Eco. Env. & Cons. 27 (November Suppl. Issue) : 2021; pp. (S204-S209)
Copyright@ EM International
ISSN 0971–765X

Metagenomics technique as new source for antimicrobial agent production

Mohamed T. Shaaban[1], Reham M. Abdelhamid[1,2], Muhammad Zayed[1] and Safaa M. Ali[3*]

[1]Botany and Microbiology Department, Menoufia University, Shebin El-Kom, Egypt
[2]Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications
[3]Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City of Scientific Research and Technological Applications, Alexandria, Egypt

(Received 6 March, 2021; Accepted 17 May, 2021)

ABSTRACT

The innovation of antimicrobial agent with elevated effectiveness in conditions agreeable to industrial processes necessities are key to successful development of chemical and drug synthesis. The rate of detection for novel biomolecules using conventional method has been really low down, as the majority of soil microorganisms cannot be cultivated by this means. The objective of the current study was to recognize of separated utilitarian gene(s) as antimicrobial specialist by utilizing metagenomics. A wealthy source of new natural resources for research is soil metagenomes. The process followed by collection of different locations in marine sediment samples from Alexandria and isolation of total DNA, fragmentation by restriction enzymes (*HindIII, BamHI, EcoRI* and *SalI*), cloning, expression of the isolated gene(s) in *E.coli* DH5α. The digested fragments were ligated and cloned into pUC_{19} vector, then transformed into *E.coli* DH5α. The 270 resulted clones were screened for plasmid with high copy numbers, only 253 clones contains plasmid. To detect the fragment size of the series of PCR products through using a universal primer (M13). Only the 131 clones which contain fragment (200-3000 bps) were selected for screening of antimicrobial agents. Only 13 clones were positive for antimicrobial agent by using diffusion method.Activity of the antimicrobial agents produced by 13 positive clones were tested towards *Bacillus, Escherichia coli* and *Candida albican*, ampicillin and tetracycline (100 mg/ml) were used as standard for comparison. Our data also proved that some of genetically modified clone produce wide spectrum antibiotic and are more effective than ampicillin and tetracycline.

Key words : *Metagenomics, DNA, Antimicrobial agents, Cloning, Restriction enzyme, Characterization*

Introduction

Anti-microbials are one of the most critical munitions in the fight against microbial infection and have benefited significantly since their session of the health-related health of humans. Mitochondrion can also be damaged by some antibiotics (Marchant, 2018). Before agreement for medical use, antibiotics are tested for any harmful properties and are commonly measured safe and well accepted (Slama *et al.*, 2005). Several antibacterial variations of below 1000 Daltons in particle size are very small particles (Dougherty and Pucci, 2011).

In a genetically competition with special, hurtful and varying environments, marine microorganisms survive (Lozupone and Knight, 2007). Marine microorganisms produce a vast variety of secondary

metabolites which could be used to supply the starved pharmaceutical market (Farnet and Zazopoulos, 2005). Over majority of the documented medical, anti-viral, anti-microbial or anti-tumor products are bacterial in origin (Berdy, 2005; Rath *et al*., 2011). Antinematodal (Donia and Hamann, 2003), anti-inflammatory (Strangman, 2007), anti-parasitic (Abdel-Mageed *et al*., 2010), and neural (Abdel-Mageed *et al.,* 2010) are other classes (Sudek *et al*., 2007). A chemo informatics study showed that 71% of the marine natural products were not represented in terrestrial natural products, and that 53% have been found only once (Montaser and Luesch, 2011). Supplementary tests investigating the spread of natural products in the chemical space have clearly demonstrated that the widest distribution of marine biomolecules contains different medication environments (Tao *et al*., 2015).

