REVIEW ARTICLE

Metagenomic Approach to Explore Microbial Diversity and Genetic Potential of Uncultured Microorganisms from Different Environment Niches

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ABSTRACT

The term metagenomics refers to genomic analysis of those microorganisms, which are difficult to cultivate in standard cultivation medium. The 16S rRNA study from various environments has provided a strong evidence for the existence of uncultured microorganism. The novel genes and gene products discovered by metagenomic approach include many hydrolytic enzymes, novel molecules and antimicrobial compounds. Furthermore, metagenomic studies from various extreme environments shed light into genomic diversity and existence of various co-operations among the microorganisms. This review discusses the concepts, basic tools and applications of metagenomic gene cloning in discovering novel molecules and microbial diversities. In addition to this, this review also highlights the impact of uncultured microorganisms on human health and environment.
Keywords: Biocatalysts,Antimicrobial, biogeochemical, metagenomics, microflora, phylogenetic, uncultivable.

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INTRODUCTION

Microorganisms (Bacteria) contribute significantly to the earth's biological diversity. They are present everywhere in the environment that includes thermal ducts, great depths of the oceans, international space station, and in general are heterogeneously distributed throughout the aggregates of soil particles [1-3]**.** Current estimates indicate that less than 1% bacteria present in most habitats are culturable [4-6]. The 16S rRNA study from diverse sources of environement has provided strong evidence for the existence of new lineages of microbes [7,8]. Assigning function to uncultured microorganisms in various environments (in absence of pure culture) present immense challenges for microbial ecologists [9, 10]. A molecular technique allows evaluation of the structure, dynamics and metabolic potential of environment samples. The word 'metagenomics' was coined to capture the notion of analysis of uncultured microorganism [5]. Metagenomic library construction and screening constitute a valuable tool for making industrial biotechnology, economically a sustainable success [11, 12]. In addition to this, understanding genomic diversity in various environment niches, especially extreme environment and human GIT (gastrointestinal tract) will shed light into their functional role in such environment. Furthermore, microorganisms play an important role in maintaining ecosystem; therefore metagenomic studies will decipher the role of unculturable microorganism in such system [13, 14]. This review article discusses the basic tools involved in metagenomic studies and further highlights the role of uncultured microorganisms in extreme environment, human and in ecosystem.

1. Tools and techniques used in metagenomic study

1.1. 16S rRNA and microbial diversity

Identification and characterization of rRNA genes help in the analysis of phylogeny and quantification of microbial diversity [15-17]. The 16S rRNA gene in bacteria consists of conserved sequences along with interspersed variable and hypervariable regions. The length of hyper variable region ranges from ~50 -100 bases long and differ with respect to variation in their corresponding utility for universal microbial identification.Various communities have been studied using metagenomic approach, here we are discussing few of them i.e. microbial diversity of a thermal environment from Yellowstone's was described by Pace et al. [18], Fierer et al. [19] have used small-subunit RNA sequence for highlighting the richness of new bacteria, archaea, fungi and viruses from soil sample. Additionally, Hallam et al. [20] have cloned a 40 Kb DNA fragment from metagenome sample of sea water, harbouring a 16S rRNA gene belonging to archaea. Moreover, a culture-independent study has revealed ~ 40 bacterial divisions, suggesting that almost 30 major bacterial divisions have no cultured representative's [21]. Meanwhile, various software's have been developed for gaining access into microbial diversity. In this context, Meyer et al. [22] have developed software RAST for studying phylogeny and function, Huang et al. [23] have developed software for the identification of rRNA genes from metagenomic fragments, based on hidden Markov models (HMMs). This software provides rRNA gene predictions with high sensitivity and specificity on artificially fragmented genomic DNAs. In addition to this, techniques like fluorescent *in situ* hybridization (FISH) targets 16S

rRNA of metabolically active bacteria, which have high cellular rRNA content, meanwhile, specific probes can be used to monitor microorganisms at different levels of taxonomic specificity [24, 25]. More recently, Hess et al. [26] have reported diversity of a cellulose and hemicellulose degrading bacteria from rumen of cow. It was demonstrated that rumen environment contains vast majority of cellulolytic mesophilic microbes, described from any habitat till date. The data obtained from such studies will substantially expand the catalogue of genes and genomes participating in the deconstruction of cellulosic biomass.

