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Research Article

ISOLATION AND CHARACTERIZATION OF MICROORGANISMS FROM SQUID LOLIGODUVAUCELIAND GENERATION OF MICROBE FREE CRUDE INK

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ABSTRACT

The main aim and objective of the study is to isolate of cephalopod ink , characterization of microorganisms from the cephalopod ink and screening of anti-microbial activity by agar well diffusion method to generation of microbe free ink. The Cephalopoda was collected from Visakhapatnam port and identified based on standard literature of the FAO Fish. Synop., (125)Vol. 3:87p.and species identified as Loligo*duvauceli* which is also known as Indian squid. The ink sac dissected to collect ink from the ink gland. For isolation of Loligoduvauceli ink, HiCrome Universal Differential Medium used. Squid ink inoculated on HiCrome Universal Differential Medium Streaked plate was incubated at 37°C for 24 hours in aerobic condition and examined the colonies grown in the plate, further incubation microbes are identified through Gram stain and characterized biochemically, confirmed as Proteus species, Klebsiella species, Enterococcus species, and Pseudomonas species.The antimicrobial activity of the Loligoduvauceli crude ink was evaluated against four bacterial species such as Proteus species, Klebsiella species, Enterococcus species, and Pseudomonas species the maximum zone of inhibition for bacterial strains were observed against Enterococcus species (14mm) and minimum against Proteus species (10mm).Result of this study concluded that the crude ink of the Loligoduvauceli contain Klebsiella species, Enterococcus species, and Pseudomonas species hence it cannot use as therapeutics and NaCl has a ability to inhibit the growth of microbes present in the cephalopod ink and can be used as an alternative method to produce microbe-free Loligoduvauceli ink.

Keywords: Loligoduvauceli, antibacterial activity, Squid ink , Microbe free ink, Crude extraction.

INTRODUCTION

Our oceanic atmosphere is a rich source in terms of biological and active natural products, many of them have not been found in earthly sources. In medicinal science fields, the marine creatures had a very impact and the current studies principally focused on their application for the management of human diseases[1]. In current years, immense attention has been paid to study the bioactivity of natural yields due to their possible pharmacological deployments. Approximately 5000 species of sponges, 11000 species of corals, jellyfish and sea anemones, 9000 species of segmented worms, 100,000 species of annelids, polychaetes, snails, clams and octopus, 6000 species of sea stars and sea cucumber and 200 species of sea squids are present in the marine atmosphere[2]. The class Cephalopoda contains the nautili cuttlefish, squids, and octopods with internal shells or without shells that are placed in the subclass coleoidea. Squid, cuttlefish, and octopus are major seawater catch other than fishes and prawns. Cephalopods from the most important things in marine fishery export from India, with increasing demand from various parts of the

world.[3-4] Cephalopods are classified into two major groups i.e. Nautiloidea (nautilus) and Coleoidea[5], which include squids, cuttlefish, octopus, and nautilus[6]. Among those, squid is the most important constituent of the Cephalopoda class (Normal et al., 2002). Currently, All over the world squid and cuttlefish are a significant fishery product especially In south Asian countries (Hoque et al., 2010). In olden times Cephalopods are used for human consumption, especially among Greeks and Egyptians, in different ways[8]. Mostly in India, cephalopods were used in the dried form[9]. In the Philippines, cephalopods are first boiled in vinegar and are then fried in oil and spices[10]. In United, Kingdome Squids are used along with fish stew to increase taste[11]. The Japanese are masters in the use of the cephalopods as food in different varieties[12]. Moreover, other varieties of cephalopods can be used as pickles[8]. Most cephalopods, apart from nautiloid, have ink sacs that produce ink[13]. Cephalopod species are living in low light and in deep-sea, produces two kinds of inks which include clouds and smokescreens which are different size and shape in inking[14]. The Cephalopod has an extremely dedicated organ which has a special mechanism to convert immature cells into mature cells respectively in inner to outer portion and, then mature cells capable to produce melanin which giving rise to particulate melanosomes after cell maturation melanin is secreted into the lumen of the gland and accumulated into the ink sac.[15] Inking of the Cephalopod is used as a defensive means to avoid enemies and risks[16]. The ejected ink helps cephalopods to confuse predators and useful to alert other cephalopods about the danger[17]. Cephalopod ink consists of a suspension of melanin granules in a viscous colorless medium. In the mantle cavity, the ink gland cells of the digestive tract degenerate and discard their content into the ink sac, which is used as a reservoir for the ink.[18] The production and ejection of the Cephalopod ink regulated by the glutamate, nitric oxide, cGMP signaling pathway located in the ink gland[19]. Cephalopod ink contains a large amount of melanin and also contains proteins, lipids, glycosaminoglycans, and various metals[20-21]. It also contains a variety of melanogenic enzymes, including tyrosine, which is a dopachrome-rearranging enzyme[22].

