SCREENING OF PKS/NRPS GENE REGIONS ON MARINE DERIVED ACTINOBACTERIA

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Abstract: Marine derived actinobacteria are very valuable organisms because of unique abillities. The goal of this study was to investigate potential of production bioactive metabolite from marine derived actinobacteria. Eight actinobacteria were screened by PCR based techniques. Results showed that many isolate positive for NRPS (62,5%) and PKS (37,5%) genes. According to the PCR screening marine erived actinobacteria from Black Sea have significant biosynthetic potential. As a results highlighted that marine sediments represented potential sources for discovery of bioactive secondary metabolites.

Keywords: PKS,NRPS, PCR screening, actinobacteria

Introduction

Actinobacteria are gram positive bacteria wih high G+C content. Actinobacteria are known for their unique ability to produce novel compounds of clinical and pharmaceutical importance (Li&Vederas,2009). Through the many actinobacterial genera, *Streptomyces*, *Micromonospora*, *Nocardiopsis*, and *Rhodococcus* are the most efficient producers of secondary bioactive metabolites (Reimer et al., 2015). Actinomycetes are found in a variety of habitats and are particularly abundant in soil. Because of the exhaustion of the usual terrestrial sources, the discovery rate of new compounds from actinobacteria has steadily decreased and the re-isolation of known compounds was subsequently increased. This progress has led to a declining trend in the discovery of unknown natural products derived from microorganisms (Khan et al., 2010; Lam, 2006; Watve et al., 2001) For this reason, efforts focused increasingly on expanding the isolation sources beyond just terrestrial soils.

The marine biosphere is one of the earth's richest innumerable habitats and is expected to be an excellent sampling point due to microbial diversity in the seawater and sediment (Berdy, 2005; Fenical and Jensen, 2006). It has been shown that marine actinobacteria species are physiologically and phylogenetically distinct from their terrestrial relatives, and were found to represent a rich source for novel, chemically multifarious bioactive secondary metabolites with potential applications in antimicrobial and anticancer therapy (Bull et al., 2005; Lam, 2006; Maldonado et al., 2005). Marine actinomycetes produced a multitude of novel lead compounds with medicinal and pharmaceutical applications (Becerril-Espinosa, 2013). Although research on marine actinobacterial natural products is still at its early stage compared to what is known about terrestrial relatives, the enormous potential of marine strains as producers of bioactive secondary metabolites has already been proven and till date it remains matchless (Lakshmipathy et al., 2010).

Various natural products belong to the chemical families polyketides and non-ribosomal peptides (Droghazi & Metcalf, 2013). Polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS) are multi-domain megasynthases that are involved in the biosynthesis of a large fraction of diverse microbial natural products known as polyketides and nonribosomal peptides, respectively (Donadio et al., 2007). Besides, PKS and NRPS gene region screening on genome use for determine bioactive secondary compounds. In this way, ability of bioactive compounds production of microorganism are determined (Pathom-Aree, 2006). Marine derived actinobacteria have PKS and NRPS pathways for secodary metabolite production. For this reason they have ability of production diverse components as terpens, terpenoids, polyketides, peptids, caprolactones, quinons and alkaloids (Solanki et al., 2008).

Marine derived actinobacteria that obtained from Black Sea sediments were investigated in terms of potency of secondary metabolite pruduction by using PCR based approach in this study.

Materials and Methods

Actinobacteria strains

Eight marine derived actinobacteria strains that have antimicrobial activity in our lab were selected for PCR screening

DNA isolation

DNA isolation from actinobacteria was done by using Fast Spin DNA Isolation Kits (MP) with instruction manual.

