Inosine at Different Primer Positions to Study Structure and Diversity of Prokaryotic Populations

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Abstract
Culture-independent methods, employed to study the diversity and complexity of microbial communities that are based on amplification of rRNA genes with universal primers, include gradient gel electrophoresis (denaturing or temperature), single-strand-conformation polymorphism, restriction fragment length polymorphism, qPCR and high-throughput DNA sequencing. Substituting one or more base(s) within or at the 3'-termi of the universal primers by inosine can overcome some of their shortcomings improving amplification capacity. Universal primer sets do not usually amplify sequences with nucleotide mismatch to the templates, particularly in the last three bases, whereas inosine-modified primers anneal and amplify a variety of rRNA gene sequences. Inosine-containing primers are therefore might be useful to detect more species in diverse prokaryotic populations. The article summarizes the pros and cons of using inosine especially at the 3' termini of universal primers in nucleic acid amplification for the study of microbial diversity.

Introduction
Phylogenetic analysis and study of prokaryotic diversity based on 16S rRNA gene comparison (Woese and Fox, 1977) was enhanced by discovery of the PCR (Weisburg, *et al*., 1991). Culture-independent methods are advantageous to investigate the occurrence and distribution of bacteria in nature, providing direct information on community structure (Daly *et al*., 2000; Steven *et al*., 2007; Su *et al*., 2012). Most of these studies require extraction of total bacterial DNA from the sample and PCR amplification of one of the rRNA genes using universal primers designed according to their conserved sequences. This approach is straightforward, but biased at almost all steps: DNA extraction, primers selection and amplification (Bru *et al*., 2008; Fujiwara *et al*., 1995; Hansen *et al*., 1998; Martin-Laurent *et al*., 2001; Polz and Cavanaugh, 1998; Suzuki and Giovanni, 1996; Wu *et al*., 2009), producing an incomplete and often distorted view (Forney *et al*., 2004) of the community structure.

The choice of universal 16S rRNA gene primers used in studies to assess the diversity of prokaryotes is not trivial becouse (Baker *et al*., 2003; Forney *et al*., 2004): (a) complementarity to a large fraction of the sequences in databases such as the ribosomal database project [RDP; (Cole *et al*., 2009)] are not necessarily optimal; (b) sequences in the database may be incomplete or ambiguous; (c) no current data base faithfully represents the estimated total number of over 10 million bacterial species, with possible high sequence divergence (Curtis *et al*., 2002; Winsley *et al*., 2012). Efforts are therefore being made to improve universality of the primers.

Substituting canonical bases by inosine
Inosine is identical to guanine lacking the N2 amino group. It is found in the 5'-nucleotide of tRNA anticodons of mRNA, known as Watson–Crick wobble position, when different triplets encode the same amino acid (Crick, 1966), and loosely pairs to either cytosine, adenosine or uridine. Inosine's annealing intensities to the four nucleotides depends on the thermodynamic stabilities of the pairings (Martin *et al*., 1985; Watkins and SantaLucia, 2005). Nevertheless, it is successfully used in a variety of PCR primers and probes that require degeneracy such as at the wobble position, to permit annealing to different but closely related sequences (Candrian *et al*., 1991; Ohtsuka *et al*., 1985). The annealing temperature and the composition of reaction mixtures can be manipulated to achieve better universality of such inosine containing primers. The presence of inosine in an oligonucleotide seems to neither disturb nor destabilize DNA duplex formation (Ohtsuka *et al*., 1985). Replacing inosine (I) in degenerate primers often yields amplification results superior than of the mixed-base degenerate primers (Liu and Nichols, 1994). In internal positions of synthetic oligonucleotides, pairing of I to thymine (T) or guanine (G) reduces the hybrid yield 10-30 fold and to a lesser extent to adenine (A) over its pairing to cytosine (C) (Case-Green and Southern, 1994). The order of stabilities of paired-inosine is: I·C > I·A > I·T ≈ I·G > I·I (Martin *et al*., 1985); it is thus the favorite base to replace two (G/T, G/A, T/A), three or four base ambiguities. At the 3'-end, I pairs preferentially in the same order, albeit with reduced discrimination between the four bases, but at the 5'-end, it shows the same signal strength when paired against each of them (Case-Green and Southern, 1994).