METHODS AND MATERIALS

The Cephalopoda was collected from Visakhapatnam port on 10th May 2018 and transported to the laboratory in Icebox and sample pictures were taken and compared the Physical characteristics of Cephalopoda according to the FAO Fish. Synop.,(125)Vol. 3:87p. and species identified as Loligo*duvauceli* which is also known as Indian squid. The ink sac dissected by manual method into a sterile plastic container and ink were collected through the gland by using a sterile needle and syringe and collected into a sterile glass bottle.

Isolation and Identification of pathogens from Squid ink:

For isolation of Loligoduvauceli ink, HiCrome Universal Differential Medium used.HiCrome Universal Differential Medium is a modified media by Pezzlo, Wilkie et al., Friedman et al., Murray et al., Soriano and Ponte, and Merlino et al..this medium is recommended for the identification of microorganisms from clinical and non-clinical specimens. This medium very helps in the identification of some gram-positive bacteria and gram-negative bacteria on the basis of chromogenic nature exhibited by a particular microorganism. Squid ink inoculated on HiCrome Universal Differential Medium Streaked plate was incubated at 37°C for 24 hours in aerobic condition and examined the colonies grown in the plate, all colonies vary in their general appearance and observed more than one type of colony, further incubation microbes are identified through Gram stain and characterized biochemically, confirmed as Proteus species, Klebsiella species, Enterococcus species, and Pseudomonas species.

Screening for antibacterial activity by agar well diffusion method

The antibacterial activity of the Loligoduvauceli crude ink was carried out by the agar well diffusion method. Four different individual pure colonies are isolated on the nutrient medium from The HiCrome Universal Differential Medium under sterile condition. Nutrient agar is weighed and mixed with distilled water and sterilized agar medium was inoculated with four bacterial species isolated and pour plated. The Petri plates are allowed to solidify and wells are prepared by metal borer and different percentages of sodium chloride solutions are loaded into different wells and incubated for overnight. The obtained microorganisms are screened with the sodium chloride solution for anti-microbial activity against microorganisms. Sodium chloride solution was prepared in different percentages i.e, in increasing concentrations and the bacterial growth was determined by measuring the diameter zone of inhibition.

