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MICROMYCÈTES PARASITES DE FRANCE

(I. *Peronosporales* et *Ascomycetes*)

GAVRIL NEGREAN

The author presents a number of 38 parasitic fungi, living on 55 plants, which were gathered in 1990 from different regions of France. Among these are 19 *Peronosporales* on 24 plants and 29 specimens and 19 *Ascomycetes* (excl. *Erysiphaceae*) on 31 plants and 38 specimens.

A l'occasion d'une visite particulière rendue en France, à l'automne de 1990, j'ai recueilli plus de 900 échantillons de champignons de différents groupes systématiques.

Les matériaux ont été recueillis dans les zones suivantes: Massif Central (dép. Haute Loire /HL/ – alentours de St.-Sigoléne, Mt. Mézenc, Le Puy; dép. Ardèche /AR/ – les sources de la Loire); Les Alpes françaises (Le Parc National Vercors /PNV/, Alpe d'Huez); Provence (dép. Vaucluse /VL/ – Caseneuve; dép. Var. /VR/ – Ile de Porquerolle); Paris, etc.

La nomenclature des champignons est celle des plus récentes œuvres à notre disposition (1-5) et celle des plantes-hôtes, conformément à la Flora Europaea (6).

Les champignons identifiés appartiennent aux groupes suivants: *Peronosporales* (19 espèces), *Ascomycetes* (69 espèces) et *Uredinales* (57 espèces), pour un total de 209 plantes-hôtes.

Le matériel entier est déposé dans la mycothèque de l'Institut de Biologie de Bucarest (BUCM).

Dans une première note nous présentons les *Peronosporales* et les *Ascomycetes* (exclusivement *Erysiphaceae*) – 38 espèces sur 55 hôtes de 67 échantillons.

Parmi ces espèces nous remarquons *Peronospora ornithopi*, pas mentionné jusqu'à présent en France. *Albugo amaranthi* sur *Amaranthus bouchonii*, est probablement «matrix nova» et *Peronospora alchemillae* n'a pas été indiqué jusqu'à maintenant en France sur *Aphanes microcarpa*. Conformément à nos informations, la plupart des champignons ont été récoltés dans des zones peu étudiées du point de vue des mycromycètes.

REMERCIEMENTS. Nous remercions beaucoup M. le pharmacien, Pierre Roux pour l'invitation de visiter la France, ainsi que pour l'appui matériel accordé.

PERONOSPORALES

1. **Albugo amaranthi** (Schwein.) Kuntze

Sur *Amaranthus bouchonii* Thell. – Drôme: Montelimar, ruderal, 44°30'N, 4°40'E, 16 X 1990, GN (BUCM 118.038).
Sur *Amaranthus hybridus* L. – HL: St.Sigolène, ruderal, 45°13'40"N, 4°19'E,alt. 800 m, 8 X 1990, GN (BUCM 117.591).

2. **Albugo candida** (J.F.Gmelin ex Hook.) Kuntze

Sur *Alyssum saxatile* L. (cult.) – HL: St.Sigolène, 45°35'N, 4°19'E, alt. 800 m, 29 IX 1990, GN (BUCM 117.285).
Sur *Capsella bursa-pastoris* (L.) Medicus – HL: Les Estables, 44°55'N, 4°14'E, alt. 1340 m, 4 X 1990, GN (BUCM 117.393). Idem 7 X 1990, GN (BUCM 117.524). St.Sigolène sud, 45°13'N, 4°19'E, alt. 800 m, 9 X 1990, GN (BUCM 117.645).

3. **Bremia lactucae** Regel

Sur *Lactuca sativa* L. (cult.) – HL: la Chaise-Dieu sud, 45°15'N,3°45'E, alt. 1100 m, 30 X 1990, GN (BUCM 117.295).
Sur *Sonchus* sp. VR: Ile de Porquerolle, 43°02'N, 6°15'E, alt. 5 m, 18 X 1990, GN (BUCM 118.061).

4. **Peponospora alchemillae** Otth

Sur *Aphanes microcarpa* (Boiss.& Reuter) Rothm. – HL: St.Sigolène, 45°13'N, 4°19'E, alt. 800 m, 10 X 1990, GN (BUCM 117.734).

5. **Peronospora alta** Fuckel

Sur *Plantago major* L. subsp. *major* – HL: St.Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.595).

6. **Peronospora boni-henrici** Gäumann

Sur *Chenopodium bonus-henricus* L. – HL: Les Estables, 44°55'N, 4°14'E, alt. 1340 m, 4 X 1990, GN (BUCM 117.386).

7. **Peronospora chenopodii** Schlecht.

Sur *Chenopodium album* L. subsp. *album* – VR: Ile de Porquerolle, 43°02'N, 6°15'E, alt. 5 m, 18 X 1990, GN (BUCM 118.075). HL: St.Sigolène nord, 45°35'N, 4°19'E, alt. 800 m, 3 X 1990 (BUCM 117.377).
Sur *Chenopodium album* L. subsp. *striatum* (Krašan) J. Murr – HL: St. Sigolène, 45°35'N, 4°19'E, alt. 800 m, 29 IX 1990, GN (BUCM 117.283). Idem 8 X 1990, GN (BUCM 117.602).

8. **Peronospora digitalidis** Gäumann

Sur *Digitalis purpurea* L. – HL: la Chaise-Dieu sud, 45°13'N, 3°46'E, alt. 850 m, 30 IX 1990, GN (BUCM 117.380). St. Sigolène nord, 45°35'N, 4°19'E, alt. 800 m, 3 X 1990, GN (BUCM 117.381).

9. **Peronospora galligena** Blumer

Sur *Alyssum saxatile* L.–HL: St. Sigolène nord, 45°35'N, 4°19'E, alt. 800 m, 3 X 1900, GN (BUCM 117.376).

10. **Peronospora grisea** (Unger) Unger

Sur *Veronica beccabunga* L.–HL: Les Estables est, Mont Mézenc, 44°54'20"N, 4°15'E, alt. 1550 m, 5 X 1990, GN (BUCM 117.456).

11. **Peronospora knautiae** Fuckel ex Schröter

Sur *Knautia ?arvensis* (L.) Coulter–HL: St. Sigolène est, 45°13'N, 4°19E, alt. 800 m, 10 X 1990, GN (BUCM 117.740).
Sur *Knautia dipsacifolia* Kreutzer subsp. *dipsacifolia* – PNV: Gresse-en-Vercors, Pas de la Ville, 44°54'N, 5°34'E, alt. 1700 m, 13 X 1990, GN (BUCM 117.894).

12. **Peronospora obovata** Bonorden

Sur *Spergula arvensis* L.–HL: St. Sigolène est, 45°13'N, 4°19'E, alt. 800 m, 10 X 1990, GN (BUCM 117.746).

13. **Peronospora ornithopi** Gäumann

Sur *Ornithopus perpusillus* L.–HL: St. Sigolène est, 45°13'N, 4°19'E, alt. 800 m, 10 X 1990, GN (BUCM 117.738).

14. **Peronospora pulveracea** Fuckel

Sur *Helleborus foetidus* L.–PNV: Gresse-en-Vercors, 44°54'N, 5°34'E, alt. 1350 m, 13 X 1990, GN (BUCM 117.933).

15. **Peronospora senneniana** Fragoso & Sacc.

Sur *Lathyrus sylvestris* L.–Alpe d'Huez, Gorge de Sarenne, 44°55'N, 5°50'E, alt. 1500 m, 15 X 1990, GN (BUCM 117.988).

16. **Peronospora symphyti** Gäumann

Sur *Symphytum officinale* L. subsp. *officinale*–HL: St. Sigolène, 45°13'40"N, 4°19'00"E, alt. 800 m, 8 X 1990, GN (BUCM 117.627).

17. **Peronospora tribulina** Pass

Sur *Tribulus terrestris* L.–VR: Ile de Porquerolle, 43°02'N, 6°15'E, alt. 5 m, 18 X 1990, GN (BUCM 118.057).

18. **Peronospora violae** de Bary ex Schröter

Sur *Viola arvensis* Murray–HL: St. Sigolène est, 45°13'N, 4°19'E, alt. 800 m, 10 X 1990, GN (BUCM 117.730).

19. **Plasmopara pusilla** (de Bary) Schröter

Sur *Geranium sylvaticum* L. subsp. *sylvaticum* – HL: Les Estables, 44°55'N, 4°14'E, alt. 1340 m, GN (BUCM 117.521).

ASCOMYCETES

20. **Claviceps purpurea** (Fr.: Fr.) Tul.

Sur *Holcus lanatus* L.–AR: St.-Cirgues-en-Montagne, 44°46'N, 4°09'E, alt. 1060 m, 6X 1990, GN (BUCM 117.504).
Sur *Lolium perenne* L.–HL: St. Sigolène, 45°13'10"N, 4°18'40"E, alt. 800 m, 10 X 1990, GN (BUCM 117.747).

21. **Coleroa alchemillae** (Grev.) Winter

Sur *Alchemilla basaltica* Buser – HL: Les Estables, Mont d'Alambre, 44°55'40"N, 4°14'E, alt. 1500 m, 5 X 1990, GN (BUCM 117.438).

22. **Coleroa chaetomium** (Kunze: Fr.) Rabenh.

Sur *Rubus idaeus* L.–PNV: Gresse-en-Vercors, 44°54'N, 5°34'E, alt. 1250 m, 13 X 1990, GN (BUCM 117.860).
Sur *Rubus* sp.–VR: Île de Porquerolle, 45°02'N, 6°15'E, alt. 5 m, 18 X 1990, GN (BUCM 118.076).

23. **Eudarluca caricis** (Fr.) O. Erikss.

Socio cum *Melampsora caprearum* Thümen sur *Salix caprea* L.–HL: la Chaise-Dieu, 45°15'N, 3°45'E, alt. 1100 m, 30 IX 1990, GN (BUCM 117.316).
Socio cum *Melampsora epitea* Thümen sur *Salix appendiculata* Vill.–AR: St.-Cirgues-en-Montagne, 44°46'N, 4°09'E, alt. 1060 m, GN (BUCM 117.501).
Socio cum *Melampsora euphorbiae* (Schubad) Cast. sur *Euphorbia cyparissias* L. – PNV: Gresse-en-Vercors, 44°54'N, 5°34'E, alt. 1250 m, 13 X 1990, GN (BUCM 117.872).
Socio cum *Puccinia pulverulenta* Grev. sur *Epilobium tetragonum* L.s.l.–AR: Vallée de la Loire, 44°50'E, 4°18'E, 6 X 1990, GN (BUCM).
Socio cum *Uromyces dactylidis* Otth sur *Dactylis glomerata* L.s.l.–HL: Les Estables, 44°55'N, 4°14'E, alt. 1340 m, 7 X 1990, GN (BUCM).
Socio cum *Uromyces laburni* (DO.) Otth sur *Cytisus scoparius* (L.) Link–HL: Montregard, 45°09'58"E, 4°26'E, alt. 1000 m, 9 X 1990, GN (BUCM 117.669); sur *Genista anglica* L.–HL: St. -Sigolène, 45°35'N, 4°19'E, alt. 800 m, 3 X 1990, GN (BUCM 117.365).

24. **Hypomyces aurantius** (Pers.: Fr.) Tul. – A

Sur *Xerocomus chrysenteron* (Bull. ex St. -Am.) Quélet – HL: la Chaise-Dieu sud, 45°15'N, 3°45'E, alt. 1100 m, 30 IX 1990, GN (BUCM).

25. **Leptotrochila astrantiae** (Ces.) Schüepp

Sur *Astrantia major* L. subsp. *carniolica* Arcangeli – PNV: Gresse-en-Vercors, 44°54'N, 5°34'E, alt. 1250 m, 13 X 1990, GN (BUCM 117.863).

26. **Leptotrochila ranunculi** (Fr.) Schüepp

Sur *Ranunculus repens* L. – la Chaise-Dieu sud, 45°15'N, 3°45'E, alt. 1100 m, 30 IX 1990, GN (BUCM 117.291).

27. **Leptotrochila sanguisorbae** (Jaap) Schüepp

Sur *Sanguisorba officinalis* L. – HL: Les Estables, Mont Mézenc, 44°54'20"N, 4°15'E, alt. 1550 m, 5 X 1990, GN (BUCM 117.473).

28. **Leptotrochila verrucosa** (Wallr.) Schüepp

Sur *Galium saxatile* L. – HL: Les Estables, Mont d'Alambre, 44°55'40"N, 4°14'E, alt. 1500 m, 5 X 1990, GN (BUCM 117.440); Mont Mézenc, 44°55'15"N, 4°15'00"E

alt. 1600 m, 7 X 1990, GN (BUCM 117.574). AR: Ville-Vieille, Vallée de la Loire, 44°52'N, 4°16'E, alt. 1400 m, 4 X 1990, GN (BUCM 117.425).

29. **Lophodermium pinastri** (Schrader: Fr.) Chev

Sur *Pinus pinaster* Aiton subsp. *atlantica* H. de Villar – Valencay, 3 X 1990, matrix leg.& det. A. Beguet, fungus comm.& det.GN. (BUCM 118.160).

30. **Mycosphaerella angelicae** Woronichin – A

Sur *Angelica ?sylvestris* L. – HL: Les Estables, Mont d'Alambre, 44°55'40"N, 4°14'E, alt. 1500 m, 5 X 1990, GN (BUCM 117.432).

31. **Mycosphaerella killiani** Petrak – A

Sur *Trifolium repens* L. – HL: St. Sigolène. 45°13'N, 4°19'E, alt. 800 m, 10 X 1990, GN (BUCM 117.713).

32. **Naemacyclus niveus** (Pers.: Fr.) Sacc.

Sur *Pinus pinaster* Aiton subsp. *atlantica* H. de Villar (voir no. 29).

33. **Phyllachora graminis** (Pers.: Fr.) Fuckel

Sur *Hordelymus europaeus* (L.) C.O. Harz – PNV: Gresse-en-Vercors, 44°54'N, 5°34'E, alt. 1250 m, 13 X 1990, GN (BUCM 117.857).
Sur *Sporobolus pungens* (Schreber) Kunth – VR: Île de Porquerolle, 43°02'N, 6°15'E, alt. 0,5 m 18 X 1990, GN (BUCM 118.117).

34. **Polystigma rubrum** (Pers.) St.-Am.

Sur *Prunus domestica* L. (cult.) – PNV: Gresse-en-Vercors, 44°54'N, 5°34'E, alt. 1250 m, 13 X 1990, GN (BUCM 117.893).

35. **Pseudopeziza trifolli** (Biv.-Bern.) Fuckel

Sur *Trifolium repens* L. – HL: la Chaise-Dieu, 45°15'N, 3°45'E, alt 1100 m, 30 IX 1990, GN (BUCM 117.310).

