Advanced Journal of Microbiology Research Vol. 2017
Available online at http://internationalscholarsjournals.org/journal/ajmr
© 2017 International Scholars Journals

Full Length Research Paper

Antibacterial activity of cyanolichen and symbiotic cyanobacteria against some selected microorganisms

R. Shyam Kumar[1]*, N. Thajuddin[2] and C. Venkateswari[3]

[1]Department of Biotechnology, PSR Engineering College, Sivakasi, India.
[2]Department of Microbiology, Bharathidasan University, Trichirapalli, India.
[3]Department of Bioinformatics, Sathyabama University, Chennai, India.

Accepted 24 February, 2017

Lichen (*Collema auriforme*) collected from Kolli Hills of Tamil Nadu, India and two symbiotic cyanobacteria (*Aphanocapsa* sp. NTK28, and *Nostoc* sp. NTK29) were taken for screening antibacterial activity. Alcohol and acetone were used as solvents for extraction of compounds from lichen and symbiotic cyanobacteria. Four clinical isolates of *Pseudomonas* sp., *Escherischia coli, Klebsiella* sp., and the Gram positive organism *Staphylococcus* sp. were used as test organisms. Solvent extracts of lichen showed antibacterial activity against three test organisms. Alcohol extract of lichen showed no inhibitory effect on *Pseudomonas* sp. Other organisms like *E. coli, Klebsiella* sp. and *Staphylococcus* sp. were highly susceptible to alcoholic extract even at low concentration. Solvent extracts (alcohol and acetone) of cyanobacteria did not show any significant effect on the selected bacterial strains.

Key words: Lichens, cyanolichens, cyanobacteria, antibiotic, antibacterial activity.

INTRODUCTION

Lichens are complex organisms involved in symbiotic relationship between a phycobiont (Cyanobacteria or Green alga, or both) and a mycobiont (a fungus), and have attracted considerable attention because of their perceived position on the ladder of evolution to land plants (Heckman et al., 2001). Lichens have a worldwide distribution, occurring in the highest, hottest, coldest, wettest and driest habitats, yet they are extremely sensitive to pollution (Hawksworth and Honegger, 1994; Rai and Bergman, 2002) As a result of symbiotic condition, extra- and intracellular substances are synthesized by variety of lichens. The secondary metabolites are heterogeneous and belong to a variety of chemical compounds including fatty acids, aromatic polyketides (depsides, depsidons, dibenzoquinones, etc.) mevalonate group components and shikimate group components (Culberson, 1969; Mosbach, 1973) . For over many centuries' lichens has been a source of useful medicinal and chemical products (Richardson, 1975).

Cyanobacteria that are originally referred as blue–green algae, a unique group of gram negative, prokaryotic organisms bridging bacteria and algae are very much akin to chloroplast of plants, whose distribution around the world delegated only by bacteria. Particularly, in the last quarter twentieth century, there has been such an information explosion about these hitherto neglected organisms, that today they seem to be neck to neck with bacteria in biotechnology race, with every promise of overtaking them by the turn of the century due to their twin potential of fixing atmospheric carbon and nitrogen (Thajuddin and Subramanian, 2005). In addition, they are used as biofertilizers, as an excellent food and feed (Sheshadri and Thomas, 1979; Venkatraman and Becker, 1985; Subramanian et al., 1994), supplement to fight malnutrition of proteins and vitamins. There are several recent promising reports on their potential applications in medicine (Sundararaman et al., 1996), pharmaceuticals (Gustafson et al., 1989) fine chemicals, enzymes, diagnostics, fuel and waste treatment, and recycling process (Subramanian and Prabhakaran, 1994). Based on the potential characteristic features of lichens and cyanobacteria, the present inves-

*Corresponding author. E-mail: kingshyam2003@yahoo.co.in.

Table 1. Antibacterial activity of lichen *C. auriforme*.

