

Anatomy and physiology features of developing cervical spine and zoological cultures as promoting of the new varieties of fishes

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Abstract

Cellular cultures can be used to investigate the role of ciliates in forming of flocs of fishes in the absence of many other microbes. Aquaculture is rapidly expanding and faces numerous difficulties in achieving growing demands while maintaining the security and suitability of fish products. The primary motivation for developing fish cellular cultures, as till recent times, the most common application of these cultures, has been the exclusion and description of fish viruses which are the causal factors of epizootics in economically valuable aquaculture or fishery. *Acinetobacter*, *Vibrio*, and *Pseudomonas* grow faster in flesh media than in artificial media. Fish-protein-hydrolysate peptones have no interaction with prions which cause bovine spongiform encephalopathy. Tissue engineering can all be combined with advanced aquaculture technologies to make marine cellular cultures as an appealing option for producing in vitro fish meat. Fish waste products could be an interesting perspective of cost effective and efficient peptones, the use of which has resulted in good growth yields for a wide range of bacterial genera. Food loss starts immediately as the fish killed, so handling should be completed as soon as possible to avoid the development of bacteria and molds on it.

Keywords: Cellular cultures, ciliates, microbes, aquaculture, fish products, sea foods.

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INTRODUCTION

High tide, earthen reservoirs for shrimp-farming are examples of brackish-water-flow through systems, whereas cage-culture is most prominent example of marine-flow-through systems and is most common method of aquaculture in several countries. A disadvantage of open -systems is that, in addition to their site specificity, they can sometimes pollute recipient waterways and thus become constrained by the environmental factors. Recirculating systems, on the other hand, include provisions for having stable quality of the water. Because water input is minimized, slight water is released, and this water can be handled, these systems have the ability to be more ecofriendly. Nevertheless, appropriate water quality control is required for recirculating system operation to be successful. So far, bio-control by bacteria and algae has been the most financially feasible strategy in this perspective [1-3].

Man has always used fish and other marine resources everywhere. Even though, most ethno biologists working and continue to focus their studies on plant usages. Moreover, in order to establish ethno biology as a beneficial restraint with own authenticity, we must broaden our scope of inquiry to include certain organisms, particularly animals. Fish has always been important constituent of human diets, and it continues to be so today [4]. *Yersinia ruckeri* causes enteric redmouth ailment in *Oncorhynchus mykiss*, and TSA and BHIA are commonly used. For primary isolation of *Y. ruckeri*, a *ferential medium*. It depends upon two properties to enhance selectivity: Tween 80 hydrolysis and the mannitol negative assimilation [4, 5].

Cellular agriculture has lately piqued the attentions of regulatory over biotechnology products and the over livestock, there's really attention in the regulations required for conceivably jurisdiction over cell based processed meat. Regardless of the fact that seafood is completely constrained by FDA with

exception of animals in order Siluriformes, cell based seafood production, and its prospect to meet an increasing demands for seafood whereas preventing the difficulties of industrial aquaculture, has received little regulatory attention [6-8].

Bacterial strains' spoilage characteristics were investigated by growing them at $28 \pm 2^\circ\text{C}$ in broth and agar media containing germ free fish and prawn flesh supernatants. The spoilers percentage found among the examined bacterial isolates, as evidenced by halo zone formation and odour production, was unaffected by the source of flesh utilized. In broth, indole and fluorescent pigment production have also been observed. *Acinetobacter*, *Vibrio*, and *Pseudomonas* grew faster in flesh media than in artificial media. The reduction in lipid and protein concentrations in clear zone of agar media indicates that spoilage bacteria are utilising the obtainable substrate [9]. Keratinase production can normally be carried out in fairly affordable growing media containing keratinous substrates as primary carbon and nitrogen source; this facilitates the production of the enzyme extremely advantageous from an economical perspective. As a result, keratinolytic microbes are thought to be involved in bio-conversion of fish scales. Whereas other studies have suggested the use of byproducts as micro-organism substrates, it's the 1st review on the use scales of fish by the *Bacillus* species. The findings of the study showed that using bioproducts in culture media is somewhat interesting when considering growth of microorganisms under the studied parameters [9-11].

