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MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF BACTERIAL SPECIES WITH POTENTIAL SCREENING ACTIVITY FROM A MARINE COASTAL ENVIRONMENT

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ABSTRACT

Marine seawater samples were collected from Rameswaram, Tamilnadu. Six bacteria were isolated and named MB_1, MB_2, MB_3, MB_4, MB_5, and MB_6. All the six isolates were identified according to morphology, biochemical tests and selective growth media. The isolates were tested for antagonistic activity against *Salmonella typhi*, *Enterobacter* sp. and *Pseudomonas* sp. Only 2 isolates of MB_2 and MB_5 showed clean growth inhibition zone against *salmonella typhi*, *Enterobacter* sp. and *Pseudomonas* sp. From the selected isolates of MB_2 and MB_5 were grown on Nutrient broth and mature broth were centrifuged at 1500 rpm for 15 minutes. The supernatant layer was collected and which was used for compound isolation studies by NMR. Two compounds of Dreschsterin - A and Cis-Sativenediol were identified in MB_2 and MB_5 respectively based on NMR spectra. It can be concluded that isolation of Marine bacterial samples can offer a numbers of microbial strains for sources of new biomolecules from Marine sources. Bacteria are known to produce bioactive substances in the Marine environment of they are specifically antibiotic principles. This study indicated that certain marine bacteria could produce potential biomolecules for treat varieties of human diseases.

KEYWORDS: Marine bacterial isolates, NMR study, Dreschsterin - A, Cis - Sativenediol.

INTRODUCTION

Marine ecosystems represent 95% of the biosphere and coastal regions are particularly promising, because of the rightly adapted species found in these harsh environments. Each of

these classes of marine bio-products has a potential multi-billion-dollar market value [Bioscience, 1996]. Thousands of unique chemical compounds have been identified from a relatively small number of the oceans biological and chemical diversity [Ireland *et.al*., 1993]. The oceans represent a virtually untapped resource for discovery of even more novel compounds with useful activity [Baslow, 1965]. In the 19th and early 20th centuries cod liver oil was used as food supplement. So far, more than 10,000 bioactive molecules have been discovered form marine sources with hundreds of new compound still being discovered every year [Porsch *et.al*., 2002]. Out of the 150, presently, commercially available antibiotics there are only six different mechanisms by which antibiotics attack bacterial cells [O'Grade *et.al*., 1997, Ristuccia and cunha 1984]. Thousands of marine organisms are known to contains antibiotic substance and less than 1% have been examined for their pharmaceutical activity [Halstead, 1965]. Bacteria are known to produce bioactive substances in the marine environment even if they are specifically antibiotic producers. Bacteria exhibiting antibacterial activities have been isolated from various water samples. In recent years marine microorganisms have become important in the study of novel microbial products exhibiting antibacterial, antiviral, antitumour as well as anticoagulant and cardioactive properties [Marderosian 1969, Austin 1989]. These active compounds may serve as model systems in the discovery of new drugs [Bernan *et.al*., 1997, finical 1997, Jaruchoktaweechai *et.al*., 2000]. For the past 50 years antibiotics have revolutionized medicine by providing cures for formerly lie threatening diseases. However strains of bacteria have recently emerged that are virtually unresponsive to antibiotics. Although many preexisting antibiotics have been modified to yield new derivatives, bacteria have the potential to mutate known resistance mechanisms to combat these [Han wek 1997; Knowles 1997; Levy 1998]. Many marine free-living inhabiting marine bacterial have been shown to produce secondary metabolites that display antibacterial properties [Burgess *et.al*., 1997]. The first antibiotic from a marine bacterium was identified and characterized in 1996 [Burkholderz *et.al*., 1966]. Recently, the marine bacterium *Alteromonas Rava* sp.was found to produce new antibiotic Thiomarinol [Shiozawa *et.al*., 1993]. The studies made by the scientists at the Scripps Institution of oceanography show that marine bacteria are capable or producing bioactive compounds that are not observed in terrestrial sources [Fenical 1993; Fenical & Jensen, 1993]. In 1947, Rosenfeld and Zo Bell (1947) at Scripps reported on antibiotic activity from marine microorganisms found as a result of study to determine why sea water was bacteriostatic (or) bactericidal to some non-marine bacteria in culture. The Department of ocean Development has brought out a vision perspective plan for 2015 and it appears that about 80% of drugs