Functional genomics is used to find complex events without the need to develop the cells that hold the pathways involved. As an unused item, Metagenomics has grown as a position area and technique to prevent genomic variations in most species and unculturability, the main essential barricades to developments in clinical and natural microbiology (National Research Council, 2007). The metagenomics discipline represents the study of population genomics at the level of microorganisms, referring to the notion that a collection of genes from a given setting is evaluated during a genome-equivalent process, contributing a broad lens to the microbial realm that is likely to change clinical sciences (Marchesi, 2012). Metagenomics sequencing has also been used to classify root-related species (Bulgarelli *et al*., 2013) for identifiable evidence of taxa associated with the human intestine (Morgan *et al.,* 2012). Side effects can indicate the antibiotic's pharmacological or toxicological properties or may include allergic reactions or hypersensitivity (Rollins *et al*., 2016). The use of antibiotics varies greatly between countries. The WHO antibiotic use monitoring study released in 2018 examined data for 2015 from 65 countries. The WHO study finds that UK antibiotic use is double that of the Netherlands 2018. Not only did these early project explain the concept of the metagenomics method, but it has revealed an immense diversity of functioning genes in the microbial environment around us. Cloning and expression of the antimicrobial agent gene has been documented in related work using built DNA derived from uncultured marine-related species.

Materials and Methods

Sample collection and preparation

The marine dregs tests were collected from distinctive area of Mediterranean Sea in Alexandria, Egypt, transported to the research facility, put away in cold room till examined.

Chromosomal DNA preparation

The genomic DNA which used in the study was isolated by a modified method of specific kit for DNA isolation from soil (QIAGEN).

DNA restriction digestion

Chromosomal DNA was digested with restriction enzymes (*Bam HI, Eco RI, HindIII, PVUI* and *Xba1*) under the reaction conditions of each one, as recommended by the companies (Fermentas).

Cloning and transformation

DNA ligation was made at that point (Processed DNA). T_4 DNA ligase (fermentase) was used for ligation between DNA and vector (pUC_{19}). The reaction was conducted overnight at 16^0C. Screening and expressed vector were scattered on specific X-Gal and IPTG-containing LB plates.

Preparation of plasmid-DNA from transforming clones

White clone (positive results of transformation) were selected for plasmid extraction. The GEBRI kit directed mini-plasmid extraction as follows: cells (1.5 ml) from overnight culture were extracted. The supernatant containing the plasmid DNA was accelerated with isopropanol and suspended in 30µl of water with 70% (v/v) ethanol at that stage.

Screening of clones carrying gene

PCR product using universal primer of pUC_{19} (M13 F: AGGCCCTGCACCTGAAG, M13 R: TCAGCGCCTGGTACC, Safaa and Nadia, 2019) were take place. At 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, the PCR was conducted for 30 cycles. Using agarose gel electrophoresis, a fraction of the PCR mixture was analyzed after completion. Molecular weight of PCR product were determined using program of gel documentation system (InGenius LHR).

Screening of antimicrobial agents

For qualitative estimation of antimicrobial agents,

pure clones which give positive PCR (200 bp-3000 bp) were screened for antimicrobial activity in NA (IPTG) inoculated with *E. coli* Dh5α using cork poorer methods. The appearance of a halo zone indicates the production of antimicrobial agents. The disk diffusion (the zone of inhibition) method is possibly the majority widely old method for the reason that, its ease and low cost. It used only small amounts of the product. The technique involves the preparation of a Petri dish containing agar medium, bacteria at a recognized concentration are stretch across the medium.

Quantitative estimation of antimicrobial activity for positive isolates was performed using cell extract against microbes and measuring inhibition zone comparing with Ampicillin and Tetracycline as reference antibiotics.

Description of Antimicrobial agent

To detect the effect of antimicrobial agents produced by each clone, ampicillin and tetracycline (100mg) were used as standard for comparison. Different categories of microorganism were used to detect the effect of each clone. *Bacillus*, *Escherichia coli* and *Candida albican* were used for detection of antimicrobial studies. All microorganism were inoculated into the LB liquid plus IPTG (inducer of antimicrobial agents).

Residual activity of the antimicrobial agents after preservation in room temperature

Preservation of antimicrobial agents were detected by standing the product at 25 ^{0}C for 1 week under aseptic condition. Activity of antimicrobial agents were detected by using *E. coli* $Dh_5\alpha$ and comparing with ampicillin freshly prepared as reference.

Results

DNA extraction

The genomic DNA from marine sediment was extracted using specific kit for DNA isolation from soil (QIAGEN). Concentration of DNA (1231 ng/µl) and purity (1.81) were measured using nano Biodrop.

DNA restriction digestion, cloning and transformation

Chromosomal DNA was digested using different restriction enzymes were used. 270 clones were obtained from DNA digested and ligated to pUC_{19} which was digested with the same restriction enzyme (white clones).