Developing the Zoological cultures for fisheries

Peptones derived from the FPH (fish-protein-hydrolysate) are a good peptide-derived nitrogen source. They may be a viable choice to peptones derived from animals or plants. Fish-protein-hydrolysate peptones have no interaction with prions which cause bovine spongiform encephalopathy, nor do they have potential for genetic alterations that plants do. Surprisingly, discovered that fish-protein-hydrolysate peptones functioned even better than beef-peptones on

many investigated micro-organisms. Because of the short length of peptides and higher amount of free amino acids in their content, MCMs enriched with FPH-based-peptones can be used by a wide range of micro-organisms. It promotes the growth of even depressed micro-organisms that require extra growth factors. Furthermore, producing fish-protein-hydrolysate from waste products of marine sources would raise the market price of fishing grounds [12-15].

Before reaching senescence, most of the new fish cellular cultures can be sub-cultured for different lengths of time. The primary motivation for developing fish cellular cultures, as till recent times, the most common application of these cultures, has been the exclusion and description of fish viruses which are the causal factors of epizootics in economically valuable aquaculture or fishery. The promising utility of fish tissue and cellular cultures as teaching aids should not be underestimated. Most fish cellular cultures are simple to establish and/or maintain. They grow in a wide range of temperatures and it can be perpetuated in most cases at house temperature [16]. Halophilic bacteria are naturally occurring pollutants in salt used in fish preservation; the better shelf consistency of salt fish produced can be influenced by the composition of salted utilised. Because anchovy ripening takes time and all these bacteria can even have lipolytic and proteolytic properties, their development can cause unwanted changes; thus, determining the halophilic bacteria content in the salted products is essential [17, 18].

When augmented with the 5-20 percentage serum, the different defined media, for example, Eagle's minimally acceptable medium, etc are highly nutritious media for the culture of most cells. It is the source of important nutrients to cells; the nutrients are present in both the solution and are bound to the proteins. Serum plays an important role in supplying proteins, such as fibronectin, that cell attachment boost t to the substrate. It also contains spreading factors, which aid in the spread of cells until they start to divide [18-20].

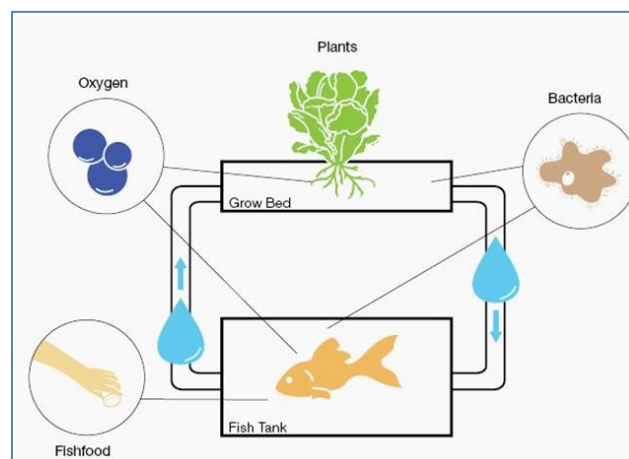


Fig-1: Shows the biological principles of fisheries and association with food chain

