

# GLOBAL JOURNAL

OF SCIENCE FRONTIER RESEARCH: G

## Bio-Tech & Genetics

Small Scale Fish Farmers

Growth Promoters by Rhizospheric

Highlights

Fish Production Technology

Microorganisms on Natural Medium

Discovering Thoughts, Inventing Future

VOLUME 20

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## Fish Production Technology of Small Scale Fish Farmers at Chaugachha Upazila under Jashore District of Bangladesh

By Md. Rasal Ali & B.M. Newaz Sharif

**Abstract-** The study was conducted on the fish farmer, which was situated at Chaugachha Upazila, Jashore, from October 2015 to April 2016. Data were collected using participatory rural appraisal (PRA) tools and personal observation. About 62% of the farmers have ponds of single, and 38% have multiple ownership. The homestead and commercial ponds were 79% and 21%, respectively. About 100% farmer carried out poly-culture fish farming though they did not know poly-culture just culture of various fishes. About 98% of the farmers control aquatic weeds manually. For controlling undesirable species, most of them (95.74%) used the netting method. Liming used 185.3-247 kg/ha and organic fertilizer, mainly cow dung used 741-1235 kg/ha. Average stocking density was found to be 12326 fry/ha. 91.5% of the farmers applied supplementary feed, such as both rice-bran and mustard oil-cake. The peak harvesting period was found from December to January. In this season, around 65% of the stocked fishes were reported to harvest, and the rest of the fish (35%) was harvested during another season.

**Keywords:** culture season; pre-stocking management, stocking density, fertilization, feed and feeding practices, fish production, harvesting and marketing.

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# Fish Production Technology of Small Scale Fish Farmers at Chaugachha Upazila under Jashore District of Bangladesh

Md. Rasal Ali <sup>α</sup> & B. M. Newaz Sharif <sup>σ</sup>

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**Keywords:** culture season; pre-stocking management, stocking density, fertilization, feed and feeding practices, fish production, harvesting and marketing.

## I. INTRODUCTION

The fisheries sector contributes 3.74% of the gross domestic product (GDP), 20.87% of agricultural resources, and 4.04% of foreign exchange earning of Bangladesh (DoF, 2009). Our country has about 40.47 lakh ha open inland water body, 5.28 lakh ha closed inland water body and marine water covers an area of 1.66 lakh ha. These water bodies are very rich in fisheries resources. Bangladesh has at least 260 freshwater fish species, and over 475 marine species (DoF, 2009). Lack of adequate and authentic information on the socio-economic condition of the target population is one of the impediments in the

successful implementation of the developmental program (Ellis, 2000). Aquaculture practice has become a promising and gainful methodology to attain self-sufficiency in the food sector and also to alleviate poverty in developing countries like Bangladesh (Ahmed *et al.* 2003).

A livelihood is sustainable when it can cope with and recover from stresses and shocks and maintain or enhance its capabilities and assets both now and in future, while not undermining the natural resource base (Chambers and Conway, 1992).

Freshwater fish farming plays a role in rural livelihoods in Bangladesh. Apart from direct self-employment opportunities from fish farming, pond fish farming offers diverse livelihood opportunities for operators farming employees of hatcheries and seed nurseries, and for seed traders and other intermediaries Fisheries is one of the sub-sector in the agricultural sectors and plays a role in the socio-economic development of the rural area, fulfilling the animal protein demand, creating employment opportunity, alleviating poverty and earning foreign exchange for the country.

Therefore, the present study was conducted on the following objectives:

1. Know to improve fish farming technology of low scale fish farmers in some selected areas of Jashore district.
2. To assess the constraints of fish production.

## II. MATERIALS AND METHODS

### a) Study area and study period

The study was conducted on the fish farmer, which was situated at Chaugachha Upazila, Jashore, from October 2015 to April 2016. Data were collected from 47 pond owners randomly selected from the study area.

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Figure 1: Map of the study area

b) Data collection method

Collecting data on livelihood and technological issues, only the questionnaire interview method was used. For collecting data, both individual and group interviews were applied with different degrees of effectiveness of the farmers' information.

c) Data analysis

All the collected data were summarized and scrutinized and analyzed by MS Excel and then presented in tabular and chart forms.

III. RESULTS

General features of fish ponds

a) Pond ownership and size

In the study area, 62% of the farmers have ponds of single and 38% have multiple ownership. The average pond size in the study area was found to be 0.10 ha.

b) Pond type and depth

In the study area, ponds were of two categories: homestead and commercial. The homestead and commercial ponds were 79% and 21% and 77% were seasonal, and 23% were perennial, respectively. The water level of perennial declined during the dry season and become unsuitable for fish culture. Some farmers pump water, during the dry season. Seasonal ponds become unsuitable for fish culture during the dry season. The average depth was 6 fit and average water was 3.45 fit.

c) Fish production technology

i. Culture season and method

The season of fish farming in the study area is from April to December. Fish fries were stocked when they become available from April to June and the cultured fishes were harvested primarily during December to January. In the study area, 100% farmer carried out poly-culture fish farming though they did not know poly-culture just culture of various fishes and feeding of a different layers of water. In poly-culture system farmer cultured mainly major Indian carps are shown in Table 1. Some farmers also culture shar punti (*Puntius sarana*), indigenous Koi, and Magur.

Table 1: Indian Major carps and Exotic carps

Species (Local Name)	Scientific Name
Silver Carp	<i>Hypophthalmichthys molitrix</i>
Catla	<i>Catla catla</i>
Rohu	<i>Labeo rohita</i>
Mrigal	<i>Cirrhinus cirrhosus</i>
Grass Carp	<i>Ctenopharyngodon idella</i>
Common Carp	<i>Cyprinus carpio var communis</i>

Farmers did not follow any scientific combination of the species are shown in Figure 2.

Farming activities	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Pond preparation		■	■									
Stocking				■	■	■	■	■	■	■	■	
Rearing				■	■	■	■	■	■	■	■	
Harvesting												■

Figure 2: Schedule of fish farming in a pond system

ii. Pre-stocking management

Pre-stocking management of ponds in the study area comprises dike repairing, aquatic weed control, and undesirable species (predator and trash fish) control. About 98% of the farmers control aquatic weeds manually. For controlling undesirable species most of them (95.74%) used the netting method. Some farmers used rotenone and phostoxin (4.26%) but did not follow any recommended dose. Then farmers used lime at the rate of 185.3-247 kg/ha and organic fertilizer mainly cow dung at the rate of 741-1235 kg/ha.

iii. Stocking density

From the survey, it was found that majority farmer's stocked hatchery- produced fry, and some wild fry. The average stocking density was found to be 12326 fry/ha.

iv. Fertilization

It was observed that the majority of the farmers used cow dung, and except one farmer used poultry droppings as organic fertilizer. Farmers used both Urea and TSP as inorganic fertilizers. In the study area, pond fish farmers generally used cow dung at the rate of 2964 kg/ha/yr regularly or four to five times in a month. The average dose of inorganic fertilizer such as urea and TSP was 741kg/ ha/yr and 370.5 kg/ha/yr, respectively. Most of the farmers used fertilizers irregularly.

v. Use of lime and its application rate

All the farmers used lime irregularly in variable doses. The average dose of liming was found to be 370.5 kg/ ha/yr in the study areas.

vi. Feed and feeding practices

It was found that 91.5% of the farmers applied supplementary feed such as both rice-bran and mustard oil-cake. Among them 13.95% farmers use marketed feed and 86.05% farmers use non marketed feeds. The average doses of rice-bran and mustard oil-cake were 2964 kg/ha/yr, and 1482 kg/ha/yr, respectively.

vii. Harvesting and marketing

Although fish are harvested throughout the year, the peak harvesting period was found from December to January. In this season, around 65% of the stocked fishes were reported to be harvested, and the rest of the fish (35%) was harvested during another season. Farmers harvested their fish using cast net and seine net locally known as ber jal. Harvested fish were kept in a plastic barrel. From the survey, it was found that

around 75% of the fishes are sold by the farmers to local packers and the rest 25% consumed by the households and given to the relatives. It was found that 82% of the farmers hired laborers for harvesting their fish.

In marketing systems, there found to be several middlemen, such as local agents, whole -sellers, local fish traders, and retailers. Market communication is being made through middlemen. It was observed that a few pond fish farmers directly sold their fish to local packers or local agents at the bank of the ponds, and the majority of the farmers brought their fish in local markets and sold them directly to local packers or consumers.

viii. Fish production

The average annual yield of fish was 4085.4 kg/ha.

ix. Problems faced by the fish farmers

Several of problems faced by the fishermen, such as poor technical knowledge, fish disease, insufficient water during the dry season, lack of money, and natural disaster (over flow of water). According to the survey, 36.17%, 10.64%, 25.53%, 12.77%, 14.895% respondent's poor technical knowledge, identified fish disease, Insufficient water during the dry season, lack of money and low price of the product to be the most problems, respectively .

Table 2: Problems faced by the fish farmers in the study areas

Problem	Total n=47
Poor technical knowledge	17 (36.17%)
Fish disease	5 (10.64%)
Insufficient water during dry season	12 (25.53%)
Lack of money	6 (12.77%)
Low price of the product	7 (14.89%)

d) Production cost

In the study area, it was found that the average total annual cost of fish production was Tk. 169645.72/ha (Table 3).



Table 3: The production cost of fish/ha/yr.

Cost items	Mean TK
Fingerlings	54782.22
Feed	66690
Fertilizers	21859.5
Lime	8151
Drugs and chemicals	895
Water pumping and electricity	6200
Human laborer	4528
Harvesting	3040
Miscellaneous	3500
Total	169645.72

e) *Net profit and cost benefit -ratio (CBR)*

It was found that the average return of fish production was Tk. 285998/ha/yr. From the survey, it was found that per hectare average profit from fish culture was 116332.28/ha/yr. The average cost-benefit ratio (CBR) was 1.46.

IV. DISCUSSION

a) *Pond feature*

i. *Pond size*

Size of the pond is a factor for fish culture because all measures regarding all management are planned considering the size of ponds. The management of small size ponds is than a large size pond in all management during the fish culture. In the study area, most of the ponds were the medium size (0.10 ha). That's why the farmers can easily manage their pond during the culture as well as harvesting. In my study, it was found that the average pond size was 0.10 ha with a range from 0.03 ha to 0.22 ha. Saha (2004) found that the average pond size in Tangail Sadar Upazila was 0.19 ha; this result was more or less same as my study. Rahman (2003) found that the average pond size in Gazipur was 0.12 ha. Saha (2003) found that the average pond size was 0.21 ha in Dinajpur sadar upazila. Saha *et al.* (1995) observed that the range of pond size was within 0.05 to 0.15 ha. Khan (1994) stated that fish culture efficiency varied with the size of ponds.

ii. *Pond ownership*

In the present study, 62% ponds were single, and 38% were multiple ownership. These results were matched with the findings of Saha (2004), who found that 52% ponds under single ownership, 21% ponds were under multiple ownership and 27% as leased ponds. Quddus *et al.* (2000) found that about 34% of the total ponds were joint ownership and 54% were single and, the rest of 12% ponds were under public or organization property in Demra, Dhaka. It is proved from many studies that multiple ownership is the main problem to improve the pond culture system as well as efficient use of resources for fish cultivation (Ali and Rahman, 1986 and Mollah *et al.*, 1990).

iii. *Type of pond*

From the survey, it was found that 77% ponds were seasonal and the remaining 23% were perennial. Saha (2004) found that 37% ponds were seasonal, and 63% were perennial in Tangail Sadar Upazila. Saha (2003) observed that 17% ponds were seasonal and 83% were perennial in Dinajpur Sadar Upazila. In the study area, the land position comparatively high from the sea level that is the cause of drying during the dry season.

b) *Fish production technology*

i. *Culture season and method*

From the survey, it was found that almost all farmers (100%) carried out a poly culture system. In the study area, the culture season was from April-December. Farmers in this area stocked carp (Indian major carp and exotic carp), punti, (Local Name) indigenous shing, and magur, koi, and tilapia. Ahmed (2003) observed that peak period of carp poly culture was from April to December. Rahman (2003) reported that the season of carp farming was from March to December. Saha (2003) stated that there were two culture seasons in Dinajpur Sadar Upazila (Fazilpur and Sunderban union). One was from June to December and another was from February to June.

ii. *Pre-stocking management*

As a traditional farmer, most of the farmers know that the pre-stocking management is to clear all aquatic weed, repair the side of the pond and fill up with water. Within the farmers, some know about the treatment of pond bottom, water and create natural food production in water trained by the Upazila Fisheries officers and some NGO under their project. In the study area, most of (98%) controlled their aquatic weed manually. For removing unwanted species, 95.74% farmers used the netting method, and 8-12% farmers did not use any chemicals or other methods. Only a few farmers (4.26%) used rotenone and phostoxin. Biswas (2003) found that the chemicals and other toxic substances used in pond farms for controlling aquatic weeds, pests, predators and undesirable species were rotenone, phostoxin, dipterex, bleaching powder, diseal, summation, endrin, copper sulphate, aldrin and DDT in 75.0, 65.0, 22.5, 10.0, 7.5 5.0, 2.5 and 2.5% farms respectively.