needed for human health care could be derived from natural sources. A great percentage of marine microorganisms have not been described [Pompori, 1999], although marine microorganisms have been shown to have an increasing interest, as a sources of new bioactive molecules [Fenical and Jensen, 1994; Bernan *et.al*., 1997]. Some marine bacteria secrete exopolysaccharides [Ikeda *et.al*., 1982; Worawattan mateckul and okutani, 1992; Raguens *et.al*.,1995] and many marine free-living bacteria produce secondary metabolites possessing antibacterial properties [Burgess *et.al*., 1991;Burgess *et.al*., 1999 and sponga *et.al*., 1999]. In this study, the distribution, Morphological characteristics and antimicrobial activities of marine bacterial species in coastal seawater of Rameswaram, was tested.\

MATERIALS AND METHODS

COLLECTION OF SAMPLES

To study the morphological characteristics and anti-microbial properties of the marine bacterial species, coastal water was collected in Rameswaram, Ramanathapuram District Tamilnadu. Sample water was collected in clean, sanitized and sterilized glass water bottles.

TOTAL VIABLE COUNT

Total viable count (TVC) of aquatic sediment sample was calculated by following spread plate technique. Each sample was serially diluted with dilutions ranging from 10^{-1}-10^{-11} and 1 ml of each dilution was spreader on the nutrient agar plates. Plates were incubated at 37°C for 48 hours in an inverted position and the colonies were counted. The count was expressed as the number of forming units in 1 ml of the original sample. The colony count below 30 was indicated as TSTC [Too few to count] and above 300 as TNTC [Too number to count].

IDENTIFICATION OF BACTERIA

The isolated bacteria were identified based on the colony morphology, gram staining property, and biochemical characteristics. Finally they were confirmed by their growth on selective media.

SAMPLE PREPARATION

10ml bacterial broth sample was centrifuged at 1500 rpm for 15 minutes. Solids were allowed to settle down by centrifugation, the supernatant was collected and transferred immediately into screw capped glass tubes. Then the supernatant from centrifuged sample was passed through the bacterial filter and the filtrate was numbered. Thus there were six samples that

contained material that did go through the bacterial filter. Filtered samples were ready to test anti-microbial activity against selected terrestrial bacteria and NMR.

ANTAGONISTIC ACTIVITY (Suryakala *et. al*., 2004**)**

Antagonistic activities of bacteria against marine bacteria were examined by agar plate assay technique. 50 micro liters of marine bacterial sample extract was placed in 2 diameter wells made in these solidified plates and 3 replicates were maintained. All plates were in incubated at 28ºC for 48 hours.

NMR (Nuclear Magnetic Resonance**)**

NMR spectra of extracts and pure compounds as a purity of check were at USIC, Madurai Kamaraj University, Madurai. The sample and the standards were analysed using nuclear magnetic resonance (NMR) Spectroscopy to investigate the chemical structural properties. The NMR experiment was conducted on a $400MH_Z$ JOEL NMR spectrophotometer with Trimethyl sulphoxide as the standard. Essentially, the sample is immerses in a magnetic field and hit with radio waves. The "spinning" nucleus is irradiated with a radio frequency, the relaxation of which causes the nucleus and its magnetic field to resonate. The signal is detected by the spectrophotometer and is generated in an interpretable form of peaks. The electron density around the proton affects the position of the peak. This is known as chemical shift.

RESULTS AND DISCUSSION

The coastal seawater sample from Rameswaram was diluted upto 10^{-6} and about six organisms were isolated and named MB_1, MB_2, MB_3, MB_4, MB_5 and MB_6. These isolated organisms were then streaked on a plate and a single colony was obtained to get a homogenous culture. These six organisms were then preserved in a slant and broth cultures, and were used in studying the colony, morphology, microscopical characteristics, gram staining, motility and other biochemical assays (Fig 1). Table 1 shows the organisms colony morphology shape and motility in medium.

Table 1: Isolated organisms bases on colony morphology and other characteristics.