Plasmid-DNA from transforming clones

White clone (positive results of transformation) were selected for plasmid extraction. From 270 clones, only 253 clones carry the true plasmid as presented in Fig 1.

Screening of clones carrying gene

Clones carrying plasmid (253) were selected to detect the fragment size inserted into puc_{19} vector. PCR products using universal primer (M13) were take place and agarose gel electrophoresis used for detection of PCR fragment and analyzed after completion to detect molecular weight of fragments (Fig. 2). Only the 131 clones which contain fragment (200-3000 bps) were selected for screening of antimicrobial agents.

Screening of antimicrobial agents

For screening of antimicrobial agents, clones carry fragment (200-3000bp) were screened for antimicrobial activity against *E. coli* Dh5α using cork poorer methods. 131 clones were tested for antimicrobial agents. 13 clones were positive for antimicrobial agent were (clear zone were detected after sonication of 5 ml cell) as appears in Fig. 3.

Description of Antimicrobial agents

Activity of the antimicrobial agents produced by 13 positive clones were tested towards *Bacillus,*

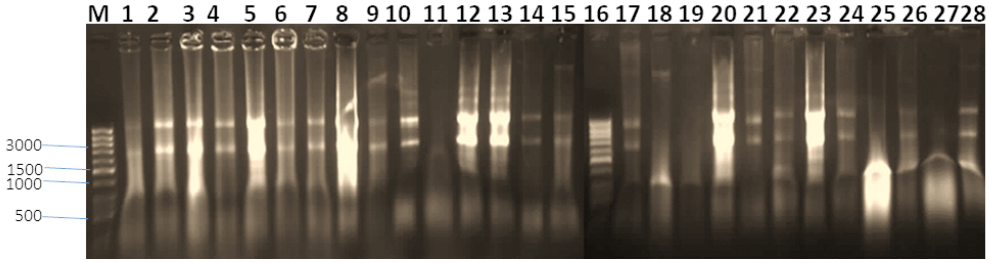

Fig. 1. Plasmid DNA profiles of tested clones using GIBRI kit. M: DNA ladder

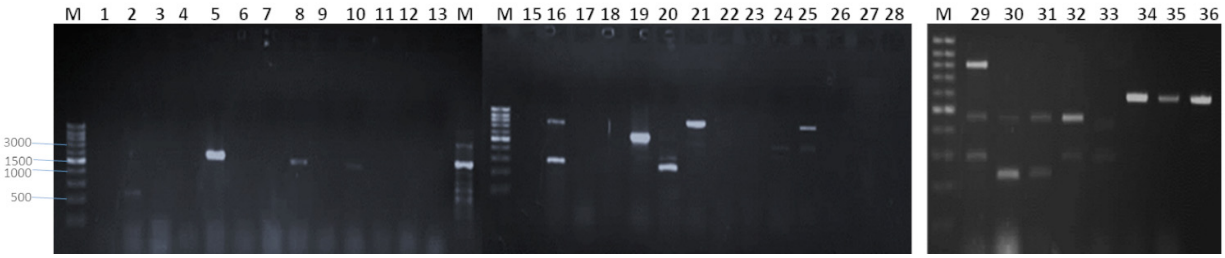

Fig. 2. PCR products of M13 using miniprep plasmid from clone having plasmid as a template

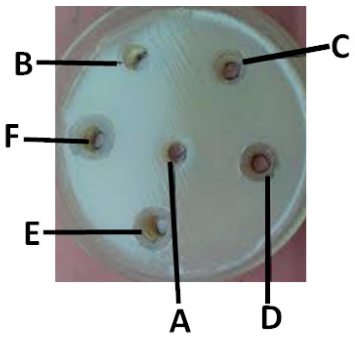

Fig. 3. Diffusion method (using *Escherichia coli*) for intracellular extract to detect antimicrobial activity. A, B: clones give negative activity. C, D, E and F: clones have antimicrobial activity (positive).

