Table 1. Data of five main US funds

Fund	Fund amount* (10^9 USD)	*WoS* papers**	*WoS h*-index**	h_E (10^{-8})
National Science Foundation (NSF)	4.506	68,133	71	1.5757
National Institutes of Health (NIH)	28.532	86,838	93	0.3259
Department of Energy (DOE)	3.807	13,331	60	1.5760
Department of Agriculture (USDA)	1.198	4796	27	2.2538
National Aeronautics and Space Administration (NASA)	10.672	9440	43	0.4029

*Source: Federal R&D Funding by Budget Function: Fiscal Years 2008–10; http://www.nsf.gov/statistics/nsf10317/
**Source: *WoS* with search strategy such as FO = ('NATIONAL SCIENCE FOUNDATION' OR 'NSF') AND CU = USA AND PY = 2008–2010.

Table 2. Normalization values and normalizing *h*-efficiencies

Fund	${}_nF$	h^3	${}_nh$	${}_nh_E$
NSF	0.158	357911	0.445	2.816
NIH	1.000	804357	1.000	1.000
DOE	0.133	216000	0.269	2.019
USDA	0.042	19683	0.024	0.583
NASA	0.374	79507	0.099	0.264

different measurement perspective. The examples above of US funds reveal that *h*-efficiency or normalizing *h*-efficiency can provide a new measure of the input–output efficiency on research funds. We hope that this indicator will enrich the performance measure of research funds.

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ACKNOWLEDGEMENTS. F.Y.Y. thanks Prof. Stefan Hornbostel and Dr Sybille Hinze, Humboldt University of Berlin, Germany for help and suggestions during his stay at Bonn. We also thank the anonymous referees for their useful comments.

Received 10 March 2011; revised accepted 13 June 2011

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Enhancement of PCR amplification of actinobacterial 16S rRNA gene using an adjuvant, dimethyl sulphoxide

Polymerase chain reaction (PCR) is one of the most widely used methods in molecular biology and it is a robust procedure for most applications and usually requires little optimization. Optimization of magnesium concentration, buffer pH, denaturing and annealing times and temperatures, and cycle number is useful in some, but not all cases. PCR, however, often yields undesired products because of the features in the sequence of the template DNA. These problems can be especially severe in the case of sequences with high GC contents[1,2]. Targets that are obstinate to amplification, despite optimization attempts, can often be amplified if the appropriate additive is included in the amplification mix. A variety of additives and enhancing agents can be included in PCR amplifications to increase the yield, specificity and consistency. Specifically, the effect of an additive, dimethyl sulphoxide (DMSO) in the PCR amplification of some GC-rich sequences is most widely studied[3–5]. DMSO has also been used to improve cycle sequencing reaction of GC-rich DNA template, although the underlying mechanism is unknown[6]. We have encountered practical problems in amplifying 16S rRNA gene of actinobacterial templates with a high GC content in PCR and overcome the difficulties by the addition of adjuvant, DMSO. The aim of the present study is to find out whether the PCR conditions could be improved for amplifiying 16S rRNA gene by the use of suitable DMSO concentration.

Genomic DNA was extracted from the cultures (*Streptomyces* sp. PM 14 and PM 17; *Nocardiopsis* sp. SH 8 and SH 9, and *Rhodococcus* sp. SH 14) grown on ISP 2 broth using the method of Ausubel *et al.*[7]. Each 50 µl amplification reaction contained 1 µl template DNA (50–