S.NO	Organisms	Colony morphology	cell shape	Motility	Gram Staining
1.	MB_1	Raised colourless, smooth edged, mucoid	Bacillus	+	+
2.	MB_2	Brown coloured, smooth edged, mucoid	Coccus	-	-
3.	MB_3	Raised edge, white coloured, beaded	Coccus	+	-
4.	MB_4	Convex, Colourless, Undulate, mucoid	Bacillus	-	+
5.	MB_5	Arborescent, light brown, mucoid, lobate	Coccus	-	-
6.	MB_6	Shiny light brown rhizoid colony (Filamentous)	Coccus	+	+

+ Indicates Positive.

- Indicates Negative.

Figure 1: SLANT

MB_1 MB_2 MB_3 MB_4 MB_5 MB_6

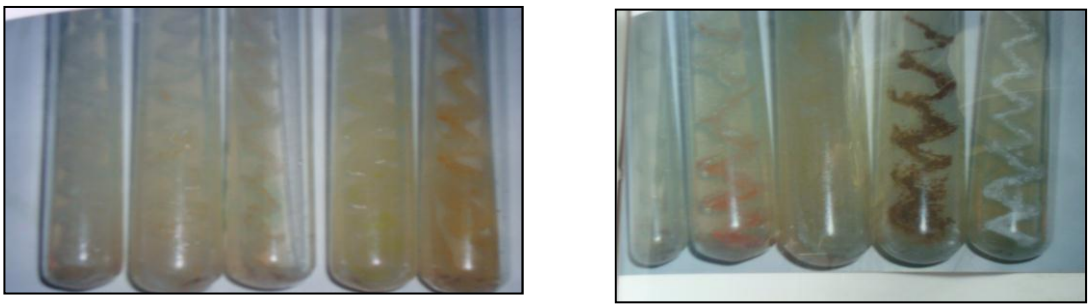


Table 2 shows IMVIC, oxidase and catalase reaction in all six bacterial organisms. MB_1, MB_4, and MB_6 were gram positive and other three of MB_2, MB_3 and MB_5 were gram negative (Fig 2).

Table 2: Biochemical characteristics.

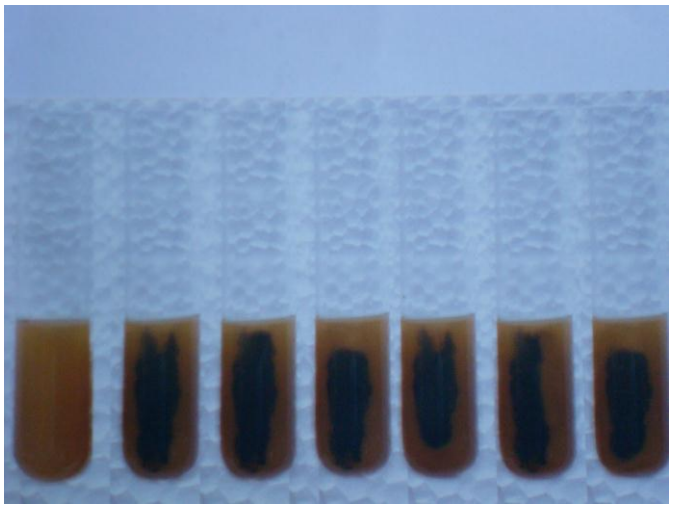
S.No	Organisms	Oxidase	Catalase	IMVIC			
				I	M	V	C
1	MB_1	+	-	-	+	-	-
2	MB_2	+	+	-	-	-	+
3	MB_3	+	-	-	-	-	+
4	MB_4	+	-	-	-	-	+
5	MB_5	+	+	-	+	-	+
6	MB_6	+	-	-	+	-	+

