Research Article

Anti-*Pectobacterium brasiliensis* activities of Macro-Algae Extracts Harvested from the Coast of El Jadida-Morocco

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ABSTRACT

Approximately 22% of potato are lost each year due to viruses, bacteria, fungi and parasites attacking either tuber or potato plant, leading to an annual loss of over 65 million tones. Bacterial soft rot represents 30-50% of this huge loss and it is one of the major post-harvest diseases of potato (*Solanum tuberosum* L). Marine algae are a great source of complex natural products that could be a promising source of new bioactive compounds that can help plant survival by providing protection against stress imposed by pathogens. In this context, extracts of 24 species of algae (Rhodophyceae, Phaeophyceae, Chlorophyceae) collected from the coast of El Jadida, Morocco, were tested for their antibacterial activity against the bacterial strain *Pectobacterium brasiliensis* that causes soft rot in potato (*Solanum tuberosum* L). To among the 24 species studied, those belonging to the Phaeophyceae and Rhodophyceae were the most active, while Chlorophyceae have a low inhibition. Maximum inhibition of the growth of *Pectobacterium brasiliensis* was obtained by extracts prepared in dichloromethane/methanol and methanol. The results obtained in this study clearly indicated that macroalgae from the coast of Sidi Bouzid can be used in the treatment of plant diseases especially soft rot of potato.

Keywords: Antibacterial activity, Algae extract, Sidi Bouzid, *Pectobacterium brasiliensis*, Potato.

INTRODUCTION

In Morocco, The potato (*Solanum tuberosum* L) is cultivable almost all the year and practically in all the regions, it has grown considerably in cultivated areas, or in eating habits.[1] The area occupied by vegetable crops varies between 180 and 200 000 hectare each year, of which about 62 000 ha/year are sown in potatoes, or more than 25% of the total market garden area. Production also increased significantly from about 150 000 tons in 1961 to a record volume of 1.6 million tons in 2010, corresponding to 28 tons/hectare. However, the potato is classified as the first seasonal vegetable crop in terms of area and production,[2] second export after tomato (early crop) and third food crop after sugar beet and corn. Its production is concentrated along the Atlantic coast, north and south of Casablanca, where the Mediterranean climate is favorable to its culture. Moroccan producers are becoming increasingly aware of the need of improving their production techniques, to better meet the demands of the local market and export market. The production of potatoes is accompanied by severe diseases caused by bacterial phytopathogens leading to enormous losses in the yield and poor quality worldwide. One of the most important diseases infecting potato is soft rot caused by pathogenic species of bacteria of the genus *Pectobacterium* that cause soft rot disease on a wide range of plant species.[3] Pectobacteria are responsible for a range of plant diseases including blackleg and soft rot in a wide range of crop and ornamental plants that include *P. atrosepticum* (Pa), *P. carotovorum* subsp. *carotovorum* (Pcc), and the recently characterized *P. wasabiae* and *P. carotovorum* subsp. *brasiliense*. Among these, Pcc has widest host range of all the soft rot bacteria.

Soft rot of potato tubers caused by Pcc can result in extensive post-harvest losses, especially during storage.[4] While in the field, the healthy plant can be infested through the microbial inoculum of Pcc found near seed tuber in soil.[5] Pcc colonizes on potato roots and makes its way into the stem and progeny tubers via the vascular system. Once in the stems, the pathogen do not necessarily cause stem rot (blackleg) but can survive in latent form.[6] Rotten mother seed tubers release the pathogen that is transmitted through soil water to contaminate neighboring progeny tubers. Long-distance transmission can also occur via flying insect vectors or via aerosols produced by rain.[7] Pcc is termed as "brute force" pathogen since it causes necrotrophic damage through physical attack on plant cell wall primarily through production of enzymes that causes the death of the host tissue. This pathogens plant is the largest competitor of agricultural crops and severely reduces the production in the range of 25–50%. About 22% of harvested potatoes are lost each year because of these plant pathogenic bacteria, leading to an annual loss of over 65 million tons.[8]

The use of pesticides to fight these diseases has increased significantly in recent years, regardless of the economic level of the country. This has led to advances in agriculture and increases in production, but continuous use of these chemical products poisons and pollutes the

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environment, negatively impacting agricultural production and reducing its sustainability.[9] The need of developing a sustainable agricultural system requires alternative methods to reduce the use of synthetic chemical pesticides for plant protection. Among the alternatives, the use of bio control agents or bio pesticides has aroused interest because of their ecological advantages.