36. **Rhytisma acerinum** (Pers. ex St.-Am.) Fr

Sur *Acer opalus* Miller – HL: Le Puy, Notre-Dame de France, 45°02'30"N, 3°55'E, alt. 630 m, 10 X 1990, GN (BUCM 117.755).
Sur *Acer platanoides* L. – HL: Les Estables, 44°55'N, 4°14'E, alt. 1340 m, 7 X 1990, GN (BUCM 117.529); St. Sigolène. 45°13'N, 4°19'E, alt. 800 m, 3 X 1990, GN (BUCM 117.350).
Sur *Acer pseudoplatanus* L. – HL: Les Estables, 44°55'N, 4°14'E, alt. 1340 m, 4 X 1990, GN (BUCM 117.392); Le Puy, Notre-Dame de France , 45°02'30"N, 3°55'E, alt. 630 m, 10 X 1990, GN (BUCM 117.750); le Chambon-sur-Lignon, 45°03'N, 4°23'E, alt. 960 m, 9 X 1990, GN (BUCM 117.675). AR: Mazan l'Abbaye, 44°45'N, 4°08'E, alt. 1200 m, 6 X 1990, GN (BUCM 117.481).

37. **Rhytisma salicinum** (Pers.) Fr

Sur *Salix appendiculata* Vill. – HL: la Chaise-Dieu, 45°15'N, 3°45'E, alt. 1100 m, 30 IX 1990, GN (BUCM 117.317).

Sur *Salix atrocinerea* Brot.-Versailles (Yvelines). VIII 1967, leg. et det. INRA Versailles sub Salix (cinerea?), matrix rev. G. Negrean, 2 III 1994 (BUCM 28.959).

38. **Venturia rumicis** (Desm.) Winter

Sur *Rumex alpinus* L. – HL: Les Estables, 44°55'N, 4°14'E, alt. 1340 m, 7 X 1990, GN (BUCM 117.533).

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EPICOCCUM PURPURASCENS
I. BIOLOGICAL PARAMETERS OF DEVELOPMENT

TATIANA ŞESAN and MARIA OPREA

Epicoccum purpurascens, known as a potential biocontrol agent usable against some phytopathogenic fungi, was isolated from wheat seeds.
The biological parameters of fungus development "in vitro" have been established, in order to be used in plant protection biotechnologies.
The most favourable conditions for optimal development of the test-fungus were: a) culture media: wheat flour and maize meal-agar, followed by oat and bean extract-agar, PDA and Czapek - Dox; b) carbon sources: starch, maltose, levulose (fructose), sucrose (saccharose), glucose (dextrose); c) nitrogen sources: DL-asparagine, potassium and ammonium nitrates, DL-norvaline; d) pH values of PDA medium: neutral (7.0), slightly alkaline (8.5 and 9.0) and slightly acid (5.5 and 5.0); e) temperatures between 21 and 28°C, with optimum at 27°C; f) photophase between 12 and 24 hours, with optimum at 24 hours; g) air relative humidity higher than 75% (78-95%).

Epicoccum purpurascens Ehrenb.ex Schlecht., syn. *E.nigrum* Desm. and *E.neglectum* Desm. is a micromycete antagonistic to more than 20 plant-pathogenic fungus species (11), producing antibiotics (1), (9) and specific pigments (4), (9). Literature data (2), (3), (5), (6), (7), (8), (9), (10) on the antagonistic properties of this fungus stimulated its biological study, in order to establish culture parameters, with a view to mass propagation and its use for biological protection against fungus diseases in some cropped plants.

MATERIAL AND METHODS

A number of 10 synthetic and natural culture media have been tested, among which the PGA medium with various carbon (16) and nitrogen (13) sources, and various initial pH values (ranging from 3.0 to 11.0), under different temperatures (between 6 and 28°C), photophases (from 0 to 24 hrs), and air relative humidity (between 60 and 95%), aiming to reveal the influence of these factores on the development of *E.purpurascens,* isolated by the authors from wheat seeds.

The isolate of this fungus has been cultivated in Petri dishes 7 and 5 cm in diameter (the latter ones for trials with nitrogen sources), in which disks 0.7 cm in diameter of the *E.purpurascens* isolate, 7-10 days old, have been centrally placed onto the culture medium surface; the colony diameters have been measured daily up to a total medium covering by the fungus. In parallel, microscope observations have been performed to evaluate sporulation. Petri dishes, in 5 replications for each variant, have been incubated at room temperature.

Scoring colony growth under the above conditions has been carried out by recording the average diameter of a colony at various times (2-8 days). Sporulation was evaluated microscopically after 7-15 days, using a notation scale with 5 levels.

Data were statistically treated by the analysis of variance.

RESULTS AND DISCUSSION

1. *Influence of culture media.* Table 1 and Figure 1 show that the best development occurred on media including wheat flour and maize meal, followed by those with oats and bean extracts, as well as PGA and Czapek-Dox; less favourable growth has been noted on malt-agar and Leonian media; the soybean extract-agar was the least suitable, on which the isolate did not grow.

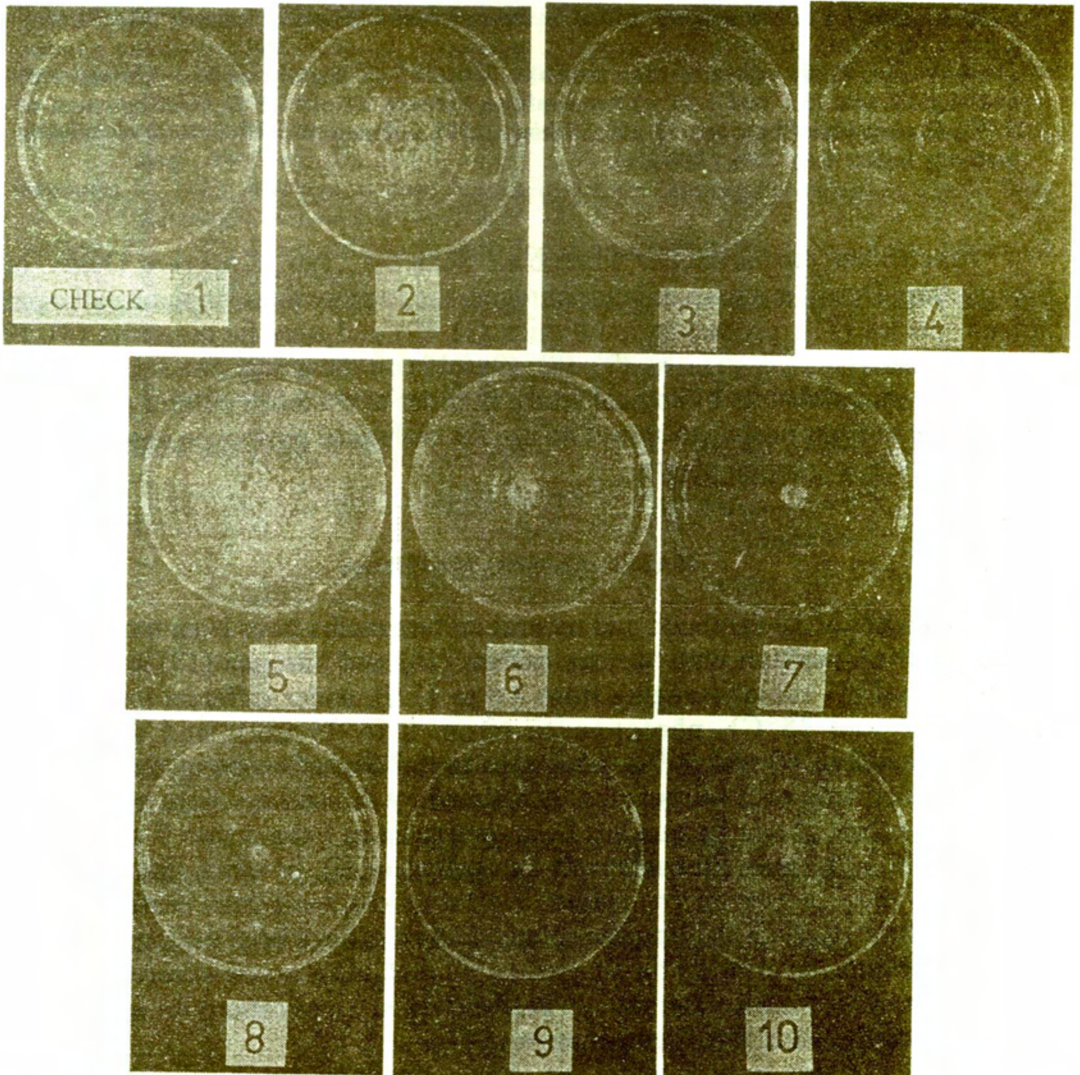

Fig. 1. - *Epicoccum purpurascens* development on different culture media: 1. Water-agar(check); 2. malt-agar; 3. Leonian-agar; 4. Czapek-Dox: 5. Wheat (flour)-agar; 6. Maize (meal)-agar; 7. Oat (extract)-agar; 8. Bean (extract)-agar; 9. Soybean(extract)-agar; 10. C.G.A.

Table 1

Epicoccum purpurascens development on different culture media

Culture media	Colony diameter (cm) after:		Colony aspect after 7 days	Pigmentation after 7 days	Sporulation
	2 days	7 days			
1. Water-agar (check)	7.0	2.94	Poor growth; white-greyish colony	–	–
2. Malt-agar	0.80 xxx	3.08	White, caespitose colony; red bottom	Intense red	+++
3. Leonian-agar	0.92 xxx	2.96	Dense growth	Yellow-orange	++
4. Czapek-Dox	0.80 xxx	3.28 x	Dense growth; caespitose colony; red bottom	Purple, diffused in the medium, which becomes orange	++
5. Wheat (flour)-agar	0.72	4.46 xxx	Abundant growth; grey, caespitose mycelium; red bottom	Purple; Bordeaux-red	++++
6. Maize (meal)-agar	0.72	4.14 xxx	Poor growth, subsequently dense growth	Intense-yellow, diffused in the medium	++++
7. Oat (extract)-agar	0.70	3.46 xxx	Poor growth; white-greyish mycelium	–	++
8. Bean (extract)-agar	0.70	3.58 xxx	Poor growth; white-greyish mycelium	–	++
9. Soybean (extract)-agar	0.70	0.70 ooo	–	–	–
10. Potato-dextrose-agar (PDA)	0.70	3.45 xx	Dense growth	Intense-red	++++
CL 5%	0.028	0.293			
CL 1%	0.038	0.393			
CL 0.1%	0.050	0.519			

* Abundant sporulation ++++
Dense sporulation +++
Moderate sporulation ++
Poor sporulation +
Without sporulation –

E. purpurascens is a fungus which rarely sporulates, therefore in our trials in no variant sporulation was noticed after 7 days, but only after 15. Sporulation was very active on PGA, wheat flour and maize meal media, good on malt-agar, moderate on Leonian, Czapek-Dox, oat and bean extracts; the isolate did not sporulate on soybean extract.

Likewise, pigmentation differences have been recorded among colonies, as depending on culture media, namely: a) bright-red on wheat flour-agar, PGA, malt-agar, Czapek-Dox; on this latter the pigment diffused very intensely through the medium, this becoming red-orange; b) yellow-orange pigmentation on Leonian and maize meal-agar media; c) without pigmentation on water-agar and oat-, bean- and soybean-agar extracts.

The pigments identified were: beta and gamma carotene, torula-rhodine and rhodoxanthine (4). Different pigmentation of test-isolate was due to its variable, specific physiology (5). Thus, existence of 4 colony-types was established (A,B,C,D), the former three ones (A,B,C) being characterized by yellow-orange coloration; however their sporulation was very variable (from very abundant to medium), while the latter type (D) did not show pigmentation. These differences were also noticed in our results (Table 1).

Influence of carbon sources. Among the 16 carbon sources tested (Table 2, figure 2), the best growth was recorded on media with starch, maltose, mannite,

Table 2

Influence of carbon sources on *Epicoccum purpurascens* development

Carbon sources	Colony diameter (cm) after:		Sporulation
	3 days	7 days	
MONOSACCHARIDES			
Mannite	0.700	2.767^{xxx}	++++
Galactose	0.800	1.033^{xxx}	+
Sorbose	0.700	0.700^{oo}	+
Levulose (fructose)	0.800	2.733^{xxx}	++++
Glucose (dextrose)	0.800	2.467^{xxx}	++++
DL-arabinose	0.700	1.400^{xxx}	++
Rhamnose	0.700	0.700^{oo}	+
Ribose	0.800	0.800	+
Dulcite	0.800	0.800	+
DISACCHARIDES			
Sucrose (saccharose)	1.433^{xxx}	2.667^{xxx}	++++
Maltose	0.933^{xxx}	2.867^{xxx}	++++
Melibiose	0.700	1.767^{xxx}	+
Trehalose	0.800	0.800	+
POLYSACCHARIDES			
Cellulose	0.700	0.700^{oo}	+
Inuline	0.700	0.733^{o}	+
Starch	1.400^{xxx}	6.567^{xxx}	+++
Without carbon sources (check)	0.700	0.800	–
CL 5%	0.102		0.058
CL 1%	0.137		0.078
CL 0.1%	0.182		0.103

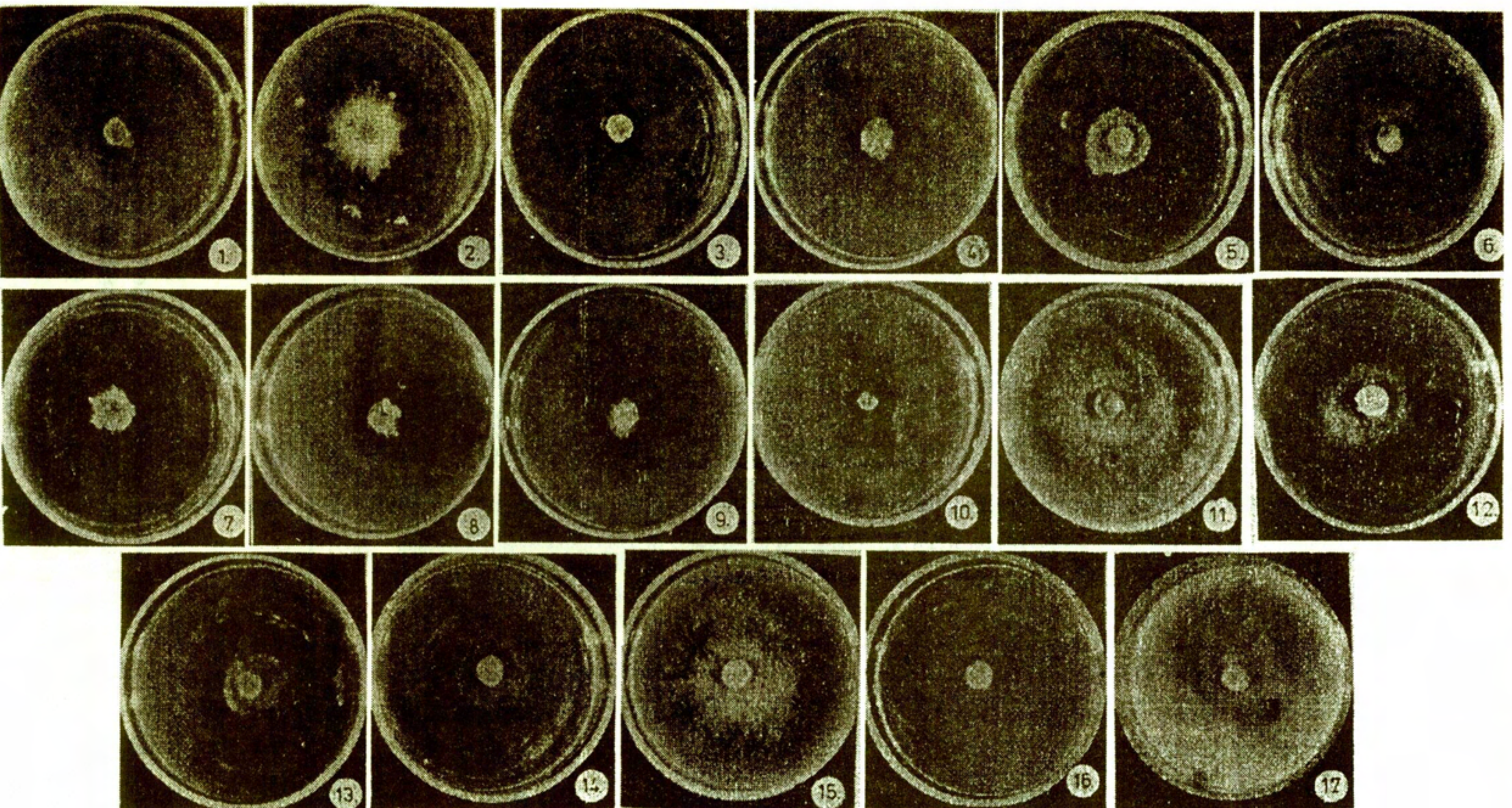

Fig. 2. - *Epicoccum purpurascens* development on Czapek medium containing different carbon sources: 1. without glucides (check); 2.mannite; 3. galactose; 4. sorbose; 5. fructose(levulose); 6. glucose(dextrose); 7. D-arabinose; 8. rhamnose; 9. ribose; 10. dulcite; 11. saccharose(sucrose); 12. maltose; 13. melibiose; 14. trehalose; 15. starch; 16. inuline; 17. cellulose.

levulose (fructose), sucrose (saccharose) and glucose (dextrose); lesser growth was noticed on media including melibiose, DL-arabinose, galactose; on other carbon sources (sorbose, rhamnose, ribose, dulcit, trehalose, cellulose, inulin) growth was poor.