Cellular cultures could also be used in coming years to investigate the role of ciliates in forming of flocs of fishes in the absence of many other microbes. In the perspective of treatment of wastewater, cultures of ciliate have been utilized for this intent. *T. thermophila* capsule secretion has shown to make a contribution to flocculation. Ciliates have been cultivated in the labs on fish homogenates, unidentified fish powder and fish cells, so fish fragments may be obligated to boost ciliate cultures. These cultures can be used to study the methods through which ciliates form floc as well as the nature of floc derived from fish samples [20-23]. Aquaculture is rapidly expanding and faces numerous difficulties in achieving growing demands while maintaining the security and suitability of fish products. The idea of generating cell based seafood is arising as a modern strategy to producing alternative animal's protein. This novel approach to animal's protein production from the fish might identify numerous key issues confronting traditional aquaculture structures and decreasing capture fisheries. This alternate solution of fish production process will alleviate the strain on ecological resources. As a result, the a whole world is shifting toward climate resistant production systems, and in vitro production of meat has arose as a cutting edge and high-priority field of research. The successful introduction of in vitro hamburger has leads to better cell based meats. The convenience of growing the fish cell lines at low temperature than animal cells may lead to reduced costs for the manufacturing of cellular fish-meat versus cellular animal-meat. Tissue engineering can all be combined with advanced aquaculture technologies to make marine cellular cultures as an appealing option for producing in vitro fish meat. Muscle cell culture of fish can be utilized for in vitro production of fish meat by taking advantage of their unique physiological characteristics such as tolerance to hypoxia, higher buffering capacity, and difference in temperature [24-26].

The aquaculture industry has experienced strong development in the recent years as a result of rising seafood demand and advancement of culture techniques. Nevertheless, the existing fisheries sector, especially high aquaculture, is experiencing numerous major challenges, including a lack of abundant and low cost protein sources for feeds, massive losses due to pathogen attack, and quality changes during storage and culture. Bacterial application may be a viable solution to such difficulties easy operation, higher protein content and diverse bioactivities. In fact, several advantageous species of bacteria have been researched and implemented to aquaculture for a variety of purposes. Nonetheless, new uses and applicable mechanistic studies are fully justified in the future to enhance conventional technologies and minimize possible risks [23, 24, 26].

Swamps are rich in biodiversity, which include sediment micro biota that can enhance the chemical and physical properties of their surroundings. *Chlorophyta*, *Streptomyces sp.* and *Bacillus sp.* are among the swamp micro biota described. *Bacillus sp.* with concentration level of 105 CFU mL⁻¹ and *Chlorophyta* micro-algae with an appropriate concentration of 10 percent of maximum density can develop in fish culture media which can be used as ecologic probiotics. *Chlorophyta* is a microbe which can be used in fish farming media as green water. *Chlorophyta* utilisation elevated the amount of dissolved O₂ in swamp water culture media by 63.63 percent and pond culture media by 60.52 percent. The increased O₂ is prompted by *Chlorophyta* performing photosynthesis process, which generates dissolved O₂ in culture media. Proteolytic bacteria *Streptomyces sp.* and *Bacillus sp.* were isolated [28-30].

Nonetheless, when these marine probiotics are commercially available, their usage to hatcheries and aquaculture plants will necessitate the manufacturing capacity of huge biomass resources and feasible cells on an industrial level. Moreover, marine medium or even marketing peptones are cost prohibitive, and the progression of a low-cost medium is critical for ensuring a constant and reliable MPB supply. Fish waste products could be an interesting perspective of cost - effective and efficient peptones, the use of which has resulted in good growth yields for a wide range of bacterial genera [30-32]. The current fishing industry, on the other hand, aims to gradually eliminate fish waste. It requires fishing boats to land all carries of commercially regulatory species. By-products are undesired catches which cannot be instantly sold for human utilization due to the absence of market [21-23]. *M. ackerel*, *gurnard*, *blue whiting*, *pouting*, *hake*, *hake*, *megrin*, *red scorpionfish*, *boarfish*, and *Atlantic horse mackerel*, are among the most important species of fish landed in Europe ports in terms of tones, but they are also among the most discredited by fishing vessels [33].