RESULTS AND DISSCUSSION

The antimicrobial activity of the Loligoduvauceli crude ink was evaluated against four bacterial species such as Proteus species, Klebsiella species, Enterococcus species, and Pseudomonas species the maximum zone of inhibition for bacterial strains were observed against Enterococcus species (14mm) and minimum against Proteus species (10mm). Klebsiella and Pseudomonas species show similar activity. Studies conducted by Nirmale et al. (2002) on Indian squid Loligoduvauceli concluded that it has good antimicrobial activity against gram-negative bacteria, Salmonella spp. Escherichia coli, Vibrio cholerae, V. parahaemolyticus, and Pseudomonas spp. on the other hand, the effects of the gram-positive bacteria Staphylococcus spp. and Micrococcus spp. are weaker than the effects against gram-negative bacteria[23] Giriji et al. (2011), isolated ink of Indian squid (Loligoduvauceli) carried out a study and reported a novel antimicrobial protein, Lolduvin-s which shows potential antibacterial and antifungal activities against different pathogens and showed promising antibacterial action against dental caries pathogens[24-25] and it also reported excellent antibacterial properties against extended-spectrum beta-lactamase (ESBL)-producing strains of E. coli and Klebsiella pneumonia[26]. Studies confirmed that squid (L. duvauceli) and soft cuttlefish (Sepioteuthislessoniana) ink have strong antimicrobial activity against biofilms causing microorganisms.[27] Cuttlefish (Sepia aculeate) ink and L. duvauceliink have antifungal effects against Fusariumspp and Aspergillus fumigates[28] and partially purified ink extracts of squid (L. duvauceli) also have antibacterial effects against Escherichia coli, Pseudomonas aerginosa, Salmonella typhi, Vibrio Cholera, Bacillus subtilis, Staphylococcus aureus, Aspergillus fumigates and Candida albicans[29] Crude and partially purified ink of squid (L. duvauceli) shows good anti-carcinogenic activity on the HepG2 cell line[30] Other properties of cephalopod ink Squid (L. duvauceli) and cuttlefish (Sepiellainermis) ink have shown strong antiretroviral activities against MMLV-RT[31].

CONCLUSION

The result of this study concluded that the crude ink of the Loligoduvauceli collected from the Visakhapatnam, Andhra Pradesh India contains Klebsiella species, Enterococcus species, and Pseudomonas species hence it cannot use as therapeutics. Previous studies on microbe-free Loligoduvauceli ink shows some beneficial therapeutic activities like antioxidant, antibacterial, anticancer, hepatoprotective, etc, Higher concentrations of NaCl shows the high activity and low concentration of NaCl shows less activity, it has been proved that NaCl has a capacity to inhibit the growth of microbes present in the cephalopod ink and can be used as an alternative method to produce microbe-free Loligoduvauceli ink.

CONFLICT OF INTREST

The authors acknowledged no possible conflicts of interest with respect to the research, authorship, and publication of this article.

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Table 1: Results of Gram staining of Loligoduvauceli crude ink

Color of colonies	Gram staining	Shape of the micro organisms
Blue	+ve	Cocci
Colorless	-ve	Rods
Blue green	-ve	Rods
Light brown	-ve	Rods

Table 2: Results of Biochemical testes of Loligo duvauceli crude ink

Color of colonies	Citrate test	Indole test	Triple sugar iron agar test	Mannitol test	Glucose fermentation test	Lactose fermentation test	Conformed microorganism
Blue	-Ve	-Ve	A/A	+Ve	+Ve	+Ve	Enterococcus species
Colorless	+Ve	-Ve	K/K	+Ve	-Ve	-Ve	Pseudomonas species
Blue green	+Ve	-Ve	A/A	+Ve	+Ve	+Ve	Klebsiella species
Light brown	+Ve	-Ve	K/A	-Ve	+Ve	-Ve	Proteus species

Table 3: Results of Antimicrobial activity of NaCL on the pathogens derived from Loligoduvauceli crude ink

Isolated pathogen	60%NaCl	70%NaCl	80%NaCl	90%NaCl	100%NaCl
Proteus species	6 mm	8 mm	10 mm	11 mm	12 mm
Klebsiella species	10 mm	10 mm	11 mm	12 mm	13 mm
Enterococcus species	8 mm	10 mm	11 mm	12 mm	14 mm
Pseudomonas species	10 mm	10 mm	11 mm	12 mm	13 mm

Table 4: Comparatives studies of the pathogens derived from Loligoduvauceli crude ink in NaCL