From our data, as well as from Schol - Schwarz (9), it resulted that maltose and starch were the most suitable carbon sources to *E. purpurascens* development.

Influence of nitrogen sources. Among 13 nitrogen sources (Table 3 and figure 3), the best growth of *E. purpurascens* was allowed by media containing DL-asparagine, cysteine, potassium and ammonium nitrate, DL-norvaline, followed by those with urea, DL-norleucine, L-norvaline, ammonium bicarbonate; the fungus grew less on peptone and leucine media, whereas the lowest development was obtained with a beta-alanine medium.

Table 3

Influence of nitrogen sources on *Epicoccum purpurascens* development

Nitrogen sources	Colony diameter (cm) after:		Sporulation
	3 days	7 days	
AMIDES			
DL-asparagine	1.133^{xxx}	3.667^{xxx}	+++
Urea	1.200^{xxx}	2.733^{xxx}	+
AMINO ACIDS			
L-leucine	0.800^{x}	1.433^{xxx}	++
Cysteine	1.500^{xxx}	3.667^{xxx}	++
DL-norleucine	1.333^{xxx}	2.733^{xxx}	++
DL-norvaline	0.967^{xxx}	3.133^{xxx}	++
L-norvaline	0.767	2.533^{xxx}	+++
Beta-alanine	0.700	0.767	+
SALTS			
Potassium nitrate	1.400^{xxx}	3.200^{xxx}	+++
Ammonium nitrate	1.433^{xxx}	3.200^{xxx}	+++
Ammonium sulphate	1.467^{xxx}	2.533^{xxx}	+
Ammonium bicarbonate	1.133^{xxx}	2.233^{xxx}	+++
PROTEINS			
Peptone	1.500^{xxx}	1.733^{xxx}	+++
Without nitrogen (check)	0.700	0.800	–
CL 5%	0.094	0.050	
CL 1%	0.127	0.068	
CL 0.1%	0.169	0.091	

In dependence of the nitrogen sources used, a very good sporulation was noticed on media including peptone, potassium nitrate, ammonium nitrate, ammonium bicarbonate, L-norvaline and DL-asparagine. Moderate sporulation was recorded on media with L-leucine, cysteine, DL-norleucine and DL-norvaline. It was poor on media containing urea, beta-alanine and ammonium sulphate.

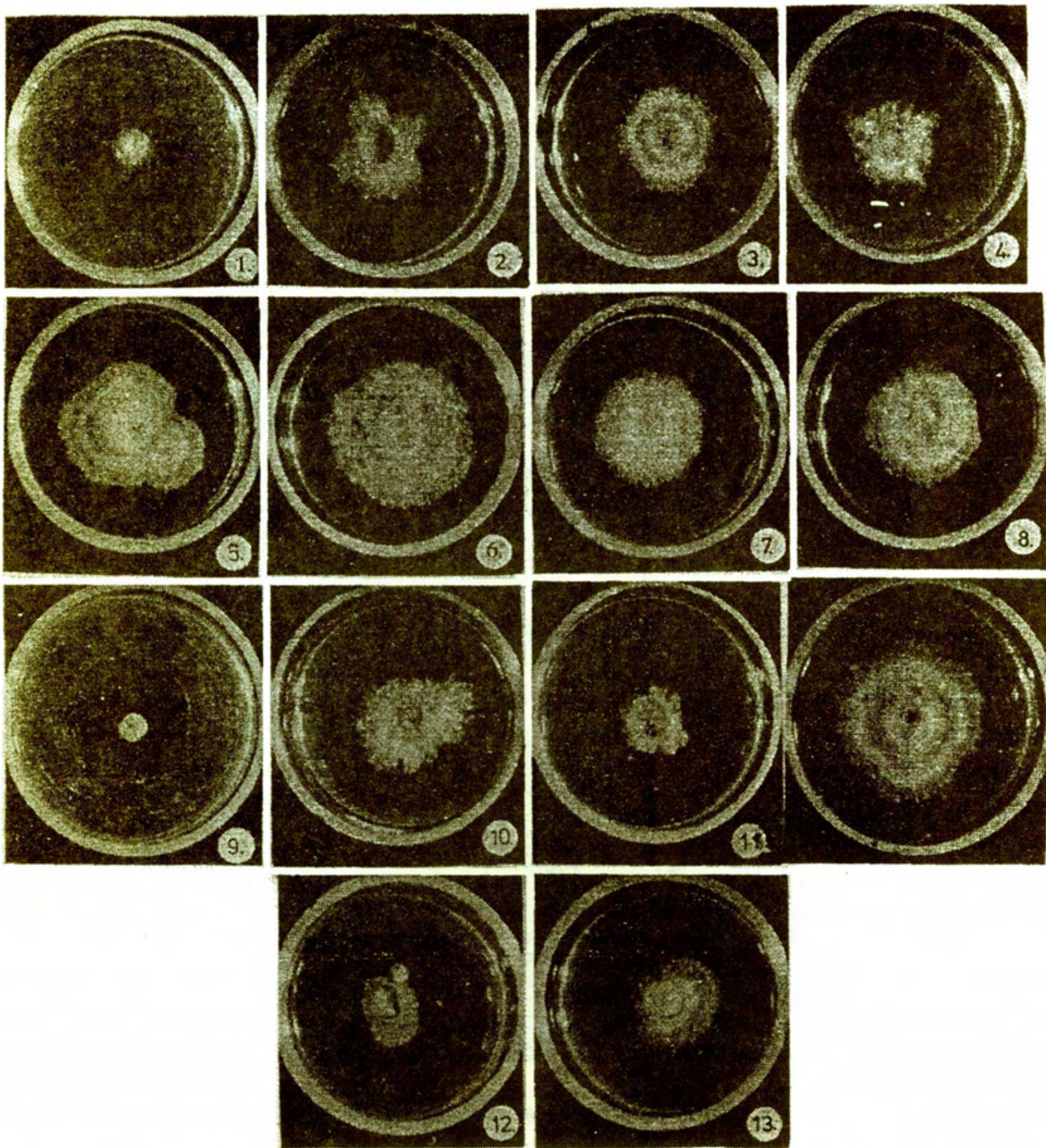

Fig. 3. - *Epicoccum purpurascens* development on Czapek medium containing different nitrogen sources: 1. without nitrogen(check); 2. DL- asparagine; 3. urea; 4. peptone; 5. potassium nitrate; 6. ammonium nitrate; 7. ammonium sulphate; 8. ammonium bicarbonate; 9. beta- alanine; 10. cysteine; 11. L-norvaline; 12. DL-norvaline; 13. L-leucine; 14. DL-norleucine.

Influence of reaction of culture medium. It resulted from Table 4 and figure 4 that the most favourable pH values were neutral, where the highest growth of the colony diameter was recorded. Slightly alkaline (pH 8.5-9.0) and slightly acid (pH 5.0-5.5) variants were also favourable.

The isolate started to grow rather well at pH 4.5, where the colony pigmentation became brightly red. Very acid (pH 3.0) or very alkaline media (pH 11.0) did not enhance fungus growth.

Table 4

Influence of pH values of PDA culture medium on *Epicoccum purpurascens* development

pH	Colony diameter (cm) after:		Colony aspect after 8 days	Pigmentation after 8 days	Sporulation
	2 days	8 days			
3.0	0.70	1.94^{ooo}	Yellow-pink, caespitose colony	Light-yellow to pink	–
4.5	0.76	4.62^{ooo}	Pink, caespitose colony; red bottom	Red	+++
5.0	0.80	4.90^{ooo}	Caespitose colony; red bottom	Red	++++
5.5	0.86	5.38^{ooo}	Pink colony; red bottom	Red	++++
6.0	0.80	5.96^{ooo}	Pink colony; red bottom	Red	++++
7.0 (check)	0.90	7.00	Pink, caespitose colony; red bottom	Red	++++
8.5	0.82	5.42^{ooo}	Poor, pink colony; red bottom	Red	+++
9.0	0.76	5.00^{ooo}	Pink colony; red bottom	Red	++
11.0	0.70	3.42^{ooo}	Pink colony; red bottom	Red	++
CL 5%	0.141	0.419			
CL 1%	0.190	0.565			
CL 0.1%	0.253	0.749			

Sporulation was abundant at pH 5.0-7.0, well at 4.5 and 8.5, moderate at 9.0 and 11.0, and absent at atrongly acid pH values (3.0).

Our results showing a good growth of the test-fungus at pH values between 7.0 and 8.5 are in disagreement with those of previous authors (9), claiming that *E. purpurascens* do not develop at pH 8.1; certainly, these differences are due to specificity of experimented isolates.

Table 5

Influence of temperature on *Epicoccum purpurascens* development

Temperature	Colony diameter (cm) after:		Colony aspect after 7 days	Pigmentation after 7 days	Sporulation
	2 days	7 days			
6°C	0.70^{ooo}	0.80^{ooo}	Pink colony; poor growth	Pink pigment, diffused in the medium, which becomes yellow	+
8°C	0.70^{ooo}	1.26^{ooo}	Pink, caespitose colony, grown only on the disc	Yellow, diffused in the medium	++
10°C	0.70^{ooo}	1.66^{ooo}	Pink, caespitose colony, grown only on the disc	Yellow, diffused in the medium	++
13°C	0.86^{oo}	4.26^{ooo}	Pink colony; red bottom	Red	++
18°C	0.86^{oo}	4.07^{ooo}	Pink colony; red bottom	Red	++
21°C	1.03	6.27	Light-pink colony; red bottom	Red	+++
25°C (check)	1.03	6.53	Pink colony; red bottom	Red	++++
27°C	1.03	7.00^{x}	Pink with yellow shades colony; red bottom	Yellow, diffused in the medium	++++
28°C	1.03	6.40	Caespitose, pink with red shades colony	Yellow, diffused in the medium	++
CL 5%	0.092	0.368			
CL 1%	0.127	0.508			
CL 0.1%	0.175	0.678			

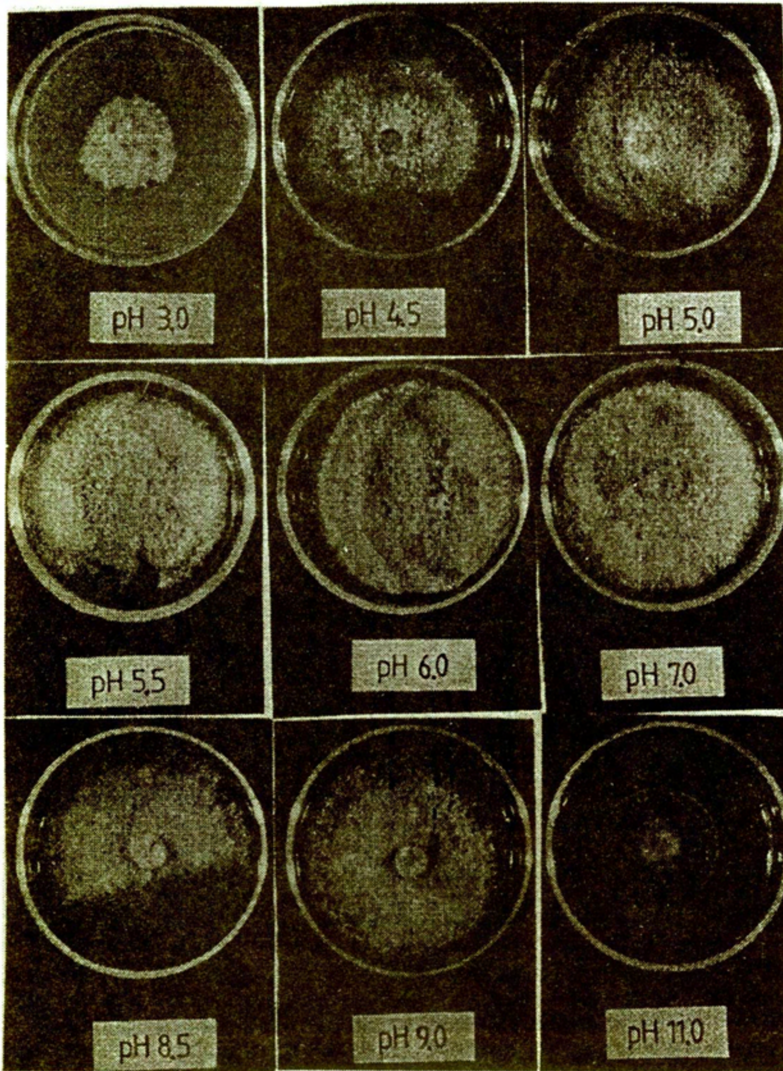

Fig. 4. - *Epicoccum purpurascens* development on P D A medium with different values of pH.

Influence of temperature. As shown in Table 5 and figure 5, temperatures between 21 and 28°C are very favourable to test-fungus development, its optimum being 27°C. Temperatures ranging between 6 and 10°C were unfavourable to development.

At different temperatures, pigmentation was variable, namely: yellow, diffused in the culture medium at 6-10°C and 25-28°C, and red at 13, 18 and 21°C.

Sporulation was very suitable at 21 - 27°C, moderate at 8-13 and 28°C, and scarce at 6 and over 28°C, this confirming other authors'data (5), (7).