PCR screening

i. NRPS gene region screening

Two primer sets A3F/A7R and ADEdom5/ ADEdom3 (Table 1) were used for PCR screening of NRPS gene region. All PCR reactions were performed 50 µl total volume. The PCR mixture was contained 10 µl 10X PCR buffer, 2 µl each primer (10 pM), 2 µl dNTPs (10 mM), 3 µl $MgCl_2$, 0.25 µl Taq DNA polymerase (5 U/µl; GoTaq Hot Start DNA polymerase), 1 µl genomic DNA, and 5 µl DMSO. To amplify the 450 bp segment encoding the NRPS, we used the primers ADEdom5 (5'- ACSGGCNNNCCSAAGGGCGT-3') and ADEdom3 (5'-CTCSGTSGGSCCGTA-3') (Busti, 2006). PCR conditions that after a 5 min incubation at 94 °C, 10 cycles (each of 1 min at 94 °C, 45 s at 50°C and 1 min at 72 °C) were followed by 20 cycles under identical conditions, except that the annealing temperature was raised to 55 °C. All PCR reactions were terminated with a 5 min elongation at 72 °C. To amplify the 700-800 bp segment encoding the NRPS, we used the primers A3F(5'-CSTACSYSATSTACACSTCSGG-3') and A7R (5'-SASGTCVCCSGTSCGGTAS-3') (Ayuso-Sacido, 2005). After a 5 min incubation at 94 °C, 30 cycles (each of 1 min at 94 °C, 1min at 58 °C and 1 min at 72 °C) All PCR reactions were terminated with a 5 min elongation at 72 °C.

ii. PKS gene region screening

K1F/M6R primers (Table 1) were used for PCR reactions. PCR reactions were performed 50 µl total volume and PCR mixture was contained 10 µl 10X PCR buffer, 2 µl each primer (10 pM), 2 µl dNTPs (10 mM), 3 µl $MgCl_2$, 0.25 µl Taq DNA polymerase (5 U/µl; GoTaq Hot Start DNA polymerase), 1 µl genomic DNA, and 5 µl DMSO. To amplify the 1200-1400 bp segment encoding the PKS, we used the primers K1F (5'-TSAAGTCSAACATCGGBCA-3') and M6R (5'-CGCAGGTTSCSGTACCAGTA-3') (Ayuso-Sacido, 2005). After a 5 min incubation at 94 °C, 30 cycles (each of 1 min at 94 °C, 2 min at 58 °C and 1.5 min at 72 °C) All PCR reactions were terminated with a 5 min elongation at 72 °C.

Table 1: Primers and their feature

primers	sequences	target gene	amplicon lenght (bp)	literature
K1F	5'-TSAAGTCSAACATCGGBCA-3'	PKS	1200-1400	Ayuso-Sacido, 2005
M6R	5'-CGCAGGTTSCSGTACCAGTA-3'			
A3F	5'-CSTACSYSATSTACACSTCSGG-3'	NRPS	700-800	Ayuso-Sacido, 2005
A7R	5'-SASGTCVCCSGTSCGGTAS-3'			
ADEdom5	5'-ACSGGCNNNCCSAAGGGCGT-3'	NRPS	450	Busti, 2006
ADEdom3	5'-CTCSGTSGGSCCGTA-3'			

Results and Discussion

PKS and NRPS screening are used as a collateral strategy for discovering bacterial natural product diversity (Pathom-Aree, 2006). Because natural product diversity are a reflection of bacterial genetic diversity. Prescreening of these target genes are beneficial approach for determine to new and useful secondary metabolites (Ketela et al., 2002). In this study eight strains were used for PCR based screening. Firsty, NRPS PCRs were done using A3F/A7R primers sets and appropriate conditions (Table 1). But PCRs were not run. For this reason other primer sets were tried for NRPS. ADEdom5/ ADEdom3 primers (Table 1) were used for NRPS PCR and 5 positive bands (≈450bp lenght) were detected on %1 agarose gel (Table 2). K1F/M6R primers (Table 1) were used for PKS PCR and 3 positive bands (≈1200-1400bp lenght) were were detected on %1 agarose gel (Table 2).

Table 2: PCR results of target genes

Actinobacteria strain	PKS PCR (K1F/M6R)	NRPS PCR (A3F/A7R)	NRPS PCR (ADEdom5/ ADEdom3)
S1	-	-	-
S2	-	-	-
S3	-	-	-
S4	+	-	+
S5	+	-	+
S6	-	-	+
S7	+	-	+
S8	-	-	+

According to results, many isolate were found positive for NRPS (62,5%) and PKS (37,5%) genes. Besides PKS and NRPS were found with together some samples (37,5%). Thus this marine derived actinobacteria have a great potantial for production of bioactive secondary metabolites.

Conclusion

PCR screening of PKS or NRPS gene regions were not functional strategy. On the contrary of fermentation based studies, active compounds were not isolated at the end of the PCR screening. But prescreening of these gene regions were found very effective approach for genetic research. Results of PCR screening can use for upper studies. Also these results might be guide similar studies

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