Journal of Algal Biomass Utilization
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ISSN: 2229 – 6905

Antibacterial Activity of Fresh Water Cyanobacteria

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Digamber Rao 2015. Antibacterial activity of fresh water Cyanobacteria. *J. Algalbiomass Utiln*. 6 (3):

Key words:*Nodularia spumigena, Anabaena oryzae,* culture crude extract, antibacterial activity

Abstract:

Acetone, Chloroform, Methanol, Petroleum ether and Water extracts of two cyanobacteria (*Anabaena oryzae*and *Nodulariaspumigena*) were tested invitro for their antibacterial activities against *Bacillus cereus, Klebsiella aerogenes, Micrococcus luteus,* and *Staphylococcus aureus* with agar well diffusion method. The Acetone extract of *Anabaena oryzae*showed maximum antibacterial activity against *Salmonella typhi* and *Nodularia spumigena* exhibited maximum antibacterial activity against *Bacillus cereus* with the zones of 15±0.35 and 12.66±0.20, respectively. These results confirms that presence of promising antibacterial compounds in the selected cyanobacteria under study

Introduction:

Microalgae are photoautotrophic organisms that are exposed to high oxygen and radical stresses and consequently have developed several efficient protective systems against free radicals (Pulz and Gross, 2004). Microalgae represent an almost untapped resource of natural antioxidants, due to their enormous biodiversity and much more diverse than higher plants. The value of microalgae as a source of natural antioxidants is further enhanced by the relative case of purification of target compounds (Lie *et.al.*,2001). Algae have a significant attraction as natural source of bioactive molecules with a broad range of biological activities, including antimicrobial, antiviral, antioxidant and anti-inflammatory effects (Tuney*et.al.,* 2006and Patra*et.al*., 2008). Algae contain minerals, polysaccharides, amino acid derivatives, carotenoids and phenolic compounds. Some of these compounds can display antioxidant properties at very low concentrations (Yuan and Walsh, 2006). Microalgae produce extracellular sulfated polysaccharide (EPS) with acidic characteristics that has a potential as a therapeutic agent(Raposo*et.al.*,2014).Additionally, the bioactive products are active against bacteria, fungi, virus (Abed *et.al.*,2011and Ramamurthy *et.al.*, 2012). Microalgae and Cyanobacteria with reference to their microbial activity and in pharmaceutical aspects have been studied by various workers (Archana Tiwari and Sharma, 2013; Kumar *et.al.*, 2013; Chandra and Rajashekar, 2013, Chandru*et.al.,* 2013; Rabia*et.al.,* 2013; Sivakami*et.al.,* 2013; Archana and Kumar, 2014; Azza*et.al.,* 2014; Battah*et.al.,* 2014; Chinnu*et.al.,* 2014; Farag*et.al.,* 2014; Fatemeh and Hosseini 2014; Kinsalin*et.al.,* 2014;MohammadShoeb, 2014; Padhi *et.al.*, 2014; Shrivastava*et.al.,* 2014; Suman Das, 2014; Mervat*et.al.,* 2015). The ability of algae to produce antibacterial substances could be used not only as a defense agent (against pathogens) but also as pharmaceutical bioactive natural compounds. Though much is known about the chemistry and the antimicrobial action of several phytochemicals, very few reports are available on the possible mechanism of action.

Materials and methods

Collection, Isolation and Growth conditions of algae:

Algal samples were collected from was collected from various locations of siddhapur village of Warangal District, Telangana State, India. Algal samples were brought to laboratory in plastic bags with water to prevent evaporation. Epiphytes wastage were removed from the sample and washed with running tap water, the final step was done by using distilled water.

Samples were isolated, identified with the help of monograph Desikachary(1959), Anand(1989) were used. BG-11 medium (Rippka*et.al.,* 1979) was used for maintenance of algae.The Cyanobacteria was cultured in a 250 ml of flask containing 100 ml of nutrient media, without shaking for 28 days. The incubation temperature was 26 ± 2^0C and illumination (2-3 lux) with white florescent light of 16 hrs light and 8 hrs dark. The cultures were harvested after 28 days by centrifugation at 4000 rpm for 10 minutes.

Preparation of algal culture crude extracts:

After 28 days cultivation period the culture was harvested by centrifugation at 4000 rpm for 10minutes by centrifugation and washed with distilled water (two times), dried under shade. Dried samples were pulverized by using mortar and pistil, the fine grinded powder (5g) extracted with 10 ml of Acetone, Chloroform, Methanol, Petroleum ether and Water. Toget extract compounds with increasing polarity by shakingovernight for complete extraction. The extracts were filteredand the filtrates were evaporated under reduced pressure at room temperature (37^0C),dried crude extracts were weighed and dissolved in 1ml of DMSO (Di Methyl Sulfoxide) and it was stored at 4^0C for further studies.

Test organisms:

Test pathogens such as,*Staphylococcus aureus, Micrococcus luteus, Bacillus cereus, Salmonella typhi, Klebsiella aerogenes* were obtained from Microbiology Department, Kakatiya university, Warangal, Telangana State, India, and maintained in Microalgal Biotechnology Laboratory.