### iii. Stocking density

Stocking density is a factor for fish culture in the case of culture technique, food habit, and measurement of lime, fertilizer use. According to DoF the stocking density of carp is 30-40 per decimal. The standard stocking density for carp culture is 35 to 40 per decimal. The average stocking density in the study area, was found 12326 fry/ha. Rahman (2003) found that, the average stocking density was 25,250/ha in Gazipur. Hassanuzzaman (1997) stated that, the average stocking density was 16,196 fry/ha in the district of Rajshahi. Hossain *et al.* (1992) observed that the range of stocking density was from 10,000-31,000/ha in a village of Mymensingh district.

### iv. Fertilization

The average dose of organic fertilizer was 2964 kg/ha/yr and inorganic fertilizer such as Urea, and TSP was 741 kg/ha/yr and 370.5 kg/ha/yr, respectively. Saha (2004) observed that the average dose of organic fertilizer was 8330 kg/ha/yr and inorganic fertilizer was urea 387 kg/ha/yr and TSP 176 kg/ha/yr. Rahman *et al.* (1998) found that doses of organic and inorganic fertilizer were 11,075 kg/ha and 739 kg/ha respectively. Hassanuzzaman (1997) observed in his study in Rajshahi district that the average dose of organic fertilizer was 2,801 and inorganic 97 kg/ha/yr. Rana (1996) found in his study in Sirajgonj district that the organic fertilizer was 8,122 kg/ha/yr and inorganic fertilizer was urea 315 and TSP 111 kg/ha/yr.

### v. Feed and feeding practices

We all know sufficient supply feeds, is important to increase fish production. In the study area, the average dose of rice-bran, and mustard-oil cake was 2964 kg/ha/yr, and 1482 kg/ha/yr, respectively. Most of the farmers do not use pellet feed because it's costly and probably beyond their capacity line. Rahman (2003) found that the dose of rice-bran and oil-cake was 2,730 and 580 kg/ha, respectively. The result of the present study is different from the report of Rahman (2003). Saha *et al.* (1995) found the average dose of rice-bran and oil-cake was 5,192 and 734 kg/ha, respectively. Hassanuzzaman (1997) found in the Rajshahi district the dose of rice-bran and oil-cake was 1,250 and 1,212 kg/ha, respectively. But the farmers in the study area did not follow any scientific methods, their feeding practice was more irregular.

### vi. Harvesting and marketing of fish

In the study area, the peak-harvesting season was from December to January because during this period, fish became marketable size and market price was high. Rahman (2003) found that the main period of harvesting was from October to January. However, Saha (2004) found that, the peak-harvesting season was from November to January. Ahmed (2003) stated that the peak-harvesting season was from December to March. Farmers harvested their fish by using the cast net and

seine net in the study area. The similar results showed by Rahman (2003) and Saha (2004). Ahmed (2003) observed that farmers harvested their fish usually by using the cast net.

### c) Cost-return analysis

The average total cost of fish production in the study area was observed Tk. 169645.72/ha/year. Ahmed (2003) found that the average fish production cost was Tk. 23,210 -Tk. 24,790/ha. Biswas *et al.* (2000) revealed that the average total cost of pond fish production was in Tk. 59,813. It was found that the average CBR was 1.46.

### d) Constraints of fish production

From the study, it was found that lack of scientific knowledge, multiple ownership, lack of feed, lack of equipment for harvesting, and lack of marketing facilities were most constraints for fish production. Moreover, besides the cultivation work, the farmer takes less care in fish production. Khan *et al.* (1998) found that lack of extension work for fisheries improvements caused the greatest difficulty in pond fish culture.

## V. CONCLUSION

On the basis of the findings of the present study, the following recommendations were made for sustainable pond fish farming and to maintain sustainable livelihoods of fish farmers in Chaugachha Upazila under Jashore district. The majority of the people are little educated, so they should be educated to develop social consciousness. People should bring under group activity, and local security system should also be developed. The problem of multiple ownership can be solved by leasing the pond to a person interested in fish culture or through the cage or pen culture by different owners. To get the proper price of fish in the market, the number of middle man should be reduced. Supply of net and other harvesting and marketing equipment to the farmers with less fare may reduce harvesting and marketing costs. For this purpose, co-operative society among fish farmers should be established to make harvesting and marketing equipment and their maintenance. Government and other organizations should play their assigned role by disseminating information to the farmers and arranging necessary training for scientific methods of fish production in a pond. Such training will assist farmers to identify and solve the problems related to the fish farming.

To supply quality fish seed to the farmers more hatcheries should be established by the help of Government and NGO. In that case, existing problems in the hatcheries should be overcome.

### Conflict of interest

None to declare.

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# Overproduction of Some Plant Growth Promoters by Rhizospheric Microorganisms on Natural Medium

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**Abstract-** Considering the nutritional values of *Mentha viridis*. L and *Aloe vera* plants, these plants can be utilized for the production of alternative cultivation media. The cost of artificial culture media is very high, and some components may be unavailable. Use of the plant-based culture media would drastically reduce the expense of the synthetic media. Fifteen bacterial isolates were isolated from *Aloe vera* rhizosphere, nine bacterial and nine actinomycetes isolates were isolated from *Mentha viridis* rhizosphere both cultivated in Sirs EL- Layan, El-Menoufia governorate, Egypt. *In-vitro* screening was done for the production of indole acetic acid (IAA) and phosphorus solubilization. Results revealed that bacterial isolate No. MB4 produced a high amount of IAA(36.51  $\mu\text{g/ml}$ ) on the *Mentha*-based culture medium, No. A6 showed maximum IAA production (16.25 $\mu\text{g/ml}$ ) on the *Aloe vera*-based culture medium and isolate No. MA6 was efficient in phosphorus solubilization (867.85 $\mu\text{g/ml}$ ) that was isolated from the *Mentha viridis*. L rhizosphere.16s rRNA analysis of these isolates revealed they are (*Pseudomonas monteilii* strain CIP 104883, *Streptomyces rochei* strain DW3 and *Kosakonia radicincitans* strain DSM 16656 respectively.

**Keywords:** plant-based culture media, menthe viridis. l, aloe vera, rhizospheric microorganisms, plant growth-promoting activities, biocontrol.

**GJSFR-G Classification:** FOR Code: 060599



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# Overproduction of Some Plant Growth Promoters by Rhizospheric Microorganisms on Natural Medium

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**Abstract-** Considering the nutritional values of *Mentha viridis*. L and *Aloe vera* plants, these plants can be utilized for the production of alternative cultivation media. The cost of artificial culture media is very high, and some components may be unavailable. Use of the plant-based culture media would drastically reduce the expense of the synthetic media. Fifteen bacterial isolates were isolated from *Aloe vera* rhizosphere, nine bacterial and nine actinomycetes isolates were isolated from *Mentha viridis* rhizosphere both cultivated in Sirs EL-Layan, El-Menoufia governorate, Egypt. *In-vitro* screening was done for the production of indole acetic acid (IAA) and phosphorus solubilization. Results revealed that bacterial isolate No. MB4 produced a high amount of IAA(36.51 µg/ml) on the *Mentha*-based culture medium, No. A6 showed maximum IAA production (16.25µg/ml) on the *Aloe vera*-based culture medium and isolate No. MA6 was efficient in phosphorus solubilization (867.85µg/ml) that was isolated from the *Mentha viridis*. L rhizosphere.16s rRNA analysis of these isolates revealed they are (*Pseudomonas monteilii* strain CIP 104883, *Streptomyces rochei* strain DW3 and *Kosakonia radicinans* strain DSM 16656 respectively. Bio-control ability of selected strains screened by antagonistic activity against pathogenic fungi revealed that *Pseudomonas monteilii* strain CIP 104883 had shown maximum zone of inhibition against *Rhizoctonia solani* (19 mm) while *Streptomyces rochei* and *Kosakonia radicinans* showed highly antifungal activity against *Fusarium solani* (18 mm and 11mm zone of inhibition, respectively).

**Keywords:** plant-based culture media, menthe viridis. I, aloe vera, rhizospheric microorganisms, plant growth-promoting activities, biocontrol.

## I. INTRODUCTION

Microbial culture media can be of different types, depending on the nutritional growth requirements of the microorganisms. Microorganisms require about ten macroelements, namely (C, O, H, N, S, P, K, Ca, Mg and Fe). The first six components are needed for the synthesis of Carbohydrates, Lipids, Proteins, and Nucleic acids, and the remaining four exist in the cell as cations and play a

variety of roles. Additionally, all microorganisms require several microelements like (Mn, Zn, Co, Mo, Ni, and Cu) that are generally part of enzymes and cofactors. They also require growth factors, which are organic compounds such as vitamins Basu *et al.* (2015). Uthayasooriyan *et al.* (2016) stated that the feasibility of evolving alternate culture media to artificial media, namely Potato Dextrose Agar and Nutrient Agar, were assessed by using locally obtainable expensive materials due to the use of synthetic culture media in colleges, researchal centers and laboratories have limitations in finance. In general, the inexpensive locally obtainable materials such as rice, chickpea, corn, and natural soy flour may serve as replacing rich nutrient media to the growth of bacteria and fungi, and reduce the cost of microbial media. Commercially available media such as Nutrient Agar and MacConkey Agar, which used for the growth of microorganisms but these, are very expensive. Plant-based culture medium is a natural environment that has been introduced for the culturing of rhizobacteria as a sole growth milieu Nour *et al.* (2012).

In this study, *Mentha* and *Aloe vera* extracts were used as a natural medium for isolation and growth of plant growth-promoting rhizobacteria (PGPR). *Mentha* and *Aloe vera* are perineal plants that are locally available, cheap plant material, and nutritionally rich. (Mainasara *et al.*, 2018) revealed that the fresh peppermint leaves contained 92.31% carbohydrates, 2.19% protein, 0.50% lipid, 1.5% fiber, 3.57% ash, 89.5% moisture, and the most predominant mineral found was potassium and sodium while the other minerals also found in low values such as calcium, magnesium, and phosphorus. *Aloe vera*, a succulent plant that grows in arid and subtropical climates, the leaves of this nutraceutical medicinal plant contains numerous vitamins, natural sugars, enzymes, and amino acids Lanka (2018).

The use of chemical fertilizers, especially nitrogenous and phosphorous, led to water, soil, and air pollution. The excessive use of these fertilizers cause harmful effects on rhizospheric microorganisms, effects on the fertility of agriculture lands, pollutes the environment, and also declines the productivity of the crops and yield Kumar *et al.* (2015) and Ahmad *et al.* (2016). In addition to its cost, the production of these

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fertilizers causes shortage in nonrenewable resources like natural gas and oil that used to produce these chemical fertilizers, and poses a lot of environmental and human dangers Prakash and Verma (2016). To achieve a clean agriculture with low cost and produce crops with desired properties, one possibility is to use soil microorganisms (bacteria, fungi, actinomycetes, algae, etc.) that increases the nutrient uptake capacity and water use efficiency. Among these potential soil microorganisms, bacteria known as plant growth-promoting rhizobacteria (PGPR) are the most promising. In this sense, PGPR may be used to enhance plant health and regulate plant growth rates without environmental contaminations (Saharan and Nehra, 2011). Plant Growth-Promoting Rhizobacteria (PGPR) are the rhizospheric bacteria that can positively affect plant growth by several mechanisms like phosphate solubilization, siderophore production, biological nitrogen fixation, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), and inhibition of biofilm formation, phytohormone production, exhibiting antimicrobial activity, induction of systemic resistance (ISR), promoting beneficial plant-microbe symbioses, and many others mechanisms Benaissa (2019).

Current studies aimed to develop and evaluate culture media from plants because the synthetic culture media have become very expensive in the local market and, most instances, are not available and also the production of plant growth promoters on this natural medium to use in clean agriculture instead of chemical fertilizers.

## II. MATERIALS AND METHODS

### a) Sampling sites

Samples from the rhizosphere of *Mentha viridis* L and *Aloe vera* plants were collected from Sirs EL- Layan, El-Menoufia governorate, Egypt, from the upper surface layer of the soil (5 cm), where maximum populations of microorganisms concentrated.

### b) Tested plants

The tested plants, *Mentha viridis* and the succulent plant's *Aloe vera*, are cultivated in Sirs EL-Layan, El-Menoufia governorate, Egypt. Both plants were perineal and rich in nutrient content, *Aloe vera* was chosen for its availability in arid and semi-arid environments as well as their copious juicy nature, while Mint is one of the most common herbs traditionally produced in Egypt for hundreds of years and widely distributed and cultivated in the temperate and sub-temperate regions of the world.