Escherichia coli and *Candida albican*. Ampicillin and Tetracycline (100 mg) were used as standard for comparison. Clear zone around tested antimicrobial agents were measured by using disk diffusion method where disk of filter paper were saturated with the tested material (Table 1 and Fig. 4). Supernatant of sonicated pellet from 20 ml of culture were applied after suspensions of pellet in 2 ml of phoshphate buffer pH7. The inhibition zone surrounding the disk was calculated and compared with zones for ampicillin and tetracycline.

Residual activity of the antimicrobial agents after preservation

Preservation of antimicrobial agents from clones (3 and 9) were detected for 1 week under aseptic condition at 30 ^{0}C. Each day, sample activity of the tested products were examined towards *E. coli*. Activity of antimicrobial agents were comparing with ampicillin prepared as reference of antibiotic. Remain activity of antimicrobial agent produced by clones no. 3 showed its stability than the others (Fig. 5).

Table 1. Antimicrobial activity of tested clones

Test	Clear zone (mm)		
	Bacillus	*Escherichia coli*	*Candida albican*
Clone 1	0.8	0.7	-
Clone 2	0.7	0.6	-
Clone 3	1.9	2	0.9
Clone 4	1	1.1	-
Clone 5	1.3	1.2	0.6
Clone 6	0.8	0.9	-
Clone 7	1.1	1.3	-
Clone 8	0.9	0.6	0.7
Clone 9	1.1	0.9	1.3
Clone 10	0.8	0.7	-
Clone 11	0.7	0.6	-
Clone 12	0.6	0.7	-
Clone 13	1	0.9	0.9
Ampicillin	1.5	1.6	0.7
Tetracycline	1.9	2.2	1.8

Discussion

By analysing sequence data obtained by pyrosequencing and/or Sanger sequence analysis of

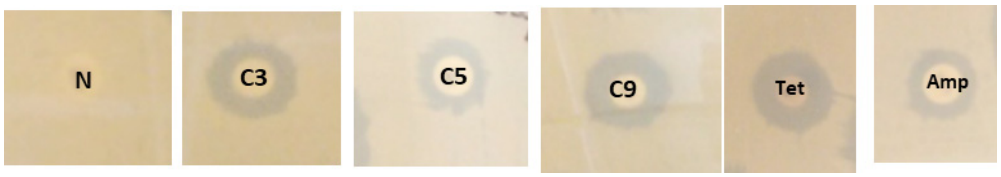

Fig. 4. Disk diffusion method (against *Escherichia coli*) for intracellular extract of clone 3, 5 and 9 (highly positive clones) to detect antimicrobial activity and comparing with AMP: ampicillin and Tet: tetracycline. N: disk with LB medium.

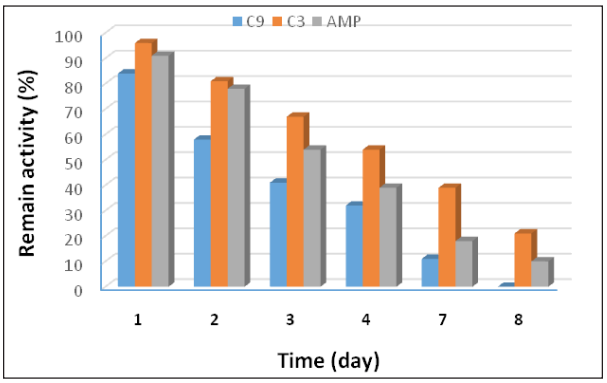

Fig. 5. Residual activity of the antimicrobial agents after preservation at 30 ^{0}C. C9: antimicrobial agent from clone 9, C3: antimicrobial agent from clone 3 and AMP: Ampicillin against *Escherichia coli.*

the present metagenomics DNA, numerous other ecosystems have been taxonomically identified. Deep sea sediments (Hallam *et al.,* 2004), including Antarctic polar Front samples collected at 500 m depth (Moreira *et al*., 2004; Moreira *et al.,* 2006), Polar Greenland Sea (Zaballos *et al.,* 2006), an acid mine biofilm (Tyson *et al.,* 2004), honey bee colonies (Cox-Foster *et al*., 2007), glacial ice (Simon *et al.,* 2009), and the Peru Margin subsea floor (Biddle *et al.,* 2008) were used for metagenomics study. The following sources are some marine related microbial examples: alkaloids (Abdelmohsen *et al*., 2012); terpenoids (Solanki *et al*., 2008); polyketides (Harunari *et al*., 2014), peptides (Chopra *et al*., 2014); and hybrids (Chopra *et al.,* 2014; Blunt *et al.,* 2015). Semi-synthetic variations of multiple natural compounds are the bulk of modern antibacterial. This include, for example, beta-lactam antibiotics, which include penicillin, cephalosporins and carbapenems (produced by fungi of the genus Penicillium). Aminoglycosides are molecules that are still isolated from live organisms, while other antibacterials are produced primarily by chemical synthesis, such as sulfonamides, quinolones, and oxazolidinones (Von Nussbaum *et al.,* 2006).