Seaweeds extract are prolific producers of biologically active compounds. Such an ability were developed as defense against numerous organisms, which coexist and interact in the same complex environment.[10] According to Smit,[11] the discovery of metabolites with biological activity from algae increased substantially in the last three decades. These substances exhibit an appreciable number of distinct biological activities such as antimicrobial activity,[12, 13, 10, 14, 15, 16] antitumoral, antiviral, antifungal, insecticidal, cytotoxic, phytotoxic and antiproliferative actions.[17, 18, 19] The majority of these compounds are terpenes and polyphenols.[10] The Moroccan coast is particularly rich in algal biodiversity and constitutes a reserve of species of considerable economic, social and ecological potential. More than 150 000 macroalgae species are found in oceans of the globe, which include green, brown and red algae, but only a few of them are identified.[20] Bibliographic inventory of benthic algae of the El Jadida coast revealed the presence of 143 species including 23 Chlorophyceae, 25 Phaeophyceae and 95 Rhodophyceae.[21, 22, 23]

The aim of our work was to search for a new antibiotic extracted from marine algae harvest on the Atlantic coast of El Jadida city (Sidi Bouzid-Morocco). In this study, tests were conducted to determine the antibacterial activity of 24 extracted of marine algae against Pectobacterium species (*Pectobacterium brasiliensis* 1347 (*Erwinia carotovora*)) using the methanol, dichloromethane and dichloromethane/methanol solvent.

MATERIALS AND METHODS

Algal materials

Seaweeds was collected by hand-picking during March to April 2013 from the coast of El Jadida (33 ° 33 °16'09''N, 8 ° 30' 8 ° 45'W) (Figure 1).

Figure 1: Localization of the collection site of Sidi Bouzid

The algae were cleaned, washed in distilled water, then dried at room temperature and crushed to a fine powder.

The algae investigated were identified from fresh species as; Chlorophyceae (green algae): *Codium elongatum, Ulva* sp., *Enteromorpha intestinalis*, Phaeophyceae (brown algae): *Bifurcaria bifurcata, Fucus spiralis, Laminaria digitata, Sargassum vulgare, Cystoseira tamariscifolia, Cystoseira humilis* and Rhodophyceae (red algae): *Halopitys incurvus, Ellisolandia elongata, Osmundea pinnatifida, Gracilaria cervicornis, Gymnogongrus norvegicus, Gelidium corneum, Gelidium pulchellum, Plocamium cartilagineum, Gigartina acicularis, hypnea musciformis, Corallina officinalis, Asparagopsis armata, Rhodymenia* sp., *Gigartina teedii, Gracilaria multipartita*.

Preparation of extracts

The separate powder from each species of dried algae was extracted in different solvents: methanol (Sigma-Aldrich®, Germany), dichloromethane (AppliChem Panreac ITW Companies, Spain) and dichloromethane/methanol (50:50), as described by Caccamese and Azolina.[24] The resulting extracts were concentrated by drying in a rotary evaporator (Heidolph instruments GmbH & Co.KG, Basis Hei-VAP Value, Germany) under reduced pressure (at 45 °C) until a crude extract was obtained, and was stored at 4 °C until utilization.

Antimicrobial Bioassays

Antibacterial assays were carried out using the agar disc-diffusion assay.[25] Three colonies of each bacterium were removed with a wire loop from the original culture plate and were introduced into a test tube containing 5 mL of Nutrient broth (Mast Group Ltd., Merseyside, U.K.). An overnight culture yielded a suspension of 10^6 bacteria per mL (evaluated by the absorbance value of 0.5 at 620 nm). This solution was diluted 100-fold and the bacterial density was then adjusted to 0.2×10^4 cells per mL with sterile water to inoculate petri dishes containing Mueller-Hinton agar culture media (Biokar diagnostics, France). Plates were dried for about 30 min before inoculation and were used within 4 days of preparation. The organic extracts were tested using cellulose disks (6 mm diameter, Filtres Fioroni, France) impregnated with the solution.

After the temperature was equalized at 4 °C, the microorganisms were incubated overnight at 26 °C. Diameters of inhibitory zones were then measured. Discs impregnated with standard antibiotics such as, streptomycin (Fluka Biochemika, Switzerland) were used at 50 or 100 µg/mL as reference in the test of antibacterial activity. In addition, control disks were prepared with each solvent and all tests were performed in triplicate. Representative halos were those measuring a diameter superior to 10 mm.[26]

Antibacterial efficiency of extracts was evaluated according to the following scale:

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Table 3: Antibacterial activity of different red seaweeds extracts against *Pectobacterium brasiliensis.*