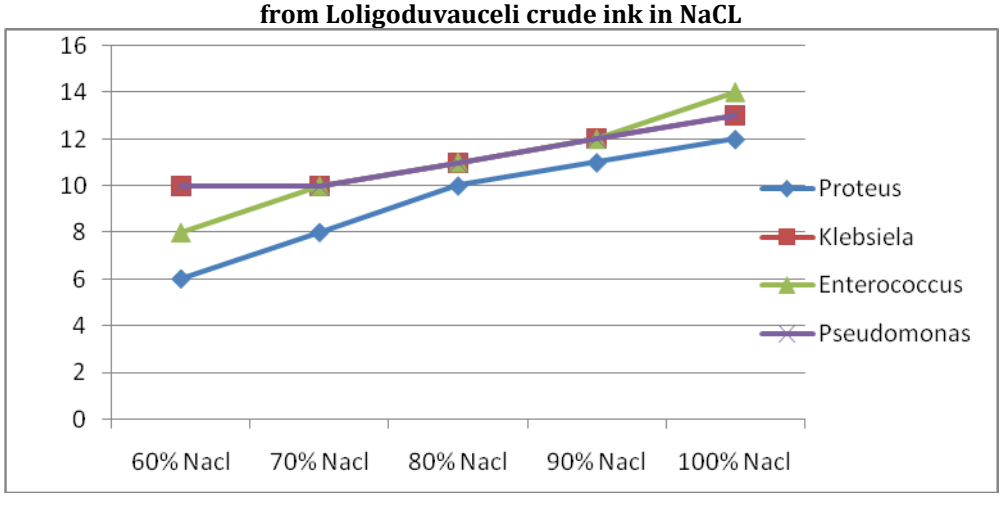

Fig. 1: Universal media shows different types of colonies.