The chemical compositions and nutritional contents of the tested succulent plant (*A. vera*) were available by Tiwari and Upadhayay (2018). Therefore, special attention was given to the analysis of the juice of Spearmint (*Mentha viridis* L) for the application in isolation of rhizobacteria. Macro- and micro-nutrients

were detected by atomic absorption analysis, total protein by Tru Spec N instrument AOAC International and Latimer (2012), carbohydrate Dubois *et al.* (1965), mineral Horwitz (2012) amino acids by performic oxidation method and vitamins by GC/MS/MS analyses Lehotay and Hajslova', (2002). Total crude fiber and ash were also determined AOAC International (1998).

### c) Preparation of plant-based culture media

Wash the succulent leaves of *A. vera* and the vegetative parts (leaves and stems) of *M. viridis*, slice, and then blend with equal aliquots of distilled water (w/v) for 5 min in the blender. The resulting slurry homogenate was coarse-filtered through cheesecloth to obtain plant juice; almost 73–82% of the plant fresh weight was recovered as juice. The plant juices from the tested plants further diluted with distilled water (v/v); 1:10, 1:20, 1:40, 1:80, and 1:100. The pH for *M. viridis* diluted juices were in the range of 5.8-6.5 and *A. vera* diluted juices 4-6.2. Exclusively, use these diluted juices to prepare the plant-based agar culture media (2% agar, w/v). Adjust the pH of all media to 7.0, then autoclave at 1.5 atm., 121°C for 20 min.

Use Serial dilution techniques to isolate rhizospheric microorganisms, prepare the suspensions of samples by adding 1g of the rhizospheric soil that obtained from Sirs El-Lian, El-Menoufia governorate, Egypt to 10 ml of sterile distilled water under aseptic conditions and shake vigorously for 10 minutes by using a vortex at 150rpm, then let to settle for a short period of time. Serial dilution sequentially made, started from stock and  $10^{-1}$  till  $10^{-6}$ . From each diluted tube, 0.1 ml was transferred into the surface of the plant-based agar media at different diluted juice of both *Mentha* and *A. vera* (in triplicate) and spread with an alcohol sterilized L shape glass rod then incubated at 30° C for 24 - 48 hours. After the successful growth of microorganisms, pick up the individual colonies and purify them.

### d) Comparison between the growth of microbial isolates on plant-based culture medium and synthetic media

Bacterial isolates that developed on plant-based culture were tested to grow in nutrient agar medium at 30°C for 24 – 48hr; on the other hand, actinomycetes isolates also developed on starch nitrate medium at 30°C for 5-7days. After the incubation period, the microbial growth and pigmentation were observed in all plates, and then compared to the plant-based culture medium.

### e) Indole acetic acid production by microbial isolates on both plant-based culture and synthetic medium

Estimate the Indole acetic acid (IAA) production quantitatively according to Salkowski method *Brick et al.* (1991). For bacterial isolates, inoculate the conical flasks (250 ml capacity), that contain 100ml of both plant-based and nutrient broth medium supplemented with

(500 $\mu$ g/ml) tryptophan (sterilized by the bacterial filter) with 1 ml of 24 h old rhizobacterial cultures, incubate the flasks on a rotary shaker (150 rpm) 36 $\pm$ 2°C for 72 h. Centrifuge the flasks at 4000 rpm for 15 min to separate supernatant. On the other hand, IAA from actinomycetes was quantified using the method of (Rafik *et al.*, 2014). Streak the actinomycetes isolates on plant-based and starch nitrate agar medium and incubate at 28°C. After five days. Transfer the agar discs of actinomycetes mycelia to conical flasks (250 ml capacity) containing 100ml of both plant-based and starch nitrate liquid medium containing 500 $\mu$ g/mL tryptophan. After seven days of culture at 30°C and stirring at 150 rpm, centrifuge at 3000 rpm for 15min. Estimate the production of IAA in the supernatants by using a colorimetric assay. Mix one milliliter of supernatant with 2 ml of the Salkowski reagent, incubate at room temperature for 30min in the dark. The appearance of pink or red color indicates to the production of IAA. Measure the absorption spectrophotometrically at 530 nm against control of 1 ml culture medium and 2 ml of Salkowski reagent (Glickmann and Dessaux, 1995). The amount of IAA produced per milliliter culture was estimated using a standard curve.

f) *Quantitative assay of phosphate solubilizing activity*

Quantitative estimation of P-solubilization was carried out as per standard methodology Mehata and Nautiyal (2001), by inoculating 1 ml of bacterial suspension and discs of actinomycetes in 50 ml of National Botanical Research Institute Phosphate broth (NBRIP) and incubating the flasks for three days at 36 $\pm$ 2°C and seven days at 30°C respectively. At the end of the incubation period, the cell suspension was centrifuged at 10,000 rpm for 10 min in aliquot 2-5 ml of barton's reagent was added, and after 10 minutes the intensity of color was measured by a spectrophotometer at 420 nm with standard KH<sub>2</sub>PO<sub>4</sub> Jackson 1967.

g) *Identification of selected bacterial and actinomycetes isolates*

The efficient bacterial isolates (MB4), (A6), and actinomycetes isolate (MA6) in IAA production and phosphorus solubilization, respectively, were further identified by 16S rRNA at Sigma Scientific Services Company, 6 of October, El Giza, Egypt.

h) *Molecular characterization of bacteria and actinomycetes*

**DNA extraction:** Add 200  $\mu$ l of the sample (liquid media that contain bacteria or actinomycetes) in microcentrifuge tube and add 95  $\mu$ l water, 95  $\mu$ l solid tissue buffer (blue) and 10  $\mu$ l proteinase K. Mix thoroughly and then incubate the tube at 55°C for 2 hours. Mix thoroughly and centrifugation at 15,000 rpm for 1 minute. Transfer supernatant to a 300  $\mu$ l tube. Add 600  $\mu$ l DNA Binding Buffer, and mix thoroughly. Move the mixture to a Zymo-Spin™ IIC-XL Column in a

Collection Tube. Centrifuge at 15,000 rpm for 60 sec. Discard the collection tube with the flow through. Add 400  $\mu$ l of DNA Pre-Wash Buffer to the column in a new Collection Tube then centrifuge at (15.000 rpm) for 60 sec. Add 700  $\mu$ l gDNA Wash Buffer and centrifuge at (12.000 xg) for 60 sec. Discard the Collection Tube. Add 200  $\mu$ l of DNA Washing Buffer to the column and centrifuge at (15.000 rpm) for 60 sec., get rid of the flow through. Add 30  $\mu$ l of elution buffer then incubate for 5 minutes, and centrifuge at (15.000 rpm) for 60 sec.

i) *PCR amplification and phylogenetic analysis*

Dissolve the extracted DNA in 20  $\mu$ l TE buffer to be used as a template in the PCR reactions. PCR amplifications is to be performed in a total volume of 50  $\mu$ l by mixing 20 ng of the genomic DNA with 2.5 mM concentrations of each dNTPs (deoxynucleotide triphosphate), 1 mM concentrations of each primer of pA (50 -AGAGTTTGATCCTGGCTCAG-30) and pH (50 -AAGGAGGTGATCCAGCCGCA-30) as described by Edwards *et al.* (1989). Also, and 3 U of Taq DNA polymerase in 10X Taq buffer A (GeNeL) is to be added to the mix. These reactions subjected to initial denaturation of 94° C for 6 min followed by 35 cycles of 94°C for 45 sec, 56°C for 45 sec and 72°C for 60 sec and a final extension step of 72° C for 5 min using GeneAmp® PCR system 9700 (Applied Biosystems). The PCR products separated using 0.8% agarose gel. 16S rRNA gene sequence of the isolate compared with 16S rRNA gene sequences available online by using BLASTN search program in the NCBI website, (<http://www.ncbi.nlm.nih.gov>). Clustal X used for multiple sequence alignment (Thompson *et al.*, 1997). The method of Jukes and Cantor (1969) used to calculate evolutionary distances. Phylogenetic analysis was constructed by the neighbor-joining manner and tree topologies were evaluated by performing bootstrap analysis of 1,000 data sets using MEGA 3.1.

j) *Effect of different incubations periods on the growth of the most efficient isolates on both natural and synthetic medium*

This study was carried out to determine the optimum incubation period at which the selected isolates showed maximum growth on both natural and synthetic medium. Inoculate *Pseudomonas monteilii* and *Kosakonia radicincitans* on the natural medium and artificial (nutrient broth) medium with pH to 7.2  $\pm$  0.2. Incubate the flasks at 30°C for different incubation periods (1, 2, 3, 4, 5, and 6 days). Determine the bacterial growth at the end of each incubation period spectrophotometrically by measuring the optical density at 600nm (OD<sub>600</sub>) Koch (1970). Inoculate the conical flasks, that contain 20ml of both *Mentha*-based broth medium and starch nitrate broth medium with *Streptomyces rochei*, then incubate it at 30°C for 1-10 days, take the culture daily, filtrate, then dry it in oven at 70°C for three days to determine the dry weight. The

growth, as indicated by dried biomasses plotted against time Sejjny (1991).

k) *In-vitro* screening of efficient microbial isolates for antagonistic activities against root rot fungi

The possible interaction between the selected bacterial strains and pathogenic fungi (*Fusarium* sp. and *Rhizoctonia* sp.) which are responsible for root rot in a variety of legume and non-legume crops was monitored on potato dextrose agar (PDA) medium using a modified agar – plate inhibition zone technique (Silosuch *et al.*, 1994). Initially, grow the pathogenic fungi (*Fusarium solani*, *Fusarium oxysporum*, and *Rhizoctonia solani*) in 15 cm Petri dishes contains PDA medium and incubate at 28° C for 48 hr. Then, cut 0.5 cm disks from the edge of the actively growing colonies, and transfer one of them on the center of a Petri dish containing PDA medium amended with (g l<sup>-1</sup>) as follows: 3.0 peptone, 0.2 CaCO<sub>3</sub>, and 0.2 MgSO<sub>4</sub>. Inoculate *Pseudomonas montellii* and *Kosakonia radicincitans* surrounded to the fungus disk. Then, incubate the dishes at 28°C for (4-7 days). Zones of inhibition of fungal growth were observed. On the other hand, Potato dextrose agar plates were prepared and inoculated with *Streptomyces rochei* by streaking in a single line at the center of the petri dish containing PDA medium. After seven days of incubation at 28°C, seed the plates with a disk of test fungi at an angle of 90° to the actinomycetes isolate and incubate at 28°C for five days, microbial interactions were recognized by the determination of the diameter of inhibition zone (Madigan *et al.*, 1997).

### III. RESULTS AND DISCUSSION

A total of fifteen bacterial isolates were isolated from *Aloe vera* rhizosphere on *Aloe vera*-based culture medium with different dilutions 1:10, 1:20, 1:40, 1:80 and 1:100 v/v (juice or sap: distilled water, v/v) (Plate 1a-d and Plate 2a), these results are harmony with

Youssef *et al.* (2015) who isolated distinctive colonies of rhizobacteria associated with the roots of *Aloe vera* developed on agar plates of plant-based culture media, that prepared from diluted juices (1:20, v/v) of *A. vera*. And nine bacterial and nine actinomycetes isolates were rhizosphere of *M. viridis* were developed on agar plates of *Mentha*-based culture media prepared from diluted juices (1:10, 1:20, 1:40, 1:80 and 1:100 v/v) of *M. viridis* (Plate 2b). The results indicated that both *Aloe vera* and *Mentha* juices were nutritionally rich enough in Table (1 and 2, respectively) to support the better growth of rhizobacterial isolates associated with the rhizosphere of both tested plants. Good bacterial growth was obtained with further dilutions up to 1:100 (juice: distilled water, v/v) (Plate 1a-d and Plate 2a and b). Such a positive dilution effect attributes to decreasing the osmotic effect of concentrated nutrients as well as minimizing the inhibitory effect of antimicrobial compounds present in the juices of tested plants Pellizzoni *et al.* (2012). The tested *Mentha* (Table 2) contains abundant nutritional content, and *Aloe vera* was reported to contain amino acids, anthraquinones, enzymes, steroids, hormones, salicylic acid, minerals, sugars, saponins, and vitamins as main ingredients Upadhyay (2018) that essential for the microbial growth. The results also showed that actinomycetes isolate (Gram-positive) developed on *Mentha*-based medium rather than *Aloe vera*-based medium. This may occur due to the presence of steroid, triterpenoid, flavonoid, phenol, tannin, alkaloid, saponin and acid in the *Aloe vera* juice responsible for its antibacterial activity. An earlier report suggested that a large number of *Aloe vera* extracts to be active against Gram-positive bacteria McCutcheon *et al.* (1992), probably due to the absence of the outer of the bacterial cell wall.