The hierarchy of hybridization yields of an octamer with two adjacent I's in the center is CC > CA > AA > AC > GC> GA > CG > TA > TC > CT = AG > AT > GT > TT (Case-Green and Southern, 1994). A neighboring pair has large influence on the stability of I·N base pair at the 5'- or 3'-end of an oligonucleotide with the following tendency's hierarchy: G·C > C·G > A·T > T·A, reflecting a complex interplay between H-bonding, nearest-neighbor stacking and mismatch geometry (Watkins and SantaLucia, 2005).

This information may be useful in designing a primer/probe of optimal stability (Martin *et al*., 1985; Watkins and SantaLucia, 2005) to enhance primer universality while retaining specificity. While these parameters can be modulated in an attempt to increase universality, it is likely to be accompanied by a tradeoff in specificity so that non-target sequences are also amplified (Forney *et al*., 2004).

Utility of inosine in culture-independent methods

Inosine at internal primer positions

Two types of primers are used to enhance their universality for amplification of related sequences from different species having more than one nucleotide at a given location: degenerate primers or those containing I (Kilpatrick *et al*., 1996; Rossolini *et al*., 1994; Wu *et al*., 2009). Replacing a wobble-position base by I substantially reduces unspecific annealing of degenerate primer sets commonly used for amplification of protein-coding or of highly-conserved (e.g., of rRNA) genes.

Introducing residues of I into the core of universal primers homologous to conserved regions in the 16S rRNA genes, for example, enabled amplification and detection of phylotypes that were not detected using the original, unmodified primers (Watanabe *et al.*, 2001). Such primers were used in analyses of microbial diversity of oil palm rhizosphere (Acevedo *et al*., 2014), cattle manure and anode biofilms (Inoue *et al*., 2013). I-containing primers for different types of human papillomavirus yield higher amounts and more specific amplicons than the corresponding degenerate primers (Rossolini *et al*., 1994). Internally-placed I in primers for 16S rRNA were recently used in next-generation DNA sequencing to catalog bacterial reads within complex, polymicrobial specimens (Salipante *et al*., 2013).

A large proportion of commonly used universal primers for 16S rRNA genes lack sequence homology to many of the “candidate” divisions, severely limiting bacterial variety assessments (Winsley *et al*., 2012) and thus display diverse coverage rates (Wang and Qian, 2009). So-called “conserved regions” in the 16S rDNA used as universal primers include many mismatches in the core (Watanabe *et al.,* 2001; Thomas *et al*., 2011) and less in their 3'-end (Ben-Dov *et al*., 2011; Brands *et al*., 2010).

Inosine at the 3′-end of primers

Inclusion of degenerate bases or I at the 3´-end is usually considered undesirable because incompatible annealing of the ultimate or penultimate base(s) can suffice to initiate PCR at wrong sites (White, 1993). On the other hand, mismatched nucleotides at these positions may be detrimental to the amplification process (Kwok *et al*., 1990; Sarkar *et al*., 1990; Wu *et al*., 2009) because loose primer hybridization affects binding stability of the DNA polymerase to a primer-template terminus (Detera *et al*., 1981; Huang *et al*., 1992; Kamtekar *et al*., 2006). This is primarily due to a need for a perfect 3'-end base pair to allow enzymatic synthesis (Batzer *et al*., 1991). A point mutation at this position in the template will therefore have a much greater effect than mutations in neighboring bases.

Inclusion of I at the 3'-end position in PCR primers of the Alu family, seminally performed by Batzer *at al*. (1991), resulted in a 150 bp amplicon from green monkey DNA, which was not amplified using identical primers without I. Using I at the 3'-termini of universal primers for 16S rDNA to study microbial diversity discovered 7 bacterial phyla whereas the original set amplified sequences belonging almost exclusively to *Proteobacteria* (Ben-Dov *et al*., 2006). I-substituted universal primers for 16S rDNA at the 3'-termini were used successfully in analyses of microbial diversity from different environmental niches such as industrial wastewater (Shapiro *et al*., 2009), coral black band diseases microbial mats (Barneah *et al*., 2007), desert soil (Angel *et al*., 2009), grapevine leaves (Bulgari *et al*., 2009), human feces (Patil *et al*., 2012; Zheng *et al*., 2009), oral plaque biofilms (Brands *et al*., 2010), hot spring ecosystems (Valverde *et al*., 2012) and gut flora of sand flies (Mukhopadhyay *et al*., 2012). This increased diversity confirms the usefulness of I-containing 3'-termini primer-pairs to expand the observed diversity of microbial communities but is not guaranteed to amplify all species existing in the examined sample.