Influence of photophase. The best growth of *E.purpurascens* isolate occurred at 12-34 hrs of continuous light (Table 6); a moderate growth was noted at 8 hrs

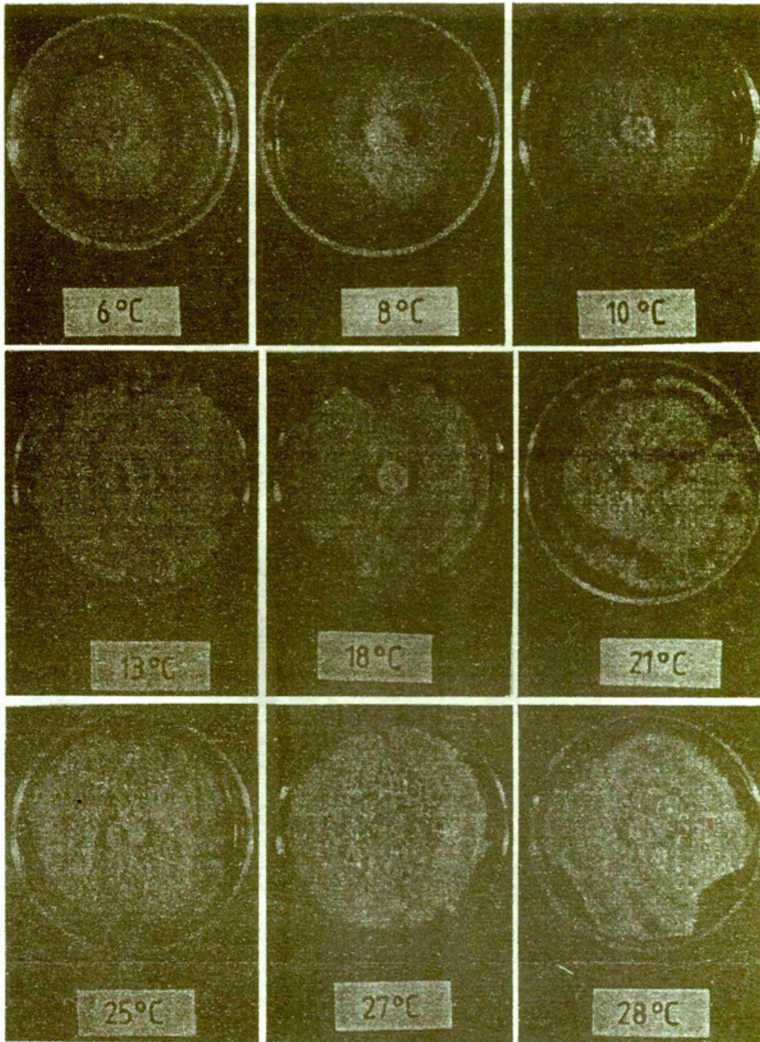

Fig. 5. - *Epicoccum purpurascens* development on P D A medium under different temperature conditions.

Table 6

Influence of photophase on *Epicoccum purpurascens* development

Photophase (hours)	Colony diameter (cm) after:		Pigmentation after 7 days	Sporulation
	2 days	7 days		
0 (check)	0.88	5.44	Purple	+++
8	0.94	6.32 ^{xxx}	Red	++
12	1.34 ^{xx}	7.00 ^{xxx}	Red	+++
16	1.32 ^{xx}	6.76 ^{xxx}	Red	+++
24	0.80	6.56 ^{xxx}	Intense-red, diffused in the medium	+++
CL 5%	0.276	0.468		
CL 1%	0.380	0.645		
CL 0.1%	0.523	0.886		

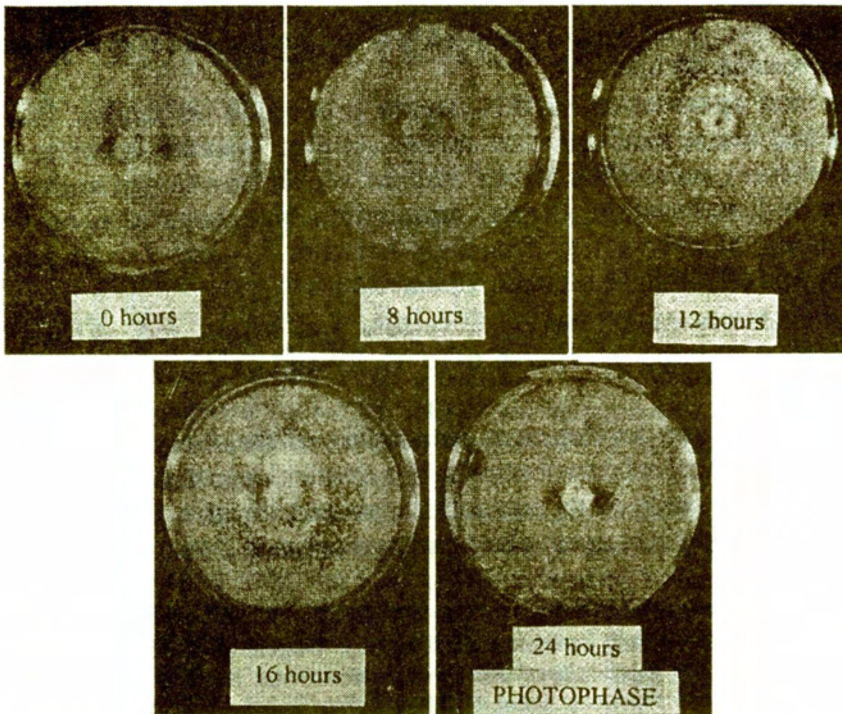

Fig. 6. - *Epicoccum development* development on P D A medium under different photophase conditions.

photophase, and the poorest under continuous dark. Under all light conditions tested, colony pigmentation was red up to bright-red. Sporulation intensified with reduced photophase, our results confirming those of other authors (9), while they contradict others (6), (10). Likewise, we are in agreement with some authors stating that sporulation occurs only if the fungus is exposed to steady illumination with low intensity, the apical area being theoretically the photoinductive part of hypha (8).

Influence of air relative humidity. At the experimental RH values ranging between 60 and 95%, development of *E.purpurascens* isolate was progressively more abundant, as humidity increased, the colony diameter gradually varying between 3.0 and 4.0 cm, for the two extreme RH values.

CONCLUSIONS

Development of the fungus *E.purpurascens* isolated from wheat seeds, a potential agent for biological control of fungus plant pathogens, was enhanced by the following factors: a) culture media with agar-oat and bean extracts, and PGA and Czapek-Dox; b) carbon sources: starch, maltose, levulose (fructose), sucrose (saccharose), glucose (dextrose); c) nitrogen sources: DL-asparagine, potassium and ammonium nitrate, DL-norvaline; d) initial pH values of the

medium between 5.0 and 9.0 ; e) temperatures from 21 to 28°C, optimum being 27°C; f) photophase between 12 and 24 hrs, optimum 24 hrs; g) high air relative humidity between 70 and 95%.

The best sporulation of this isolate proceeded on media: PGA, wheat flour-agar and maize meal-agar; on carbon sources: mannite, levulose (fructose), glucose (dextrose), sucrose (dextrose), maltose, starch; on nitrogen sources: peptone, potassium and ammonium nitrate, ammonium bicarbonate, L-norvaline and DL-asparagine; at pH values of medium between 5.0 and 7.0; at temperatures between 21 and 27°C; light between 12 and 24 hrs.

Pigmentation varied between yellow and bright red, diffusing in the culture medium at temperatures of 6-10°C, 27 and 28°C, and red colour at 13, 18 and 21°C.

Setting up these development parameters of the fungus *E. purpurascens* constitutes the first original contribution in this country, approaching this micromycete fungus through the angle of its antagonistic properties, as a potential prospective biological agent to prevent some plant pathogenic fungi.

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ÉTUDE PHYTOCOENOLOGIQUE ET DE LA BIOMASSE DE L'ASSOCIATION *ARO ORIENTALIS – CARPINETUM* Täuber 92 DU PLATEAU CENTRAL MOLDAVE

T.CHIFU, N. ŞTEFAN et M.COROI

Studying the phytocoenoses of *Aro orientalis-Carpinetum* Täuber 92 association in the Moldavian Central Plateau, we deal with the coenotaxonomic framing of the association. With that end in view references have been made on the floristic structure and composition, which comprise numerous species characteristic for suball. *Aro orientalis-Carpinenion* Täuber 92, all.*Lathyro hallersteinii-Carpinion* (Soó 64) Boşcaiu 79, ord.*Fagetalia sylvaticae* Pawl., Sok.et Wall.28 and cls. *Carpino-Fagetea* (Br.-Bl. et Vlieg.37) Jakucs 67.

Our researches have dealt with biometric and biomass problems, too. In the arborescent layer measures of height, diameter medium density at various wooden species have been made. At the same time, the total quantity of aerial bomass, herbaceous and wooden biomass is determined for various species which make up the phytocoenose. The wooden biomass is analysed on various organs (trunks, branches, leaves). In the annexed tables there are data which synthesize the result of our researches from three habitats: Gheorghiţoaia, Strunga and Mădârjac.

Depuis 1989, on a commencé une étude écologique sur les forêts du Plateau Central Moldave (1,2,3,4,7) et à la suite des investigations effectuées dans cette période, on présente dans cet ouvrage les résultats concernant les phytocoenoses appartenant à l'association *Aro orientalis-Carpinetum.*

CONDITIONS STATIONNELLES

Les recherches ont été effectuées du côté Nord et surtout Nord-Ouest du Plateau Central Moldave, dans les localités Popeşti (altitude 160-180 m; exposition E, NE, inclinaison 5-15°), Strunga (altitude 220-240m, exposition E-NE, inclinaison 5-10°), Gheorghiţoaia-Mădârjac (altitude 220-240 m, exposition SV, NE, inclinaison 10-20°) et Mădârjac (altitude 230-260 m, exposition E-SE, inclinaison 2-5°), qui sont situées sur le territoire de l'arrondisement sylvicole Podu Iloaei.

Le relief est représenté par un ensemble de collines à pantes et orientations diverses. Les versants sont très affectés par des glissements de terrain qui créent un microrelief caractéristique. Les phytocoenoses occupent surtout la base des versants.

Le climat est tempéré-continental à nuance excessive. La température moyenne annuelle est de 8°4C à Popeşti, 8°3-8°6C à Strunga, 8°5-8°8C à

Gheorghiţoaia et Mădârjac et les précipitations moyennes annuelles varient entre 520-550 mm à Strunga et 550-570 mm à Popeşti, Gheorghiţoaia et Mădârjac.

Le sol est varié, représenté par les types : brun luvique pseudo-gleyifié (Popeşti, Gheorghiţoaia et Mădârjac), brun argillique pseudo-gleyifié (Strunga, Gheorghiţoaia et Mădârjac), brun eumésobasique et tchernozem cambique typique (Strunga)*.

COMPOSITION ET STRUCTURE PHYTOCOENOLOGIQUE

Les phytocoenoses étudiées ont été comprises dans l'association *Aro orientalis-Carpinetum* Täuber 92, qui, récemment, à cause des exigences du Code International de phytocoenologie, a synonymisé l'association *Querco robori-Tilio-Carpinetum* Dobrescu et Kovacs 73 (9). Le même auteur encadre l'association dans une nouvelle sous-alliance – *Aro orientalis - Carpinenion* Täuber 92, dont, dans les phytocoenoses

Tableau 1

Aro orientalis-Carpinetum (Dobrescu et Kovacs 73) Täuber 92

Numero du relevé	1	2	3	4	5	6	7	8**	9	10	K
Recouvrement de la strate arborescente %	90	80	80	90	85	75	90	85	75	75	
Recouvrement de la strate juvenile + arbustive %	5	10	3	5	15	50	30	15	7	7	
Recouvrement de la strate herbacée %	25	20	45	55	40	45	55	45	40	30	
Caract. ass.											
Carpinus betulus	3	3	1	2	1	1	3	1	1	1	V
Tilia tomentosa	1	1	+	1	+	1	+	2	1	2	V
Arum orientale	+	+	+	-	+	+	+	+	-	-	IV
Aro orientalis-Carpinenion											
Scutellaria altissima	-	+	+	-	+	+	+	+	-	-	III
Carpesium cernuum	-	-	+	+	-	-	-	-	-	-	I
Lathyro hallersteinii-Carpinion											
Galium schultesii	+	-	+	+	+	+	+	+	+	+	V
Stellaria holostea	1	1	2	1	+	2	2	2	2	+	V
Carex pilosa	+	1	1	+	+	+	+	+	-	-	IV
Campanula trachelium	-	-	+	+	+	+	+	+	+	-	IV
Ranunculus auricomus	-	-	+	+	+	+	-	+	+	+	IV
Tilia cordata	-	+	+	-	-	+	+	+	+	-	III
Cerasus avium	-	-	+	-	-	+	+	+	+	+	III
Dactylis polygama	-	-	-	+	-	+	+	+	+	+	III

* = Les données sur le climat et le sol ont été fournies par G. Davidescu et V. Cazacu, de l'Institut de Recherches Biologiques de Iaşi.
** = relevé néotypique

Tableau 1 (suite)

Numero du relevé	1	2	3	4	5	6	7	8	9	10	K
Lathyrus venetus	+	-	-	-	-	+	+	-	-	-	II
Fagetalia silvaticae											
Ulmus minor	-	+	+	+	+	+	+	+	+	+	V
Acer platanoides	+	+	+	+	+	+	+	-	+	+	V
Stachys sylvatica	-	+	+	+	1	+	+	+	+	+	V
Euphorbia amygdaloides	+	+	+	+	+	+	+	+	+	+	V
Geranium robertianum	+	+	+	+	+	+	+	+	+	+	V
Asarum europaeum	+	+	+	+	1	+	+	1	+	+	V
Allium ursinum	+	-	1	1	+	1	1	+	+	+	V
Galium odoratum	1	+	1	1	1	+	1	+	+	1	V
Milium effusum	+	-	+	+	+	-	+	+	+	+	IV
Galeobdolon luteum	+	-	+	+	+	+	-	+	+	+	IV
Hordelymus europaeus	-	+	+	+	+	+	+	+	-	-	IV
Dentaria bulbifera	+	-	+	1	+	-	+	+	1	1	IV
Sanicula europaea	+	-	+	+	+	+	+	+	-	-	IV
Pulmonaria officinalis	-	-	+	-	+	+	+	+	+	+	IV
Lathyrus vernus	-	-	+	+	+	+	+	+	-	-	III
Carex sylvatica	-	-	1	+	+	+	-	+	+	-	III
Carex digitata	-	-	-	+	+	-	-	+	+	+	III
Fagus sylvatica	-	-	+	+	-	+	-	-	+	-	II
Mercurialis perennis	2	-	-	-	-	-	-	+	+	-	II
Lamium maculatum	-	+	-	-	-	+	-	+	-	-	II
Dryopteris filix-mas	+	-	+	+	+	-	-	-	-	-	II
Cephalanthera longifolia	-	+	+	+	+	-	-	-	-	-	II
Dentaria glandulosa	-	-	+	+	+	-	+	-	-	-	II
Cardamine impatiens	-	-	+	+	+	-	-	-	-	-	II
Salvia glutinosa	-	-	-	+	-	-	-	+	+	-	II
Paris quadrifolia	+	-	-	+	-	-	-	-	-	-	I
Maianthemum bifolium	+	-	-	+	-	-	-	-	-	-	I
Actaea spicata	+	-	-	-	+	-	-	-	-	-	I
Atropa bella-donna	-	-	-	+	-	-	-	-	-	-	I
Galeopsis speciosa	-	-	-	+	+	-	-	-	-	-	I
Epilobium montanum	-	-	-	+	+	-	-	-	-	-	I
Chaerophyllum aromaticum	-	-	-	-	-	+	+	-	-	-	I
Isopyrum thalictroides	+	-	-	-	-	-	-	-	-	1	I
Arctium nemorosum	+	-	-	-	-	-	-	-	-	-	I
Carpino-Fagetea											
Quercus robur	3	2	3	3	4	2	2	2	2	3	V
Quercus dalechampii	+	1	+	+	+	+	1	1	2	+	V
Fraxinus excelsior	+	1	1	1	1	1	+	+	1	+	V

