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ISOLATION OF MICROBIAL GROUPS FROM A SEAWEED EXTRACT AND COMPARISON OF THEIR EFFECTS ON A GROWTH OF PEPPER CULTURE (*CAPSICUM ANNUUM L.*)

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This work describes the results of the microbiological characterization of seaweed extract called ALGAENZIMSв that is produced by the company PalauBioquim (Mexico) as a biofertilizer. ALGAENZIMSв production is performed by means of a seaweed treatment on alkaline solution and addition of some other natural extracts from Mexican desert plants and salts. The results obtained in this work demonstrated that this product contains a great variety of microorganisms at a high concentration (>2.0E+9 CFU/ml). The goal of this study is to isolate from the product some microbial fractions (1,- Mesophilic Aerobic Bacteria, 2,- Fungi, 3,- Microorganisms growing in medium without addition of nitrogen sources and 4,- Microorganisms tolerant to high salt concentration), apply them on agricultural test on pepper culture and compare the observed effects. During assay kinetics of growth, the plant height at the end of experiment, the length of time before initial harvest and the fruit weights were evaluated. This work indicates the importance of the interactions between plant and microorganisms isolated from ALGAENZIMSв.

Intensive application of chemical fertilizers in agriculture has caused damage to the ecological state of the agricultural systems [1]. The use of biofertilizers is an alternative to improve the conditions of Mexican fields and world-wide. Biological fertilizers do not contaminate the soil and atmosphere and help to produce healthy foods [2].

Seaweeds and their derivatives are used in agriculture as potential plant growth regulators. The main types of seaweeds are: *Chlorophyta, Euglenophyta, Pyrrophyta, Chrysophyta* and *Rhodophyta*. Biological regulators are substances that in small concentrations cause a physiological effect in plants inhibiting or stimulating specific function. Some of them are phytohormones (absicines, auxins, cytokines, gibberellins, ethylene) and microbial enhancers.

Agricultural enhancers are defined as biological or nonbiological agents which reduce the time of growth, increase the production and/or quality of the agricultural products as well as the time of the flowering and fruition, the fruit size, etc. [3]. The participation of microorganisms as enhancers is related to their enzymatic activity. For example, potential for nitrogen fixing is a property of some prokaryotes [4].

It is known, that during the growth, the plant interacts intimately with microorganisms of soils. The interaction between the microorganisms and the plants can be beneficial, neutral or detrimental [5]. Three classes of microorganisms beneficial for the plants may be defined [5–11]: 1) as microorganisms that can increase the supplement of mineral nutrients essential for growth such as nitrogen and phosphorus; 2) microorganisms that stimulate the growth of the plants at an indirect form by means of the repression of pathogenic organisms (for example *Bacillus thuringensis* produces a toxin lethal for the phytopathogenic insects). These microorganisms are utilized for biocontrol, and are called such to differentiate it from the use of chemical insecticide. 3) Microorganisms that direct the biological growth of the plants, for example by phytohormones. The investigations of this form of growth promotion have begun recently and offer potential opportunities. A great number of bacteria such as *Azotobacter sp*, *Pseudomonas sp.*, *Azosporillium sp.* and some fungi such as the *Fusarium sp*. are able to produce a plant growth regulators such as gibberellins, auxins and cytokines.

This study focuses on the microbial characterization of the seaweed extract (ALGAENZIMS®) and the role of microorganisms present in this bioproduct for its potential as fertilizer. ALGAENZIMS® is produced by the company PalauBioquim (Mexico) as a biofertilizer. Its production is performed by seaweed treatment on alkaline solution and an addition of some other natural extracts from Mexican desert plants and salts. Previously it was demonstrated that this product applied at low concentrations (1–4 L/Ha) enhanced the growth and productivity of farming cultures [10]. However, the microbial components of ALGAENZIMS® responsible for its agricultural effectiveness were not characterized.

MATERIALS AND METODOS

Isolation of the microbial fractions from ALGAENZIMS®

Four microbial groups: mesophilic aerobic bacteria (MAB), fungi (Fung), nitrogen fixing bacteria (N2 Fixers) and microorganisms tolerant to high salt concentrations (HALO) were quantified and isolated from ALGAENZIMS®. The extract samples were diluted in peptone water (from 10^{-2} to 10^{-12}) and inoculated in the appropriate medium: nutritive agar for MAB isolation, Sabouraud agar for fungi isolation and agars (one without nitrogen source and other with NaCl at30 g/L) selective to nitrogen fixing and salt tolerant microorganisms.