In media for the culture and isolation of *Renibacterium salmoninarum*, the causal agent of bacterial renal disease in salmonid fish, charcoal is an appropriate substitute for serum. KDM-C medium have 1 gram of L-cysteine hydrochloride, 0.5 grams of yeast extract, 1 gram of activated charcoal, 10 grams of peptone, and 15 grams of agar per litre and is maintained to pH 6.8 before sterilizing with NaOH. Though fish has many nutritional benefits, as previously stated, fresh seafood has a very limited shelf life. Food loss starts immediately as the fish killed, so handling should be completed as soon as possible to avoid the development of bacteria and molds on it. Fermentation by reducing the pH), Cooking by boiling, frying or roasting, and curing by smoking, drying or

salting, are all methodologies for preserving fish. Gas, electricity, Solar, firewood and charcoal are all examples of sources of energy [34, 35]. Different types of cultures have been used for testing and analysis of different metals and detection of various infectious diseases. Diseases from aquaculture populations to wild fish is possible, infections of concern to fish culturists and aquarists may be caused by *Streptococcus* and that also depends on various factors such as fish density, temperature, water quality and nutritional status of the host, and virus strain influence susceptibility. Overall, use of different cultures have been adopted in order to control the biological and pathological infections in such a way that management of infections and strategies through biological conservation [35-40].

CONCLUSION

Dryers come in a variety of shapes and sizes, but they all operate on same basic principles, that include power production, power distribution, humidity migration, and the drying process. Cabinet (tray) dryers, belt dryers, bin (silo) dryers, fluidized bed dryers, tunnel (truck) dryers, pneumatic (flash) dryers, drum dryers, rotary dryers, vacuum dryers, freeze dryers and spray dryers, are all popular ones dryers. The process of drying is mass and heat transfer phenomenon in which moisture relocates from the interior of the product to surface and vaporizes via diffusion.

REFERENCES

1. Van Rijn, J. (1996). The potential for integrated biological treatment systems in recirculating fish culture—a review. *Aquaculture*, 139(3-4), 181-201.
2. Summerfelt, S. T. (1998, July). An integrated approach to aquaculture waste management in flowing water systems. In *Roanoke (USA): Proceedings of the Second International Conference on Recirculating Aquaculture* (pp. 87-97).
3. Arbiv, R., & van Rijn, J. (1995). Performance of a treatment system for inorganic nitrogen removal in intensive aquaculture systems. *Aquacultural Engineering*, 14(2), 189-203.
4. Svanberg, I., Locker, A. Ethnoichthyology of freshwater fish in Europe: a review of vanishing traditional fisheries and their cultural significance in changing landscapes from the later medieval period with a focus on northern Europe. *J Ethnobiology Ethnomedicine* 16, 68 (2020). <https://doi.org/10.1186/s13002-020-00410-3>