An additional approach to obtain expanded microbial diversity uses substitutions of the two last bases at the 3′-end of each of the primers by two inosines (Ben-Dov *et al*., 2011). This primer-pair expands somewhat the observed diversity of a bacterial community at the family/class level, but significantly shifts the composition of the resulting 16S rDNA libraries from that obtained by two other primer-pairs, one with a single I substitution at the 3'-end and the other with no substitution. The most obvious shift in composition came with the detection of a higher abundance of sequences related to the class *Clostridia*. When two I's substituted AG in the two last 3′-end bases of the forward primer, the resulting hybridization (I·T-I·C) apparently destabilized primer-template-polymerase complex, and since the base adjacent to the 3'-end in the 16S rDNA of *Clostridia*'s template contains a high frequency of G's (98.8%) the formation of first pairing (C·G) by Taq polymerase stabilized the initial amplification efficiency and thus biased it toward this class (Ben-Dov *et al*., 2011).

The base adjacent to the 3′-end of universal PCR primers targeting 16S rDNA is variable and thus impacts the initial phase of PCR amplification: A or T decrease PCR efficiency whereas G or C stabilize primer-template-enzyme-nucleotide complex and thus increase amplification rates (Ben-Dov *et al*., 2012). I-modified primers preferentially (but not exclusively) amplify sequences containing G or C next to the 3′-end of the primer over sequences containing A or T (Brands *et al*., 2010). Such next-base effects can distort data used to draw conclusions about structures of microbial communities in a PCR-based approach, due to bias in estimating the relative ratios between phylotypes having different template-contiguous bases to the 3′ end primers (Ben-Dov *et al*., 2012).

Concluding remarks

High-throughput or 'next generation' DNA sequencing platforms dramatically increase sequencing depth of phylogenetic analyses of explored microbial communities, but limitation in universality of commonly used primers can still provide only a narrow view of these communities. PCR amplification of DNA is a key preliminary step in many applications of high-throughput sequencing technologies,

yet design and taxonomic analysis of novel universal or near-universal primers remains a challenging task (Walters *et al*., 2011). Universal primers containing inosine can reveal more varied templates and thus expand the observed diversity of microbial communities, but does not necessarily amplify all heterologous sequences of different species existing in explored niches. The importance of adopting multiple approaches to design universal primers for PCR analyses to assess microbial biota in complex environments is emphasized and substantiated.

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References

Acevedo, E., Galindo-Castañeda, T., Prada, F., Navia, M., and Romero, H.M. (2014). Phosphate-solubilizing microorganisms associated with the rhizosphere of oil palm (*Elaeis guineensis* Jacq.) in Colombia. Appl. Soil Ecol. *80*, 26–33.

Angel, R., Soares, M.I.M., Ungar, E.D., and Gillor, O. (2009). Biogeography of soil archaea and bacteria along a steep precipitation gradient. ISME J. *4*, 553–563.

Baker, G.C., Smith, J.J., and Cowan, D.A. (2003). Review and re-analysis of domain-specific 16S primers. J. Microbiol. Methods *55*, 541–555.

Barneah, O., Ben−Dov, E., Kramarsky−Winter, E., and Kushmaro, A. (2007). Characterization of black band disease in Red Sea stony corals. Environ. Microbiol. *9*, 1995–2006.

Batzer, M.A., Carlton, J.E., and Deininger, P.L. (1991). Enhanced evolutionary PCR using oligonucleotides with inosine at the 3'-terminus. Nucleic Acids Res. *19*, 5081.

Ben−Dov, E., Shapiro, O.H., and Kushmaro, A. (2012). 'Next−base'effect on PCR amplification. Environ. Microbiol. Rep. *4*, 183–188.

Ben-Dov, E., Shapiro, O.H., Siboni, N., and Kushmaro, A. (2006). Advantage of using inosine at the 3′ termini of 16S rRNA gene universal primers for the study of microbial diversity. Appl. Environ. Microbiol. *72*, 6902–6906.