The quantification of CFU/ml was performed after incubation of nutritive agar for 48 hours at 37°C, Sabouraud agar for 24 hours at 37°C followed by incubation at 25°C for 72 hours and selective agars were incubated for 2 weeks at 25°C at darkness.

The macroscopically different microbial colonies were selected and characterized microscopically.

Propagation of the microbial fractions

To obtain the amount of biomass sufficient for application in an agricultural test, the propagation of the isolated microbial fractions was performed using the enriching non-selective medium (CPMA). The strains of each group were incubated separately for 7 days at 25°C and then in mixture for 72 hours at 37°C under agitation at 75 rpm. The CFU/ml of obtained biomass was defined by the standard technique in petri dishes containing the mentioned solid media.

Isolation of the fraction free of microorganisms

To define the role of plant growth regulators, distinct to the microbial flora of ALGAENZIMS, the filtrate of product free of microorganisms was obtained and applied in agriculture test. The filtration was performed using Millipore filters.

Table 1

Microbial characteristics of ALGAENZIMS®

Group of microorganisms	Concentration (CFU/ml)
Mesophilic aerobic bacteria	2.9×10^{8}
Fungi	2.7×10^{8}
Nitrogen fixed microorganisms	1.81×10^{10}
Microorganisms tolerant to salt concentration	$>10^{8}$

Table 2

Quantification of microorganisms in each treatments (CFU per plant)

ALGAENZIMS	MAB	FUNGI	N2 Fixer	Filt	HALO
4.64×10^{6} UFC of MAB 4.32×10^{6} UFC of FUNGI 2.90×10^{8} UFC of N2 Fixer	2.4×10^{7} UFC	1.5×10^{9} UFC	2.2×10^{5} UFC	0× UFC	1.4×10^{6} UFC

Study of the effect of isolated fractions in agricultural test

The environmental conditions in a greenhouse are similar to those in the field. Its advantage is that some environmental factors can be controlled. In this assay the growth of pepper *Capsicum annuum* in greenhouse conditions was evaluated. During assay kinetics of growth, the plant height at the end of experiment, the length of time before initial harvest and the fruit weights were monitored. The plants were maintained in controlled conditions of greenhouse. The abovementioned microbial treatments, ALGAENZIMS®, water (BCO), CPMA medium and fraction without microorganisms (obtained from ALGAENZIMS® by filtration on microspore filter) were applied directly to soil at concentration 0.04% v/v obtaining by dilution in the water of the first irrigation. Each treatments and controls were applied to 15 different pepper plants.

RESULTS AND DISCUSSION

The results obtained in this study demonstrates that the seaweed extract ALGAENZIMS contains a great variety of microorganisms at a high concentration (Table 1). Applying selective media and conditions, four microbial groups were isolated and proliferated to be used in assay with pepper culture: 1) Mesophilic aerobic bacteria, 2) Fungi, 3) Microorganisms growing in medium without addition of nitrogen sources and 4) Microorganisms tolerant to high salt concentration. The 24 strains of microorganisms were isolated. The presence of microorganisms that possessed the ability to fix nitrogen from air, was demonstrated. The microscopic study of isolates did not confirm the presence of viable algae. Only seaweed fragments were detected.

The amount of each group of microorganisms applied in each treatment per plant is presented in Table 2.

During the agricultural assay performed under controlled conditions of greenhouse, the kinetics of growth, the plant height at the end of experiment, length of the time before initial harvest and the fruit weights were evaluated.

