International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.1, pp 351-355, Jan-March 2014

Screening for Enzyme Inhibitors in Marine Bacteria

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Abstract: In a screening program, a total of 633 strains were isolated from various marine samples from Tuticorin, southeastern coast of India. In these strains 94 strains were identified as *Streptomyces* sp. and they were screened for enzyme inhibitors using a general screening method. Angiotensin converting enzyme inhibitors (reduce hypertension either by the suppression of Angiotensin II biosynthesis or by the stimulation of bradykinin breakdown) and Adenosine deaminase inhibitors (ADA inhibitors are responsible for alteration in adenosine and deoxyadenosine levels and lymphocytic growth and functions and also enhance the effects of chemotherapeutic effects of adenosine analogs) were also screened. Among 94 *Streptomyces*strains screened, 8 and 4 strains were positive for ACE and ADE inhibitors respectively
Keywords: marine bacteria, enzyme inhibitors, ADA inhibitors, ACE inhibitors.

Introduction

Marine organisms have the highest prospective yielding of natural compounds with exciting biological activity[1,2]. Marine microorganisms, in particularly the bacteria have had a profound effect on the development of drugs in the pharmaceutical industries[3,4,5].. In the past several decades, microorganisms have provided us with many antibiotics useful as chemotherapeutic agents. It has been considered that many enzymes have important roles in the maintenance of homeostasis in living organismsand that disease results form a breakdown of homeostasis. The correlation between the biological functions of several enzymes and disease process has been classified[6].

Some inhibitors of enzymes in which the correlation with disease processes has been established are very important as pharmacologically active drugs. Many analogs of the normal substrates of various enzymes have been synthesized chemically and subjected to the investigation of structure activity relationships[7,8].

Drug discovery in industry today has evolved to the use of specific assays with target receptors and enzymes involved in pathogenesis of disease rather than cellular or tissue assays. For example the targeted approach in the antitumor areaincludes enzyme inhibitors assays such as topoisomerase inhibitors[9]. Marine

natural products were also been screened for various enzyme inhibitors[10-11]. The Angiotensin-converting enzyme inhibitors act as antihypertensive drugs. Many commercially available synthetically developed ACE inhibitors are in market which includes drugs like teprotide and captopril etc[12]. Two important enzyme systems, the rennin-angiotensin (hypertensive) system, are involved in the regulation of blood pressure in mammals. Renin cleaves one peptidebond in angiotensinogen to release the biologically inactive decapeptide angiotensinI. Angiotensin - I converting enzyme causes removal of the C-terminal histidylleucine moiety from angiotensin I to give the octapeptide angiotensin II, which is a potent vasoconstrictor and stimulant of adrenocortical aldosterone secretion. The same enzyme inactivates the vasodilator bradykinin by the hydrolytic release of one or more carboxyl - terminal dipeptide residues. These ACE inhibitors can reduce hypertension either by suppression of angiotensin II biosynthesis or by the stimulation of bradykinin breakdown[6].

Adenosine deaminase inhibitors are responsible for alteration in adenosine and deoxyadenosine levels and lympocytic growth and functions and enhance the chemotherapeutic effects of adenoside analogs which are important in cancer chemotherapy[13.] In this present study, marine Streptomyces sp. were screened for Angiotensin-converting enzyme inhibitors (ACE inhibitors) and Adenosine deaminase inhibitors employing a simple plate assay technique.

Materials and Methods

The ACE inhibitors were screened following the plate assay method of O'Conner and Sommers[14.] This agar plate assay involves two aspects, such as applying the sample to be tested to an enzyme-containing layer and overlaying with a substrate-containing layer. The lower agar layer contains 1% agarose, 20 mMHEPES buffer, 100mM Nad, 200 urn CoCI2 and ACE of 400 ug protein ml"1 with the pH set at 6.5. The broth cultures of the Streptomyces strains were centrifuged and352the supernatant was impregnated on to 7mm filter paper discs and allowed to dry. The paper discs were placed onto the first layer of agar and given at 2 h diffusion time. Then this agar layer with disc was overlaid with the second layer of agar containing 1% agrose, 20 mM HEPES buffer, 100mM Nad, 200 urn CoCI2 and NBGCG (p-nitrobenzyloxycarbonylglycyl - {S-4-nitrobenzo - 2-ox-1,3-diazole) -L- cystinglycine) at 200 pg ml"1. The plates were incubated for 2 hrs at 37°C and flooded with 0.1 N NaOH for 10 min. Then the solution on the agar was drained and inhibition zones were noted. The zones initially seen as colourless on an amber background turn pink after 30 min and fade completely in 2h. The zones were measured and tabulated.

ADA inhibitors were screened using an agar plate method containing a pH indicator developed by Katsuragi et al[13.] Agar plates containing a medium of 6 pg of adenosine deaminase, 0.5 m mol of adenosine, 0.25 m mol of potassium phosphate buffer, 20 mg of phenol red and 1 g of agar in 100 ml of distilled water at pH 5.9 was used for the assay. The culture broth supernatants were impregnated on to the paper discs and were placed on the agar plates. After few hours of incubation at room temperature, yellow zones developed around the paper discs and were considered positive for ADE inhibitors and while the background colour turned to a purplish red. Colour change by the pH indicator depends on ammonia liberation in deamination reaction and increased pH.