Tableau 1 (suite)

Numero du relevé	1	2	3	4	5	6	7	8	9	10	K
Acer campestre	+	-	1	+	+	1	1	+	+	+	V
Crataegus monogyna	+	+	+	-	+	1	+	+	+	+	V
Viola reichenbachiana	+	-	+	+	+	+	+	+	+	+	V
Glechoma hirsuta	-	+	1	1	+	1	2	1	1	+	V
Mycelis muralis	+	+	+	+	+	-	+	+	+	+	V
Scrophularia nodosa	+	-	+	+	+	+	+	+	+	+	V
Melica uniflora	+	+	+	+	+	+	+	1	+	+	V
Quercus petraea	-	+	+	+	-	1	+	+		-	IV
Sorbus torminalis	-	+	-	-	+	+	+	+	+	+	IV
Euonymus europaeus	-	+	-	+	-	+	+	+	+	+	IV
Geum urbanum	+	+	+	+	+	+	+	+	-	-	IV
Brachypodium sylvaticum	+	-	+	+	1	+	+	+	-	-	IV
Lapsana communis	+	-	+	+	+	+	+	+	-	-	IV
Convallaria majalis	+	+	+	-	+	+	+	+	-	-	IV
Hepatica nobilis	+	-	+	+	+	+	+	+	-	-	IV
Hedera helix	+	-	-	+	+	+	+	1	-	-	III
Poa nemoralis	-	-	+	+	+	+	+	-	-	-	III
Torilis japonica	-	-	+	+	+	-	+	+	-	-	III
Ficaria verna	-	-	-	-	-	+	+	+	+	1	III
Clematis vitalba	-	-	-	-	+	+	1	-	-	-	II
Corylus avellana	-	-	-	-	+	+	+	-	-	-	II
Polygonatum latifolium	+	-	-	-	-	-	-	-	+	+	II
Viola mirabilis	-	+	+	-	+	-	-	-	-	-	II
Vicia sepium	-	-	+	-	+	+	-	+	-	-	II
Moehringia trinervia	-	-	+	+	+	-	-	-	-	-	II
Fragaria vesca	-	-	-	+	+	+	-	-	-	-	II
Anemone raunculoides	-	-	-	-	-	+	-	-	+	+	II
Rubus hirtus	-	-	-	-	+	-	+	-	-	-	I
Cornus mas	-	+	-	-	-	1	-	-	-	-	I
Lonicera xylosteum	+	-	-	-	-	+	-	-	-	-	I
Ligustrum vulgare	-	-	-	+	-	-	-	+	-	-	I
Viola odorata	+	-	-	-	-	-	-	-	-	-	I
Platanthera bifolia	+	-	-	-	-	-	-	-	-	-	I
Epipactis helleborine	+	-	-	-	-	-	-	-	-	-	I
Athyrium filix-femina	-	-	-	+	-	-	-	-	-	-	I
Cruciata glabra	-	-	-	-	-	+	-	-	-	-	I
Diverses espèces											
Circaea lutetiana	+	+	+	+	+	+	+	+	+	+	V
Euonymus verrucosus	-	-	+	+	+	1	+	+	+	-	IV
Sambucus nigra	-	+	+	-	+	+	+	+	+	+	IV

Tableau 1 (suite)

Numéro du relevé	1	2	3	4	5	6	7	8	9	10	K
Galium aparine	-	-	+	+	+	+	+	+	+	+	IV
Alliaria petiolata	+	+	+	+	+	+	+	+	-	-	IV
Anthriscus cerefolium ssp. trichosperma	+	+	+	+	+	+	+	+	-	-	IV
Polygonatum odoratum	-	-	+	+	+	+	+	+	+	+	IV
Ajuga reptans	+	-	-	+	+	+	+	+	-	-	III
Viburnum lantana	-	-	+	-	+	+	+	-	-	+	III
Rosa canina	-	-	+	+	-	+	+	+	-	+	III
Fallopia dumetorum	-	+	+	-	+	+	+	+	-	-	III
Urtica dioica	+	+	+	+	+	+	-	-	-	-	III
Acer pseudoplatanus	-	-	-	-	-	-	+	+	+	+	II
Veronica hederifolia	-	-	-	-	-	+	+	+	-	-	II
Swida sanguinea	-	-	-	-	+	+	+	+	-	-	II
Lathyrus niger	+	-	-	-	+	+	+	-	-	-	II
Galeopsis tetrahit	-	-	+	-	-	+	-	+	-	-	II
Viola hirta	-	-	+	-	+	+	+	-	-	-	II
Chelidonium majus	-	-	-	+	+	+	-	+	-	-	II
Veronica chamaedrys	-	-	-	-	-	+	-	+	+	+	II
Viburnum opulus	-	-	+	-	-	-	+	-	-	-	I
Fraxinus angustifolia	-	-	-	-	+	-	-	-	-	-	I
Rhamnus catharticus	-	-	-	-	-	+	-	-	-	-	I
Chaerophyllum temulum	+	-	-	-	-	-	-	-	-	-	I
Lunaria annua	-	+	-	-	-	-	-	-	-	-	I
Carex pendula	-	+	-	-	+	-	-	-	-	-	I
Solanum dulcamara	-	-	-	+	-	-	-	-	-	-	I
Impatiens noli-tangere	-	-	-	+	-	-	-	-	-	-	I
Stellaria nemorum	-	-	-	-	-	-	+	+	-	-	I
Festuca gigantea	-	-	-	+	+	-	-	-	-	-	I
Astragalus glycyphyllos	-	-	+	-	-	-	-	+	-	-	I
Hypericum maculatum	-	-	+	-	+	-	-	-	-	-	I
Ranunculus polyanthemos	-	-	-	+	-	-	-	-	-	-	I
Eupatorium cannabinum	-	-	-	+	+	-	-	-	-	-	I
Veronica officinalis	-	-	-	+	-	-	-	-	-	-	I
Asparagus officinalis	-	-	-	-	-	-	-	-	-	-	I

Localité des relevés: 1-2 = Popeşti - Cenuşa
3-4-5 = Gheorghiţoaia
6-7-8 = Strunga
9-10 = Mădârjac

du Plateau Central Moldave font partie les espèces suivantes: *Carpinus betulus*, *Tilia tomentosa*, *Arum orientale*, *Scutellaria altissima* et *Carpesium cernuum*.

La strate arborescente est constituée surtout par les espèces: *Quercus robur, Carpinus betulus, Tilia tomentosa,* auxquelles s'ajoutent = *Fraxinus excelsior, Acer campestre, Acer platanoides,* etc.La densité de la strate arborescente varie surtout en fonction de l'âge du peuplement forestier: 750-900 arb./ha dans les forêts agées

Tableau 2

La densité totale et par stades de développement (arb./ha)

Espèces	Stades de développ. (diamètre moyen cm)	Popeşti 60-80 ans	Mădârjac 80-100 ans	Strunga 100-120 ans	Gheorghiţoaia 120-140 ans
Quercus robur	6-10	-	-	-	-
	11-20	9	10	3	1
	21-35	158	130	3	111
	>35	53	24	78	81
Total		220	164	84	193
Quercus petraea + Q.dalechampii	6-10	-	-	-	-
	11-20	13	22	-	-
	21-35	226	112	-	6
	>35	43	12	20	10
Total		282	146	20	16
Carpinus betulus	6-10	20	24	141	30
	11-20	16	238	218	90
	21-35	-	16	10	53
	>35	-	-	-	3
Total		36	278	369	176
Tilia tomentosa	6-10	56	-	15	-
	11-20	106	64	54	9
	21-35	105	52	46	21
	>35	-	-	-	14
Total		267	116	115	44
Fraxinus excelsior	6-10	4	-	7	-
	11-20	24	2	22	3
	21-35	30	28	5	21
	>35	-	-	-	26
Total		58	30	34	50
Acer campestre + A.platanoides	6-10	58	2	47	1
	11-20	18	6	97	14
	21-35	3	-	5	7
	>35	-	-	3	1
Total		79	8	152	23
Sorbus torminalis	6-10	-	2	-	-
	11-20	-	2	-	-
	21-35	-	-	2	-
	>35	-	-	-	-

Tableau 2 (suite)

Total		-	4	2	-
Ulmus minor	6-10	20	2	1	-
	11-20	24	2	5	2
	21-35	3	-	-	1
	>35	-	-	-	-
Total		47	4	6	3
Cerasus avium	6-10	-	4	4	-
	11-20	-	6	23	2
	21-35	-	-	5	1
	>35	-	-	-	-
Total		-	10	32	3
Total general		989	760	814	502

de 75-100 ans (Popeşti et Mădârjac) et 500-800 arb./ha dans les forêts plus de 100 ans (Strunga et Gheorghiţoaia) (Tableau 2). La grande densité de la strate arborescente des phytocoenoses de Strunga se réalise surtout par le peuplement forestier jeune, qui représente plus de 70% de la totalité des arbres. Dans toutes les autres phytocoenoses prédominent les arbres adultes (qui ont plus de 20 cm en diamètre), ce qui imprime à la forêt une structure relativement équienne, presque unistratifié (Fig.1).

La strate arbustive est à peine développée, étant formée des espèces suivantes: *Crataegus monogyna, Euonymus europaeus, Corylus avellana, Cornus mas, Ligustrum vulgare* etc.

Dans la strate herbacée, qui est diversifiée, dominent *Dentaria bulbifera, Dentaria glandulosa, Galium odoratum, Ficaria verna, Anemone ranunculoides, Aegopodium podagraria* etc.

BIOMASSE AÉRIENNE DES STRATES ARBORESCENTE ET HERBACÉE

Pour déterminer la biomasse des strates arborescente et herbacée, nous avons pris en considération une série d'ouvrages se référant spécialement à ce type de forêt (5,6,8). Pour la strate arborescente, on a agi comme suit:

- on a déterminé d'abord la densité totale pour chaque phytopopulation et par état de développement, en même temps avec le diamètre et la hauteur (8);

- de chaque phytopopulation on a coupé (au mois d'octobre) deux arbres de dimensions moyennes: l'un d'âge jeune (6-20 cm en diamètre) et l'autre d'âge adulte (plus de 20 cm en diamètre);

- on a pesé séparément les troncs, les rameaux et les feuilles (5,6) dont on a prélevé des échantillons pesant 0,250 kg, qui ont été séchés à une température de 105°C;

- ensuite, on a déterminé la biomasse séchée partielle par ha (par phytopopulation et par stade de développement) et totale (ou chaque phytocoenose).

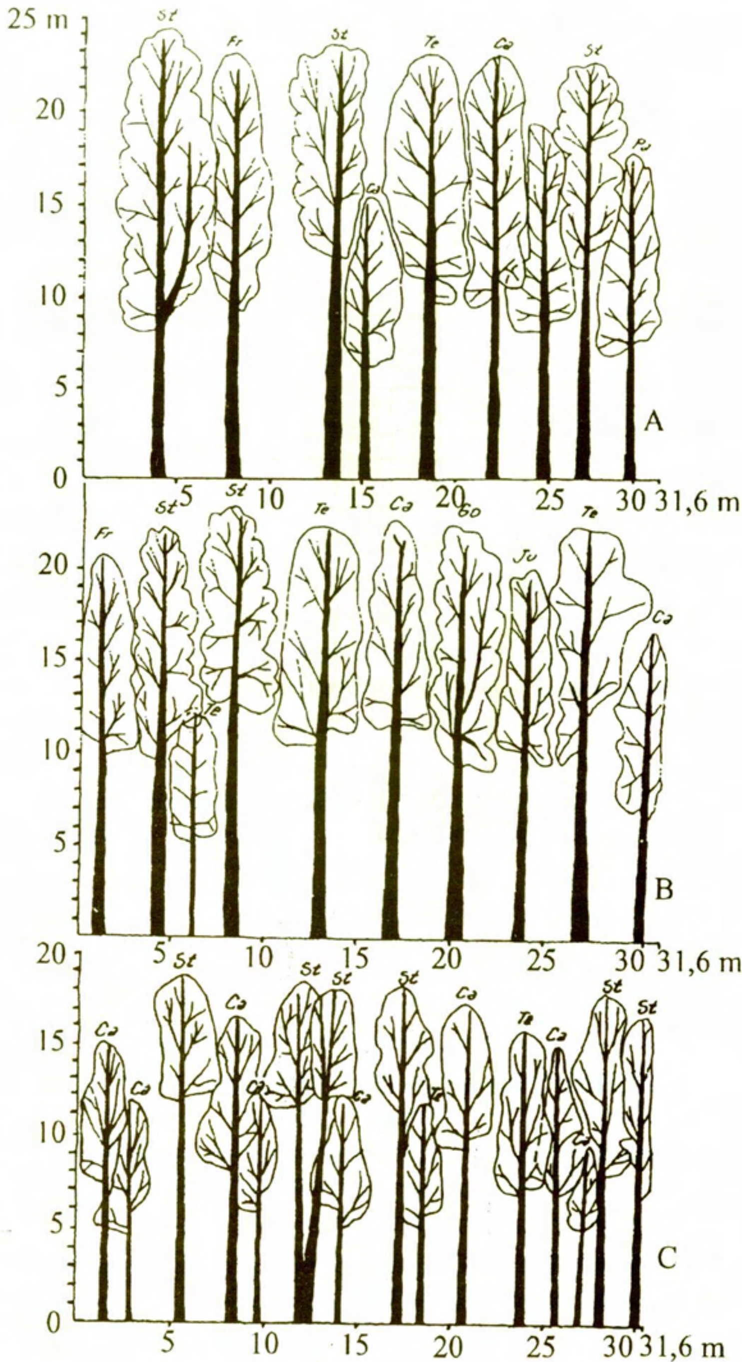

Fig. 1. - Structure verticale du peuplement forestier:
A = Gheorghițoaia; B = Strunga; C = Mădârjac.

Pour la biomasse de la strate herbacée on a procédé ainsi:

- on a choisi les trajets parallèles aux courbes de niveau, sur lesquels on a récolté les herbes sur des placettes ayant une surface de 1 m^2, de 10 en 10 m, à l'aspect vernal et estival; on en a récolté 15 à 20 surfaces d'un m^2;

- on a séparé les herbes par phytopopulations principales et on les a pesées séparément; on a prélevé des échantillons (0,250 kg) en vue du séchage à 105°C;

- puis, on a calculé la biomasse séchée pour chaque phytopopulation, aspect et phytocoenose.