S. no	Test organisms	Control	Streptomycin	Concentration (μg/ml)							
				Alcohol				Acetone			
				15	30	45	60	15	30	45	60
1	*Pseudomonas* sp.	–	19	–	–	–	–	–	–	–	–
2	*E. coli*	–	31	–	–	15	17	–	–	–	–
3	*Staphylococcus* sp.	–	17	19	20	20	22	18	20	20	20
4	*Klebsiella* sp.	–	17	-	17	18	20	–	15	18	18

Zone of inhibition in 'mm' '–' Resistance.

tigation focus on antibacterial activity of lichens *Collema auriforme*, symbiotic cyanobacteria *Aphanocapsa* sp. (NTK28) and *Nostoc* sp. (NTK29) was carried out.

MATERIALS AND METHODS

Collection of samples

Lichen (*C. auriforme* (With.)) Coppins and Laundon.) collected from Kolli Hills and samples were brought to laboratory, debris were cleaned and then sun-dried. These samples were used for extraction process.

Cultivation of symbiotic cyanobacteria

The lichen thalli were washed in a washing chamber for about 30 min (Renner, 1982). Sections of 30 - 40 m thickness were cut with a microtome, placed in 6 cm petri dishes on BG11 medium containing 1.5% agar (Waterbury and Stanier, 1978; Boissiere, 1987) and incubated at 20˚C under continuous light at 2,000 lux (Osram, universal white, fluorescent light, 40 W). After 6 - 8 weeks, the first free colonies of the cyanobiont were observed and they were transferred to fresh agar plates and incubated under the same conditions. Colonies of the phycobiont were harvested after approximately 4 months. Cyanobacterial isolates were identified using the taxonomic publications of Geitler (1932); Desikachary (1959); Starmach (1966).

Antimicrobial activity of lichen and symbiotic cyanobacteria

In vitro antibacterial studies were carried out with agar–disc diffusion method against different test organisms (Bauer et al., 1956)

Biomass preparation of symbiotic cyanobacteria

The symbiotic C. *Aphanocapsa* sp. (NTK28) and *Nostoc* sp. (NTK29) isolated from lichens *C. auriforme* (With.) Coppins and Laundon, and *Leptogium milligranum* (Swartz ex Ach.) Nyl., respectively. Both were cultivated in large quantity with BG11 medium by providing 2000 Lux light intensity at 16.8 L/D cycle, in large sterilizable polyethylene bags.

Solvent extraction of lichen and symbiotic cyanobacteria (NTK28 and NTK29)

Dried samples of lichens and partially dried samples of symbiotic cyanobacteria were ground thoroughly and then (5 g) were extracted with alcohol and acetone by shaking overnight. Extracts were filtered through sterile cotton wool and stored at -20˚C (Perry et al., 1999). The concentration was adjusted to 1 mg/ml by using the same solvent used for extraction.

Antibacterial assay

For antibacterial assay, from the standard extracts of lichen and cyanobacteria (15, 30, 45, and 60 l) were dried onto 6 mm sterile (Commercial disc – Himedia laboratories, Mumbai), which were then placed on seeded agar petri plates. Gram positive bacterium *Staphylococcus* sp. and Gram negative organisms, such as *E. coli, Pseudomonas* sp., *Klebsiella* sp., were obtained from Meenakshi Mission Hospital, Madurai, Tamil Nadu, India. Activity showed as a zone of inhibition around the disc (Bauer et al., 1966). Its width was recorded from the edge of the disc in millimeters. Positive control was Streptomycin (30 g). Muller Hinton agar (Himedia, Mumbai) was used for this analysis.