Table 1: The chemical compositions and nutrient contents of *Aloe vera* Tiwari and Upadhyay (2018)

Chemical Group	Constituents
Amino Acids	Provides 20 of the 22 required amino acids and 7 of the 8 essential ones.
Enzymes	Anthranol, barbaloin, chrysophanic acid, ethereal oil, ester of cinnamic acid, isobarbaloin, resistannol
Anthraquinones	Provides aloe emodin, aloe tic acid, alovin, Anthracine.
Steroids	Cholesterol, lupeol, camp sterol, sistosterol
Hormones	Auxins and gibberellins
Salicylic Acid	Aspirin like compounds
Saponins	Glycosides
Minerals	Calcium, chromium, copper, iron, manganese, potassium, sodium and zinc
Sugars	Monosaccharide's: Glucose and Fructose Polysaccharides: Glucmannans/polymnrose
Vitamins	A, B, C, E, choline, B12, folic acid

Table 2: The chemical compositions and nutritional contents of *Mentha*

parameters	mg/ 11g fresh weight of mint	parameters	mg/ 11g fresh weight of mint
<b>Protein and Amino Acids</b>		Carbohydrate	850
Protein	350	Fiber	790
Tryptophan	5.3	Ash	175
Threonine	15.1	<b>Fats &amp; Fatty Acids</b>	
Isoleucine	15.1	Fat	190
Leucine	26.8	Omega-3 fatty acids	38.2
Lysine	15.4	Omega-6 fatty acids	5.8
Methionine	5.1	<b>Vitamins</b>	
Cysteine	4.2	Vitamin C	1.5
Phenylalanine	18.6	Niacin	0.1
Tyrosine	11.0	Folate	0.012
Valine	17.5	Retinol	0.022
Arginine	17.2	<b>Minerals</b>	
Histidine	7.1	Calcium	21.4
Alanine	18.7	Iron	1.3
Aspartic acid	43.4	Magnesium	7.0
Glutamic acid	40.0	Phosphorus	6.4
Proline	15.2	Potassium	50.5
Serine	14.4	Sodium	3.2
Glycine	17.4	Zinc	0.1
Phytosterols	1.1	Manganese	0.1

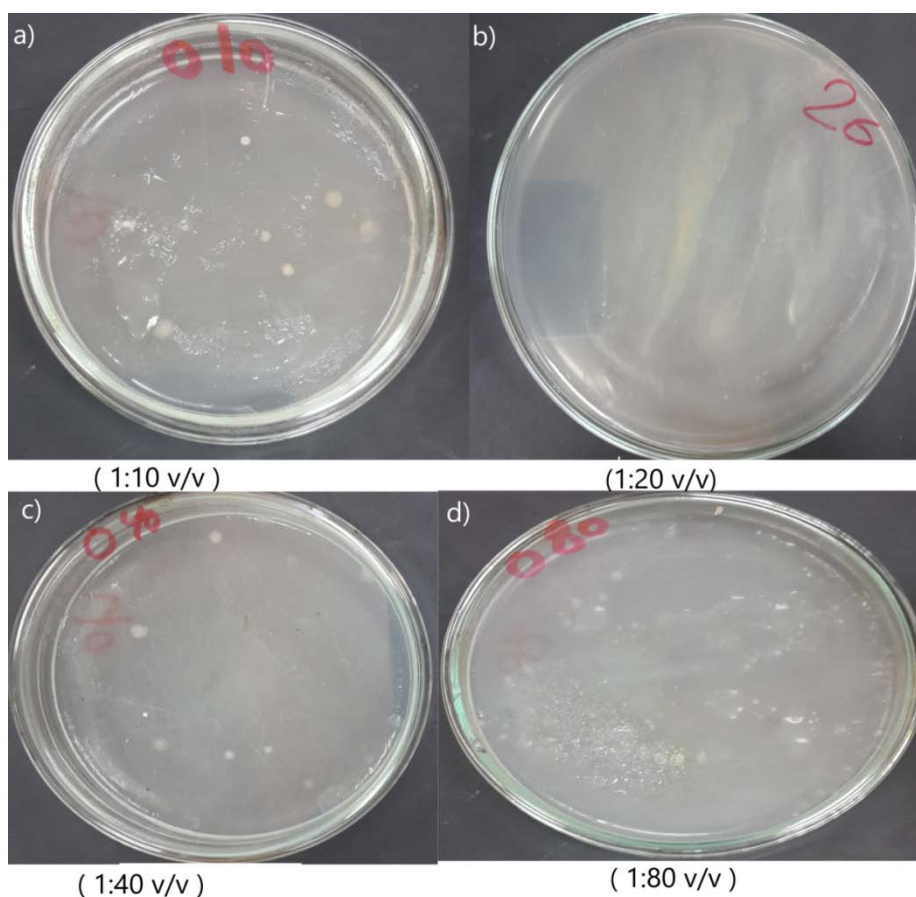
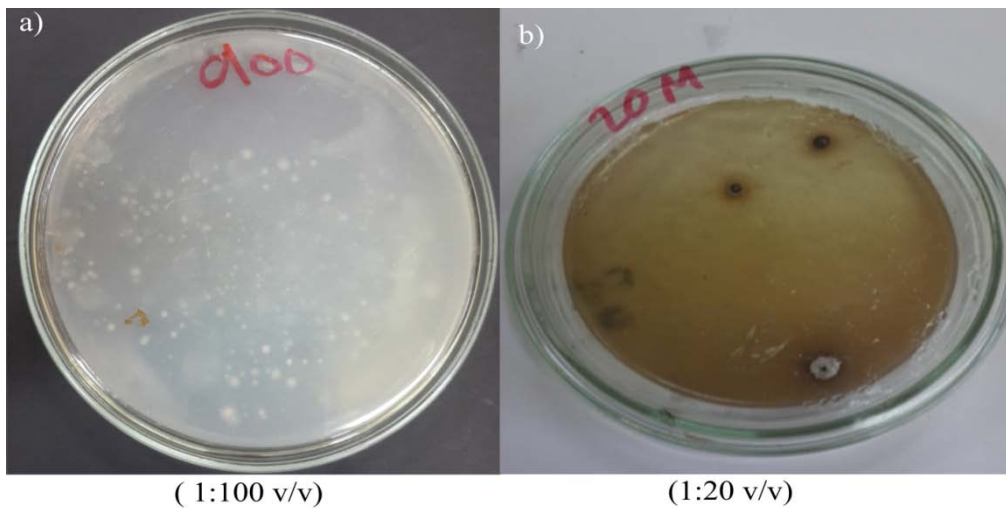


Plate 1: a-d) Colonies of rhizobacteria associated with the roots of *Aloe vera* developed on agar plates of plant-based culture media prepared from diluted juice (1:10, 1:20, 1:40, 1:80 v/v) of *A. vera*



**Plate 2:** a) Colonies of rhizobacteria associated with the roots of *Aloe vera* developed on agar plates of plant-based culture media prepared from diluted juice (1:100 v/v) of *A. vera*. b) Colonies of microorganisms associated with the roots of *Mentha viridis* developed on agar plates of plant-based culture media prepared from diluted juice (1:20, v/v) of *M. viridis*.

a) Comparison between the growth of microbial isolates on plant-based culture medium and synthetic media

The actinomycetes isolates showed higher rate of growth on *Mentha*-based agar plates than starch nitrate agar plates (Plate 3). This may be due to *Mentha*-based medium possesses a considerable amount of nutritional contents like proteins, carbohydrate and fats and also growth factors such as vitamins and amino acids (Table 2) that essential for their growth while synthetic media provides only limited growth factors. In general, the growth of actinomycetes isolates on the plant-based culture media was good enough and very much comparable to the standard culture medium (starch nitrate medium). It is clear from the results that the growth is greatly influenced by the nature and type of the nitrogen source supplied in the culture medium. In comparison with inorganic nitrogen sources, organic nitrogen sources (protein and amino acids) induced relatively higher biomass yield. This is in accordance by the findings of Yu *et al.* (2008) who found that the *Streptomyces* spp. records maximum growth rates when peptone use as nitrogen source while ammonium chloride showed moderate growth when it supplied as inorganic nitrogen source in medium. Amendment of amino acids, in combination with carbon source, promoted the growth of actinomycetes, which correlated with Kumar and Kannabiran (2010). Although *Mentha* contains antibacterial and phenolic compounds, the growth of actinomycetes was not affected by them; this may be because actinomycetes are resistant to these compounds or their concentrations are not enough to inhibit their growth. On the other hand, some bacterial isolates exhibited maximum growth and pigmentation on nutrient agar plates like A13 (Plate 4) than *Mentha* and *Aloe vera*-based culture medium because nutrient agar

medium provides balanced salt solution with specific pH and osmotic pressure. While other bacterial isolates showed maximum growth on *Aloe vera*-based culture medium and high pigmentation on nutrient agar like (A6 isolate) (Plate 4). In addition, MB4 (Plate 5) isolate also yielded significant growth on *Mentha*-based culture medium but less biomass yield when compared with nutrient agar medium. The phenolic compounds in *Mentha* and *Aloe vera* juice may limit the growth of bacterial isolates. These results are in line with similar findings of previous reports of (Jadhav *et al.*, 2018).

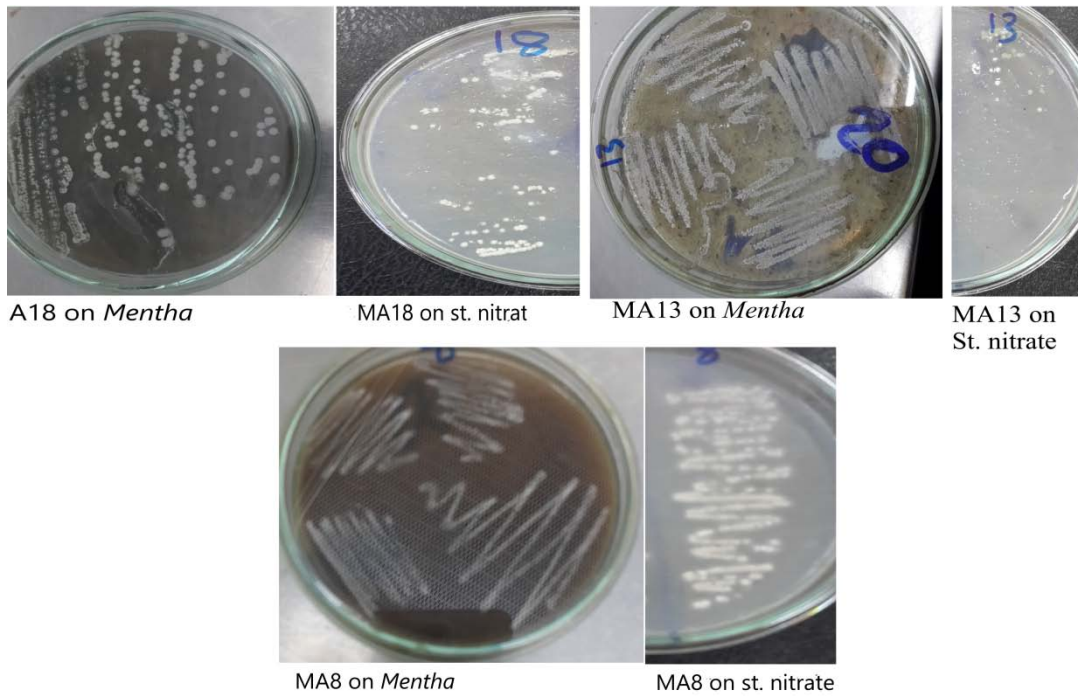


Plate 3: Comparison between the growth of actinomycetes isolates on *Mentha viridis*-based medium and starch nitrate medium

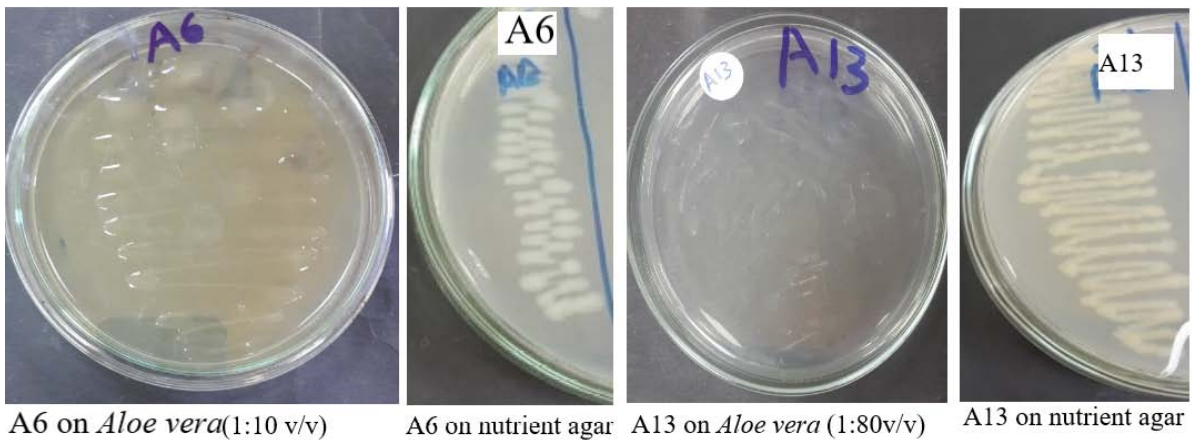


Plate 4: Comparison between the growth of rhizobacterial on *Aloe vera*-based medium and nutrient agar medium