+ Indicates Positive

- Indicates Negative

OXIDASE TEST

CON MB_1 MB_2 MB_3 MB_4 MB_5 MB_6



CATALASE TEST

CON MB_2

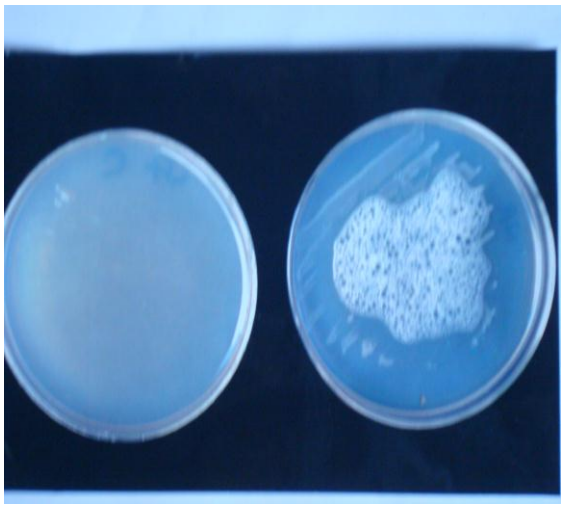
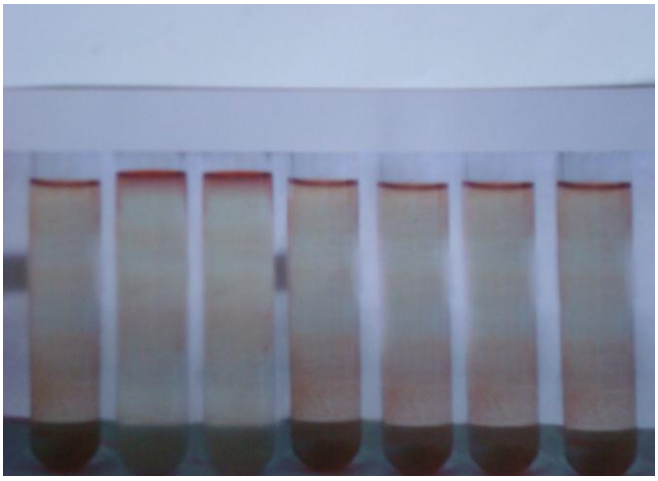


Figure 2: Biochemical tests.