RESULTS AND DISCUSSION

Solvent extracts of lichen showed antibacterial activity against three test organisms. Alcohol extract of lichen showed no inhibitory effect on *Pseudomonas* sp. Other organisms like *E. coli, Klebsiella* sp. and *Staphylococcus* sp. were highly susceptible to alcoholic extract even at the lower concentration. Zones of inhibition (mm) observed were 15 and 17, in 45 and 60 g against *E. coli.* Inhibition zones (mm) were 17, 18, 20 and 20 against *Klebsiella* sp. and 19, 20, 20 and 22 against *Staphylococcus* sp., respectively, for the extract concentrations 15, 30, 45 and 60 g. The antibacterial activity is depicted in (Table 1) . Acetone extract of lichen also showed some noticeable zone of inhibition except against *Pseudomonas* sp. and *E. coli.* Zone of inhibition (in mm) were 15, 15, 18 and 18 against *Klebsiella* sp. and 18, 20, 20 and 20 against *Staphylococcus* sp. for concentrations 15, 30, 45, and 60 g. Both alcohol and acetone extracts of lichen thus showed some noteworthy inhibitory effect against selected clinical isolates (Table – a).Both alcohol and acetone extracts of lichen samples had activity against tested microbes even in lower concentration. So this may be due to the presence of some organic compounds in lichen. According to the available reports *Collema* species such as *Collema*

Table 2. Antibacterial activity of C. *aphanocapsa* sp. (NTK28).

S. no	Test organisms	Control	Streptomycin	Concentration (µg/ml)							
				Alcohol				Acetone			
				15	30	45	60	15	30	45	60
1	*Pseudomonas* sp.	−	20	−	−	−	−	−	−	−	−
2	*E. Coli*	−	32	−	−	−	−	−	−	−	−
3	*Staphylococcus* sp.	−	15	−	−	−	−	−	−	2	2
4	*Klebsiella* sp	−	30	−	−	−	−	−	−	−	−

Zone of inhibition in 'mm' '−': Resistance.

Table 3. Antibacterial activity of C. *nostoc* sp. (NTK29).

S. no	Test organisms	Control	Streptomycin	Concentration (µg/ml)							
				Alcohol				Acetone			
				15	30	45	60	15	30	45	60
1	*Pseudomonas* sp.	−	21	−	−	−	−	−	−	−	−
2	*E.Coli*	−	30	−	−	−	−	−	−	−	−
3	*Staphylococcus* sp.	−	16	−	−	−	−	−	−	−	−
4	*Klebsiella* sp	−	31	−	−	−	−	−	−	1	1

Zone of inhibition in 'mm' '−': Resistance, Control: solvent (alcohol and acetone).

furfuraceum and *Collema subnigrescens* have no such activity against any microorganisms, except against *Staphylococcus aureus. C. subnigrescens* shows some activity against *S. aureus* (Rowe et al., 1999) which is related to this present report. Moreover, some lichens have usnic acid that acts against certain type of gram positive and gram-negative pathogenic organisms (Bustinza, 1951). Reportedly, there are more than 20 species of *Pseudocyphellaria*, and *Petligera* contain phenolic compounds which have antibacterial activity also belong to cyanobacterial lichen (Galloway, 1985; 1988). It is reported that 47 extracts of 69 lichen species that has no anti-fungal activity in contrast to boiled solvents ((Perry et al., 1999; Calder et al., 1986).