Ben-Dov, E., Siboni, N., Shapiro, O.H., Arotsker, L., and Kushmaro, A. (2011). Substitution by inosine at the 3′-ultimate and penultimate positions of 16S rRNA gene universal primers. Microb. Ecol. *61*, 1–6.

Brands, B., Vianna, M.E., Seyfarth, I., Conrads, G., and Horz, H.P. (2010). Complementary retrieval of16S rRNAgene sequences using broad-range primers with inosine at the 3'-terminus: implications for the study of microbial diversity. FEMS Microbiol. Ecol. *71*, 157-167.

Bru, D., Martin-Laurent, F., and Philippot, L. (2008). Quantification of the detrimental effect of a single primer – template mismatch by real-time PCR using the 16S rRNA gene as an example. Appl. Environ. Microbiol. *74*, 1660–1663.

Bulgari, D., Casati, P., Brusetti, L., Quaglino, F., Brasca, M., Daffonchio, D., and Bianco, P. A. (2009). Endophytic bacterial diversity in grapevine (*Vitis vinifera* L.) leaves described by 16S rRNA gene sequence analysis and length heterogeneity-PCR. J. Microbiol. *47*, 393–401.

Candrian, U., Furrer, B., Hofelein, C., and Luthy, J. (1991). Use of inosine-containing oligonucleotide primers for enzymatic amplification of different alleles of the gene coding for heat-stable toxin type I of enterotoxigenic *Escherichia coli*. Appl. Environ. Microbiol. *57*, 955–961.

Case-Green, S.C., and Southern, E.M. (1994). Studies on the base pairing properties of deoxyinosine by solid phase hybridisation to oligonucleotides. Nucleic Acids Res. *22*, 131-136.

Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Marsh, T., Garrity, G.M., *et al*. (2009). The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. *37*, D141–D145.

Crick, F.H.C. (1966). Codon-anticodon pairing: the wobble hypothesis. J Mol Biol *19*, 548–555.

Curtis, T.P., Sloan, W.T., and Scannell, J.W. (2002). Estimating prokaryotic diversity and its limits. Proc. Natl. Acad. Sci. USA *99*, 10494-10499.

Daly, K., Sharp, R.J., and McCarthy, A.J. (2000). Development of oligonucleotide probes and PCR primers for detecting phylogenetic subgroups of sulfate-reducing bacteria. Microbiology *146*, 1693–1705.

Detera, S.D., Becerra, S.P., Swack, J.A., and Wilson, S.H. (1981). Studies on the Mechanism of DNA Polymerase alpha. J. Biol. Chem. *256*, 6933–6943.

Forney, L.J., Zhou, X., and Brown, C.J. (2004). Molecular microbial ecology: land of the one-eyed king. Curr. Opin. Microbiol. *7*, 210–220.

Fujiwara, H., Fujiwara, K., and Hashimoto, K. (1995). PCR with deoxyinosine-containing primers using DNA polymerases with proofreading activity. Genome Res. *4*, 239-240.

Hansen, M.C., Tolker-Neilson, T., Givskov, M., and Molin, S. (1998). Biased 16S rDNA PCR amplication caused by interference from DNA flanking template region. FEMS Microbiol. Ecol. *26*, 141–149.

Huang, M.M., Arnheim, N., and Goodman, M.F. (1992). Extension of base mispairs by Taq DNA polymerase: implications for single nucleotide discrimination in PCR. Nucleic Acids Res. *20*, 4567–4573.

Inoue, K., Ito, T., Kawano, Y., Iguchi, A., Miyahara, M., Suzuki, Y., and Watanabe, K. (2013). Electricity generation from cattle manure slurry by cassette-electrode microbial fuel cells. J. Biosci. Bioeng. *116*, 610–615.

Kamtekar, S., Berman, A.J., Wang, J., Lázaro, J.M., de Vega, M., Blanco, L., Salas, M., and Steitz, T.A. (2006). The φ29 DNA polymerase: protein-primer structure suggests a model for the initiation to elongation transition. EMBO J. *25*, 1335–1343.

Kilpatrick, D.R., Nottay, B., Yang, C.-F., Yang, S.-J., Mulders, M.N., Holloway, B.P., Pallansch, M.A., and Kew, O.M. (1996). Group-specific identification of polioviruses by PCR using primers containing mixed-base or deoxyinosine residues at positions of codon degeneracy. J. Clin. Microbiol. *34*, 2990–2996.