1.2. Metagenomic library construction and screening

Metagenomic library construction and screening constitutes a valuable tool for exploring novel biocatalysts and other molecules, and is constructed by extraction and purification of heavy molecular weight DNA [27-29]. The DNA obtained is digested with suitable restriction enzyme, followed by cloning of gene of interest in suitable vector molecules (vector choice depend upon the size of insert to be cloned) (Table1). After successful construction of a metagenomic library, it is screened either based on the function of gene or based on sequence method. The latter is dependent on the hybridization of the conserved DNA sequences of the target genes [30, 31]. Furthermore, screening based upon function always resulted in novel sequences (readers are referred to Table 2 for more detail). In addition to this, new screening methods like micro-array further help in identification of active clones among millions of clones [32-34]. Genome enrichment is another method which is used to target the active components of microbial population [35]. Another useful technique SIGEX (substrate-induced gene expression screening) is further used to select catabolic active genes, induced by various substrates using green fluorescent protein (GFP), in concert with fluorescence activated cell sorting [36]. Implementation of such screening method will provide comprehensive functional information about the unknown molecules in shorter timescale.

Table1: Vectors used for construction of metagenomic library and insert size

Different vectors used in library construction cloned	Fragment size to be
Plasmid vectors (pUC, pBR322)	1-9kb
Fosmid and Cosmid vector	35-40kb
Phagemid vector	7-20kb
BAC vector	80-120kb
YAC vector	200-800kb

1.3. PCR based cloning

Polymerase chain reaction (PCR) is another useful technique that is utilized for exploring the genomic diversity of uncultured microbes. PCR allows highly selective amplification of target DNA under optimized conditions. Here, we would like to discuss some of the important genes cloned by PCR e.g. two β-ketoacyl genes were cloned from environmental DNA by PCR amplification [37], by designing oligonucleotides against conserved domain of known keto synthase and acyl carrier protein encoding genes. In another study from East China Sea, PCR

was utilized successfully for isolation of new polyketide synthase gene, by designing primer against two most conserved motifs, DPQQR and HGTGT [38]. These polyketide synthases (PKS) are multifunctional enzymes and catalyzes the formation of a polyketide assembled from sequential condensation of short chain acyl coenzymes. Some of them possess antitumor or antibiotic activity. Another modified PCR technique followed by genome walking, resulted in the isolation of two complete genes of 2, 5,-diketo-acid reductase. The gene product obtained showed valuable properties like lower k_m and high thermostability, as compared to earlier reported genes [39]. Recently, an inverse PCR technique (I-PCR) technique followed by pre-amplified I-PCR (PAI-PCR) was performed, to obtain two novel full length xylanases genes from digestive tract of horse, which displayed ~49-64% amino acid sequence similarities to the known xylanases [40]. A novel cytochrome b5 gene was amplified and cloned from a metagenome sample, by designing degenerate primers from conserved motifs of cyt b5 [41]. Additionally, taking the advantage of most conserved regions of the lipase genes i.e. catalytic triad and oxyanion hole, a novel lipase gene was amplified and cloned, that showed ~20% homology to the existing gene [42]. PCR technique has also been utilized to explore the functional diversity of chitinase genes in unculturable marine bacteria [43].