Antimicrobial activity of lichens is due to the production of several kinds of secondary metabolites (Dayan and Romagni, 2001). *Cetraria aculeate*—extracts showed antimicrobial activity against most of the tested bacteria, except *Pseudomonas syringae, Klebsiella pneumoniae* and *Yersinia olitica* (Turk et al., 2003). In the present study, *C. auriforme* extract showed significant results against *E. coli*, *Klebsiella* sp. and *Staphylococcus* sp. Solvent extracts (alcohol and acetone) of cyanobacteria did not show any significant effect on the selected bacterial strains. Acetone extract of *Aphanocapsa* sp. (NTK28) showed little activity against *Staphylococcus* sp. at higher (45 and 60 g) concentration. However, no organism showed significant susceptibility to extracts (Table 2). Acetone extracts of *Nostoc* sp. (NTK29) showed no effect on *Pseudomonas* sp., *E. coli* and *Staphylococcus* sp. However, slighter inhibitory effect was shown against *Klebsiella* sp. in 45 and 60 g concentrations (Table 3). Antibacterial activities of symbiotic cyanobacterial extracts did not show any significant effect. But acetone extract of *Nostoc* sp. (NTK28) showed minimal activity against *S. aureus* at higher (45 and 60 g) concentration. Similarly, acetone extract of *Nostoc* sp. (NTK29) showed minimal activity against *Klebsiella* sp. at higher concentration. Recent report stated that, extracted mixture of unsaturated fatty acids of cyano-bacteria *Oscillatoria redekei* has antimicrobial activity against gram positive bacteria while no activity was found against gram negative bacteria (Mundt et al., 2001; Sabin et al., 2003). Multi resistant S*. aureus* were not suscep-tible to cyanobacterial extracts. But in present study, clinical isolates *Staphylococcus* sp. was susceptible to higher concentration level at (45 and 60 g) of acetone extracts. Usha Pandey and Pandey (2002) reported that 22 cyanobacterial samples could be isolated from freshwater and terrestrial environment and its extracts showed activity against gram positive bacteria but no activity on gram negative bacteria. This observation is comparable to the results of the present study. Acetone extract of *Nostoc* sp. (NTK28) had antagonistic activity on gram positive bacteria. The higher concentration of *Nostoc* sp. (NTK29) acetone extract showed minimal effect against gram-negative bacteria. Cytotoxic activity exhibited by the cyanobacterial extracts could be of 38.6% (Mian et al., 2003). Three strains of cyanobacteria such as *Lyngbya majuscula, Microcystis aeruginosa* and *Plectonema boyamum* were tested for the antibacterial properties towards *Xanthomonas vesicatoria* (Usha

Pandey and Pandey, 2002; Volk and Furkert, 2006).

REFERENCES

Bauer AW, Kirby WMM, Scherris JKC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disc method, Am. J. Clin. Pathol. 45: 493-496.

Boissiere MC (1987). Ultrastructural evidence for polyglucoside reserves in *Nostoc* cells in *Peltigera* and *Collema* and the effect of thallus hydratation. Bibliotheca Lichenologica. 25: 109-116.

Bustinza F (1951). A note on the Antimicrobial activity of C*ladonia rangiferina* Web, *C. sylvatica* (L.) Hoff. emend. Sandst, and *C. impexa* Harm. Antibiotics Chemother. 1: 443-446.

Calder VL, Cole ALJ, Walker JRL (1986). Antibiotic compounds from New Zealand plant. III : a survey of some New Zealand plants for antibiotic substance, J. Royal. Soc. New. Zealand 47: 169-181.

Culberson CF (1969). Chemical and botanical guide to lichen products. Univ of North Carolina Press, Chapel Hill.

Dayan EF, Romagni G (2001). Lichens as a potential source of pesticides, J. Roya.l Soc. Chem. Chem. pp. 229-232.

Desikachary TV (1959). Cyanophyta. Indian Council of Cultural Resarch, New Delhi, India.

Galloway DJ (1985). Flora of New Zealand Lichens Wellington; P.D. Hasselberg, New Zealand Govt. printers.

Galloway DJ (1988). Studies in *Pseudocyphellaria* (Lichen) The New Zealand species. Bulletin of the British Museum (Natural History), Botany. 17: 1-267.

Geitler L (1932). Cyanophyceae. Rabenhorsts Kryptogamonflora von Deutschland, Osterreich und der Schweicz. Akademische Verlagsgesellschaft, Lepizig, Germany.

Gustafson KR, Cardellina JHII, Fuller RW, Weslow OS, Kiser RF, Snader KM, Paterson GM, Boyd MR (1989). AIDS – antiviral sulfolipids from cyanobacteria (Blue green algae). J. Natl. Cancer Inst. 81: 1254-1258.