Les déterminations de la biomasse aérienne de la strate arborescente de deux phytocoenoses d'âges différents relèvent le fait que les valeurs obtenues varient selon l'âge, de 274 t/ha dans les forêts plus jeunes à 390 t/ha dans les forêts adultes

Tableau 3

La biomasse aérienne de la strate arborescente (t/ha)

Espèces	diam. moyen (cm)	hauteur moyenne (m.)	densité moyenne (arb/ha)	Biomasse					
				totale	troncs	rameaux			feuilles
						total	> 7 cm	< 7 cm	
Gheorghițoaia									
Quercus robur + Q.petraea	52.1	25.3	68	170.75	95.70	71.26	30.09	41.16	3.80
Carpinus betulus	32.0	22.2	102	70.78	42.90	26.75	21.65	5.10	1.13
	17.5	14.9	88	21.52	14.50	6.45	4.35	2.10	0.57
Total	-	-	190	92.30	57.40	33.20	26.00	7.20	1.70
Tilia tomentosa	37.4	22.3	78	59.00	40.65	17.75	14.00	3.75	0.60
	19.3	17.1	10	0.66	0.50	0.15	0.12	0.03	0.01
Total	-	-	88	59.66	41.15	17.90	14.12	3.78	0.61
Fraxinus excelsior	45.9	24.5	68	62.85	50.65	10.40	9.55	0.85	1.80
Acer platanoides + A. campestre	40.0	21.8	2	1.72	1.25	0.45	0.35	0.10	0.02
	8.0	9.2	2	0.08	0.05	0.02	-	0.02	0.01
Total	-	-	4	1.80	1.30	0.47	0.35	0.12	0.03
Ulmus minor	32.3	21.7	4	2.96	2.30	0.60	0.50	0.10	0.06
	16.0	13.1	2	0.09	0.06	0.02	0.01	0.01	0.01
Total	-	-	6	3.05	2.36	0.62	0.51	0.11	0.07
Total général	-	-	424	390.41	248.56	133.84	80.62	53.22	8.01
Mădârjac									
Quercus robur + Q. dalechampii	33.1	20.8	268	183.20	134.65	46.45	41.35	5.10	2.10
	20.0	19.1	12	2.65	2.08	0.50	0.40	0.10	0.07
Total	-	-	280	185.85	136.73	46.95	41.75	5.20	2.17
Carpinus betulus	25.4	19.4	24	10.50	8.40	1.95	1.70	0.25	0.15
	16.5	16.1	336	58.80	44.95	12.95	9.45	3.50	0.90
Total	-	-	360	69.30	53.35	14.90	11.15	3.75	1.05
Tilia tomentosa	28.4	18.9	32	9.45	6.90	2.45	2.10	0.35	0.10
	17.2	16.6	44	3.59	2.90	0.65	0.50	0.15	0.04
Total	-	-	76	13.04	9.80	3.10	2.60	0.45	0.14
Fraxinus excelsior	26.1	20.5	8	4.99	3.80	1.10	0.90	0.20	0.09
Acer campestre	10.2	11.8	8	0.33	0.25	0.07	0.02	0.05	0.01
Umus minor	12.0	12.3	4	0.23	0.18	0.04	0.02	0.02	0.01
Total général	-	-	736	273.74	204.11	66.16	56.44	9.72	3.47

(Tableau 3). Une importante contribution à la constitution de la biomasse est apportée par les espèces édificatrices principales: *Quercus robur, Quercus petraea, Carpinus betulus, Tilia tomentosa,* auxquelles s'associent d'habitude *Fraxinus excelsior* et *Acer campestre.*

Parmi les organes végétatifs, les troncs réalisent la plus grande quantité de biomasse, mais on observe qu'aux populations végétales plus jeunes, le pourcentage de participation de la biomasse des troncs est plus élevé (70-75 %) qu'aux populations végétales adultes (60-65 %). De même, le pourcentage de la biomasse des rameaux, qui ont un diamètre moyen au-dessous de 7 cm, varie de 18-20 % dans les jeunes peuplements forestiers à 60-65 % dans les peuplements forestiers adultes. En ce qui concerne la participation de la biomasse des feuilles à la constitution de la biomasse aérienne de la strate arborescente, on observe que celle-ci ne représente que 1,2-2,0 % de la biomasse totale, des pourcentages plus grands étant réalisés par *Quercus robur, Fraxinus excelsior* et *Acer campestre*, surtout dans les peuplements forestiers adultes.

Tableau 4

Biomasse aérienne herbacée (t/ha)

Espèces	Subst. séchée moyenne %	Biomasse fraiche		Biomasse séchée	
		Mădârjac	Gheorghiţoaia	Mădârjac	Gheorghiţoaia
a. Biomasse vernale					
Stellaria holostea	14.3	0.281	0.038	0.040	0.005
Dentaria bulbifera	13.7	0.301	0.259	0.041	0.036
Viola sp.	18.1	0.113	0.084	0.020	0.015
Galium aparine	12.6	0.093	-	0.012	-
Asarum europaeum	15.6	0.073	0.054	0.011	0.008
Polygonatum odoratum + P. latifolium	16.5	-	0.049	-	0.008
Isopyrum thalictroides	17.8	-	0.153	-	0.027
Pulmonaria officinalis	21.1	-	0.013	-	0.003
Ficaria verna	11.7	-	0.113	-	0.013
Allium ursinum	7.6	-	0.148	-	0.011
Diverses especes	15.7	0.057	0.057	0.009	0.009
Total a.	-	0.918	0.966	0.113	0.135
b. Biomasse estivale					
Galium odoratum	13.6	0.076	0.188	0.010	0.026
Glechoma hederacea	17.1	0.140	0.075	0.024	0.013
Melica uniflora	28.4	0.054	0.090	0.015	0.003
Sanicula europaea	17.8	0.010	0.021	0.002	0.004
Galeobdolon luteum	22.8	0.011	-	0.003	-
Aegopodium podagraria	15.0	-	0.077	-	0.011
Mycelis muralis	12.5	-	0.016	-	0.002

Tableau 4 (suite)

Veronica chamaedrys	19.8	-	0.023	-	0.005
Carex pilosa + *C. sylvatica*	26.6	0.187	0.082	0.050	0.022
Carex digitata	27.4	0.023	0.089	0.006	0.024
Salvia glutinosa	17.6	0.055	-	0.010	-
Diverses espèces	20.3	0.015	0.062	0.003	0.013
Total b.		0.571	0.723	0.123	0.128
Total a. + b.		1.489	1.689	0.256	0.263

La biomasse aérienne de la strate herbacée a des valeurs relativement réduites dans toutes les phytocoenoses (0,25-0,26 t/ha) (Tableau 4). On remarque une participation plus active à la constitution de la biomasse, surtout des herbes vernales, parmi lesquelles nous citons *Dentaria bulbifera, Isopyrum thalictroides, Stellaria holostea, Ficaria verna,* etc. Parmi les espèces estivales, une contribution plus importante est réalisée par les espèces: *Carex pilosa, Carex sylvatica, Glechoma hederacea, Melica uniflora, Galium odoratum* etc.

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DEVELOPMENTAL ELECTRONMICROSCOPY OF EXOGENOUS PROGESTERONE INFLUENCES ON DIRECT SOMATIC EMBRYOGENESIS FROM LEAF AND COTYLEDON EXPLANT OF *Nicotiana tabacum* cv. *xanthi*

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The influence of progesterone on the cytological and histological events of direct somatic embryogenesis of leaf and cotyledon explant of *Nicotiana tabacum* L. cv. *xanthi* is presented in the paper. Histological analyses strongly indicated that the presence of progesterone in the culture medium caused the decrease of the ability of somatic cells to perform the embryogenetic program but without abolishing it. The extent of the decrease was directly correlated with the concentration of progesterone. The highest concentration used (25 mg/l) proved to have toxic effects. The ultrastructural analysis of the cells during somatic embryogenesis induction and the evolution of somatic embryos did not exhibit any qualitative modifications due to the presence of progesterone.

INTRODUCTION

The presence and the effects of steroid hormones in plants have been intensively studied during the last decades (4), (5), (8), (9), (10). Despite this, their physiological function is still little known. Previous *in vivo* and *in vitro* experiments on the plant *Nicotiana tabacum* cv. *xanthi* (1), (2) have demonstrated different effects of progesterone on the growing and cytodifferentiation processes.

It is well known that somatic embryogenesis represents an ideal experimental system for the study of growth and differentiation processes in plant ontogeny, as well as their inducing factors. Somatic embryogenesis is also used as a biotechnology for *in vitro* plant micropropagation of valuable genotypes. In most experimental reports the investigators have concentrated almost exclusively on the physiological and morphological aspects of embryo development (3,5,11,13,14,15). No studies have been devoted to the ultrastructural characterization of the effects of the treatment with exogenous progesterone.

This paper considers the effect of progesterone on direct somatic embryogenesis in *Nicotiana tabacum* cv. *xanthi* using morphological, histological and electronmicroscopal analyses, with special reference to the globular and torpedo stages during somatic embryo differentiation.

MATERIALS AND METHODS

INDUCTION OF SOMATIC EMBRYOGENESIS

Plants of *Nicotiana tabacum* cv. *xanthi* germinated aseptically from seeds on basal Murashige and Skoog culture medium (6) deprived of hormones, in the

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phase of 4-6 expanded leaves were used as explant sources. Segments of cotyledons and leaves, with an average weight of 1.5 mg were used as inoculum and placed on basal Murashige Skoog culture medium supplemented with NAA (0.1 mg/l) and BAP (1 mg/l). The cultures were incubated at 25±3°C for 36 days. The photoperiod was 16 hours light and 8 hours dark. Each variant included 30 samples and the entire experiment was repeated twice.

Progesterone treatment

Progesterone (P) powder was dissolved in absolute ethanol and introduced into the culture medium before autoclaving. The following experiments were done: control (C) in the absence of progesterone, P_I with 0.025 mg P/l, P_{II} with 0.25 mg P/l, P_{III} with 2.5 mg P/l and P_{IV} with 25 mg P/l.

Histological study

For the histological observations of the different stages of embryo differentiation samples were prepared and examined using the standard method which involved material fixation in Navashin fixator, paraffin inclusion, sectioning at 8-10 μm, and hematoxylin staining.

Electronmicroscopy

For ultrastructural studies foliar and cotyledonar explant grown for three and six days were examined under stereomicroscope and embryos in globular, torpedo and bycotyledonary stages were excised into a small drop of fixative solution (3% glutaraldehyde in 0.2M sodium phosphate buffer, pH 6.8). Globular stages embryos were fixed intact while the torpedo and bycotyledonary stages were divided into apical and basal region. The samples were prefixed at 4°C overnight and then transferred in fixative (2% OsO_4 solution) for two hours at room temperature followed by infiltration in Epon 812 resin. The ultrasections were done with a Tesla ultramicrotome and were contrasted according to the Reynolds method. The examination was made with a Tessla BS 600 and Philips electronmicroscope at an acceleration of 80 kV.

The effects of the progesterone on the growth and the initiation of morphogenetic processes were appreciated by successive determination at 3, 6, 9 and 36 days after inoculation of the wet and dry weight of the explant, as well as of the percentage of the explant that started morphogenesis.

RESULTS AND DISCUSSIONS

THE EFFECT OF EXOGENOUS PROGESTERONE ON DIRECT SOMATIC EMBRYOGENESIS

The introduction of progesterone in the specific culture medium for somatic embryogenesis induction did not generate significant effects regarding the pattern

of morphogenetic processes. The morphogenetic reactivity of the inoculum was different for the two kinds of explant, but only in the first phase of the incubation period (Figure 1). After six days the percentage of foliar explant that started

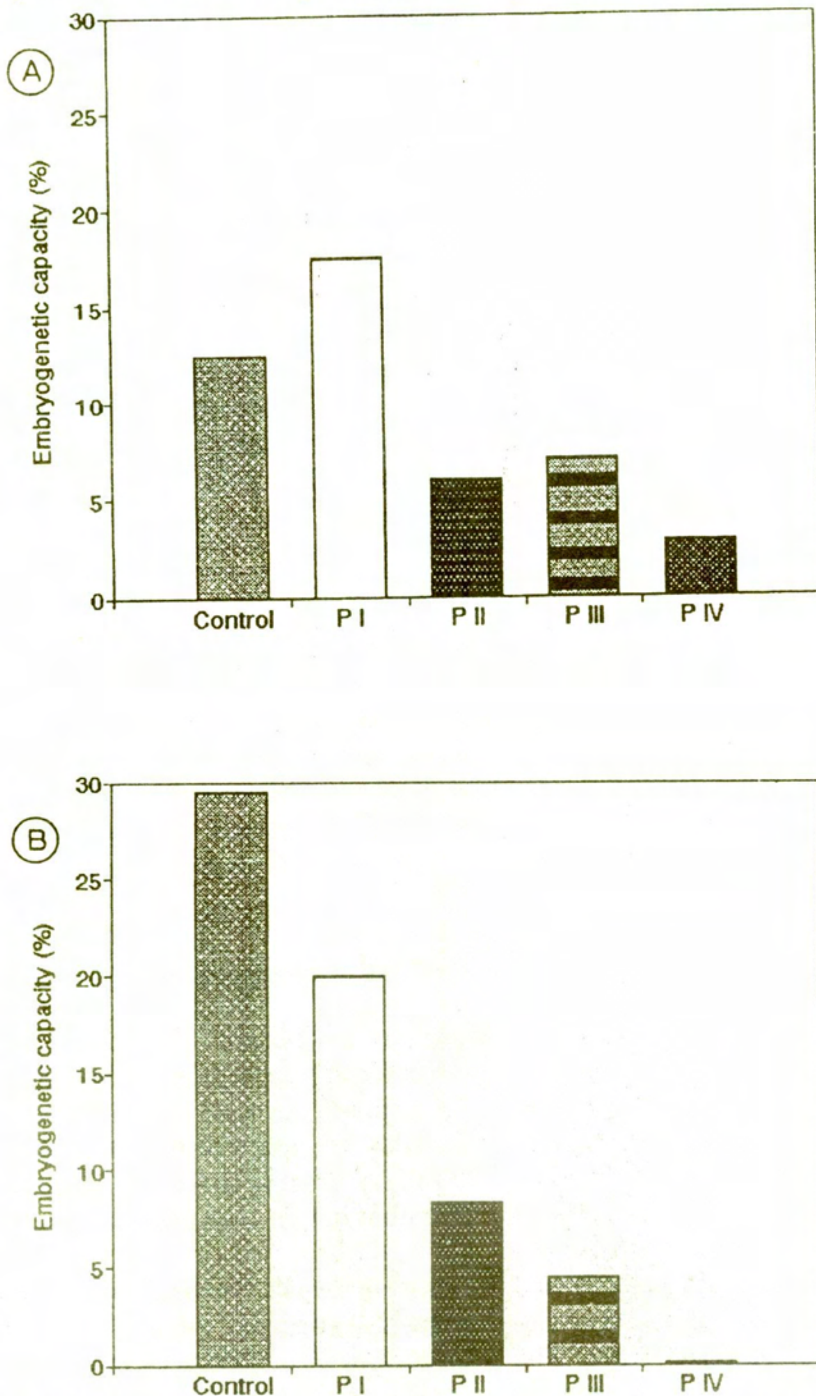

Fig. I.- Percentual evaluation of the embryogenetic capacity of the leaf (A) and cotyledon explants (B) under the influence of exogenous progesterone in different concentrations.

morphogenesis is slightly diminished in the presence of progesterone, with one exception, variant P_I (0.025mgP/l) that surpassed the control. In the variant P_{IV} (25 mgP/l) the answer is insignificant. The cotyledonary explant is more sensitive towards the treatment with progesterone. The presence of progesterone, experimentally identified in seeds (7), suggests that at the level of the cotyledon an overdose of it can result from an exogenous source faster than at the leaf level, causing inhibition or toxic effects. For the leaf explant the only concentration of progesterone that does not cause inhibition is 0.025 mgP/l.