2. Application of metagenomic gene cloning

2.1. Discovery of novel biocatalysts/molecules from different environment niches

The range of the organic reactions performed by the biocatalysts is enormous; therefore identification of suitable enzyme candidate is very much important. Among different class of enzymes, the most exploited enzyme class belongs to hydrolases.Using metagenomic approach, many novel genes encoding enzymes and antibiotic molecules had been reported. Here, we would like to discuss some of the novel lipases with potential application in industries. Recently, a careful selection of metagenome sample resulted in detection of 2661 lipolytic clones, which shared very less homology (~40-60%) with the earlier reported lipases/estereases. These lipases showed high specific activity against long chain triacylglycerols [44]. Recently, a metagenomic library constructed from Antarctic soil resulted in number of hypothetical, putative and novel cold adaptive functional enzymes, and many other mobile genetic elements [45]. In another study, a metagenomic library screened, resulted in ~350 novel lipases and esterases from environmental DNA samples, which showed high affinity for the synthesis of 1, 2-Oleoyl-3-palmitoyl-sn-glycerol (OOP) and 1, 3-Oleoyl-2-palmitoyl-sn-glycerol (OPO) [46]. Lipase plays an important role in biosynthetic reaction, a metagenomic derived lipase that showed its promising application in hydrolyzing stereo-selectively ibuprofen-*p*NP ester, with a high preference for the (*R*) enantiomer of >91% ee, was discovered from forest soil [47]. The discovered lipase showed ~ 90% identity with the enzymes from *P. fluorescens*LS107d2, B52 or *Pseudomonas* sp. KB700A.Table 2 will further summarize diverse class of novel molecules and biocatalysts reported from various metagenome sources. From Table 2 it is evident that these enzymes are novel and shared very less homology with the existing enzymes.

Table 2: Diverse class of biocatalysts and novel molecules from various source of metagenome

Source of MetagenomeGene	Name of Enzyme	% Homology with the previous	Reference
Deep sea sediments	Lipase/esterases	33-58%	[48]
Mangrove sediment	Lipase	25-52%	[6]
Deep golden mine	Esterases	55%	[49]
Active sludge	Lipase	52-71%	[50]
Oil contaminated soil	Llipase	90-98%	[51]
Bovine rumen microflora	Polyphenol oxidases	42-69	[52]
Deep sea environment	Aminotransferase	≥45	[53]
Forest soil	Antifungal	24-90	[54]
Hot spring	Cyclomaltodextrinase45-85		[55]
Human gut	Xylanases	53	[56]
Lake	Cellulase and esterase	68-95	[57]
Pacific deep-sea sediment	Alkane hydroxylases	56-72	[58]
Acid mine drainage	Ni resistance gene	36-74	[59]
Sludge	Aromatic degrading enzyme	51-99	[60]
Soil	Amylase	47-78	[61]
Soil	2-Deoxy-*scyllo*-inosose (DOI) synthase	97	[62]
hot spring	Neopullulanase	45-48	[63]
Tidal flat	Lipase	34	[64]
Compost Soil	RNase H	40–72%	[65]
Mangrove soil	Laccase	42-52%	[66]
Gobi and Death Valley Deserts	Serine protease	53%	[67]
Antarctic coastal sediment	Protease	41-51	[68]
Antarctic soil	Carboxymethylcellulase	47%	[69]
Marine sponge goat skin surface	DNA polymerase	53	[70]
metagenome	Serine protease	98	[71]
Glacier Soil	Lipase	51-82%	[72]
pitcher fluid of the carnivorous plant Nepenthes hybrid	Lipase	32-41	[73]
Metagenome	Mettaloproteases	49-92	[74]
Rumenof Chinese Holstein	Glycoside Hydrolases	35	[75]
Soil	Xylanase	56-71%	[76]
yak rumen	Xylanase/endoglucanase	37-72	[77]
cow manure	Xylanases	45	[78]
soil	Turbomycin A and B	47-97	[79]
Soil	Aminotransferase	68-96	[80]

2.2. Exploring genomic diversity from various extreme environment niches

Genes in extreme environment are mostly related to catabolism, transport and degradation of complex organic molecules, and therefore provide valuable information about the abundance of cooperation, source of energy and aggregation among the microorganisms. Recent study from deep Mediterranean region has revealed presence of diverse class of metabolic genes that included lux, dehydrogenases and cox genes. Presence of these quorum sensing genes in deep Mediterranean environment, at least suggest existence of abundance of co-operation between different organisms, for shelter or food [81]. Furthermore, most abundant marine proteobacteria SAR 11 reported from Sargasso Sea revealed ~71% identity (at amino acid level) to earlier reported genome, and further observed a significant evolutionary divergence between the coastal isolates and Sargasso Sea populations. Additionally, this study has documented that genome rearrangements in SAR11 are not random but are concentrated at a particular site, which are often operon boundaries [82]. Interestingly, largest metagenomic study from one of such extreme environment has acquired 17 million new ORF, which has significantly changed the landscape of current protein space [83]. Furthermore, there are many *Archaeal*groups which are able to survive in most extreme environments, i.e., deep in sea, at temperatures, over 100ºC, hot springs, and in extremely alkaline or acid waters. However, little is known about their physiology and biochemistry and therefore it provides an opportunity for the researchers, to study such aspects. In this context, several metagenomic studies from different extreme environment (springs) has revealed the presence of many bacterial groups involved in anoxygenic type of photosynthesis, sulfur reduction, anaerobic fermentation, ammonia oxidation, viruses and many novel delta-proteobacterial groups [84-87]. Additionally, a unique kind of DNA polymerase has been characterized from uncultivated psychrophillicarcheon*Canarchaeumsymbiosum*, which lives in specific association with marine sponge. This DNA polymerase gene of *Canarchaeumsymbiosum* exhibits greatest specific activity towards gapped duplex DNA, and towards single stranded DNA, as a substrate with 3'-5' exonuclease and minor 5'-3'exonuclease activity [70] (Schleper et al. 1997).