5. Sanders, J. E., & J. L. Fryer. (1980). *Renibacterium salmoninarum* gen. nov., sp. nov., the causative agent of bacterial kidney disease in salmonid fishes. *International Journal of Systematic Bacteriology* 30: 496–502.
6. Waltman, W. D., & Shotts Jr, E. B. (1984). A medium for the isolation and differentiation of *Yersinia ruckeri*. *Canadian Journal of Fisheries and Aquatic Sciences*, 41(5), 804-806.
7. Stephens, N., Di Silvio, L., Dunsford, I., Ellis, M., Glencross, A., and Sexton, A. (2018). Bringing cultured meat to market: technical, socio-political, and regulatory challenges in cellular agriculture. *Trends Food Sci. Technol.* 78, 155–166. doi: 10.1016/j.tifs.2018.04.010
8. Tacon, A. G. J., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture* 285, 146–158. doi: 10.1016/j.aquaculture.2008.08.015
9. Chandrasekaran, M., Lakshmanaperumalsamy, P., & Chandramohan, D. Fish flesh agar medium — a suitable experimental medium for the detection of spoilage bacteria. *Antonie van Leeuwenhoek*, 51, 219–225 (1985).
10. Verma, A., Singh, H., Anwar, S., Chattopadhyay, A., Tiwari, K. K., Kaur, S., & Dhilon, G. S. (2017). Microbial keratinases: industrial enzymes with waste management potential. *Critical reviews in biotechnology*, 37(4), 476-491.
11. Vittori, J., Schocken-Iturrino, R. P., Poiatti, M. L., Pigatto, C. P., Chioda, T. P., Ribeiro, C. A. M., ... & Ragazani, A. V. F. (2008). Qualidade microbiológica de leite UHT caprino: pesquisa de bactérias dos gêneros *Staphylococcus*, *Bacillus* e *Clostridium*. *Ciência Rural*, 38(3), 761-765.
12. Beaulieu, L., Desbiens, M., Thibodeau, J., & Thibault, S. (2009). Pelagic fish hydrolysates as peptones for bacterial culture media. *Canadian journal of microbiology*, 55(11), 1240-1249.
13. Beaulieu, L., Desbiens, M., Thibodeau, J., & Thibault, S. (2009). Pelagic fish hydrolysates as peptones for bacterial culture media. *Canadian journal of microbiology*, 55(11), 1240-1249.
14. Horn, S. J., Aspmo, S. I., & Eijsink, V. G. H. (2005). Growth of *Lactobacillus plantarum* in media containing hydrolysates of fish viscera. *Journal of Applied Microbiology*, 99(5), 1082-1089.
15. Vecht-Lifshitz, S. E., Almas, K. A., & Zomer, E. (1990). Microbial growth on peptones from fish industrial wastes. *Letters in applied microbiology*, 10(4), 183-186.
16. Nicholson, B. L. (1989). Fish cell culture: an update. *Advances in cell culture*, 7, 1-18.
17. Felix, M., Amezttoy, I., Ramirez, E., & Yeannes, M. (2004). Incidence of different genus of halophilic *Arqueobacteria* in samples of matured salted anchovy (*Engraulis anchoita*). *Biocell*, 28(2), 226.
18. Félix, M. M. L., Amezttoy, I., & Yeannes, M. I. (2008). Evaluación microbiológica de anchoíta (*Engraulis anchoíta*) salada madurada y filetes en aceite. In *Publicación anual del X Congreso Rosarino. XXVIII Reunión Anual de la Sociedad de Biología de Rosario*.