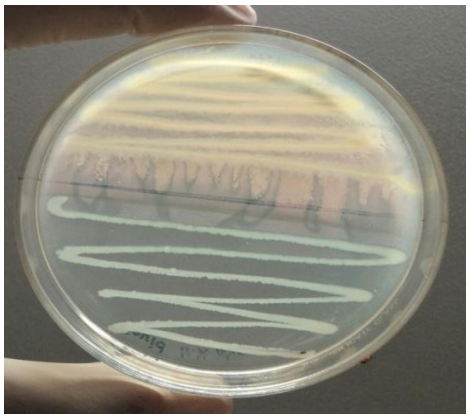

Fig. 2: Blue and Colorless Colonies on Nutrient Agar

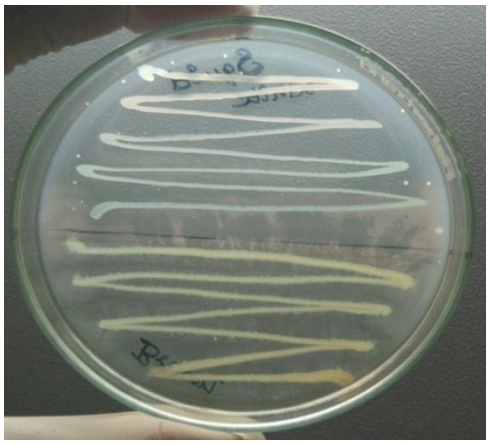

Fig. 3: Blue green and Light brown Colonies on Nutrient Agar

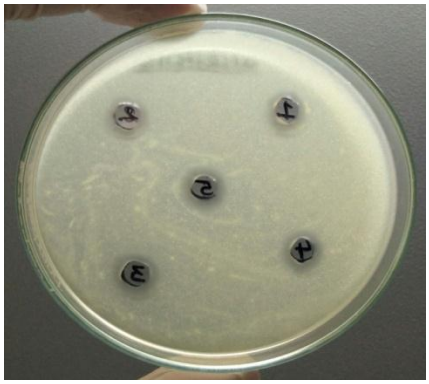

Fig. 4: Klebsiella specieson Nutrient Agar Medium

Fig. 5: Enterococcus species growth on Nutrient Agar Medium

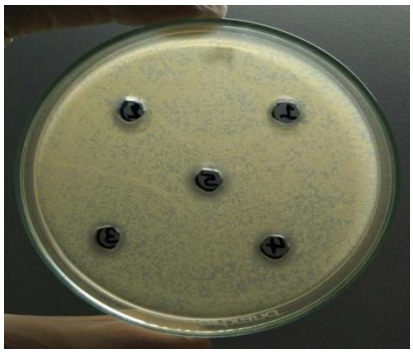

Fig. 6: Proteus specieson Nutrient Agar Medium

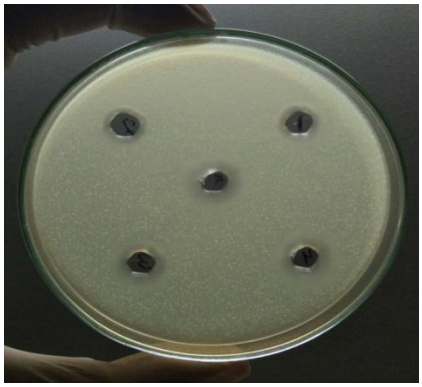

Fig. 7: Pseudomonas specieson Nutrient Agar Medium

REFERENCES

1. Boyle P and Redhouse P. Cephalopod's ecology and fisheries. Blackwell Science. Ames, Iowa. 2005;1-452
2. Love MS Probably More Than You Want to Know About the Fishes of the Pacific Coast. 2nd Edn., Really Big Press, Santa Barbara, CA. 1996;381.
3. Hanlon RT and Messenger JB. Cephalopod Behaviour. Cambridge University Press, Cambridge, UK. 1996.
4. Norman M and Reid A. A Guide to Squid, Cuttlefish and Octopuses of Australasia. CSIRO Publishing, Victoria. 2000.
5. Derby CD. Cephalopod ink: production, chemistry, functions and applications. Marine Dugs. 2014;12(5):2700-2730. https://doi.org/10.3390/ md12052700.
6. Nair P and Sherief PM. Antibacterial activity in the accessory nidamental gland extracts of the Indian squid, LoligoduvauceliOrbigny. Indian Journal of Marine Science. 2010;39(1):100-104.
7. Hoque MS, Benjakul S and Prodpran T. Effect of heat treatment of film-forming solution on the properties of film from cuttlefish (Sepia pharaonis) skin gelatin. Journal of Food Engineering. 2010;96(1):66-73. https://doi.org/10.1016/ j.jfoodeng.2009.06.046.
8. Sundaram S. Various uses of cephalopods. Fishing Chimes. 2009;29(8):23-25.
9. Rao KV. Biology and fishery of the Palk-Bay squid, Sepioteuthisarctipinnis Gould. Indian Journal of Fisheries. 1954;1(1 and 2):37-66.
10. Voss GL. Cephalopods of the Philippine Islands. Vol. 234, p. 1-180. United States National Museum: Bulletin of the United States National Museum. 1963. https://doi.org/10.5479/si.03629236.234.1.
11. Cornell JJ and Handy R. Trends in fish utilization. 1982;30-31. England: Fishery news books.
12. Silas EG and Pillai PP. Resources of tunas and related species and their fisheries in the Indian Ocean. In Central Marine Fisheries Research Institute Bulletin. 1982;32:1-174. India: Indian Council of Agricultural Research.
13. Hanlon RT and Messenger JB. Cephalopod behaviour. 1st ed. United Kingdom: Cambridge University Press. 1996.
14. Bush SL and Robison BH. Ink utilization by mesopelagic squid. Marine Biology. 2007;152(3): 485494. https://doi.org/10.1007/s00227-007-0684-2
15. Ortonne JP, Voulot C, Khatchadourian C, Palumbo A and Prota G. A reexamination of melanogenesis in the ink gland of cephalopods. In Seiji, M. (Ed.) Pigment Cell 1981: Phenotypic Expression in Pigment Cells. 1981;49-57. Tokyo: University of Tokyo Press.
16. Liu H, Luo P, Chen S and Shang J. Effects of squid ink on growth performance, antioxidant functions and immunity in growing broiler chickens. Asian-Australasian Journal of Animal Sciences. 2011;24(12):1752-1756. https:// doi.org/10.5713/ajas.2011.11128
17. Lucero MT, Farrington H and Gilly WF. Quantification of L-dopa and dopamine in squid ink: implications for chemoreception. The Biological