2.3. Deciphering role of uncultivable microorganism present in human

Metagenome studies from humans is generally focused on structure and dynamic co-operations of microbial communities with human cells, and provide valuable insights into improvement of nutrition, drug discovery and preventative medicine. Recently, culture independent comparisons for mice and humans have revealed that despite sharing common bacterial phyla, most genera and species found in mice were not seen in humans. This study has further suggested the possible role of these microbial communities in producing many digestive disorders, skin diseases, gum diseases and even obesity [88]. Yet, in another study, ametagenomic library prepared from fecal microbiota demonstrated that BSH (bile salt hydrolyzing) activity is a conserved microbial adaptation to human gut environment, with a high level of redundancy in this ecosystem. Phylogenetic analysis illustrated that there must be some selective pressure in form of conjugated bile acid, which has resulted in evolution of abundance of BSH related microorganisms in human gut flora [89]. Metagenomic study will further allow the evaluation of surface marker involved in the interactions of microbes with the

eukaryotic cells [90]. Additionally, viral community i.e. phages present in human gut has huge impact on bacterial microflora. In one such study, a viral community analyzed from DNA of human feces resulted in ~ 1200 viral genotypes with great variation in the bacteriophages present in human feces and another environment [91]. Metagenomic study from human has further established role of microorganism in inflammatory bowel disease [92].

2.4. Depicting role of uncultured microorganisms in recycling of nutrients

Microorganisms play an important role in recycling of nutrients to the environment. Advancement made in molecular biology have demonstrated significant role of uncultured microorganisms in maintaining ecology. Recent study has demonstrated role of microbial associationsin anaerobic cycling of carbon [93]. Therefore, there is a pressing need to explore these associations, to better understand the microbial processes. Newly introduced techniques, like shot gun sequencing and clone free pyrosequencing provides valuable insight into the metabolic potential of such microorganisms [94, 95]. Additionally, microorganisms play an important role by acting as a sink for various chemical reactions along with biogeochemical cycling of sulphur, phosphorous and iron. Previously, such processes were reported to occur abiotically [96]. Microbial activities play an important role in maintaining the biogeochemistry of the planet, in this regard, a metagenomic profiling of 45 microbiomes and 42 viromessuggest their repository role in storing and sharing genes among their microbial hosts, and influences the global evolutionary and metabolic processes [97].Using metagenomic approach certain microorganisms have been studied which hold the capability to use selenite as a terminal electron acceptor, by a process, known as dissimilatory reduction of selenate (DSeR) [98]. Yet another study reported from three water bodies showed anaerobic bioconversion of selenium in such environment [99].

CONCLUSION

Conclusions and future directions

Metagenomics can provide the tools to balance the abundance of knowledge attained from culturing, with an understanding of the uncultured majority of microbial life. Metagenomics may further increase our understanding of many of the exotic and familiar habitats that are attracting the attention of microbial ecologists. It includes deep sea thermal vents; acidic hot springs; permafrost; temperate; desert; cold soils; Antarctic frozen lakes and eukaryotic host organs. However, a number of barriers have limited the discovery of new genes that provide insight into microbial community structure and function, and can be used to solve medical, agricultural, or industrial problems. Furthermore, most DNA extraction methods have been tested on a limited number of soil types, so their general applicability is unknown for comparative ecological studies. Additionally, given the profound utility and importance of microorganisms to all biological systems, methods are needed to access the wealth of information within the metagenome. Finally, it will expand and continue to enrich our knowledge about unexplored microorganisms and their wide applications.