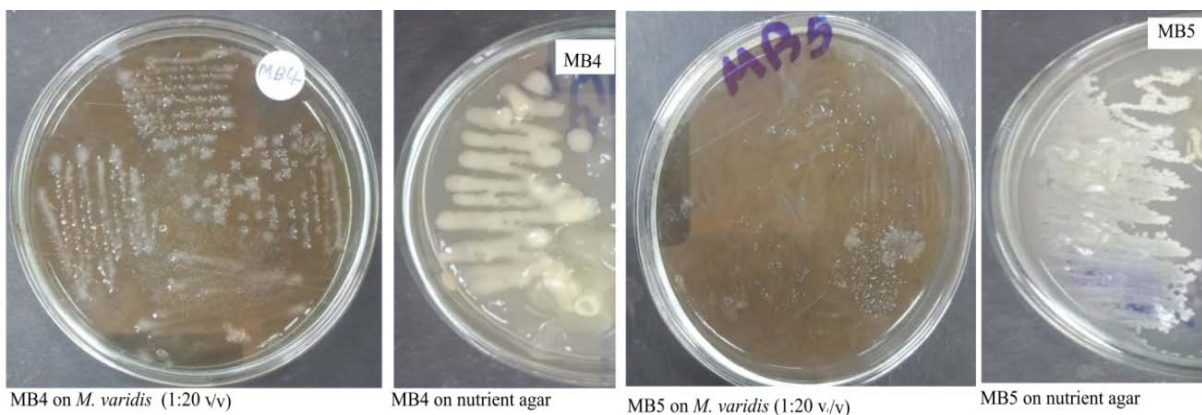
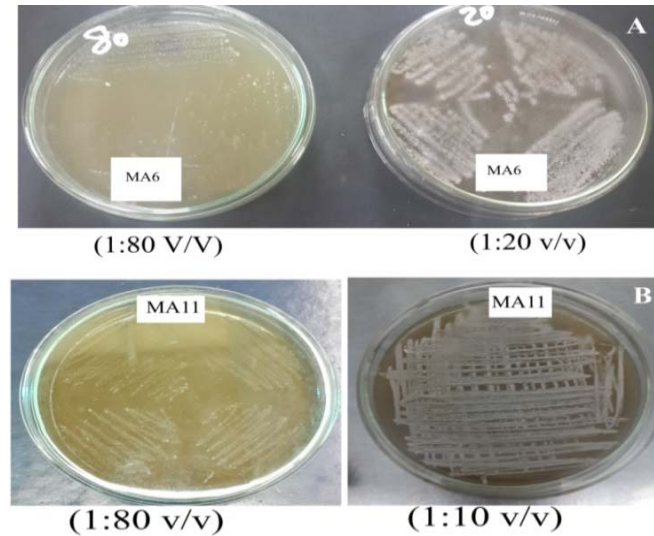


Plate 5: Comparison between the growth of rhizobacterial isolates on *Mentha viridis*-based medium and nutrient agar medium



Not only the growth of actinomycetes affected by the type of medium but also by the concentration of the nutritional content. The growth and pigmentation of actinomycetes isolate (MA6) and (MA11) were high at dilution (1:20 v/v) and (1:10 v/v) respectively while both growth and pigmentation of the two isolates were

decreased at dilution (1:80 v/v) (Plate 6). This due to the nutritional content was high at lowest dilutions and low with highest dilutions of plant extract. These results are in agreement with Saleh *et al.* (2017), who found that the diluted plant juice (1:10, v/v) supported better growth compared to further diluted plant juices.



**Plate 6:** The growth of actinomycetes isolates (MA6&MA11) at different dilutions of *Mentha* extract

*In-vitro* screening of isolates for production of some plant growth-promoting (PGP) activities

b) Indole acetic acid (IAA) production by microbial isolates on both plant-based culture and synthetic medium

Auxin is the most investigated hormone among plant growth regulators. The most common, best characterized, and physiologically most active auxin in the plant is indole-3-acetic acid (IAA). IAA is known to stimulate both a rapid response (e.g., increased cell elongation) and a long term response (e.g., cell division and differentiation) in plants. The production of IAA was examined with the use of Salkowski reagent. The development of pink color was first visible within minutes and continued to increase in its intensity for 30 min in the dark, so the optical density was then measured. All bacterial and actinomycetes isolates produce IAA on both plant-based medium and synthetic medium. MB4 and A6 were efficient producers of IAA (36.51 and 16.25 ppm, respectively) on *Mentha* and *Aloe vera*-based culture medium respectively Table (3), while the quantity of IAA decreases (10.53 and 8.69 ppm) on nutrient broth medium, this due to the presence of tryptophan, vitamins, salt, carbon source and nitrogen in a plant-based culture medium that were contributing factors in the IAA biosynthesis which correlated with (Apine and Jadhav, 2011) and also the tryptophan supplemented in the medium. It was observed that the ideal concentration of tryptophan needed for maximum IAA production differed between the isolates (Sergeeva *et al.*, 2007). (Narayana *et al.*, 2009; Malhotra and

Srivastava, 2009) mentioned that plant extracts influence bacterial IAA biosynthesis, and organic nitrogen sources observed to promote IAA production better than inorganic nitrogen sources. Furthermore, isolate no. MA13 produce the smallest amount of IAA (2.64ppm) on *Mentha*-based culture and the highest amount on nutrient broth medium (25.05ppm) Table (3), this due to the high concentration of tryptophan in *Mentha*-based culture (*Mentha* content Table (2) and 0.5 mg/ml supplemented to medium) reduce the production of IAA, while nutrient broth medium contains the optimum amount of tryptophan. This result is in consistence with (Shokri and Emtiazi, 2010) where they revealed that some strains grown on high concentrations of tryptophan actually reduced IAA production.

**Table 3:** Production of IAA on *Mentha* and *Aloe vera* –based medium and synthetic medium by bacterial and actinomycetes isolates isolated on *Mentha* and *Aloe vera* extract medium

<i>Mentha</i> extract medium		Synthetic medium		<i>Aloe vera</i> extract medium		Synthetic medium	
No of isolates	IAA ( $\mu\text{g/ml}$ )	No of isolates	IAA ( $\mu\text{g/ml}$ )	No of isolates	IAA ( $\mu\text{g/ml}$ )	No of isolates	IAA ( $\mu\text{g/ml}$ )
MB1	16.77	MB1	12.35	A1	6.02	A1	11.47
MB2	6.17	MB2	8.74	A2	4.84	A2	18.32
MB3	9.75	MB3	10.64	A3	3.55	A3	8.66
MB4	36.51	MB4	10.53	A4	0.79	A4	0.82
MB5	8.2	MB5	7.88	A5	14.27	A5	4.19
MA6	10.06	MA6	7.47	A6	16.25	A6	8.69
MA7	4.35	MA7	2.82	A7	0.69	A7	5.86
MA8	10.06	MA8	10.77	A8	0.74	A8	0.9
MA9	31.31	MA9	15.26	A9	3.34	A9	8.43
MA10	6.48	MA10	9.75	A10	0.27	A10	6.51
MA11	3.15	MA11	1.52	A11	1.18	A11	7.68
MA12	31.83	MA12	16.32	A12	0.53	A12	0.58
MA13	2.64	MA13	25.05	A13	1.08	A13	4.19
MB14	15.42	MB14	12.71	A14	0.97	A14	5.39
MB15	5.02	MB15	5.13	A15	5.94	A15	4.82
MB16	6.92	MB16	14.17				
MB17	23.52	MB17	7.02				
MA18	12.56	MA18	10.79				

c) *Quantitative assay of phosphate solubilizing activity*

In NBRIP, broth phosphate solubilization activity of microbial isolates was varied from 44.69 to 867.85 $\mu\text{g/ml}$  using tricalcium phosphate as a source of insoluble P. Soil microorganisms can solubilize insoluble mineral phosphate by producing various organic acids (Illmer *et al.*, 1995; Jones, 1998). In our study all bacterial and actinomycetes isolates were able to solubilizing phosphorus, which shows an agreement with the estimation of (Kucey, 1983) and (Chabot *et al.*, 1993) and according to them 20 to 40% of the cultivable bacterial population of soil solubilize P. Our results showed that bacterial isolate No A6 was shown maximum phosphorus solubilization on NBRIP broth medium after grown on *Aloe vera*-based culture as compared to after grown on nutrient broth medium (Figure 1), furthermore actinomycetes No MA6 and bacterial No MB5 isolates were efficient in phosphorus solubilization on NBRIP broth medium after grown on *Mentha*-based culture 867.85 and 767.2  $\mu\text{g/ml}$  respectively than after grown on starch nitrate and nutrient broth medium 648 and 613.46 $\mu\text{g/ml}$  respectively (Figure 1). The high phosphorus solubilization on *Mentha*-based culture and *Aloe vera*-based due to the lower pH of *M. viridis* juices was in the range of 5.8-6.5 and *A. vera* juices 4-6.2 that lowers the pH of the medium and increases phosphorus solubilization by microorganisms that isolated on *Mentha* and *Aloe vera*-based media, indicating the organic acid secretion that similar with the estimation of Humaira and Asghari (2011). The inverse relationship between pH and soluble phosphate was reported earlier by (Rashid *et al.*, 2004).

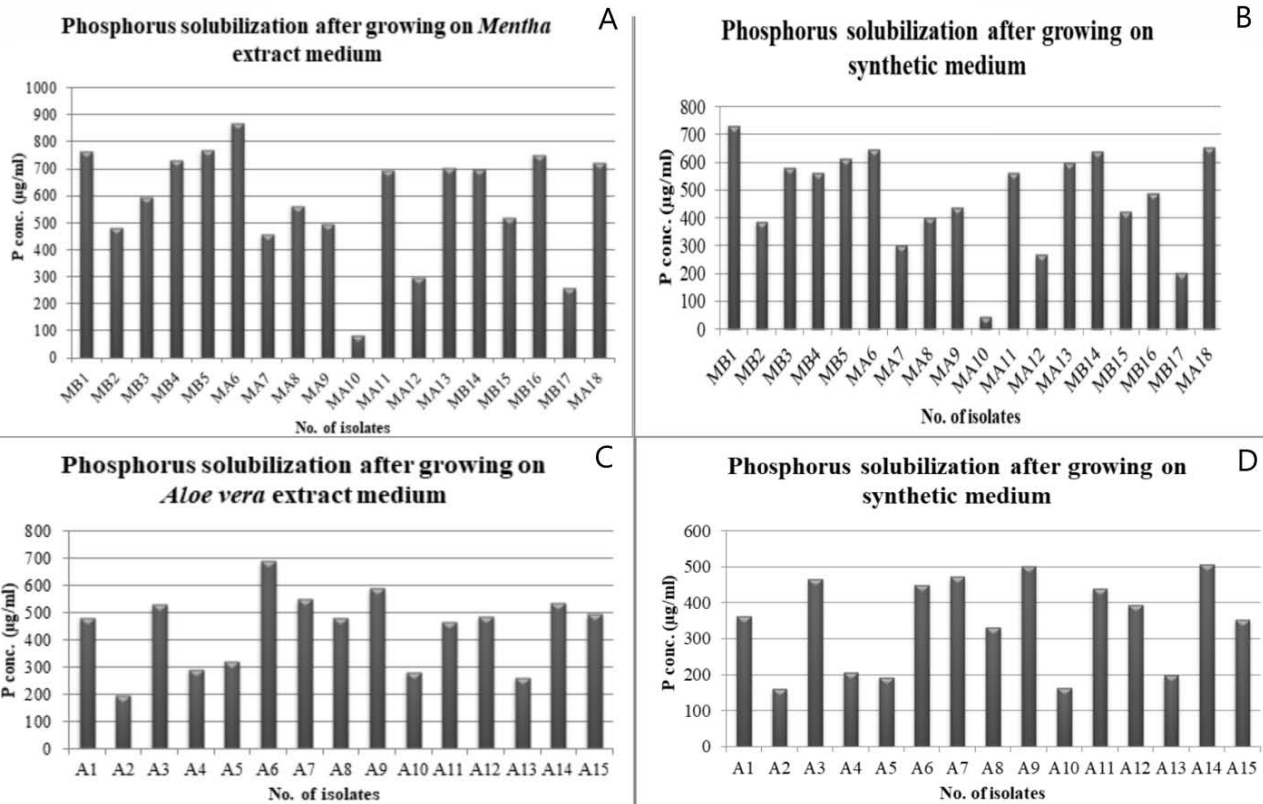


Figure 1: Phosphorus solubilization on NBPIP medium by A and B) bacterial and actinomycetes isolate isolated from *Mentha* rhizosphere after growing on *Mentha* extract and synthetic medium C and D) bacterial isolates isolated from *Aloe vera* rhizosphere after their growth *Aloe vera* extract and synthetic medium

d) Identification of Selected plant growth-promoting rhizobacteria isolates (PGPR) by 16s rRNA Gene Sequencing

The sequences of 16S rRNA gene of our isolates compared with the sequences in GenBank through BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST>). The results of the BLAST were, MA6 showed

98.9% similarity with *Streptomyces rochei* strain DW3 while the MB4 showed 91.4% similarity with *Pseudomonas monteilli* strain CIP 104883 and A6 showed 99.36% similarity with *Kosakonia radicincitans* DSM 16656. These quences from strains MA6, MB4, and A6 have been deposited in the Gen Bank and accession numbers were obtained (Table4).