Bulletin. 1994;187(1):55-63. https://doi.org/10.2307/1542165.
18. Palumbo A, Gesualdo I, Di Cosmo A and De Martino L. The Ink Gland of Sepia officinalis as Biological Model for Investigations of Melanogenesis. In New Developments in Marine Biotechnology. 1998;147-149. US: Springer. https://doi.org/10.1007/978-1-4757-5983-9_32.
19. Lei M, Wang JF, Wang YM, Pang L, Wang Y, Xu W and Xue CH. Study of the radioprotective effect of cuttlefish ink on hemopoietic injury. Asia Pacific Journal of Clinical Nutrition. 2007;16(S1): 239-243.
20. Liu H, Luo P, Chen S and Shang J. Effects of squid ink on growth performance, antioxidant functions and immunity in growing broiler chickens. Asian-Australasian Journal of Animal Sciences. 2011;24(12):1752-1756. https://doi.org/10.5713/ajas.2011.11128.
21. Zhong JP, Wang G, Shang JH, Pan JQ, Li K, Huang Y and Liu HZ. Protective effects of squid ink extract towards hemopoietic injuries induced by cyclophosphamine. Marine Drugs. 2009;7(1):9-18. https://doi.org/10.3390/md7010009.
22. Palumbo A, Gesualdo I, Di Cosmo A and De Martino L. The Ink Gland of Sepia officinalis as Biological Model for Investigations of Melanogenesis. In New Developments in Marine Biotechnology. 1998;147-149. US: Springer. https://doi.org/10.1007/978-1-4757-5983-9_32.
23. Nirmale V, Nayak BB, Kannappan S and Basu S. Antibacterial effect of the Indian squid, Loligoduvauceli (d'Orbigny), ink. Journal of the Indian Fisheries Association. 2002;29:65-69.
24. Giriji S, Priyadharshini JV, Suba PK, Hariprasad G and Raghuraman R. Isolation and characterization of lolduvin-S: A novel antimicrobial protein from the Ink of Indian Squid Loligoduvauceli. International Journal of Current Research. 2011;3(7):4-14.
25. Girija AS, Suba KP, Hariprasad G and Raghuraman R. A novel study on the antibacterial effect of the crude squid ink extracts from the Indian squid against four bacterial pathogens isolated from carious dentine. International Journal of Current Microbiology and Applied Sciences. 2014;3(4): 904-911.
26. Smiline Girija AS, Vijayshree Priyadharshini J, Pandi Suba K, Hariprasad P and Raguraman R. Antibacterial effect of squid ink on ESBL producing strains of Escherichia coli and Klebsiellapneumoniae. Indian Journal of Geo-Marine Sciences. 2012;41(4):338-343.
27. Nicomrat D and Tharajak J. Antimicrobial Effect of Squid Ink on Common Microbial Causing Biofilm Attaching to Silicone. In Yimnirun, R. (Ed.) Applied Mechanics and Materials. 2015;804: 191194. Switzerland: Trans Tech Publications. https://doi.org/10.4028/www.scientific.net/AMM.804.191.
28. Vennila R, Kanchana S, Arumugam M and Balasubramanian T. Investigation of antimicrobial and plasma coagulation property of some molluscan ink extracts: Gastropods and cephalopods. African Journal of Biochemistry Research. 2010;5(1):14-21.
29. Diaz JHJ and Thilaga RD. Screening of Antimicrobial Activities In The Ink of Cephalopods Against Human Pathogens. Drugs. 2016;1(2):3-4.
30. Diaz JHJ, Thilaga RD and Sivakumar V. Cytotoxic activity of crude and partially purified ink of L. duvauceli towards HepG2cell line. International Journal of Pharma Research and Review. 2014;3(6):19-23.
31. Rajaganapathi J, Thyagarajan SP and Edward JK. Study on cephalopod's ink for anti-retroviral activity. Indian Journal of Experimental Biology. 2000;38:519-520.