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REFERENCES

[1]   Alain K, Querellou J, Lesongeur F et al. Int J SystEvolMicrobiol 2002; 52: 1317-132.
[2]   Novikova N, Boever PD, Poddubko S et al. Res Microbiol 2006; 157:5-12.
[3]   Robe P, Nalin R, Capellano C et al. Eur J Soil Biol 2003; 39: 183-190.
[4]   Amann RI, Ludwig W, Schleifer KH. Microbiol Rev 1995; 59: 143-169.
[5]   Handelsman J, Rondon MR, Brady SF, Clardy J, Goodman RM. ChemBiol 1998; 5:245-249.
[6]   Couto GH, Glogauer A, Faoro H et al.Gen Mol Res 2010; 9: 514-523.
[7]   Pace NR A. Science 1997; 276: 734-740.
[8]   Lagesen K et al.Nucleic Acids Res 2007; 35: 3100–3108.
[9]   Fuhrman JA, McCallum K, Davis AA. Appl Environ Microbiol 1993; 59:1294-1302.
[10]   Kaeberlein T, Lewis K, Epstein SS. Sci. 2002; 296: 1127-1129.
[11]   Daniel R.CurrOpinBiotechnol2004; 15:199-204.
[12]   Lorenz P, Liebeton K, Niehaus F, Eck J. CurrOpinBiotechnol 2002; 13: 572-577.
[13]   Leininger S, Urich T, Schloter M et al. Nat 2006; 442: 806–809.
[14]   Michael K, Alfred B, Anke M et al. SystApplMicrobiol2005; 287: 287–294.
[15]   Tringe SG, Rubin EM. Nat Rev Genet 2005; 6:805–814.
[16]   Gans J, Wolinsky M, Dunbar J. Sci. 2005; 309: 1387–1390.
[17]   Hong SH Bunge J, Jeon SO, Epstein SS. ProcNatlAcadSci USA 2006; 103:117–122.
[18]   Pace NR, Stahl DA, Lane DJ, Olsen GJ. AdvMicrobiol Eco 1986; 9: 1–55.
[19]   Fierer N, Breitbart M, Nulton J et al. Appl Environ Microbiol 2007; 73: 7059–7066.
[20]   20. Hallam SJ, Konstantinidis KT, Putnam N, Schleper C et al.Proc Nat AcadSci USA 2006; 103: 18296–18301.
[21]   Ludwig W, Schleifer KH. FEMS Microbiol Rev 1994; 15: 155–173.
[22]   Meyer F, Paarmann D, Souza MD.BMC Bioinformatics 2008; 9: 386.
[23]   Huang Y, Gilna P and Li W. Bioinformatics 2009; 25: 1338-1340.
[24]   López-Archilla AI, Gerard E, Moreira D,López-Garcia P FEMS MicrobiolLett2004;235: 221-228.
[25]   Grenni P, Gibello A, Caracciolo AB et al.Water Res2009;43:2999-3008.
[26]   Hess et al. Sci 2011; 331: 463-467.
[27]   Zhou J, Bruns MA, Tiedje JM. Appl Environ Microbiol1996; 62: 316-322.
[28]   Lakay FM, Botha A, Prior BA. J ApplMicrobiol 2006; 100: 1365-1373.
[29]   Sharma PK, Capalash N, Kaur J. MolBiotechnol2007; 36: 61-3.
[30]   Beja O, Spudich EN, Spudich JL et alProteorhodopsinphototrophy in the ocean. Nature 2001; 411:786–789.
[31]   Meng J, Wang F, Wang F, Zheng Y et al.ISME J 2009; 3: 106–116.
[32]   Zhou J. CurrOpinMicrobiol 2003; 6: 288–294.
[33]   LehnerA,Loy A,BehrT,GaengeH et al.FEMS MicrobiolLett2005;246: 133–142.