Hawksworth DL, Honegger R (1994). The lichen thallus a symbiotic phenotype of nutritionally specialized fungi and its response to gall producers. In: Plant galls. (eds. Williams MAJ) : 77-98. Clarendon Press, Oxford.

Heckman DS, Geiser DM, Eidell BR, Stauffer RL, Kardos NL, Hedges SB (2001). Molecular evidence for the early colonization of land by fungi and plants, Science, 293: 1129-1133.

Mian P, Heilmann J, Burgi HR, Sticher O (2003). Biological screening of terrestrial and fresh water cyanobacteria for Antimicrobial activity, Brine shrimp lethality and cytotoxicity. Pham. Biol. 41(4): 243-247.

Mosbach K (1973). Biosynthesis of lichen substances. In Ahmadjian V, Hale ME (eds). The Lichens. Academic Press, London New York pp. 523-246.

Mundt S, Kreitlow S, Nowotny A, Effmert U (2001). Biochemical and pharmacological investigations of selected cyanobacteria. Int. J. Hygiene. Environ. Health 203: 327-334.

Perry BN, Benn HM, Brennan JN, Burgess JE, Ellis G, Galloway JD, Lorimer DS, Tangeny SR (1999). Antimicrobial, Antiviral and Cytotoxic activity of New Zealand lichens, Lichenologist 31(6): 627-636.

Rai A, Bergman B (2002). Cyanolichen. In: Biology and Environment: Proceedings of the Royal Irish Academy. 102B(1): 19-22.

Renner B (1982). The presence or absence of secondary metabolites in cephalodia and their possible implications. J. Hatt. Bot. Lab. 52: 367-377.

Richardson DHS (1975). The Vanishing Lichens: Their history, biology and importance. Newton Abbot: David and Charles.

Rowe JG, Garcia Gimenez MD, Saenz Rodriguez MT (1999). Some lichen products have Antimicrobial activity. Z. Natureforsch. 54c: 605-609.

Sabin M, Susann K, Jansen R (2003). Fatty acids with antibacterial activity from the cyanobacterium *Oscillatoria redekei* HUB 051. J. Appl. Phycol. 15 (2-3): 263-267.

Sheshadri CV, Thomas S (1979). Mass culture of spirulina using low-cost nutrients. Biotechnol. Lett. pp. 287-291.

Starmach K (1966). *Cyanophyta – Since, Glaucophyta-Glaukofity,* Flora Slodkowodna Polski, tom 2, Warszawa, pp. 800-806.

Subramanian G, Uma L, Prabaharan D, Sundararaman M, Thajuddin N (1994). In: Abstracts, Second Asia – Pacific Conference on Algal Biotechnology, National University of Singapore, Singapore.

Sundararaman M, Subramanian G, Averal HI, Akbharsha MA (1996). Evaluation of the bioactivity of marine cyanobacteria on some biochemical parameters of rat serum. Phytothe. Res. 10: 9-12.

Thajuddin N, Subramanian G (2005). Cyanobacterial biodiversity and potential applications in biotechnology, Curr. Sci. 89(1): 47-57.

Turk OA, Yilmaz M, Kivanc M, Turk H (2003). The Antimicrobial activity of extracts of the lichens *Cetraria aculeate* and its protolichesternic acid constituent. Z. Natureforsch. 58c: 850-854.

Usha Pandey Pandey J (2002). Antibacterial properties of cyanobacteria: A cost – effective and ecofriendly approach to control bacterial leaf spot disease of chilli*,* Curr. Sci. 7(2): 262-264.

Venkatraman LV, Becker EV (1985). In: Biotechnology and utilization of Algae, CFTRI, Mysore, India.

Volk BR, Furkert HF (2006). Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. Microbiol. Res. 161(2): 180-186.

Waterbury JB, Stanier RY (1978). Patterns of growth and development in pleurocapsalean cyanobacteria. Microbiol. Rev. 42: 2-44.