Kwok, S., Kellogg, D.E., McKinney, N., Spasic, D., Goda, L., Levenson, C., and Sninsky, J.J. (1990). Effects of

primer-template mismatches on the polymerase chain reaction: human immunodeficiency virus type 1 model studies. Nucleic Acids Res. *18*, 999-1005.

Liu, H., and Nichols, R. (1994). PCR amplification using deoxyinosine to replace an entire codon and at ambiguous positions. Biotechniques *16*, 24–26.

Martin, F.H., Castro, M.M., Aboul-ela, F., and Tinoco, I. (1985). Base pairing involving deoxyinosine: implications for probe design. Nucleic Acids Res. *13*, 8927–8938.

Martin-Laurent, F., Philippot, L., Hallet, S., Chaussod, R., Germon, J.C., Soulas, G., and Catroux, G. (2001). DNA extraction from soils: Old bias for new microbial diversity analysis methods. Appl. Environ. Microbiol. *67*, 2354–2359.

Mukhopadhyay, J., Braig, H.R., Rowton, E.D., and Ghosh, K. (2012). Naturally occurring culturable aerobic gut flora of adult *Phlebotomus papatasi*, vector of *Leishmania major* in the Old World. PLoS One *7*, e35748.

Ohtsuka, E., Matsuki, S., Ikehara, M., Takahashi, Y., and Matsubara, K. (1998). An alternative approach to deoxyoligonucleotides as hybridization probes by insertion of deoxyinosine at ambiguous codon positions. J. Biol. Chem. *260*, 2605–2608.

Patil, D.P., Dhotre, D.P., Chavan, S.G., Sultan, A., Jain, D.S., Lanjekar, V.B., Gangawani, J., Shah, P.S., Todkar, J.S., Shah, S., *et al*. (2012). Molecular analysis of gut microbiota in obesity among Indian individuals. J. Biosci. *37*, 647–657.

Polz, M.F., and Cavanaugh, C.M. (1998). Bias in template-to-product ratios in multi-template PCR. Appl. Environ. Microbiol. *64*, 3724–3730.

Rossolini, G.M., Cresti, S., Ingianni, A., Cattani, P., Riccio, M.L., and Satta, G. (1994). Use of deoxyinosine-containing primers vs degenerate primers for polymerase chain reaction based on ambiguous sequence information. Mol. Cell Probes *8*, 91–98.

Salipante, S.J., SenGupta, D.J., Hoogestraat, D.R., Cummings, L.A., Bryant, B.H., Natividad, C., Thielges, S., Monsaas, P.W., Chau, M., Barbee, L.A., *et al*. (2013). Molecular diagnosis of *Actinomadura madurae* infection by 16S rRNA deep sequencing. J. Clin. Microbiol. *51*, 4262–4265.

Sarkar, G., Cassady, J., Bottema, C.D.K., and Sommer S.S. (1990). Characterization of polymerase chain reaction amplification of specific alleles. Anal. Biochem. *186*, 64-68.

Shapiro, O.H., Kushmaro, A., and Brenner, A. (2009). Bacteriophage predation regulates microbial abundance and diversity in a full-scale bioreactor treating industrial wastewater. ISME J. *4*, 327–336.

Steven, B., Briggs, G., McKay, C.P., Pollard, W.H., Greer, C.W., and Whyte, L.G. (2007). Characterization of the microbial diversity in a permafrost sample from the Canadian high Arctic using culture−dependent and culture−independent methods. FEMS Microbiol. Ecol. *59*, 513–523.

Su, C., Lei, L., Duan, Y., Zhang, K.Q., and Yang, J. (2012). Culture-independent methods for studying environmental microorganisms: methods, application, and perspective. Appl. Microbiol. Biotechnol. *93*, 993–1003.

Suzuki, M.T., and Giovanni, S.J. (1996). Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. Appl. Environ. Microbiol. *62*, 625–630.

Thomas, M.C., Thomas, D.K., Selinger, L.B., and Inglis, G.D. (2011). SPYDER, a new method for in silico design and assessment of 16S rRNA gene primers for molecular microbial ecology. FEMS Microbiol. Lett. *320*, 152–159.