Table 4: Genetic similarity of the selected strains with different species of the bacteria and actinomycetes determined by 16S rRNA gene sequencing

Isolate code	Source of isolation, Plant	Identified as	Similarity (%)	ACC number
MA6	Rhizophere soil, <i>Mentha viridis</i>	<i>Streptomyces rochei</i> strain DW3	98.9	MN135856.1
MB4	Rhizophere soil, <i>Mentha viridis</i>	<i>Pseudomonas monteilli</i> strain CIP 104883	91.4	NR114223.1
A6	Rhizophere soil, <i>Aloe vera</i>	<i>Kosakonia radicincitans</i> DSM 16656	99.36	NR117704.1

e) A phylogenetic relationship based on 16S rRNA nuclear gene

The construction of the phylogenic tree (Figure 2, 3&4) described the phylogenetic relationship of the three species, namely *Streptomyces rochei* strain DW13, *Pseudomonas monteilli* strain CIP 104883 and *Kosakonia radicincitans* DSM 16656.

The above results are agree with those of Meliani *et al.* (2017) who investigate that the plant growth-promoting traits of a PGPR *P. fluorescence* and *P.*

*putida* like production of IAA, siderophore and phosphate solubilization and found that *P. fluorescence* and *P. putida* can produce 3-indole-acetic acid (IAA) *in-vitro*, at concentrations of 89 µg.ml<sup>-1</sup> and 116 µg.ml<sup>-1</sup>, respectively. The above results were harmony with Jog *et al.* (2012), who found that *Streptomyces rochei*, from the wheat rhizosphere, can produce IAA and phosphate solubilization and improve plant growth by increases seed germination, root elongation, and root dry weight and PO<sub>4</sub>. The results of Zamoum *et al.* (2017) showed

that *Streptomyces rochei* was positive for IAA (100.3  $\mu\text{g ml}^{-1}$ ). This not harmony with our results. Furthermore, the *in-vitro* analyses, which demonstrated by Schilling *et al.* (1998) showed that *Kosakonia*

*radicincitans* can solubilize rock phosphates. Moreover, this strain produces phytohormones as auxin and cytokine-like compounds Scholz and Ruppel (1992).

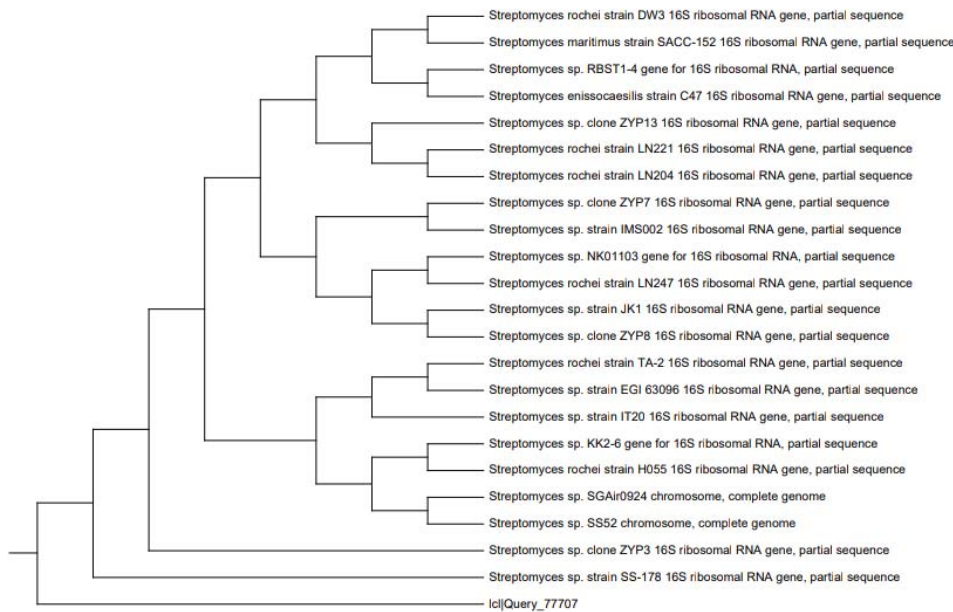


Figure 2: The phylogenetic tree based on the sequencing of the 16S rRNA gene using MEGA4 software (Tamura *et al.*, 2007) illustrating the genetic relationship of *Streptomyces rochei* and closely related *Streptomyces* species

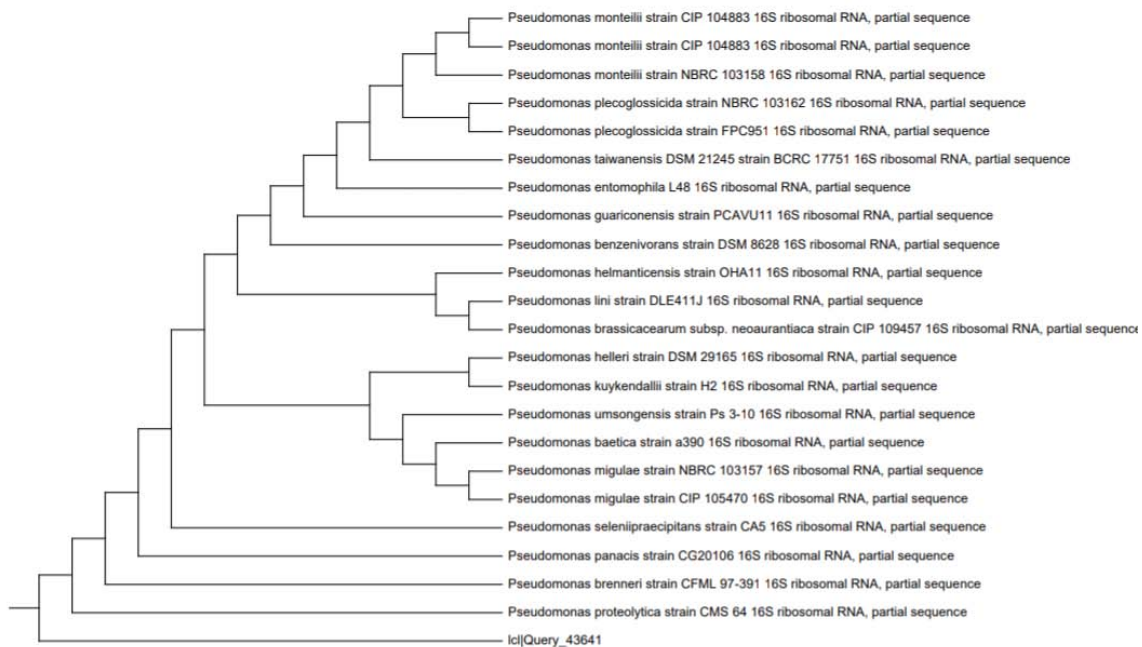


Figure 3: The phylogenetic tree based on the sequencing of the 16S rRNA gene using MEGA4 software (Tamura *et al.*, 2007) illustrating the genetic relationship of *Pseudomonas montellii* strain CIP 104883 and closely related *Pseudomonas* species.

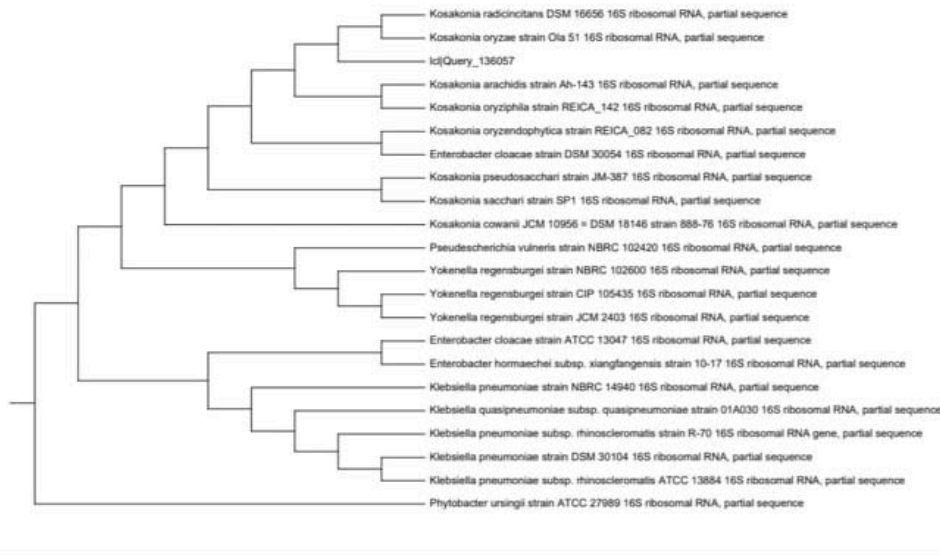


Figure 4: The phylogenetic tree based on the sequencing of the 16S rRNA gene using MEGA4 software (Tamura et al., 2007) illustrating the genetic relationship of *Kosakonia radicinicans* and closely related *Kosakonia* species

When the growth of *Pseudomonas monteilii*, *Kosakonia radicinicans*, and *Streptomyces rochei* on plant extract-based medium was compared with nutrient and starch nitrate respectively, it was found that maximum growth was in plant-based medium and less growth recorded on synthetic medium. It is clear from (Figure 5) that the *Pseudomonas monteilii*, and *Kosakonia radicinicans* grow exponentially during two

and three days of incubation on both natural and synthetic media. The highest growth was observed on the third day of incubation. After the exponential phase at the fourth and fifth day, then slightly decreases (stationary phase). On the other hand, *Streptomyces rochei*, and grow exponentially during the first 4 to 7 days of incubation and at the eighth day, the growth slightly decreased.

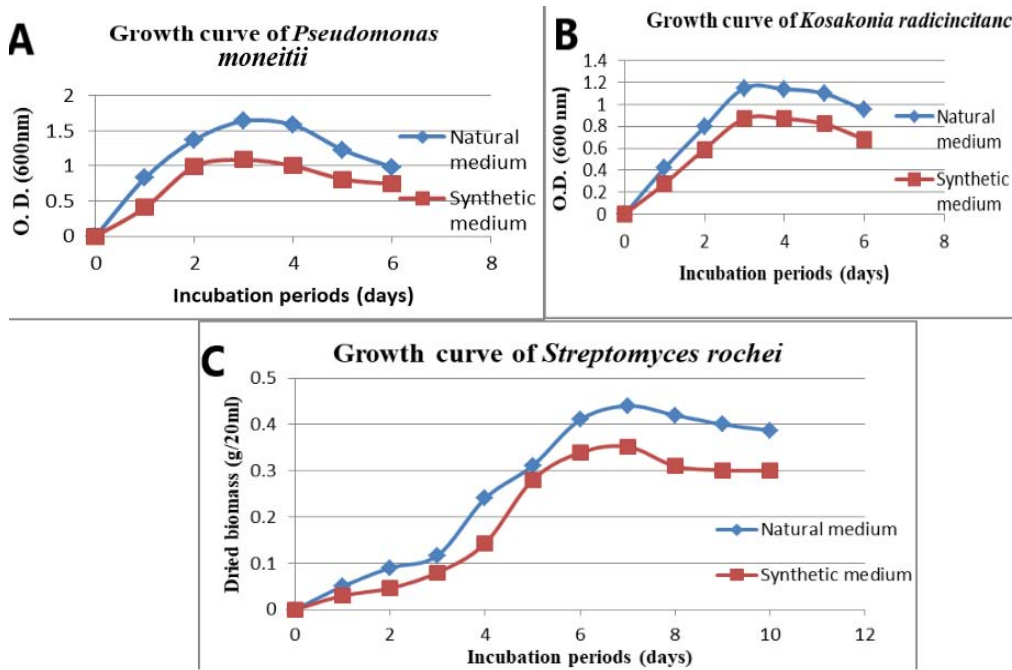


Figure 5: Growth curves of A) *Pseudomonas monteilii*, B) *Kosakonia radicinicans* and C) *Streptomyces rochei*

The three efficient isolates tested for their ability to produce inhibitory activity against pathogenic fungi (*Fusarium solani*, *Fusarium oxysporum*, and *Rhizoctonia*

*solani*). Rhizobacteria can produce plant growth-promoting substances that enhance the growth of plants, and also have an antagonistic activity against

pathogenic fungi. The results showed that *Pseudomonas monteilii* and *Streptomyces rochei* (Plate 7) were more active against *Rhizoctonia solani* and *Fusarium solani* respectively, that shown inhibition zone 19 and 18mm respectively Table (5), which in line with similar findings of previous reports (Asgharet al.,

2019). Additionally, *Kosakonia radicincitans* was suppressing the growth of *Fusarium solani* due to *K. radicincitans* have genes that responsible for salicylic acid or the jasmonate/ethylene signaling pathways to protect plants against potential pathogen attack Brock et al. (2013).