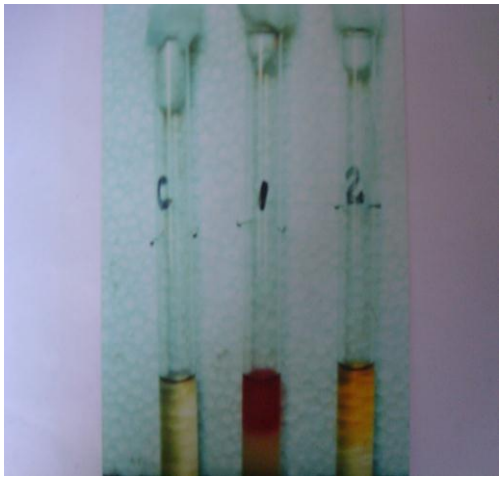
INDOLE PRODUCTION TEST

CON MB_1 MB_2 MB_3 MB_4 MB_5 MB_6



METHYL RED TEST

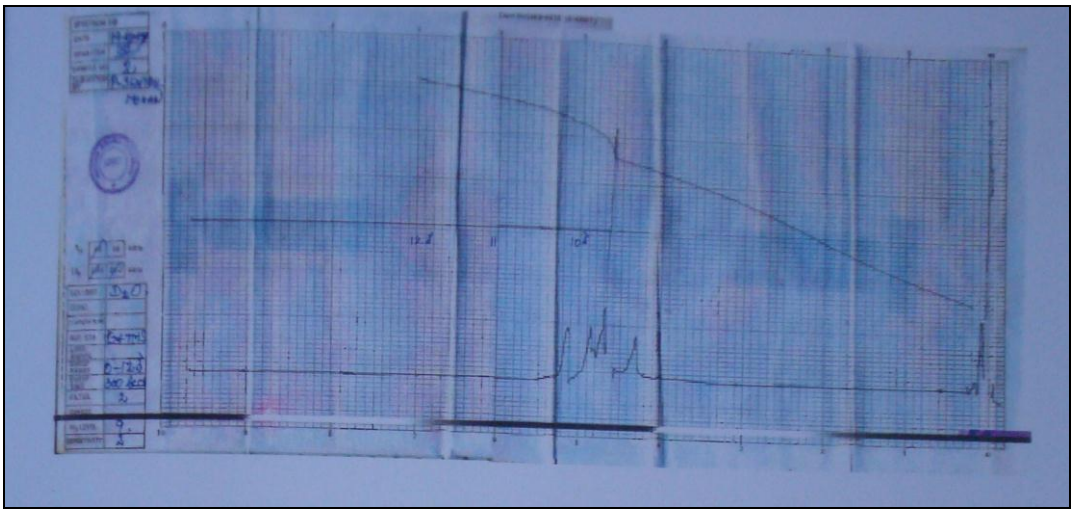
CON MB_1 MB_2



MB_1 and MB_4 were rod shaped. MB_2, MB_3 MB_5 and MB_6 were spherical shaped. Only MB_1, MB_3 and MB_6 showed motility. All bacterial organisms showed oxidase positive. But only in MB_2 and MB_5 were catalase positive. All organisms were negative for indole and voges-proskauer tests. In citrate utilization test, except MB_1 all organisms were positive. In methyl red test MB_1, MB_5 and MB_6 showed a positive result. Using various selective media and biochemical tests these microorganisms having anti-microbial activity were identified as *Alteromonas* sp. (MB_2) and R*hodopseudomonas* sp. (MB_5). Two broth cultures of MB_2 and MB_5 were centrifuged and the supernatant was taken immediately. These were tested to

identify the antibacterial compounds using detracted water as a solvent. NMR studies were carried out. (Fig 3).

MB_2



MB_5

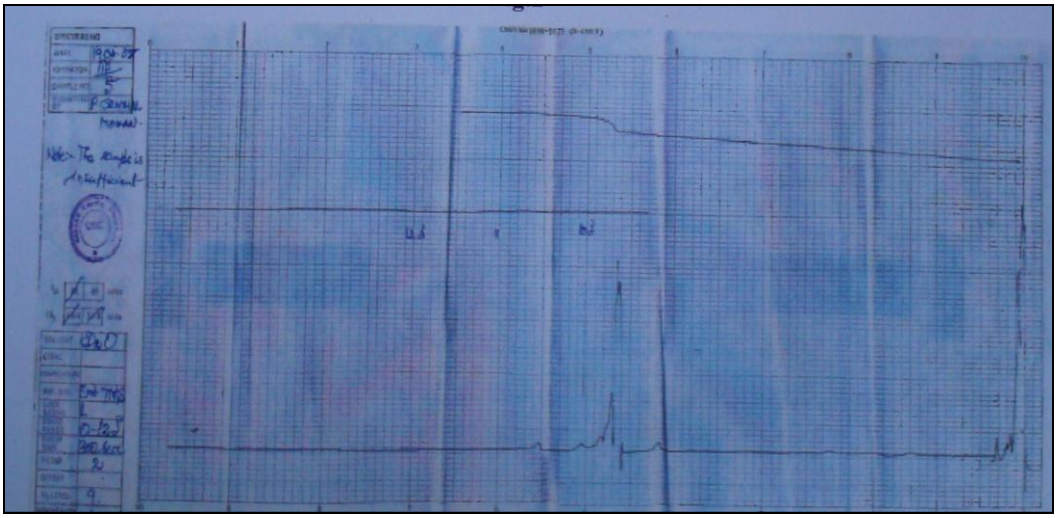


Figure 3: NMR Result.

Using all six organisms, anti-microbial activity test against human pathogens like *salmonella typhi*, *Enterobacter* sp. and *Pseudomonas* sp. were done. Only MB_2 and MB_5 showed clear zone of anti-microbial activity against these organisms (Table 3) (Plate1) (Fig 4). According to Osterhage (2001), the compounds isolated were found to be identified as Dreschsterin-A and Cis-Sativenediol presents in MB_2 and MB_5 respectively.

Table 3: Antimicrobial activity of isolated colonies.