[34]   Wu Z, Irizarry RA, Gentleman R, Murillo FM, Spencer F. J Am Stat Assoc 2004; 99: 909–917.
[35]   Wu C, Sun B.J MicrobiolBiotechnol2009; 19:187-193.
[36]   Manefield M, Whiteley AS, Griffiths RI, Bailey MJ. Appl Environ Microbiol 2002; 68: 5367–5373.
[37]   Uchiyama T, Abe T, Ikemura T, Watanabe K. Nat Biotechnol2005; 23: 88-93.
[38]   Seow KT, Meurer G, Gerlitz M et al. J Bacteriol 1997; 179:7360–7368.
[39]   Jiao YL, Wang LH, Dong XY et al. ApplBiochemBiotechnol2008;149: 67-78.
[40]   Eschenfeldt WH, Stols L, Rosenbaum H. et al. Appl Environ Microbiol 2001; 67: 4206–4214.
[41]   Yamada K, Terahara T, Kurata S, Yokomaku T, Tsuneda S, Harayama S.Environ Microbiol 2008; 10: 978-987.
[42]   Roh C, VillatteF,Kim BG,Schmid RD. LettApplMicrobiol2003; 44: 475-480.
[43]   Bell PJL, Sunna A, Gibbs MD et al (2002) Prospecting for novel lipase genes using PCR. Microbiol 148: 2283-2291.
[44]   Arnaldo G, Viviane PM, Helisson F et al. Microbial Cell Fact 2011; 10:54.
[45]   Renaud B, Delphine P, Maud D et al Revista Argentina de Microbiología 2011;43: 94-103.
[46]   Mark B, Petra H, David PW, Jesal SP, Flash B, Timothy SH, Uwe TB. JAOCS 2008; 85:47–53.
[47]   Elend C, Schmeisser C, Hoebenreich H, Steele HL, Streit WR. J Biotechnol 2007; 130: 370-77.
[48]   Jeon JH, Kim TJ, Lee HS et al. Evidence-Based complementary and alternative medicine 2011; doi:101155/2011/271419
[49]   Abbai NS, Heerden EV, Piater LA, D Litthauer D African J Biotechnol2011; 10: 6090-6100.
[50]   Gang LJ, Gui ZK, Jun, HW et al. Microbial Cell Fact 2010; 9: 83.
[51]   Zuo K, Zhang L, Yao H, Wang J. ActaBiochmi Polin 2010;57: 305–311.
[52]   Beloqui A, Pita M, Polaina J et al. J BiolChem 2006; 281:22933-22942.
[53]   Aoki R, Nagaya A, Arakawa S, Kato C, Tamegai H. BiosciBiotechnolBiochem 2008; 72: 1388-1393.
[54]   Chung EJ, Lim HK, Kim JC et al. Appl Environ Microbiol2008; 74: 723-730.
[55]   Tang K, Utairungsee T, Kanokratana P et al.FEMS MicrobiolLett 2006; 260:91-99.
[56]   Hayashi H, Abe T, Sakamoto M et al. Can J Microbiol2005; 51: 251-259.
[57]   Rees HC, Grant S, Jones B, Grant WD, Heaphy S.Extremophiles 2003;7: 415-421.
[58]   Meixiang X, Xiao X, Wang F. Extermophiles 2007; 12: 255-262.
[59]   Mirete S, Figueras CG, Pastor JEG. Appl Environ Microbiol2007; 73: 6001-6011.
[60]   Suenaga H, Ohnuki T, Miyazaki K. Environ Microbiol2007; 9: 2289-2297.
[61]   Yun J, Kang S, Park S, Yoon H, Kim MJ, Heu S et al. Appl Environ Microbiol2004; 70: 7229-7235.
[62]   Tamegai H, Nango E, Koike-Takeshita A, Kudo F, Kakinuma K. BiosciBiotechnolBiochem 2002; 66:1538-1545.
[63]   Tang K, Kobayashi RS, Champreda V, Eurwilaichitr L, Tanapongpipat S. BiosciBiotechnolBiochem 2008; 72: 1448-1456.