Conclusion

Using metagenomics application is offers good and fast screening procedure and important to find new antimicrobial agents. Also the method is time saving and maintain convincing information on each component.

Acknowledgements

The authors acknowledge City of Scientific Research and Technological Applications, Alexandria, Egypt for support and facilitating this study.

References

Abdel-Mageed, W. M., Milne, B. F., Wagner, M., Schumacher, M., Sandor, P., Pathom-aree, W. and Ebel, R. 2010. Dermacozines, a new phenazine family from deep-sea dermacocci isolated from a Mariana Trench sediment. *Organic & Biomolecular Chemistry.* 8(10) : 2352-2362.

Abdelmohsen, U. R., Szesny, M., Othman, E. M., Schirmeister, T., Grond, S., Stopper, H. and Hentschel, U. 2012. Antioxidant and anti-protease activities of diazepinomicin from the sponge-associated Micromonospora strain RV115. *Marine Drugs.* 10(10) : 2208-2221.

Berdy, J. 2005. Bioactive microbial metabolites. *The Journal of Antibiotics*. 58 (1) : 1-26.

Biddle, J.F., Fitz-Gibbon, S., Schuster, S. C., Brenchley, J. E. and House, C.H. 2008. Metagenomic signatures of the Peru Margin subseafloor biosphere show a genetically distinct environment. *Proceedings of the National Academy of Sciences.* 105 (30) : 10583-10588.

Blunt, J., Copp, B., Keyzers, R., Munro, M. and Prinsep, M. 2015. Marine natural products. *Nat. Prod. Rep.* 32 : 116-211.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E. V. L. and Schulze-Lefert, P. 2013. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology.* 64 : 807-838.

Chopra, L., Singh, G., Choudhary, V. and Sahoo, D.K. 2014. Sonorensin: an antimicrobial peptide, belonging to the heterocycloanthracin subfamily of bacteriocins, from a new marine isolate, Bacillus sonorensis MT93. *Applied and Environmental Microbiology.* 80 (10) : 2981-2990.

Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J. D., Moran, N.A. and Geiser, D.M. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science.* 318 (5848) : 283-287.

Donia, M. and Hamann, M.T. 2003. Marine natural products and their potential applications as anti-infective agents. The Lancet infectious diseases. 3(6): 338-348.

Dougherty, T.J. and Pucci, M.J. 2011. *Antibiotic Discovery and Development*: Springer Science & Business Media.

Farnet, C. and Zazopoulos, E. 2005. Improving Drug Discovery From Microorganisms in Natural Products: *Drug Discovery and Therapeutic Medicine*, ed. L. Zhang and AL Demain: Humana Press, Totowa, NJ.

Hallam, S. J., Putnam, N., Preston, C. M., Detter, J. C.,

Rokhsar, D., Richardson, P. M. and DeLong, E.F. 2004. Reverse methanogenesis: testing the hypothesis with environmental genomics. *Science.* 305 (5689) : 1457-1462.

Harunari, E., Imada, C., Igarashi, Y., Fukuda, T., Terahara, T. and Kobayashi, T. 2014. Hyaluromycin, a new hyaluronidase inhibitor of polyketide origin from marine Streptomyces sp. *Marine Drugs.* 12 (1) : 491-507.

Lozupone, C. A. and Knight, R. 2007. Global patterns in bacterial diversity. *Proceedings of the National Academy of Sciences.* 104 (27) : 11436-11440.

Marchant, J. 2018. When antibiotics turn toxic. *Nature.* 555 (7697) : 431-433.

Marchesi, J.R. 2012. Metagenomics: current innovations and future trends. *Future Microbiology.* 7 (7) : 813-814.