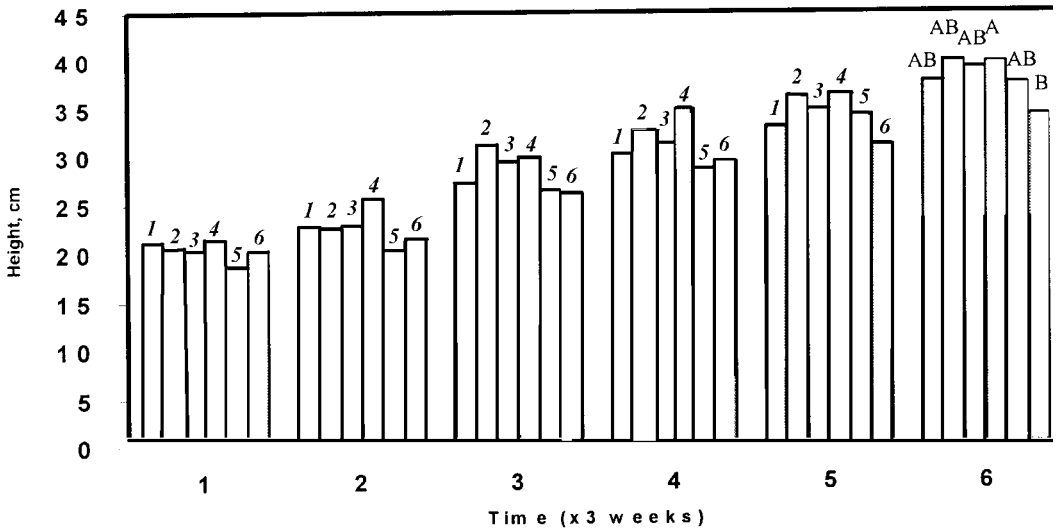

Fig. 1. Growth kinetics of *Capsicum annuum* in the agricultural test performed by using of different treatments. The data marked by different letters are statistically significant difference (0.05 of probability according to SNK test)

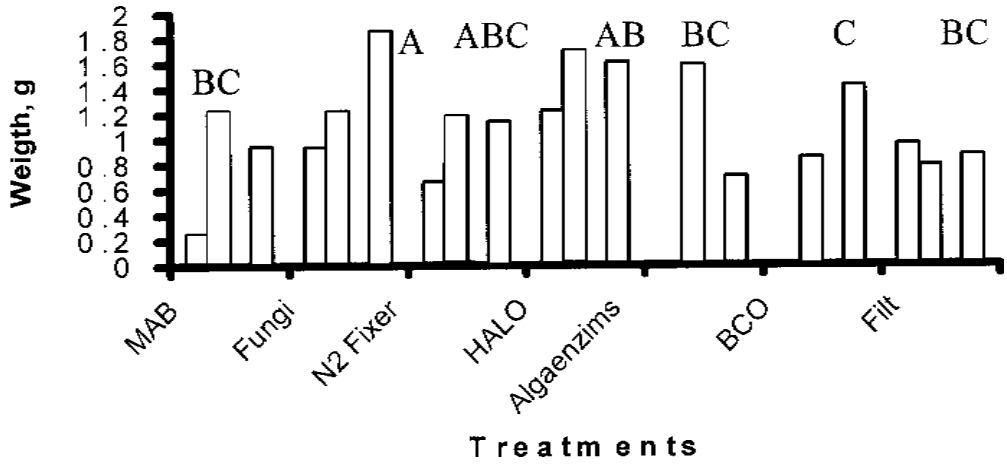

Fig. 2. Weight of dry biomass of pepper *Capsicum annuum* fruits determined for 4 cuts realized from plants treated with ALGAENZIM?s fractions and control treatments. The data marked by different letters are statistically significant difference (0.05 of probability according to SNK test)

The Fig. 1 shows that the treatments with Microorganisms tolerant to high salt concentration (HALO) and with Fungi caused the effect to accelerate the growth kinetics. However, considering the final height of the plants only microorganisms in fourth group demonstrated the effect statistically verified by SNK analysis. Data of this ANOVA test is presented in Table 3.

It was also observed that treatment also caused a reduction of time to initial harvest. The fist fruits of plants treated with Microorganisms tolerant to high salt concentration were obtained 15 days earlier than in the other treatment. This effect has importance to the pepper farmers since it may help to obtain the advantage in agricultural market [10]. Treatment with Fungi demonstrated an increase in the weight of dry fruits (Fig. 2). The data of ANOVA test is presented in the Table 4. However, there was no significant difference in the fresh biomass of

Table 5

Summary of significant effects observed in the agricultural test of ALGAENZIMS and its fractions

Parameter evaluated	Treatment with better effect over *Capsicum annuum* (pepper)
Growth	HALO and Fungi
Final height of plants	HALO
Fresh weight of fruit	No differences detected
Dry weight of fruit	Fungi