[64]   Kim EY, Oh KH, Lee MH, Kang CH, Oh TK, Yoon JH. Appl Environ Microbiol 2009; 75: 257–260.
[65]   Kanaya E, Sakabe T, Nguyen NT et al.J ApplMicrobiol2010;109:974–983.
[66]   Ye M, Gang Li, Liang WQ, Liu YH. ApplMicrobiolBiotechnol2011; 87: 1023-1031.
[67]   Neveu J, Regeard C, DuBow MS. ApplMicrobiolBiotechnol 2011; 91: 635–644.
[68]   Zhang Y, Jing Zhao J, Zeng R. Extremophiles 2011; 15: 23–29.
[69]   Berlemont R, Delsaute M, Pipers D et al. The ISME Journal 2009; 3: 1070–1081.
[70]   Schleper C, Swanson RV, Mathur EJ, Delong EF.J Bacteri 1997; 179: 7803–7811.
[71]   Pushpam PL, Rajesh T, Pushpam PG et al. AMB Express 2011; 1:3.
[72]   Zhang Y, Shi P, Liu W, Meng K, Bai1 Y, Wang G, Zhan Z, Yao. J MicrobiolBiotechnol 2009; 19: 88-897.
[73]   Morohoshi T, Oikawa M, Sato S et al. J Biosci and Bioengi 2011; 112: 315–320.
[74]   Waschkowitz T, Rockstroh S, Daniel R. Appl Environ Microbiol2009; 75:2506-2516.
[75]   Hu Y, Zhang G, Li A, Chen J, Ma L. ApplMicrobiolBiotechnol 2008; 80: 823–830.
[76]   Zhao S, Bu D, Wang J, Liu K, Zhu Y, Dong Z, Yu Z.Appl Environ Microbiol2010; 76: 6701-6705.
[77]   Chang L, Ding M, Bao L et al. ApplMicrobiolBiotechnol 2011; 90: 1933–1942.
[78]   Li R, Kibblewhite R, Orts WJ, Charles C Lee CC. World J MicrobiolBiotechnol 2009; 25:2071–2078.
[79]   Gillespie DE, Brady SF, Bettermann AD et al. Appl Environ Microbiol 2002; 68: 4301-4306.
[80]   Nagaya A, Takeyama S, Tamegai H. BiosciBiotechnolBiochem 2005; 69: 1389-1393.
[81]   Cuadrado ABM, Garcia PL, Alba JC et al. PLoS One 2007; 2: e914.
[82]   Wilhelm LJ, Tripp HJ, Givan SA, Smith DP, Giovannoni SJ. Biol Direct 2007; 2: 27.
[83]   Weizhong L, Adam G. Bioinformatics 2006; 22: 887-888.
[84]   Mostafa S, Najar FZ, Aycock M. et al. Appl Environ Microbiol2005; 71: 7598-7602.
[85]   Cavicchioli R, DeMaere MZ, Thomas T. Bioassays 2006; 29:11-14.
[86]   Schoenfeld T, Patterson M, Richardson PM et al.Appl Environ Microbiol2008; 74: 4164-4174.
[87]   Moreira D, Valera FR, Garcia PL.Microbiol2006; 152: 505-517.
[88]   Lay RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. ProcNatlAcadSci USA 2005; 102: 11070–11075.
[89]   Jones BV, Begley M, Hill C, Gahan CGM,Marchesi JR. ProcNatlAcadSci USA2008; 105: 13580-13585.
[90]   Marcy Y, Ouverney C, Bik EM et al.ProcNatlAcadSci USA 2007;104: 11889-11894.
[91]   Breitbart M, Hewson I, Felts B et al. J Bacteriol2003; 185:6220-6223.
[92]   Manichanh C,Rigottier-GoisL, Bonnaud E et al. Gut 2006; 55: 205-211.
[93]   SchinkB. Antonie van Leeuwenhoek 2002;81: 257-261.
[94]   Tringe SG,Mering CV, Kobayashi A et al. Sci2005; 308: 554-557.
[95]   Biddle JF, Gibbon S, Schuster SC et al.ProcNatlAcad Sci. 2008; 105:10583-10588.
[96]   Hinrichs KU, Hayes JM, Bach W et al. ProcNatlAcadSci USA 2006; 103: 14684-14689.
[97]   Dinsdale EA, Edwards RA, Hall D et al. Nat 2008; 452: 629-632.
[98]   Nisan AS, Oremland RS. Appl Environ Microbiol 1990; 56:3550-3557.
[99]   Narasingarao P, Haggblom MM. Appl Environ Microbiol 2007; 73: 3519-3527.