S.NO	Organisms	Zone of Inhibition in mm		
		Salmonella Typhi	*Enterobacter* sp.	*Pseudomonas* sp.
1	MB_1	-	-	-
2	MB_2	10	-	30
3	MB_3	-	-	-
4	MB_4	-	-	-
5	MB_5	11	12	25
6	MB_6	-	-	-

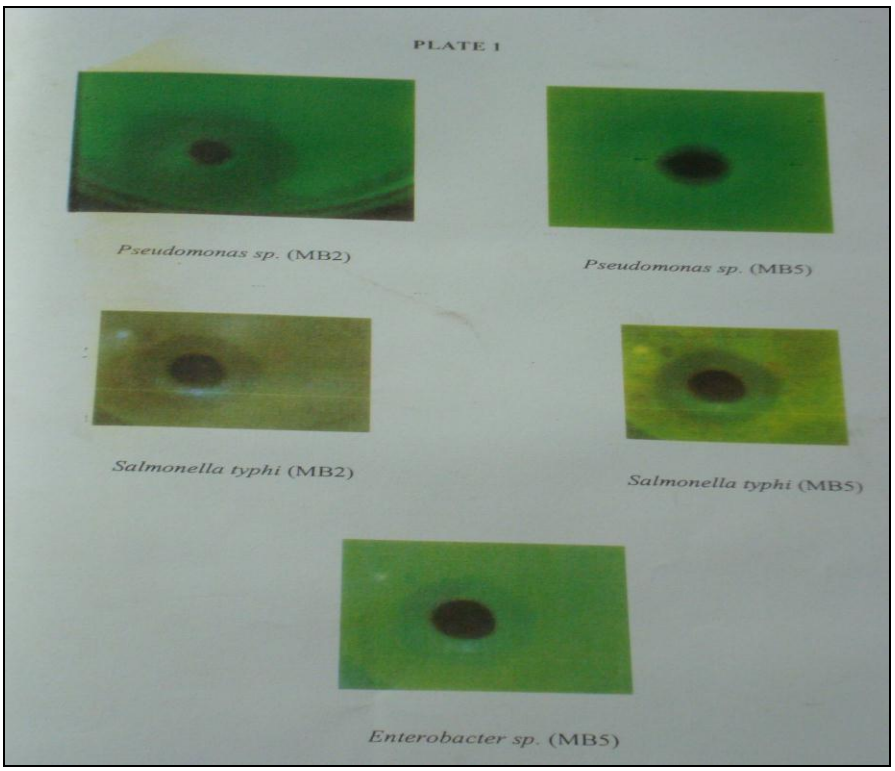


Figure 4: Antimicrobial activity.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from microorganisms, many based on their use in tradition medicine. The studies made by the scientists at the Scripps institution of Oceanography show that marine bacteria are capable of producing unusual bioactive compounds that are not observed in terrestrial sources (Fencical 1993; Fenical and Jensen 1993). Nair and Simidu (1987) have also reported that the absence (or) reduction in number of bacteria with antibacterial activity in Tokyo bay is due to its eutrophic nature. Likewise, in the six isolate organisms only in MB_1 and MB_5 were showed clear zone in the antibacterial activity test against human pathogens of *salmoella typhi*, *Enterobacter* sp. and *Pseudomonas* sp. Other samples showed zones but they were not as clean as MB_2 and MB_5 organisms.

These MB_2 and MB_5 organisms were tentatively identified as *Alteromonas* sp. and *Rhodopseudomonas* sp. Several workers have also record high incidence of *Alteromonas* and their inhibitory activity against many bacteria (Gauthier *et.al*., 1975; Nair and Simidu 1987, Austin 1989). Recently the marine bacterium *Alteromonas rava* was found to produce new abtibiotic thiomarinol (Shiozawa *et.al.*, 1993). Stierile, 2002 stated that a microbial metabolite from *Alteromonas* sp. has been developed with anti HIV potential as reverse transcriptase inhibitor, from marine microbes isolated from the tissues of Bermudian marine sponge. In the present study gram negative bacteria showed antibacterial activity compare to gram positive bacteria Okazake and Okami (1972) and Pisma *et.al*., (1989) also reported a similar from while screening *Actinomycetes* isolated from marine sediments. The biological evaluation of marine derived extracts and pure bunds of pharmaceutical development have been based on assays sped for the high throughput screening of large libraries of synthetic pounds. (Ireland *et.al*., 1993 Mc Connell *et.al*., 1994 Munro *et.al.,* 1994). Marine microorganisms as model systems offer the potential to understand and develop treatments for disease based on the normal physiological role of their secondary metabolites. (Hopkins et al., 1995, t al., 1997) and are currently being applied to the development of new drugs. In order to find more novel structures, new ways of screening of these compounds should be applied. This study indicated that certain strains of bacterial it could be induced to produce antibiotics.

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