, 2009 DEC	Applied And Environmental Microbiology	Slovenia; Austria; Canada; United Kingdom; USA	10.1128/AEM.01541-09	
Blackbox Chimera check (B2C2) (Gontcharova et al. 2010)	Black Box Chimera Check (B2C2): a Windows-Based Software for Batch Depletion of Chimeras from Bacterial 16S rRNA Gene Datasets.	Gontcharova V, 2010 Aug	Open Microbiol J.	USA	10.2174%2F1874285801004010047	http://www.researchandtesting.com/B2C2
Perseus (Quince et al. 2011b)	Removing Noise from Pyrosequenced Amplicons	Quince, Christopher et al, 2011 JAN	Bmc Bioinformatics	Norway; United Kingdom; USA	10.1186/1471-2105-12-38	
ChimeraSlayer *(Haas, Gevers, Earl, Feldgarden, Ward, Giannoukos, Ciulla, Tabbaa, Highlander, Sodergren, Methé, DeSantis, Consortium, et al. 2011a)*	Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons	Haas, Brian J et al, 2011 MAR	Genome Research	United Kingdom	10.1101/gr.112730.110	
Uchime (Edgar et al. 2011b)	UCHIME improves sensitivity and speed of chimera detection	Edgar, Robert C et al, 2011 AUG	Bioinformatics	United Kingdom ; USA	10.1093/bioinformatics/btr381	http://drive5.com/uchime.
ChimeraScan (Iyer et al. 2011)	ChimeraScan: a tool for identifying chimeric transcription in sequencing data	Iyer, Matthew K et al, 2011 OCT	Bioinformatics	USA	10.1093/bioinformatics/btr467	http://chimerascan.googlecode.com
Decipher (Wright et al. 2012)	DECIPHER, a Search-Based Approach to Chimera Identification for 16S rRNA Sequences	Wright, Erik S et al, 2012 FEB	Applied And Environmental Microbiology	USA	10.1128/AEM.06516-11	http://DECIPHER.cee.wisc.edu
EBARDEnov o (Chu et al. 2013)	EBARDenovo: highly accurate de novo assembly of RNA-Seq with efficient chimera detection	Chu, Hsueh-Ting et al, 2013 APR	Bioinformatics	Taiwan, Canada	10.1093/bioinformatics/btt092	http://ebardenovo.sourceforge.net/
FunFrame (Weisman et al. 2013)	FunFrame: functional gene ecological analysis pipeline	Weisman, David et al, 2013 MAY	Bioinformatics	USA	10.1093/bioinformatics/btt123	http://faculty.www.umb.edu/jennifer.bowen/software/FunFrame.zip.

CATCh (Mysara et al. 2015)	CATCh, an Ensemble Classifier for Chimera Detection in 16S rRNA Sequencing Studies	Mysara, Mohamed et al, 2015 MAR	Applied And Environmental Microbiology	Belgium	10.1128/AEM.02896-14	-
Uchime 2 (Edgar 2016)	UCHIME2: improved chimera prediction for amplicon sequencing	Robert C. Edgar et al, 2016 Sept	BioRxiv	USA	10.1101/074252	-
VSEARCH(Rognes et al. 2016)	VSEARCH: a versatile open source tool for metagenomics	Rognes, Torbjorn et al. 2016 Oct	PEERJ	Germany; France; Norway; United Kingdom	10.7717/peerj.2584	-
ChimeraMiner (Lu et al. 2019b)	ChimeraMiner: An Improved Chimeric Read Detection Pipeline and Its Application in Single Cell Sequencing.	Lu N et al, 2019 Apr	Int J Mol Sci.	China	10.3390/ijms20081953	-

Table 2: Characteristics of chimera detection tools

Tools	Software type	Interface	Restrictions to use	Biological technology	Implementation	Operating system	Computer skills	Stability	Maintained	Version
Bellerophon(Huber et al. 2004a)	Pipeline/ Workflow	Command line interface	None		Python	Unix/Linux	Advanced	Stable	Yes	-
CCode (Gonzalez et al. 2005)	-	-	-		C		-	-	-	-
Pintail (Ashelford et al. 2005b)	Achievable	Graphical interface			Java	Linux, Windows et OS X	Advanced	Stable	Yes	10.2
Mallard (Ashelford et al. 2006b)					Java					-
Mothur (Schloss et al. 2009a)	Package/ Module	Command line interface	None		C++	Unix/Linux	Advanced	Stable	Yes	1.38.0
Blackbox Chimera check (B2C2) (Gontcharova et al. 2010)	Achievable	Graphical interface		Roche	C++	Linux/Windows	Advanced	Stable	Yes	0.62.x
Perseus (Quince et al. 2011b)										
ChimeraSlayer *(Haas, Gevers, Earl, Feldgarden, Ward, Giannoukos, Ciulla, Tabbaa, Highlander, Sodergren, Methé, DeSantis, Consortium, et al. 2011a)*	Package/ Module	Command line interface	None	Roche	Perl	Unix/Linux	Advanced	Stable	Yes	-
Uchime (Edgar et al. 2011b)	Package/ modules	Command line interface	None		C++	Unix/Linux	Advanced	Stable		4.2.40
ChimeraScan (Iyer et al. 2011)	Package/ Module	Command line interface	Academic or non-commercial use		Python	Unix/Linux	Advanced	Stable	Yes	0.4.5

Decipher (Wright et al. 2012)	Package/ Module	R software	None		R programing language	Unix/Linux, Mac OS, Windows	Advanced		Yes	-
EBARDEnovo (Chu et al. 2013)	Application/Script, Package/Module	Command line interface	Academic or non-commercial use		C	Unix/Linux, Mac OS, Windows	Advanced	Stable	Yes	2.0.1
FunFrame (Weisman et al. 2013)	Pipeline/ Workflow	Command line interface	None		R	Unix/Linux, Mac OS, Windows	Advanced	Stable	Yes	0.9.3
CATCh (Mysara et al. 2015)	Package/ Module	Command line interface	None	Illumina, Roche	-	Unix/Linux	Advanced	Stable	Yes	-
UCHIME2 (Edgar 2016)	Package/ Module	Command line interface	None		C++	Unix/Linux	Advanced	Stable	Yes	
VSEARCH	Package/ Module	Command line interface	None		C, C++	Unix/Linux, Mac OS, Windows	Advanced	Stable	Yes	2.3.4
ChimeraMiner (Lu et al. 2019b)	Package/ Module	Command line interface	None		Perl	Unix/Linux, Mac OS	Advanced	Stable	Yes	-