19. Karnieli, O., Friedner, O. M., Allickson, J. G., Zhang, N., Jung, S., Fiorentini, D., ... & Oh, S. (2017). A consensus introduction to serum replacements and serum-free media for cellular therapies. *Cytotherapy*, 19(2), 155-169.
20. Fekete, N., Rojewski, M. T., Lotfi, R., & Schrezenmeier, H. (2014). Essential components for ex vivo proliferation of mesenchymal stromal cells. *Tissue Engineering Part C: Methods*, 20(2), 129-139.
21. Piazzon, M. C., Wiegertjes, G. F., Leiro, J., & Lamas, J. (2011). Turbot resistance to *Philasterides dicentrarchi* is more dependent on humoral than on cellular immune responses. *Fish & Shellfish Immunology*, 30(6), 1339-1347.
22. Castro, R., Paramá, A., Barja, J. L., Leiro, J., Sanmartin, M. L., & Lamas, J. (2007). Culture of the histophagous ciliate *Philasterides dicentrarchi* (Ciliophora: Scuticociliatia) in fish tissues. *Journal of fish diseases*, 30(4), 239-242.
23. Arregui, L., Serrano, S., Linares, M., Pérez-Uz, B., & Guinea, A. (2007). Ciliate contributions to bioaggregation: laboratory assays with axenic cultures of *Tetrahymena thermophila*. *International Microbiology*, 10(2), 91.
24. Zaraska, M. (2013). Lab-grown beef taste test: 'Almost' like a burger.
25. Rubio, N., Datar, I., Stachura, D., Krueger, K. (2019). Cell-based fish: a novel approach to seafood production and an opportunity for cellular agriculture.
26. Wang, C., Chuprom, J., Wang, Y., & Fu, L. (2020). Beneficial bacteria for aquaculture: nutrition, bacteriostasis and immunoregulation. *Journal of Applied Microbiology*, 128(1), 28-40.
27. Rurangwa, E., & Verdegem, M. C. (2015). Microorganisms in recirculating aquaculture systems and their management. *Reviews in aquaculture*, 7(2), 117-130.
28. Pérez-Sánchez, T., Ruiz-Zarzuola, I., de Blas, I., & Balcázar, J. L. (2014). Probiotics in aquaculture: a current assessment. *Reviews in Aquaculture*, 6(3), 133-146.
29. Saraswati, N. (2018). Actinomycetes isolation for bioremediation of swamp water polluted by organic matter].
30. Safari, R., Motamedzadegan, A., Ovissipour, M., Regenstein, J. M., Gildberg, A., & Rasco, B. (2012). Use of hydrolysates from yellowfin tuna (*Thunnus albacares*) heads as a complex nitrogen source for lactic acid bacteria. *Food and Bioprocess Technology*, 5(1), 73-79.
31. Safari, R., Motamedzadegan, A., Ovissipour, M., Regenstein, J. M., Gildberg, A., & Rasco, B. (2012). Use of hydrolysates from yellowfin tuna (*Thunnus albacares*) heads as a complex nitrogen source for lactic acid bacteria. *Food and Bioprocess Technology*, 5(1), 73-79.
32. Gildberg, A., Dahl, R., Mikkelsen, H., & Nilsen, K. (2010). Peptones from Atlantic cod stomach as nitrogen sources in growth media to marine bacteria. *Journal of Aquatic Food Product Technology*, 19(2), 75-83.
33. Iñarra, B., Bald, C., Cebrián, M., Antelo, L. T., Franco Uría, A., Vázquez, J. A., ... & Zufía, J. (2019). What to do with unwanted catches: Valorisation options and selection strategies.
34. Daly, J. G., & Stevenson, R. M. (1985). Charcoal agar, a new growth medium for the fish disease bacterium *Renibacterium salmoninarum*. *Applied and Environmental Microbiology*, 50(4), 868-871.
35. Mumtaz, Muzammil, Justin Mendoza, Ardalan Seyed Vosoughi, Anthony S. Unger, and Vijay K. Goel. (2021). "A Comparative Biomechanical Analysis of Various Rod Configurations Following Anterior Column Realignment and Pedicle Subtraction Osteotomy." *Neurospine* 18, no. 3: 587.
36. Hinz, Boris, Sem H. Phan, Victor J. Thannickal, Marco Prunotto, Alexis Desmoulière, John Varga, Olivier De Wever, Marc Mareel, and Giulio Gabbiani. (2012). "Recent developments in myofibroblast biology: paradigms for connective tissue remodeling." *The American journal of pathology* 180, no. 4: 1340-1355.
37. Nishida, Norihiro, Muzammil Mumtaz, Sudharshan Tripathi, Amey Kelkar, Takashi Sakai, and Vijay K. Goel. (2021). "Biomechanical Analysis of Posterior Ligaments of Cervical Spine and Laminoplasty." *Applied Sciences* 11, no. 16: 7645.
38. Mumtaz, Muzammil. (2017). "Finite element analysis of cervical spine & finite element modeling of knee joint." Master's thesis, Fen Bilimleri Enstitüsü.
39. Ehrlich, Melanie. (2002). "DNA methylation in cancer: too much, but also too little." *Oncogene* 21, no. 35: 5400-5413. Harvard
40. Mumtaz, Muzammil, Iman Zafarparandeh, Paniz Taherzadeh, Saliha Zeyneb Akıncı, and Deniz Ufuk Erbulut. (2016). "Effect of U-shaped implant on the biomechanics of the cervical spine." In 2016 20th National Biomedical Engineering Meeting (BIYOMUT), pp. 1-3. IEEE.
41. Strogatz, Steven H. (2018). *Nonlinear dynamics and chaos: with applications to physics, biology, chemistry, and engineering*. CRC press.