Antibacterial assay:

Five bacterial strains,*Staphylococcus aureus*, *Bacillus cereus, Micrococcus luteus*, *Salmonella typhi*,and *Klebsiella aerogenes* were tested using Agar well diffusion method. Bacterial strains were grown overnight in nutrient broth at 37^0C . Muller Hinton Agar(MHA) medium werepoured into 15 cm diameter Petri dishes were allowed to cool and solidify and then 100 µl of bacterial suspension were spread on MHA plates with a sterile cotton swab and made 5 wells in each plate with equal distance with the help of 6 mm borer in the plates.The wells filled with 50 µl of the crude extracts with sterile pipette on Muller Hinton Agar plates. Plates were incubated forbacteria at 37 ^{0}C for a period of 24 hrs. In the present study Gentamycin 10µg/mlwas used as standard control.

RESULTS AND DISCUSSION:

The results were observed to find out the antibacterial activity in five extracts of microalgae against five pathogenic bacteria. The antibacterial activity exhibited maximum zone of inhibition (15.50 mm) was found in Acetone culture crude extract of *Anabaena oryzae* against strain of *Salmonella typhi* followed by (12.60 mm) was noticed in Petroleum Ether extract against *Bacillus cereus,* 10.33 mm inhibition zonewas found in Methanol extract against *Klebsiella aerogenes* 9.66 mm of inhibition zone was observed Chloroform extract against *Micrococcus luteus* under study. There is no antibacterial activity was found in Petroleum Ether extract against *Salmonella typhi* and Water extract was also failed to express antibacterial activity against all the pathogenic bacteria. In the *Nodularia spumigena* maximum zone of inhibition 13.83 mm was found in Acetone culture crude extract against *Klebsiella aerogenes* , followed by 13.66 mm against *Staphylococcus aureus,* 11.66 mm was found in the Chloroform culture extract against *Micrococcus luteus,* 7.66 mm was noticed in Petroleum Ether extract against *Salmonella typhi.* There is no zone of inhibition was found in Methanol extract and Water extracts of *Nodularia spumigena.* There have been a number of reports that demonstrate the antibacterial activity of microalgae and cyanobacterial species, Pratt *et al.,* (1944) were the first to isolate an antibacterial substance from *Chlorella* and was followed by *in vitro* antimicrobial activity along with biomass production in waste water by cyanobacteria,*Spirulina platensis*(Suman Das, 2014),antibacterial activity of two blue-green algae against pathogenic bacteria, *Proteus vulgaris, Bacillus cereus, Ecoli* (Kumar *et.al.,* 2006), Blue-green algae against pathogenic bacteria *Staphylococcus aureus* (Bhateja*et.al.,* 2006), *Phormidium,Lyngbya* extracts against pathogenic bacteria *Staphylococcus aureus, S. epidermis, Bacillus cereus, B.bravis*(Priyadarshini *et.al.,*2012) and the member like *Anabaena* sp. was studied by Chauhan *et.al., (*2011) and on *Pithophora* sp. *Oedogonia* sp.(green algae) by Singh and Chaudhary(2010) were studied on their antimicrobial activity.

Antibacterial activity of *Nodularia spumigena* and *Anabaena oryzae*

Algae	Culture crude extract	Bacterial Pathogens(Zone of inhibition diameter in mm)				
		Staphylococcus aureus	*Bacillus cereus*	*Micrococcus luteus*	*Salmonella typhi*	*Klebsiella aerogenes*
Nodularia spumigena	i)Acetone ii)Chloroform iii)Methanol iv)Petroleum Ether v)Water extract	13.66±0.20 - - - -	12.66±0.20 7.83±0.20 - 7.33±0.20 -	12.33±0.40 11.66±0.20 - 7.13±0.16 -	7.33±0.20 8.26±0.17 - 7.66±0.20 -	13.83±0.20 - - - -
Anabaena oryzae	i)Acetone ii)Chloroform iii)Methanol iv)Petroleum Ether v)Water extract	8.16±0.20 8.66±0.20 9.66±0.40 11.66±0.20 -	12.50±0.35 9.33±0.54 9.50±0.35 12.60±0.12 -	11.33±0.20 9.66±0.20 7.33±0.20 8.33±0.20 -	15.50±0.35 8.23±0.17 7.50±0.35 - -	7.60±0.20 9.33±0.20 10.33±0.20 11.83±0.20 -
Control	Gentamycin (10µg/ml)	25.33±0.20	24.16±0.40	28.00±0.35	26.16±0.40	24.16±0.75

Results including diameter of the well(mean ± standard error), (-)= not found.

Conclusion

Over the past fifty years, a wealth of studies has clarified the importance of secondary metabolites in the ecology of organisms from microorganisms to mammals. Though currently rather limited , growing evidence continues to support a functional role of toxic or otherwise biologically active secondary metabolites from freshwater and marine Cyanobacteria in the ecology of these organisms . In particular, these metabolites have seemingly complex roles in the defense against potential grazers and allelopathic interactions with competing photosynthetic microalgae. Still much remains to be elucidated with respect to these roles, and their implications to the evolution of aquatic systems. In addition ,however, it has become equally clear that many of these metabolites may have potential for commercial development of, not only biomedically relevant compounds,but also those with applications for control of algae, and control of mosquito larvae and pests (particularly those that act as potential vectors of disease). The present study proved that microalgae possess antibacterial bioactivic compounds and it confirms that the chosen microalgae *Nodularia spumigena* and *Anabaena oryzae* were potential source of bioactive compounds against various pathogens which can be used as natural non-toxic preservative and may be more acceptable to consumers. Further work is needed to identify the active compounds of these algae.

Acknowledgements:

Authors are thankful to the Head, Department of Botany,Kakatiya University for providing research facilities to carryout the present investigation.

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