Table 5: Antimicrobial activity of *Pseudomonas monteilii*, *Kosakonia radicincitans* and *Streptomyces rochei* isolates

Pathogenic fungi Isolates	Diameter of zone of Inhibition (mm)		
	<i>Fusarium solani</i>	<i>Fusarium oxysporum</i>	<i>Rhizoctonia solani</i>
<i>Pseudomonas monteilii</i>	17	15	19
<i>Kosakonia radicincitans</i>	11	3	5
<i>Streptomyces rochei</i>	18	13	12

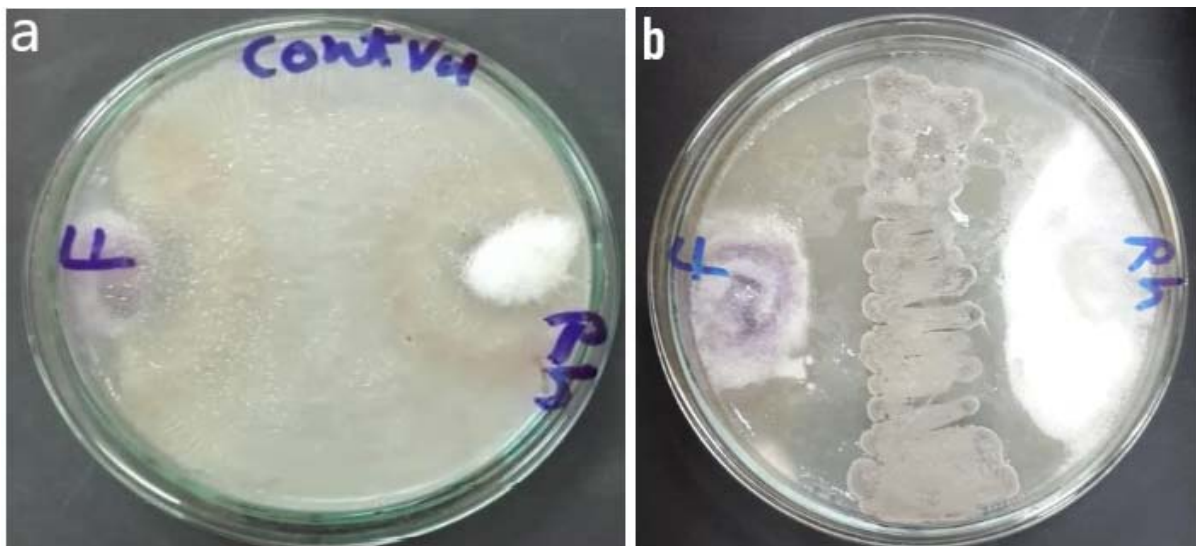


Plate 7: Streptomyces isolates showed antimicrobial activity against the test organisms a) control plate with (*Fusarium* sp. and *Rhizoctonia solani*), b) assay plate with (*Fusarium* sp. and *Rhizoctonia solani*) and potential *Streptomyces rochei*

As shown in (Table 5), the cost of natural liquid media is drastically less than the synthetic liquid media. The natural media containing agar for solidification is also very cost-effective as compared to synthetic solid media. The rise in the price of natural agar media is due to the addition of agar, which raises the cost. The finding cheap source instead of agar as solidifying agents needs to found to low this cost.

Table 6: Economic comparison of the media

Media type	Cost in E£/ 100L	
	Synthetic medium	Natural medium
Solid media	4600	4100
Liquid Media	600	100

#### IV. CONCLUSIONS

The natural media of *Mentha* and *Aloe vera* extracts supported the growth of bacteria and actinomycetes such as *Pseudomonas monteilii*,

*Kosakonia radicincitans*, and *Streptomyces rochei*, respectively. The plant-based culture media could use as inexpensive alternate medium for the routine practical experiments. The plant-based culture increases the cultivability of rhizobacteria than synthetic medium, and was highly cost-effective. Results suggest that PGP isolates developed on plant-based culture medium, which can produce multiple PGP activities like IAA production, solubilize the phosphate, and existed antagonistic activity against pathogenic fungi may improve the growth of plants. Moreover, in the present study, PGPRs were used with a lower dose of fertilizer; thus, it is an environmentally-friendly technology that can minimize soil pollution and maximize crop returns.

#### ACKNOWLEDGMENTS

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## Showcase to Illustrate how the web-server iATC\_Deep-mISF is working

By Kuo-Chen Chou

*Gordon Life Science Institute*

*Introduction-* In 2020, a very powerful web-server predictor has been established for identifying the Anatomical Therapeutic Chemicals [1], in which a same chemical may occur in two or more classes and hence needs to be marked with the multi-label approach [2].

The web-server predictor is called “iATC\_Deep-mISF”, where “Deep” means the web-server has been further improved by the “Deep Learning” technique [3-6], and “m” means the capacity able to deal with the multi-label systems. To learn how the web-server is working, please do the following.

*Step 1:* Click the link at [http://www.jci-bioinfo.cn/iATC\\_Deep-mISF/](http://www.jci-bioinfo.cn/iATC_Deep-mISF/), the top page of the iATC\_Deep-mISF web-server will appear on your computer screen, as shown in **Fig.1**. Click on the Read Me button to see a brief introduction about the predictor.

*GJSFR-G Classification: FOR Code: 100499, 080505*



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# Showcase to Illustrate how the web-server iATC\_Deep-mISF is working

Short Title: Anatomical Therapeutic Chemicals

Kuo-Chen Chou

## INTRODUCTION

In 2020, a very powerful web-server predictor has been established for identifying the Anatomical Therapeutic Chemicals [1], in which a same chemical may occur in two or more classes and hence needs to be marked with the multi-label approach [2].

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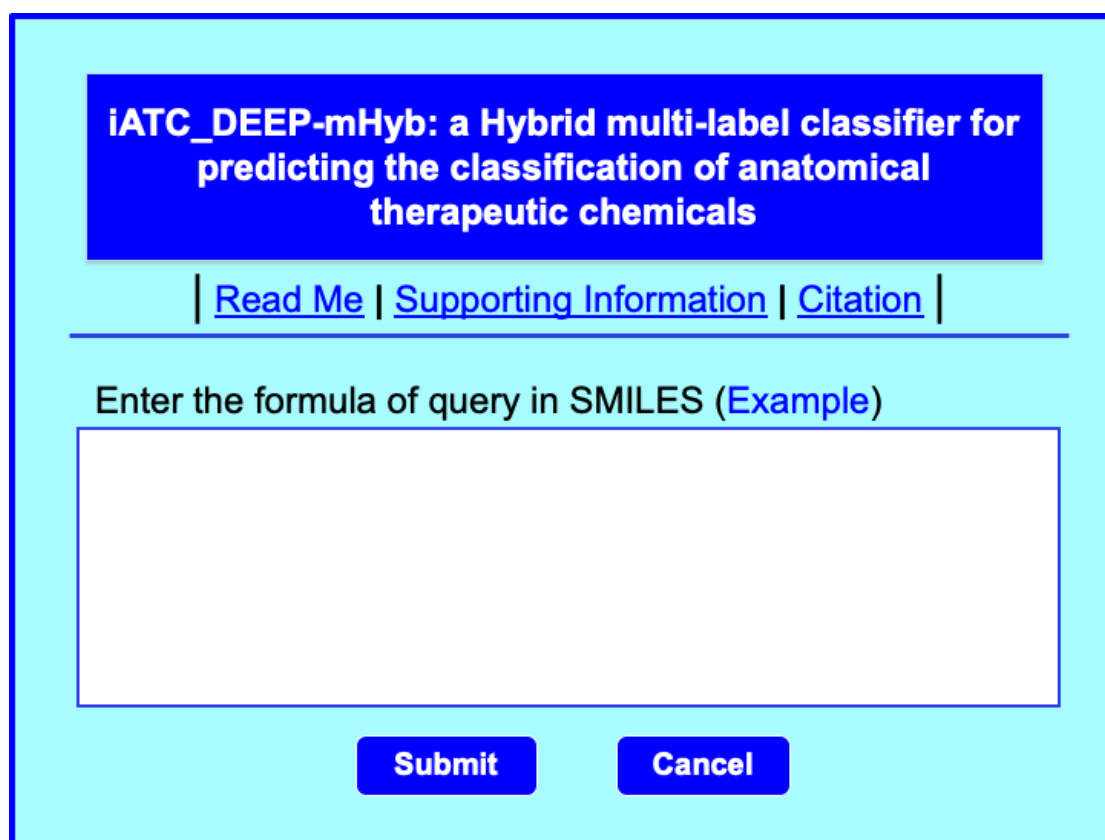


Figure 1

*Step 2:* Either type or copy/paste the Anatomical Therapeutic Chemicals into the input box at the center of **Fig. 1**. The input sample should be in the FASTA format. For the examples of FASTA format, click the [Example](#) button right above the input box.

*Step 3:* Click on the [Submit](#) button to see the predicted result. For instance, if you use the four samples in the [Example](#) window as the input, after 10 seconds or so, you will see a new screen (**Fig. 2**) occurring. On its upper part

*Author:* Gordon Life Science Institute, Boston, MA 02478, USA. e-mails: [kcchou@gordonlifescience.org](mailto:kcchou@gordonlifescience.org), [kcchou38@gmail.com](mailto:kcchou38@gmail.com)

are listed the names of the samples (1) to (14) covered by the current predictor. On its lower part are the predicted results: the query compound-1 of example-1 corresponds to “3, 5, 9” the query compound-2 corresponds to “3,” and so forth. All these results are perfectly consistent with experimental observations.

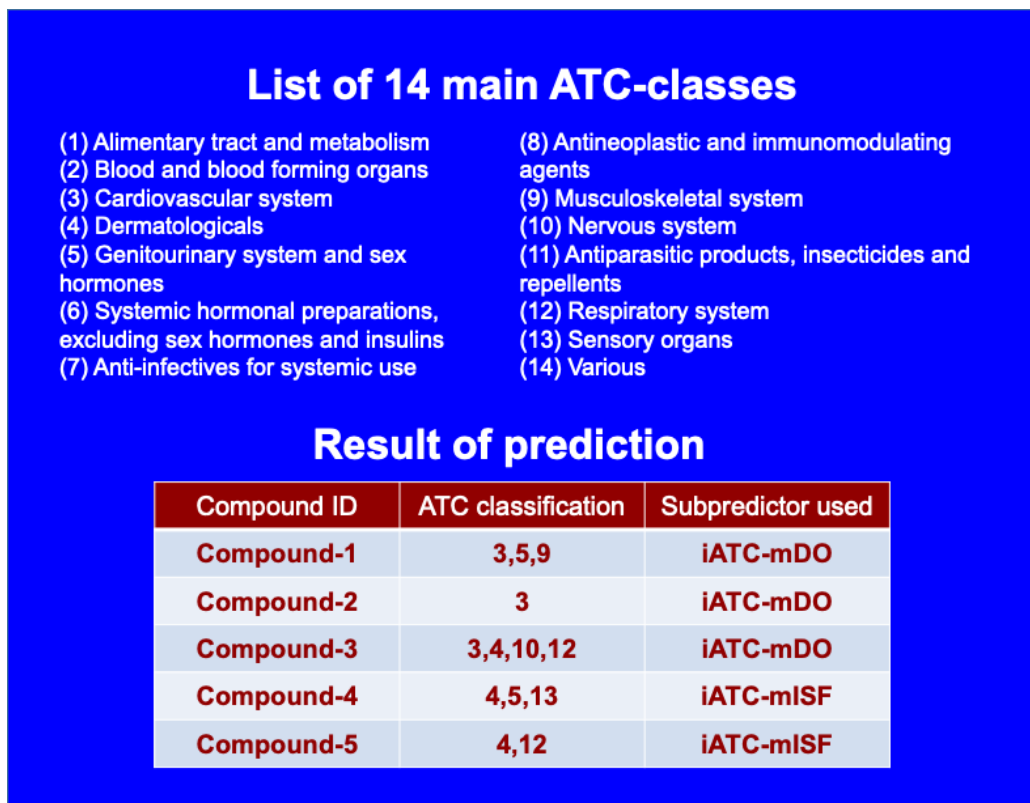


Figure 2

*Step 4:* As shown on the lower panel of **Fig. 2**, you may also choose the batch prediction by entering your e-mail address and your desired batch input file (in FASTA format of course) via the [Browse](#) button. To see the sample of batch input file, click on the button [Batch-example](#). After clicking the button [Batch-submit](#), you will see “Your batch job is under computation; once the results are available, you will be notified by e-mail.”

*Step 5:* Click on the [Citation](#) button to find the papers that have played the key role in developing the current predictor of iATC\_Deep-mISF.

*Step 6:* Click the Supporting Information button to download the Supporting Informations mentioned in this paper.