Valverde A., Tuffin, M., and Cowan, D.A. (2012). Biogeography of bacterial communities in hot springs: a focus on the actinobacteria. Extremophiles *16*, 669–679.

Walters, W.A., Caporaso, J.G., Lauber, C.L., Berg-Lyons, D., Fierer, N., and Knight, R. (2011). PrimerProspector: de novo design and taxonomic analysis of barcoded polymerase chain reaction primers. Bioinformatics *27*, 1159-1161.

Wang, Y., and Qian, P.Y. (2009). Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS One *4*, e7401.

Watanabe, K., Kodama, Y., and Harayama, S. (2001). Design and evaluation of PCR primers to amplify 16S ribosomal DNA fragments used for community fingerprinting. J. Microbiol. Methods *44*, 253–262.

Watkins, N.E., and SantaLucia, J. (2005). Nearest-neighbor thermodynamics of deoxyinosine pairs in DNA duplexes. Nucleic Acids Res. *33*, 6258–6267.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. (1991). 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. *173*, 697–703.

White, B.A. (1993). PCR Protocols, Current Methods and Applications. (Humana Press, Totowa, New Jersey).

Winsley, T., Van Dorst, J.M., Brown, M.V., and Ferrari, B.C. (2012). Capturing greater 16S rRNA gene sequence diversity within the domain bacteria. Appl. Environ. Microbiol. *78*, 5938–5941.

Woese, C.R., and Fox, G.E. (1977). Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc. Natl. Acad. Sci. USA *74*, 5088–5090.

Wu, J.H., Hong, P.Y., and Liu, W.T. (2009). Quantitative effects of position and type of single mismatch on single base primer extension. J. Microbiol. Methods *77*, 267–275.

Zheng, G., Yampara−Iquise, H., Jones, J.E., and Carson, A. (2009). Development of *Faecalibacterium* 16S rRNA gene marker for identification of human faeces. J. Appl. Microbiol. *106*, 634-641.

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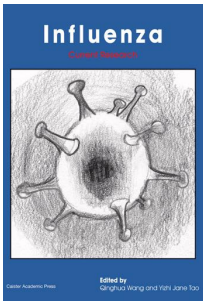

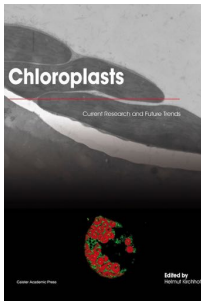

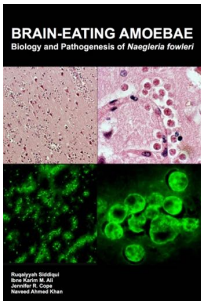

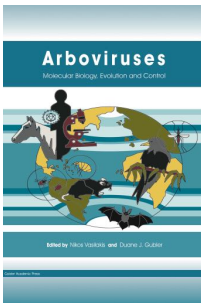

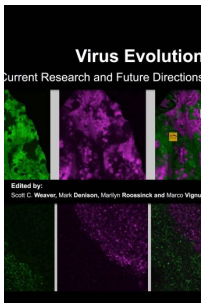

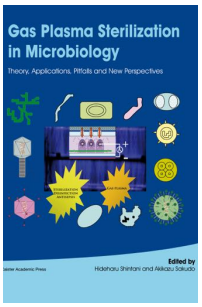

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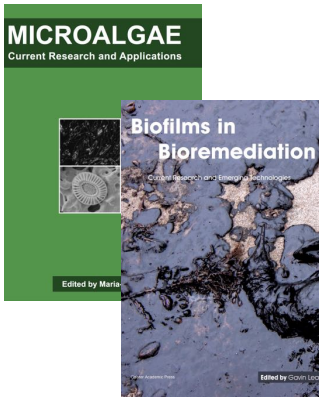

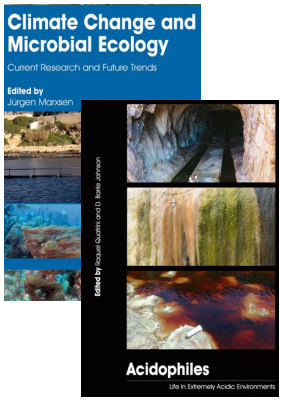

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