Table 3

Statistical SNK-analysis of data described the final height of pepper plants treated by different fractions of ALGAENZIMS and control treatments: 1.- MAB; 2.- Fungi; 3.-N2 Fixer; 4.- Halo; 5.- CPMA; 6.- Algaenzims; 7.- Control (Bco)

Dependent Variable: A7

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	34	21.37599267	0.62870567	3.02	0.0001
Error	70	14.56260022	0.20803715		
Corrected Total	104	35.93859289			

R-Square	C.V.	Root MSE	A2 Mean
0.594792	6.782053	0.45611089	6.72526254

SNK Grouping	Mean	No.	TREATMENT
A	6.9812	15	4
A			
B A	6.9218	15	2
B A			
B A	6.8302	15	5
B A			
B A	6.7006	15	3
B A			
B A	6.6269	15	1
B A			
B A	6.5516	15	7
B			
B	6.4646	15	6

Table 4

Statistical analysis of data of total dry biomass of fruits of pepper *Capsicum annuum* applying in the agricultural test of ALGAENZIMS and its fractions: 1.- MAB; 2.- Fungi; 3.-N2 Fixer; 4.- Halo; 5.- CPMA; 6.- Algaenzims; 7.- Control (Bco)

Dependent Variable: PS2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	12.50521834	1.25052183	3.72	0.0003
Error	94	31.62080624	0.33639156		
Corrected Total	104	44.12602458			

R-Square	C.V.	Root MSE	PS2 Mean
0.283398	34.79968	0.57999272	1.66666126

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRAT	6	11.01133763	1.83522294	5.46	0.0001
REP	4	1.49388071	0.37347018	1.11	0.3564

Tukey Grouping	Mean	No.	TREATMENT
A	2.2713	15	2
A			
B A	1.8821	15	4
B A			
B A C	1.7635	15	3
B C			
B C	1.5901	15	6
B C			
B C	1.5502	15	1
B C			
B C	1.4295	15	5
C			

pepper fruits. These data demonstrates that the applied treatments improve the quality of the pepper fruits.

Table 5 summarizes the results obtained in the agricultural test and indicates that the treatments with Fungi and Microorganisms tolerant to high salt concentration results in statistically available effect to the growth, productivity and quality of testing plants.

In all acquired data the treatment with ALGAENZIMS demonstrated statistically significant difference to the data obtained in control treatments with water (BCO) or microbial medium CPMA (Tables 3 and 4). However, its effects were less than those induced by treatments with microbial fractions.

The fraction free of microorganisms also showed the positive effects in comparison with control treatments (Tables 3 and 4). This result shows that ALGAENZIMS contains the plant growth regulators differ to the microorganisms. The results obtained in the present study indicate the importance of the interactions between plant and microorganisms of ALGAENZIMS®. The studied seaweed extract may be considered as the source of the microorganisms that can be useful to development of new agricultural technologies.

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ВЫДЕЛЕНИЕ МИКРООРГАНИЗМОВ, ПРИНАДЛЕЖАЩИХ РАЗЛИЧНЫМ ГРУППАМ, ИЗ ЭКСТРАКТА МОРСКИХ ВОДОРОСЛЕЙ И СРАВНЕНИЕ ИХ ВЛИЯНИЯ НА РОСТ КУЛЬТУРЫ ПЕРЦА (*CAPSICUM ANNUUM L.*)

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Получены микробиологические характеристики экстракта морских водорослей ALGAENZIMST (Альчаэнзимс). Этот продукт содержит щелочной гидролизат водорослей, соли, а также экстракты из некоторых растений мексиканских пустынь. Показано, что ALGAENZIMST содержит высокую концентрацию разных микроорганизмов (> 2.0×10^9 КОЕ/мл). Использование селективных сред для роста клеток позволило выделить из продукта ALGAENZIMST следующие микробиологические фракции: 1) мезофильные аэробные бактерии; 2) грибки; 3) микроорганизмы, способные расти на средах без добавления источников азота, 4) микроорганизмы, выдерживающие высокие концентрации соли. Изучено влияние отдельных групп микроорганизмов на рост культуры перца при внесении продукта и его фракций в почву. Определяли кинетику роста растений, время, необходимое для сбора первого урожая, и вес плодов. Полученные результаты показывают важность взаимодействий, существующих между растениями и микроорганизмами, выделенными из продукта ALGAENZIMST.