## REFERENCES RÉFÉRENCES REFERENCIAS

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- S. Khan, M. Khan, N. Iqbal, T. Hussain, S.A. Khan, K.C. Chou, A Two-Level Computation Model Based on Deep Learning Algorithm for Identification of piRNA and Their Functions via Chou's 5-Steps Rule (2019) <https://doi.org/10.1007/s10989-019-09887-3>, *Human Genetics* (2019).
- I. Nazari, M. Tahir, H. Tayari, K.T. Chong, iN6-Methyl (5-step): Identifying RNA N6-methyladenosine sites using deep learning mode via Chou's 5-step rules and Chou's general PseKNC, *Chemometrics and Intelligent Laboratory Systems (CHEMOLAB)*, <https://doi.org/10.1016/j.chemolab.2019.103811> (2019).
- Z.U. Khan, F. Ali, I.A. Khan, Y. Hussain, D. Pi, iRSpot-SPI: Deep learning-based recombination spots prediction by incorporating secondary sequence information coupled with physio-chemical properties via Chou's 5-step rule and pseudo components, *Chemometrics and Intelligent Laboratory Systems (CHEMOLAB)*, 189 (2019) 169-180.



## Showcase to Illustrate how the web-server pLoc\_Deep-mGpos is working

By Kuo-Chen Chou

*Gordon Life Science Institute*

*Introduction-* In 2020, a very powerful web-server predictor has been established for identifying the subcellular localization of human proteins based on the sequence information alone [1], in which a same protein may occur or move between two or more location sites and hence needs to be marked with the multi-label approach [2].

The web-server predictor is called “pLoc\_Deep-mGpos”, where “Deep” means the web-server has been further improved by the “Deep Learning” technique [3-6], and “m” means the capacity able to deal with the multi-label systems. To learn how the web-server is working, please do the following.

*Step 1:* Click the link at [http://www.jci-bioinfo.cn/pLoc\\_Deep-mGpos/](http://www.jci-bioinfo.cn/pLoc_Deep-mGpos/), the top page of the pLoc\_bal-mGpos web-server will appear on your computer screen, as shown in **Fig.1**. Click on the Read Me button to see a brief introduction about the predictor.

*GJSFR-G Classification: FOR Code: 100499, 080505*



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# Showcase to Illustrate how the web-server pLoc\_Deep-mGpos is working

Short Title: Showcase for pLoc\_Deep-mGpos

Kuo-Chen Chou

## INTRODUCTION

In 2020, a very powerful web-server predictor has been established for identifying the subcellular localization of human proteins based on the sequence information alone [1], in which a same protein may occur or move between two or more location sites and hence needs to be marked with the multi-label approach [2].

The web-server predictor is called “pLoc\_Deep-mGpos”, where “Deep” means the web-server has been further improved by the “Deep Learning” technique [3-6], and “m” means the capacity able to deal with the multi-label systems. To learn how the web-server is working, please do the following.

*Step 1:* Click the link at [http://www.jci-bioinfo.cn/pLoc\\_Deep-mGpos/](http://www.jci-bioinfo.cn/pLoc_Deep-mGpos/), the top page of the pLoc\_bal-mGpos web-server will appear on your computer screen, as shown in Fig.1. Click on the [Read Me](#) button to see a brief introduction about the predictor.

**pLoc\_Deep-mGpos: predict subcellular localization of gram-positive proteins by deep learning**  
| [Read Me](#) | [Supporting information](#) | [Citation](#) |

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**Enter query sequences**

Enter the sequences of query proteins in FASTA format ([Example](#)): the number of proteins is limited at 10 or less for each submission.

**Or, upload a file for batch prediction**

Enter your e-mail address and upload the batch input file ([Batch-example](#)). The predicted result will be sent to you by e-mail once completed; it usually takes 1 minute or so for each protein sequence

Upload file:    
Your Email:

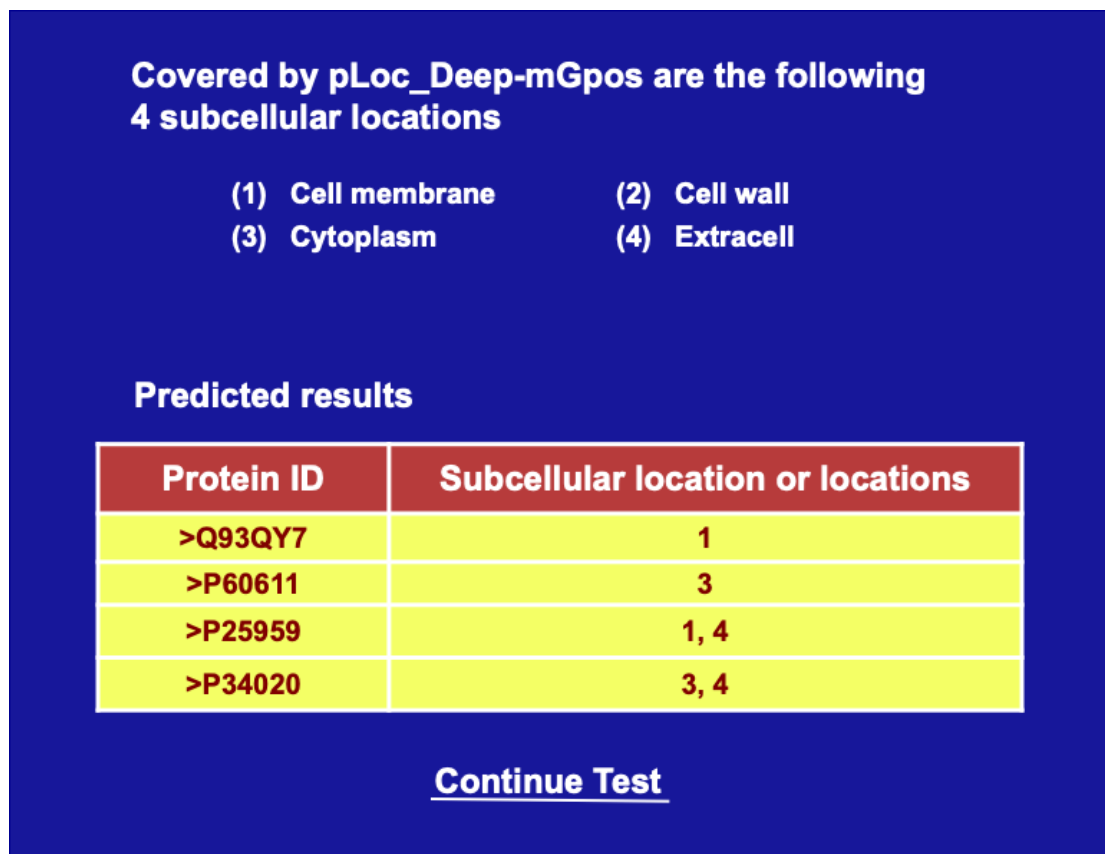
 

Figure 1

*Step 2:* Either type or copy/paste the sequences of query human proteins into the input box at the center of Fig.1. The input sequence should be in the FASTA format. For the examples of sequences in FASTA format, click the [Example](#) button right above the input box.

*Step 3:* Click on the [Submit](#) button to see the predicted result. For instance, if you use the four protein sequences in the [Example](#) window as the input, after 10 seconds or so, you will see a new screen (Fig.2) occurring. On its upper

part are listed the names of the subcellular locations numbered from (1) to (8) covered by the current predictor. On its lower part are the predicted results: the query protein P22340 of example-1 corresponds to “2,” meaning it belongs to “Cell outer membrane” only; the query protein P04032 of example-2 corresponds to “8” meaning it belongs to “Periplasm”; the query protein P04825 of example-3 corresponds to “1, 3”, meaning it belongs to “Cell inner membrane” and “Cytoplasm”; the query protein P22251 of example 4 corresponds to “4, 6”, meaning it belongs to “Extracellular” and “Flagellum”. All these results are perfectly consistent with experimental observations.



*Figure 2*

*Step 4:* As shown on the lower panel of **Fig.2**, you may also choose the batch prediction by entering your e-mail address and your desired batch input file (in FASTA format of course) via the [Browse](#) button. To see the sample of batch input file, click on the button [Batch-example](#). After clicking the button [Batch-submit](#), you will see “Your batch job is under computation; once the results are available, you will be notified by e-mail.”

*Step 5:* Click on the [Citation](#) button to find the papers that have played the key role in developing the current predictor of pLoc\_Deep-mGpos.

*Step 6:* Click the Supporting Information button to download the Supporting Informations mentioned in this paper.

### REFERENCES RÉFÉRENCES REFERENCIAS

1. Z. Lu, K.C. Chou, Showcase to illustrate how the web-server pLoc\_Deep-mGpos is working, Journal of Biomedical science and Engineering (JBiSE) 13 (2020) 55-65.
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3. A. Maxwell, R. Li, B. Yang, H. Weng, A. Ou, H. Hong, Z. Zhou, P. Gong, C. Zhang, Deep learning architectures for multi-label classification of intelligent health risk prediction. , Bmc Bioinformatics, 18 (2017) 523.
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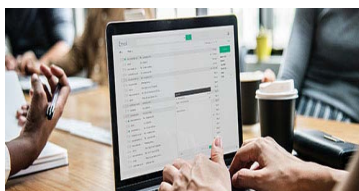
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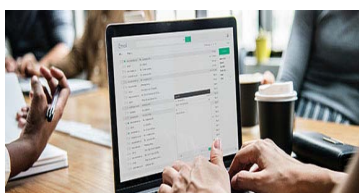


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4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s) names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
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It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

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Plagiarism is not acceptable in Global Journals submissions at all.

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- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures



- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

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2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

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The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

### Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

### Declaration of funding sources

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## PREPARING YOUR MANUSCRIPT

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.



### ***Manuscript Style Instruction (Optional)***

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

### ***Structure and Format of Manuscript***

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

- a) A title which should be relevant to the theme of the paper.
- b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
- c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
- d) An introduction, giving fundamental background objectives.
- e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
- f) Results which should be presented concisely by well-designed tables and figures.
- g) Suitable statistical data should also be given.
- h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

- i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
- j) There should be brief acknowledgments.
- k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.



## FORMAT STRUCTURE

***It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.***

All manuscripts submitted to Global Journals should include:

### **Title**

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

### **Author details**

The full postal address of any related author(s) must be specified.

### **Abstract**

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

### **Keywords**

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in a research paper?" Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

### **Numerical Methods**

Numerical methods used should be transparent and, where appropriate, supported by references.

### **Abbreviations**

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

### **Formulas and equations**

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

### **Tables, Figures, and Figure Legends**

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.



## Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

## PREPARATION OF ELETRONIC FIGURES FOR PUBLICATION

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

## TIPS FOR WRITING A GOOD QUALITY SCIENCE FRONTIER RESEARCH PAPER

Techniques for writing a good quality Science Frontier Research paper:

**1. Choosing the topic:** In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

**2. Think like evaluators:** If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

**3. Ask your guides:** If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

**4. Use of computer is recommended:** As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

**5. Use the internet for help:** An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.



**6. Bookmarks are useful:** When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

**7. Revise what you wrote:** When you write anything, always read it, summarize it, and then finalize it.

**8. Make every effort:** Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

**9. Produce good diagrams of your own:** Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

**10. Use proper verb tense:** Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

**11. Pick a good study spot:** Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

**12. Know what you know:** Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

**13. Use good grammar:** Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

**14. Arrangement of information:** Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

**15. Never start at the last minute:** Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

**16. Multitasking in research is not good:** Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

**17. Never copy others' work:** Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

**18. Go to seminars:** Attend seminars if the topic is relevant to your research area. Utilize all your resources.

**19. Refresh your mind after intervals:** Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.





**20. Think technically:** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

**21. Adding unnecessary information:** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

**22. Report concluded results:** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

**23. Upon conclusion:** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

## INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

### **Key points to remember:**

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

### **Final points:**

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

*The introduction:* This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

### **The discussion section:**

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

### **General style:**

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

**To make a paper clear:** Adhere to recommended page limits.



### *Mistakes to avoid:*

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

### **Title page:**

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

**Abstract:** This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

*Reason for writing the article—theory, overall issue, purpose.*

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

### **Approach:**

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

### **Introduction:**

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.



*The following approach can create a valuable beginning:*

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

#### **Approach:**

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

#### **Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

#### **Materials:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

#### **Methods:**

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

#### **Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

#### **What to keep away from:**

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



**Results:**

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

**Content:**

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

**What to stay away from:**

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

**Approach:**

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

**Figures and tables:**

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

**Discussion:**

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."



Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

**Approach:**

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

*Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.*

*Segment draft and final research paper:* You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

*Written material:* You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.



CRITERION FOR GRADING A RESEARCH PAPER (COMPILATION)  
BY GLOBAL JOURNALS

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Topics	Grades		
	A-B	C-D	E-F
<i>Abstract</i>	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form  Above 200 words	No specific data with ambiguous information  Above 250 words
<i>Introduction</i>	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
<i>Methods and Procedures</i>	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
<i>Result</i>	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
<i>Discussion</i>	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



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