After nine days from inoculation, the embryogenic reactivity of the leaf explant is between 92.5-98.3% for all variants and for the cotyledonal explant is 100%, with one exception, variant P_{IV} 96.6%. These results suggest that the excess of progesterone was quickly metabolized. After 36 days the percentage of embryogenic explant is 100% for all variants.

The measurement of wet and dry weight of the explant after 36 days indicates that if for wet weight the presence of progesterone causes a gradual decrease in weight, for dry weight all experimental variants exceeded the control (Figure II). This suggests a difference between the physiological state and the biosyntheses involved in growth and differentiation.

HISTOLOGICAL ANALYSES

Recent studies (11) indicate that the development of somatic embryo by direct morphogenesis in *Nicotiana tabacum* is achieved through typical embryogenesis stages, characteristic to zygotic development. Our histological analyses confirmed this fact suggesting that somatic embryos were formed on leaf or cotyledon surface explants as anatomically independent units, free of vascular connexions with the original explant (Figure III.1. and III. 2). The presence of progesterone does not modify the developmental patterns, causing only a high percentage of asynchronies in the development.

ELECTRONMICROSCOPE OBSERVATIONS

Comparative ultrastructural studies of the embryogenetic process also revealed that in our experiment the addition of progesterone in the culture medium did not affect the pattern of the developmental process. The characteristics of the tissues used as inoculum proved to play an essential role on the dynamics of the induction process. In all variants the successive cytological changes during embryogenesis are similar.This suggests that in this case progesterone had mainly a quantitative effect.

Several ultrastructural peculiarities were revealed in the somatic embryogenetic cells in the different stages of development, especially the globular, torpedo and incipient cotyledonary stage. The embryogenic protuberances in the inoculum are formed mainly by meristematic-like cells, originating from parenchymatic cells through dedifferentiation. The cells are rich in cytoplasm (Figure III.1) with a reduced vacuole, and a round or elongated nucleus. The cytoplasm region bordering

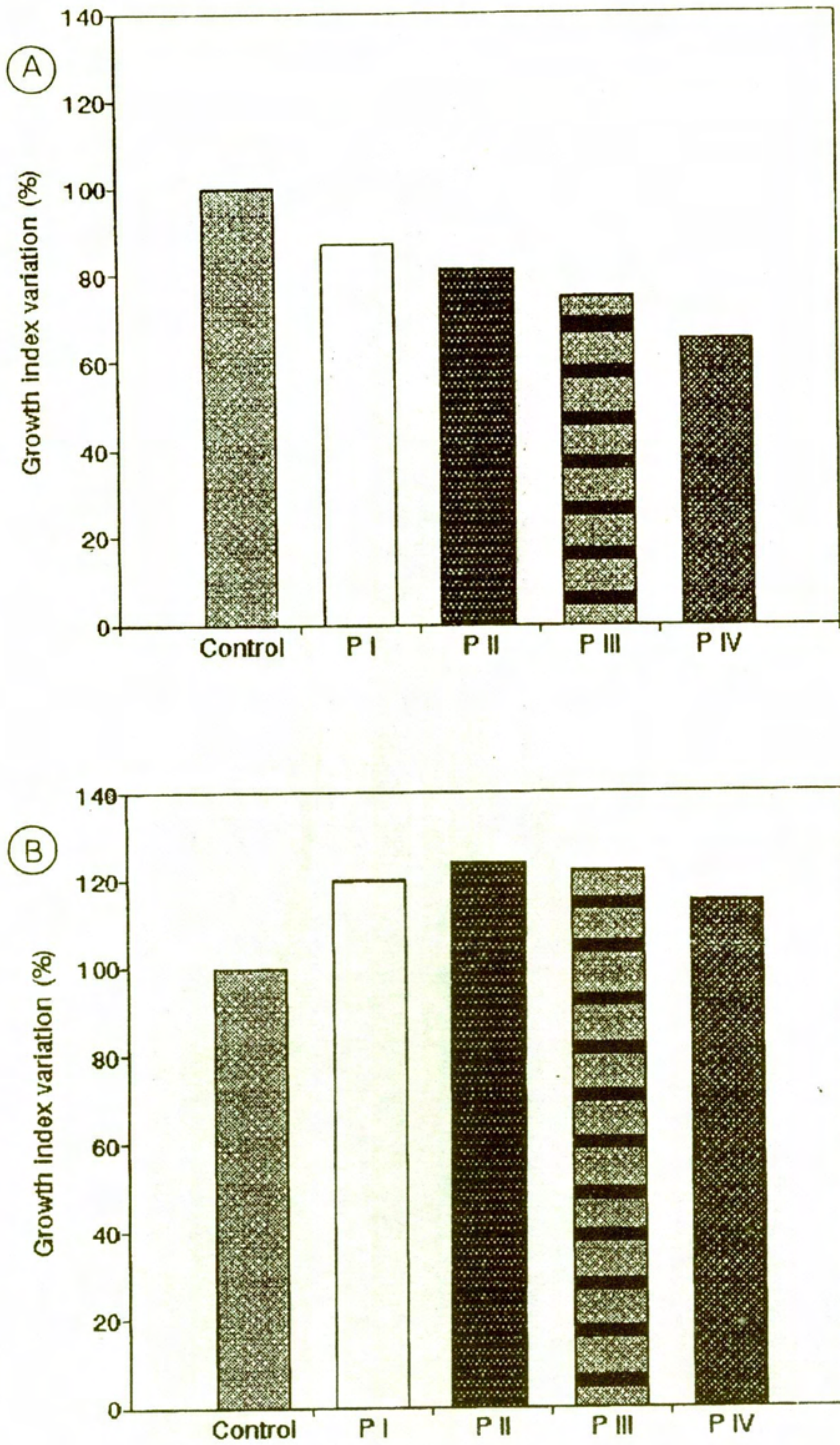

Fig. II.- Variation of the growth of the leaf (A) and cotyledon (B) explants under the influence of progesterone in different concentrations.

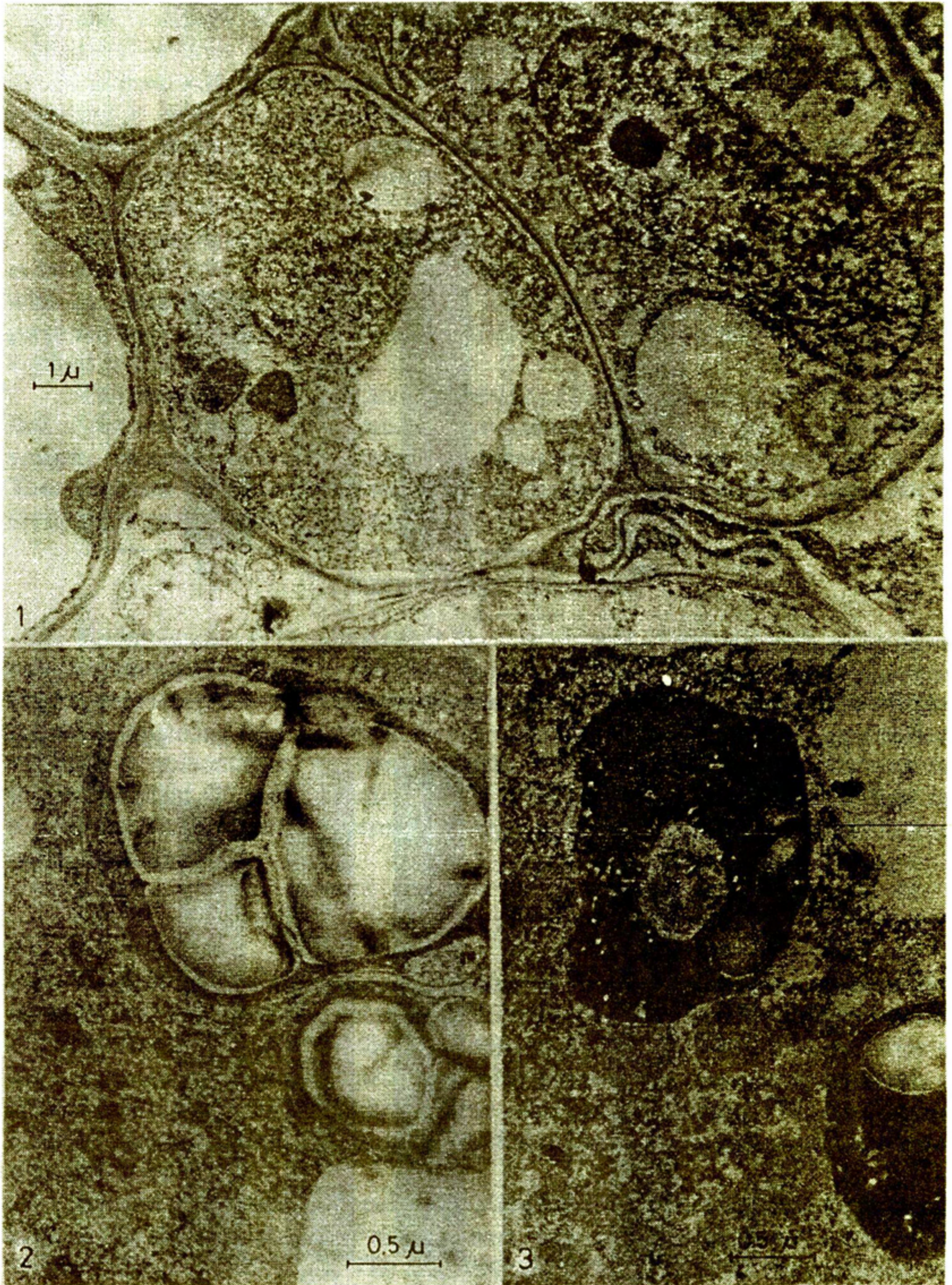

Fig. III.- Ultrastructural peculiarities of the proembryogenic cells differentiation in the cotyledonary tissue.

the vacuole has no organelles. The plastids are represented by two populations. One of them has an electronodense stroma, irregular shape, with small starch grains and poorly developed thylakoids. The other contains large, multiple starch inclusions and a small quantity of stroma (Figure III.2). The presence of numerous profiles of the endoplasmic reticulum, most of them of the rough type, as well as mitochondrias with normal structure indicate an intensive metabolic activity. Microbodies with a crystalline core (probably peroxisomes) are also frequent (Figure III.1, 2).

The globular stage is characterized by a more uniform cell population (Figure IV, 1, rich in cytoplasm with a large nucleus and reduced vacuole,

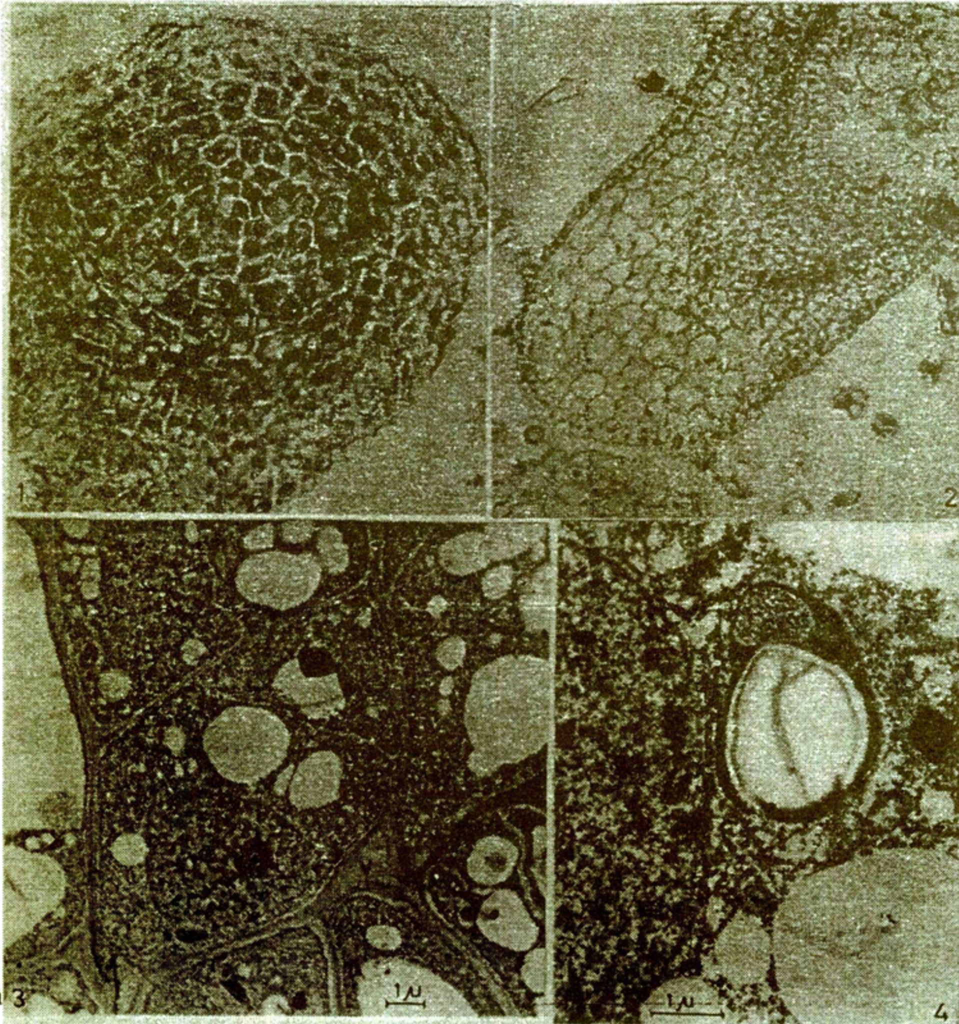

Fig. IV.- Histological aspect of the somatic embryo in globular stages (1) and torpedo/initial cotyledonary (2) on the leaf explants. (3)- electronomicroscopical peculiarities of cells from globular stages which present an active mitotic activity (arrow indicated new cell wall formation);(4)- a plastid with large starch granule and poor lamelae system in interaction of contiguity with endoplasmic reticulum and microbodies with crystalline core.

probably resulted during the initial embryogenic cell division (Figure 4c). A plastidial polymorphism is also present, plastids in an incipient stage of differentiation coexisting with amyloplasts with large starch inclusions, electronodense stroma and poorly developed thylakoids. Cell divisions were frequently encountered