Results

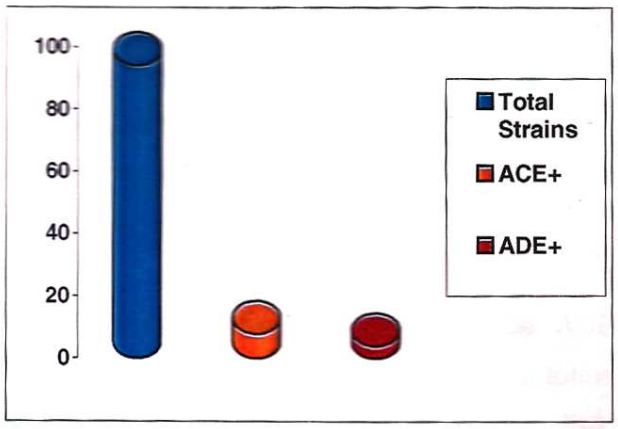
A total of 94 Streptomyces strains were screened for ACE inhibitors and ADE inhibitors. In the initial screening, 10 and 15 strains exhibited ACE and ADE inhibitor activity but in the second confirmational screening only 8 and 4 strains exhibited ACE and ADE activity respectively. A total of 8 strains out of 94 were positive for ADE inhibitors and were SF1, AH7, CC7, BFC21, GMB 10, SCL18, STCL 20 and SPE5.The strains SF1 (sponge derived), CC7 (Crab derived), BFC21 (Biofilm derived) gave an inhibition zone of 3mm (+++). The strains GMB10 (Gut microflora) exhibited a 2 mm zone (++) around the disc. The other strains AB12, AH7 (algae derived), AH7, SCL 18, STCL20 (Coral derived) and SPE 5 {Cephalopod egg derived) exhibited trace activity(+).

Only 4 strains (SA4, AE7, BFB 18, and GE 2) were found to be positive for ADE inhibitor. The strains were derived from sponge,algae, biofilm and gastropod egg respectively (Table 1 & Fig. 1).

Table 1: Enzyme inhibitor activity of marine Streptomyces strains.

Strain	ACE inhibitor (inhibition zone in mm)	ADE inhibitor (Yellow zone formation)
SA4	-	+
SF1	+ + +	-
AB12	+	-
AE7	-	+
AH7	+	-
CC7	+ + +	-
BFB18	-	+
BFC21	+ + +	
GMB10	+ +	-
GE2	-	+
SCL18	+	-
STCL20	+	-
SPE5	+	-

(+ + + = 3mm, + + = 2mm, + = trace)



Discussion

The screening of enzyme inhibitors from microorganisms requires the knowledge and techniques obtained in many fields of natural sciences microbiology, biochemistry, chemistry and pharmacology. These disciplines concern the isolation and cultivation of microorganisms, measurement of enzyme reactions, isolation and characterization of the inhibitors and their evaluation in animals etc [15-17]. During the pastthree decades, about 300 new bioactive compounds have been discovered during screening for enzyme inhibitors. Among them, bestatin has been used as a drug for the treatment of cancer in Japan. Since 1987, HMG - Co A reductase inhibitors such as lavastatin, pravastatin and simvastatin have been used for the treatment of hyperchole sterolemia in the world[6]

In the present study few Streptomyces strains were found to produce ACE and ADE inhibitors. In the marine side enzyme inhibitor screening has been in focus for the past one decade in organizations such as National Cancer Institute, USA, where enzyme inhibition drugs were used to screen for antitumor activity[9]. Regarding screening for marine bacteria for ACE and ADE inhibitor, there is a lack of any prior reports. But from the terrestrial side few works have been reported by different authors. For ACE inhibitors, compounds coded A58365A and A58365B were discovered using the agar plate assay method from terrestrial microorganisms[18]. Other ACE inhibitors reported from terrestrial microorganisms wasPhenacein, K-4, K-13, and K-2[19-20].

Regarding ADE inhibitors the reports from the terrestrial side was also few. Cofromycin, an ADE inhibitor was isolated from *Streptromyceskaniharaensis*[21]. Deoxycoformycin another ADE inhibitor was isolated from strain of Streptomycesantibioticus[21]. Omura et al [23-24] isolated two new ADE inhibitors adechlorin and adecypenol from culture broths of Actinomadura sp. and Streptomyces sp.

Both ACE as well as ADE inhibitors reported from the terrestrial side was from actinomycetes. The ACE and ADE inhibitors positive strains of the marine Streptomyces screening may yield potential novel metabolites of this nature.

Acknowledgment

Authors thank sincerely the Dr Patterson Edward, Director, Suganthi Devadason Marine Research Institute, Tuticorin, for the sample collection and laboratory support and Chairman and Director, Vel Tech Multi Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Chennai, for their unremitting encouragement.

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