Algae/Solvent	Diameter of inhibition (mm) [a]		
	Dichloromethane	Methanol	Dichloromethane/Methanol
Gigartina acicularis	9±1	8±2	11±3
Hypnea musciformis	8±3	9±3	8±3
Corallina officinalis	11±2	11±3	12±3
Asparagopsis armata	10±3	11±3	13±3
Rhodymenia sp.,	10±1	15±3	14±3
Gigartina teedii	7±2	7±2	11±3
Gracilaria multipartita	11±3	8±3	12±3
Ellisolandia elongata	12±1	11±2	16±2
Osmundea pinnatifida	10±3	11±3	15±2
Gracilaria cervicornis	12±3	16±2	13±2
Gymnogongrus crenulatus	10±3	12±3	11±4
Gelidium corneum	11±3	11±3	11±3
Gelidium pulchellum	10±3	11±2	10±1
Plocamium cartilagineum	10±3	12±3	14±3
Halopitys incurvus	13±2	25±3	20±4
Streptomycin (Control)	12 ± 1		

[a] The results reported in the tables are the average of values obtained for three tests

Marine organisms are a rich source of structurally novel and biologically active metabolites.[28] Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the different industry.[29] To date, many chemically unique compounds of marine origin with various biological activities have been isolated, and some of them are under investigation and are being used to develop new product.[26]

According to the Zbakh et al.[30] the Moroccan marine biodiversity including macroalgae remains partially unexplored in term of their potential bioactivities. Antibacterial activity of methanolic extracts from 20 species of macroalgae (9 Chlorophyta, 3 Phaeophyta and 8 Rhodophyta) collected from Moroccan Mediterranean coasts was evaluated against *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*. The extracts of the studied Rhodophyceae inhibited considerably the growth of the three tested bacterial strains and gave inhibition zones between 20 and 24 mm. The results indicate that these species of seaweed present a significant capacity of antibacterial activities, which makes them interesting for screening for natural products. The results of research on the biological properties of marine algae harvested from the coast of El Jadida are promising avenues of development in various fields in the longer term. This study has made it possible to isolate many substances who exhibit an appreciable number of distinct biological activities such as antimicrobial activity, antitumoral, antiviral, antifungal, cytotoxic, antiparasitic, anti-inflammatory and antiproliferative actions.[12, 31, 13, 32, 33, 10, 14, 34, 15, 27, 16] The majority of these compounds are terpenes and polyphenols.[10]

The methanol extracts of *Padina pavonica* and *Enteromorpha compressa* show an important antibacterial activity against *Bacillus subtilis*.[35] The in vitro antimicrobial effect of seaweed extracts belonging to *Ascophyllum nodosum* and *Laminaria digitata* were tested against some pathogens (*Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candidaalbicans*). The organic solvent extracts of *Ascophyllum nodosum* and *Laminaria digitata* exhibited high bacterial activity against all the test microorganisms. Higher inhibitory effect of organic solvent extracts was found against Gram-positive bacteria than Gram-negative bacteria.[36]

Antibacterial activity of red, brown and green algae against both Gram positive and Gram negative bacteria has been established by several scientists. The best antibacterial activity was recorded with *Gracilaria edulis* extract.[37] *Calorpha peltada* was showed inhibitory activity against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus faecalis*.[38]

Methanolic extracts of six marine algae belongs to Rhodophyceae (*Corallina officinalis*), Phaeophyceae (*Cystoseria barbata, Dictyota dichotoma, Halopteris filicina, Cladostephus spongiosus, Fucus verticillatus*) and Chlorophyceae (*Ulva rigida*) from North Aegean sea (Turkey) were studied for the antibacterial activity against pathogenic microbes, three Gram positive bacteria viz *Staphylococcus aureus, Micrococcus luteus, Enterococcus faecalis* and three Gram negative bacteria viz *Escherichia*

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coli, Enterobacter aerogenes, Escherichia coli in vitro. Extracts of the test marine algae except *Clostridium officinalis* showed inhibition against *Staphylococcus aureus* and highest inhibition activity among all the extracts was shown to *Enterobacter aerogenes*.[39]

According to Pandian,[40] the methanolic extract of *Acanthaphora spicifera* was showed higher antibacterial and antifungal activity compare to other two extracts. The minimum inhibitory concentration of methanolic extract of *Acanthaphora spicifera* was found to the range of 100 $\mu g.mL^{-1}$ to 50 $mg.mL^{-1}$ on test organisms. The antibacterial and antifungal activity of methanolic extracts showed similar activity to that of ciprofloxacin and amphotericin respectively.

CONCLUSION

These results show that the Atlantic coast of Sidi Bouzid (El Jadida-Morocco) are a source of great biological diversity with an almost unexplored potential to provide significant therapeutic, agricol, as well as nutritional, benefits for humans. The investigation and exploitation of the potential of marine algae will have significant health implications for current and future generations, not only for local people but also for people from all around the world.

From all these results, we can conclude that macroalgae from Atlantic Moroccan coasts represent a potential source of bioactive compounds and must be studied for the production of natural antibiotics. Biochemical analysis are being undertaken to determine the nature of compounds responsible of the bioactivity of the extracts with high antibacterial activity, which makes these organisms interesting.

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