Montaser, R. and Luesch, H. 2011. Marine natural products: a new wave of drugs? *Future Medicinal Chemistry.* 3 (12) : 1475-1489.

Moreira, D., Rodríguez Valera, F. and López García, P. 2004. Analysis of a genome fragment of a deepsea uncultivated Group II euryarchaeote containing 16S rDNA, a spectinomycin like operon and several energy metabolism genes. *Environmental Microbiology.* 6 (9) : 959-969.

Moreira, D., Rodrýguez-Valera, P. and López-Garcýa, P. 2006. Genome fragments from mesopelagic Antarctic waters reveal a novel deltaproteobacterial group related to the myxobacteria. *Microbiology.* 152 : 505-517.

Morgan, X.C., Tickle, T.L., Sokol, H., Gevers, D., Devaney, K.L., Ward, D.V. and Huttenhower, C. 2012. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biology.* 13 (9) : 1-18.

National Research Council, Committee on the Nutrient Requirements of Small Ruminants, Board on Agriculture, Division on Earth, and Life Studies, 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids.

Rath, C.M., Janto, B., Earl, J., Ahmed, A., Hu, F.Z., Hiller, L. and Wolff, J.J. 2011. Meta-omic characterization of the marine invertebrate microbial consortium that produces the chemotherapeutic natural product ET-743. *ACS Chemical Biology.* 6 (11) : 1244-1256.

Rollins, K.E., Varadhan, K.K., Neal, K.R. and Lobo, D.N. 2016. Antibiotics versus appendicectomy for the treatment of uncomplicated acute appendicitis: an updated meta-analysis of randomised controlled trials. *World Journal of Surgery.* 40 (10) : 2305-2318.

Safaa M. Ali and Nadia A. Soliman, 2019. New source of cellulase production using a metagenomic technique. *Novel Research in Microbiology Journal*. 3 (6).

Slama, T.G., Amin, A., Brunton, S.A., File Jr, T.M., Milkovich, G., Rodvold, K.A. and Appropriate, C. 2005. A clinician's guide to the appropriate and accurate use of antibiotics: the Council for Appropriate and Rational Antibiotic Therapy (CARAT) criteria: Elsevier.

Strangman, W.K. 2007. Anti-inflammatory capabilities of compounds from marine bacteria in a mouse model of allergic inflammation and asthma. UC San Diego.

Sudek, S., Lopanik, N. B., Waggoner, L. E., Hildebrand, M., Anderson, C., Liu, H. and Haygood, M.G. 2007. Identification of the putative bryostatin polyketide synthase gene cluster from "*Candidatus Endobugula sertula*", the uncultivated microbial symbiont of the marine bryozoan Bugula neritina. *Journal of Natural Products.* 70 (1) : 67-74.

Tao, L., Zhu, F., Qin, C., Zhang, C., Chen, S., Zhang, P. and Chen, Z. 2015. Clustered distribution of natural product leads of drugs in the chemical space as influenced by the privileged target-sites. *Scientific Reports.* 5 (1) : 1-10.

Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R. J., Richardson, P. M. and Banfield, J.F. 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature.* 428 (6978) : 37-43.

Simon, C., Wiezer, A., Strittmatter, A. W. and Daniel, R. 2009. Phylogenetic diversity and metabolic potential revealed in a glacier ice metagenome. *Applied and Environmental Microbiology.* 75 (23) : 7519-7526.

Solanki, R., Khanna, M. and Lal, R. 2008. Bioactive compounds from marine actinomycetes. *Indian Journal of Microbiology.* 48 (4) : 410-431.

Von Nussbaum, F., Brands, M., Hinzen, B., Weigand, S. and Häbich, D. 2006. Antibacterial natural products in medicinal chemistryxodus or revival? *Angewandte Chemie International Edition.* 45 (31) : 5072-5129.

Zaballos, M., Lopez-Lopez, A., Ovreas, L., Bartual, S.G., D'Auria, G., Alba, J. C. and Rodríguez-Valera, F. 2006. Comparison of prokaryotic diversity at offshore oceanic locations reveals a different microbiota in the Mediterranean Sea. *FEMS Microbiology Ecology.* 56 (3) : 389-405.