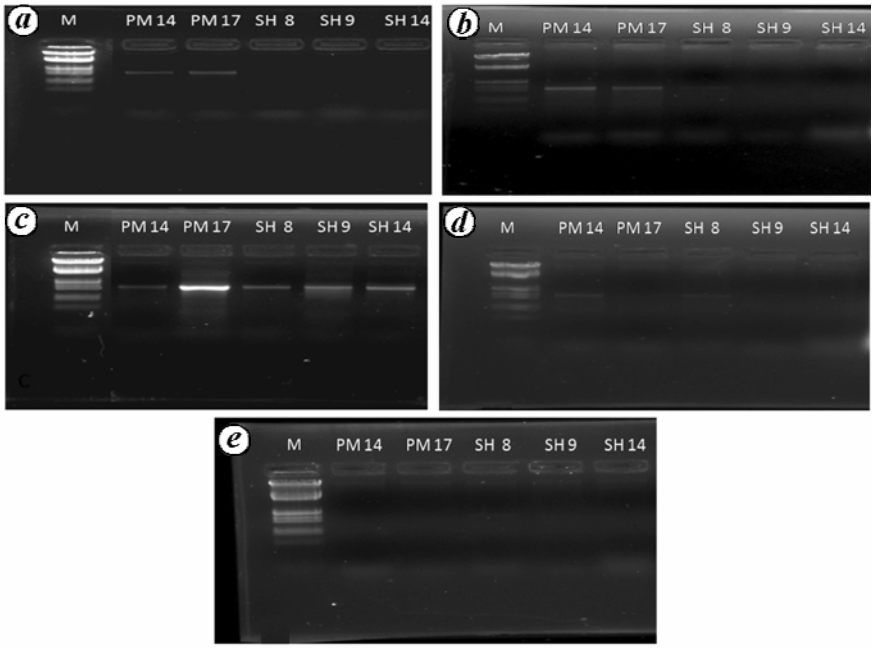

Figure 1. Optimization of DMSO concentration of PCR amplification of 16S rRNA gene of Actinobacteria: *Streptomyces* sp. PM 14 and PM 17; *Nocardiopsis* sp. SH 8 and SH 9, and *Rhodococcus* sp. SH 14. ***a***, PCR condition without DMSO. ***b***–***e***, PCR condition with 2.5%, 5%, 7.5% and 10% DMSO respectively.

200 ng), 5 µl 10× PCR buffer, 1 µl each PCR primer (20 mM) (27F, 1492R), 1 µl dNTP mix (10 mM), 6 µl $MgCl_2$ (25 mM), 2.5 U *Taq* DNA polymerase, 5 µl DMSO (2.5%, 5%, 7.5% and 10%) and 29 µl sterile MilliQ water. The reaction conditions were initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 90s. A final extension was performed at 72°C for 10 min. Reaction products were electrophoresed on a 1% agarose gel and checked with ethidium bromide under UV light.

In the present study, the amplification reaction used the same universal primers for amplifying five different templates of three different genera (*Streptomyces* sp., *Nocardiopsis* sp. and *Rhodococcus* sp.). The use of DMSO has been proposed in certain PCR applications[7–9]. However, we found that the optimum DMSO concentration required depended on the template. Increasing concentrations of DMSO were tested in the amplification reactions. We have observed that the amplification efficiency and/or specificity of PCR are changed by the concentration of DMSO. To amplify five different actinobacterial templates, the PCR condition with 5% DMSO gave the best results (Figure 1 *c*). PCR conditions with 2.5% gave better results, although only smeary PCR products were observed (Figure 1 *b*). However, the PCR condition with 7.5% DMSO (Figure 1 *d*) and 10.0% DMSO (Figure 1 *e*) gave poor results.

Several mechanisms of PCR enhancement with DMSO have been suggested. DMSO may affect the Tm of the primers, the thermal activity profile of *Taq* DNA polymerase and the degree of product strand separation achieved at a given denaturation temperature[10]. The first two mechanisms seem unlikely with our materials because different DMSO effects were observed with five actinobacterial DNA templates, even though the same temperature profile and primers were used. The effects that we observed may be related in part to the destabilizing influence of DMSO on dsDNA. The presence of a high GC ratio would stabilize dsDNA in both PCR products and intramolecular secondary structures, and could inhibit PCR[11]. In this context, our results have also suggested that the use of DMSO is effective in the amplification of 16S rRNA gene of the high GC content genomic DNA of Actinobacteria. We anticipate that the modified method described here will be applicable in the amplification of actinobacterial genomic DNA templates with high GC content.

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ACKNOWLEDGEMENTS. We thank the Director and Dean, Centre of Advanced Study in Marine Biology, and the authorities of Annamalai University, Parangipettai for providing the necessary facilities. V.K. and K.S. thank the Ministry of Environment and Forests, Government of India for funds. This is CASMB contribution number 2/2011.

Received 31 March 2011; accepted 6 June 2011

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