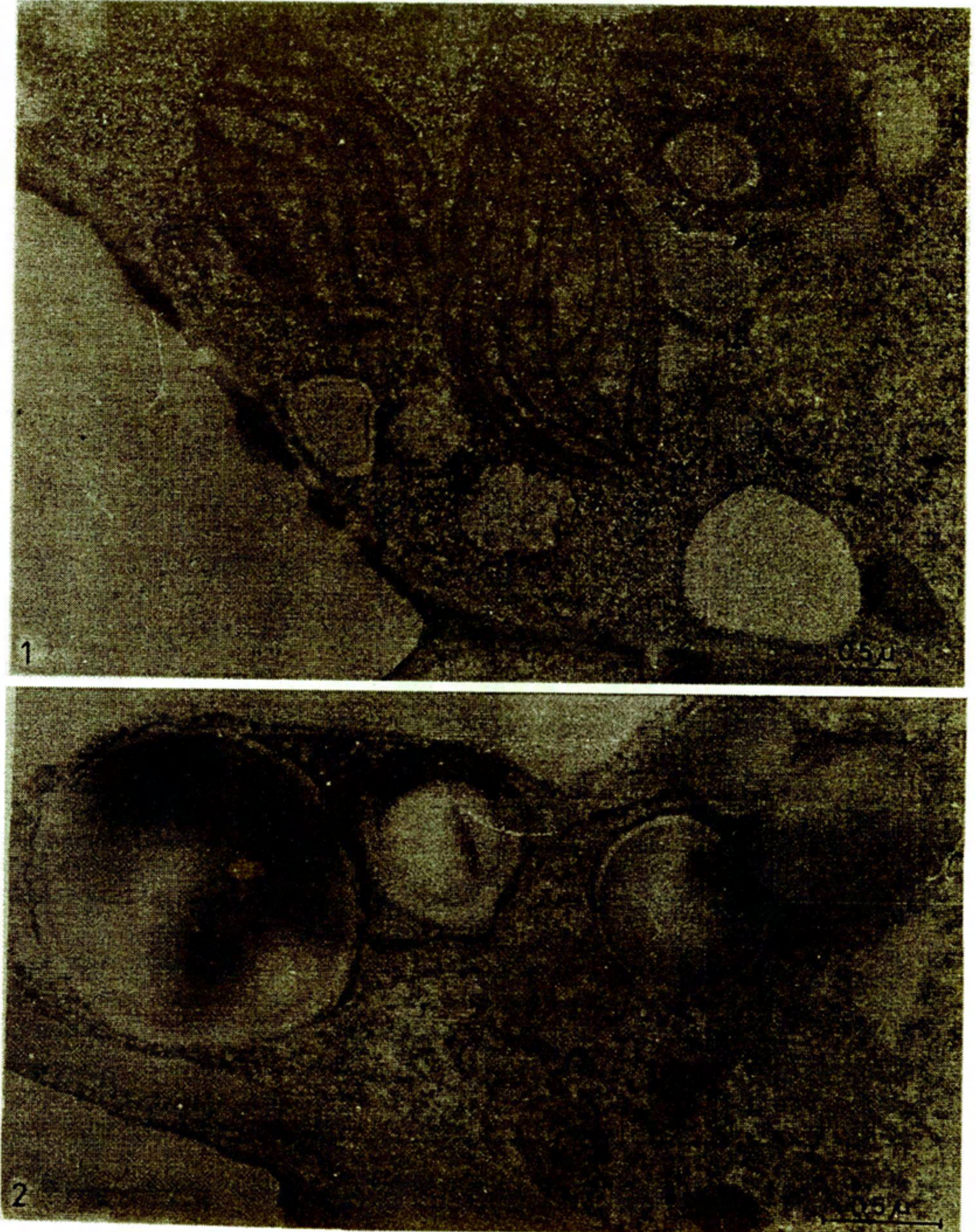

Fig. V.- Embryocells ultrastructure from the torpedo stages. The chloroplasts with typical structure as well as amyloplasts with large and numerous starch granules are present.

(Figure IV, 3 - arrow).

In the torpedo and mainly during the beginning of the dicotyledonary stage different cell type populations were observed, depending on the area of cutting. Close to cells of parenchymatous type that lack any differentiation (originating from the basal area) are cells of the mesophile (originated from apical area) that

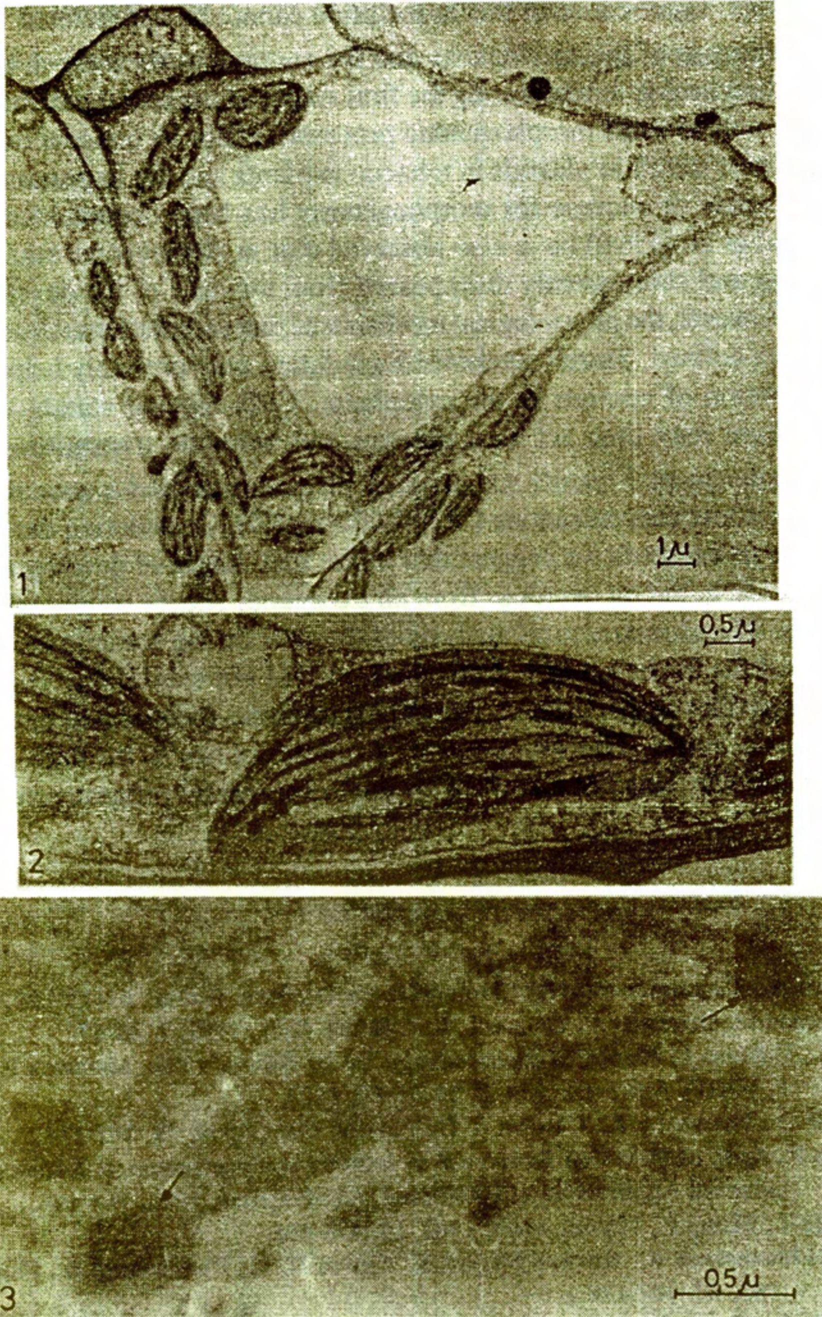

Fig. VI.- Ultrastructural peculiarities of the cells from cotyledonary area of the somatic embryo (on cotyledon explant). The mesophyll like structure of the cells is revealed.

contain chloroplasts with a characteristic structure (Figure V,1 VI,1 VI,2), contiguous, with mitochondria with electronotransparent stroma and extended cristae, peroxisomes with large crystalline inclusions (Figure V,1, 2, VI 3) and frequent profiles of endoplasmic reticulum. Sometimes myelinic figures are closely associated with the external membrane of the plastidial envelope (Figure V,1- arrow).The exact metabolic significance of this association cannot be appreciated.

The presence of the amyloplasts and the continuous increase in both number and size of the starch granules during the first stages of the somatic embryo dedifferentiation as well as in all morphogenetic processes is not an unusual event. Intense accumulation of starch in plastids in tobacco explants and the importance of carbohydrates, glucose or sucrose for morphogenesis has been documented (12), (13), (14). Mangal et al. (1990) have also observed that in *Begonia rex* starch was first accumulating and subsequently disappeared from the cells of the developing shoot primordia. However, the physiological mechanisms that stimulate starch accumulation have not been defined. Starch synthesis/degradation may probably help to stabilize the concentration of sucrose in the cytoplasm. Cells lacking starch, close to the culture medium may thus act as a nutrient and hormone transmitting layer (7).

Our experimental results suggest that progesterone had a quantitative effect mainly at very low concentrations (P_I= 0.025 mgP/l). Considering the presence of progesterone in the seeds we suggest that in the first stages of development it has a physiological role, but an overdose from an exogenous source causes inhibition and toxic effects. The ultrastructural analysis of the cells during somatic embryogenesis induction and the evolution of somatic embryos did not exhibit any changes of the main cell organelles due to the presence of progesterone, independent of the analyzed developmental stage. The evolution of somatic embryos through the normal morphogenetic way was not disturbed by the experimental progesterone treatment.

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ARSENIUM SOLUBILIZATION FROM AN AURIFEROUS PYRITE CONCENTRATE BY *THIOBACILLUS FERROOXIDANS* BACTERIA. TESTS ON FACTORS INFLUENCING THE PROCESS

V. OROS, P. ILIE

Laboratory experiments done in different working conditions have proved the technical possibility and the ability of a *Thiobacillus ferrooxidans* culture to achieve arsenium dissolution from a gold-bearing pyrite concentrate which cannot be processed by conventional technologies.
The factors influencing the process have been found to be temperature, agitation, solid/liquid ratio and initially addition of ferrous iron sulphate in solution. Previous adaptation of bacteria on the substrate has a positive influence of the process but only over the initial period of the experiments.

In some precious metal ores, arsenopyrite accompanies the metal sulphides, particularly the pyrite. It can appear both as mineral grains proper and as extremely fine inclusions in pyrite. By flotation processing of ores arsenium is accumulating in the concentrate of gold-bearing pyrite. Such concentrate has a refractory behaviour in metallurgical processing for extraction of gold. High arsenium contents cause technological troubles at the metallurgical processing and the emission of gas containing arsenium is very dangerous for the environment. For this reason arsenium removal from such concentrates becomes a necessity.

The ability of *Thiobacillus ferrooxidans* bacteria to oxidize the mineral sulphides is well known [8, 9, 11, 14]. Their capacity to oxidize arsenopyrite is also established [2, 8]. During the last period researches concerning the use of these bacteria in biotechnological processing of auriferous refractory concentrates have been developed [7, 9, 10, 11, 13, 14, 16, 17]. The first researches accomplished in Romania for arsenium removal from metal concentrates are presented here.

The purpose of researches was to prove the ability of our own bacterial culture to dissolute arsenium from gold-bearing pyrite concentrates. To establish the main factors influencing the process was also intended and a future aim was to establish a base for technological scale-up experiments.

MATERIALS AND METHODS

A concentrate sample of auriferous pyrite concentrate provided from the current flowsheet of flotation processing of the Suior ore was used in the experiments. Its contents were: S 46.08 %; Fe 39.60 %; As 1.50 %; Cu 0.68 %; Zn 0.27 %; Pb 0.38 %; SiO_2 5.40 %; Au 12g/t; Ag 48 g/t. The grain sizes were 60 % less than 0.070 mm in diameter.

The bacteria used in experiments were *Thiobacillus ferrooxidans* from a laboratory culture isolated from Suior ore and maintained by passing on mineral liquid 9K medium Silverman and Lundgren (1959) with ferrous iron sulphate as energy source [15].

METHODS

Tests in different working conditions have been made as table 1 shows.

Table 1

Experimental conditions at the tests for bacterial solubilization of arsenium from the pyrite concentrate

No. of the test	Variants	Concentrate amount (g)	9K medium (ml)		Temp. (°C)	S/L	Bacterial inoculum (ml)	Notes
			with $FeSO_4$	without $FeSO_4$				
1.	Control without bacteria and without $FeSO_4$	40	-	400	35	1/10	-	Add. of tymol 1% Stationary cond.
2.	Test with bacteria, S/L ratio 1/100, medium without $FeSO_4$	4	-	400	35	1/100	4	Stationary conditions
3.	Test with bacteria, S/L ratio 1/10 medium without $FeSO_4$	40	-	400	35	1/10	4	Stationary conditions
4.	Test with bacteria, S/L ratio 1/5 medium without $FeSO_4$	80	-	400	35	1/5	4	Stationary conditions
5.	Control without bacteria, medium with $FeSO_4$	40	400	-	35	1/10	-	Add. of tymol 1% Stationary cond.
6.	Test with bacteria S/L ratio 1/100, medium with $FeSO_4$	4	400	-	35	1/100	4	Stationary conditions
7.	Test with bacteria, S/L ratio 1/10, medium with $FeSO_4$	40	400	-	35	1/10	4	Stationary conditions
8.	Test with bacteria, S/L ratio 1/5, medium with $FeSO_4$	80	400	-	35	1/5	4	Stationary conditions
9.	Test under agitating at ambiental temperature	40	400	-	18-25	1/10	4	Agitating
10.	Test for adaptation of bacteria	40	400	-	35	1/10	4	Agitating
11.	Test with adapted bacteria	40	400	-	35	1/10	4	Agitating

Samples of liquid were taken periodically and a chemical analysis has been made. Arsenium concentrations were determined by precipitating with hypophosphite, re-dissolving with iodine and titrating the excess of iodine with

thiosulphate. From the residual solid, arsenium was determined by the same procedure after the material disintegration with hydrochloric and nitric acids.

In the control tests a solution of 1 % tymol was added instead of inoculum. Tests under agitating conditions have been done in flasks on a magnetical stirrer.

RESULTS AND DISCUSSIONS

The series of tests, as presented in Table 1, have the purpose to study and prove the following aspects:

1 - the role of bacteria in arsenium solubilization;

2- the influence of initial addition of ferrous iron sulphate in solution;

3 - the influence of solid/liquid ratio;

4 - the influence of pulp agitation;

5 - the influence of temperature;

6 - the importance of bacteria adaptation.

1. The active role of *Thiobacillus ferrooxidans* bacteria in arsenium solubilizing from the arsenious pyrite concentrate is evident as it clearly results from the diagrams presented in figure 1 (test 1 compared with tests 2 or 3) and figure 2 (test 5 compared with tests 6 or 7). The results show that in the presence of bacteria arsenium dissolution increases 5 - 8 times as much as in controls without bacteria where only small arsenium extractions are achieved, produced by some chemical dissolution processes.

2. The influence of ferrous iron sulphate addition in the initial solution can be watched by comparing the results presented in figure 1 with those in figure 2. Opposed to the supposition that without ferrous iron bacteria will be obliged to

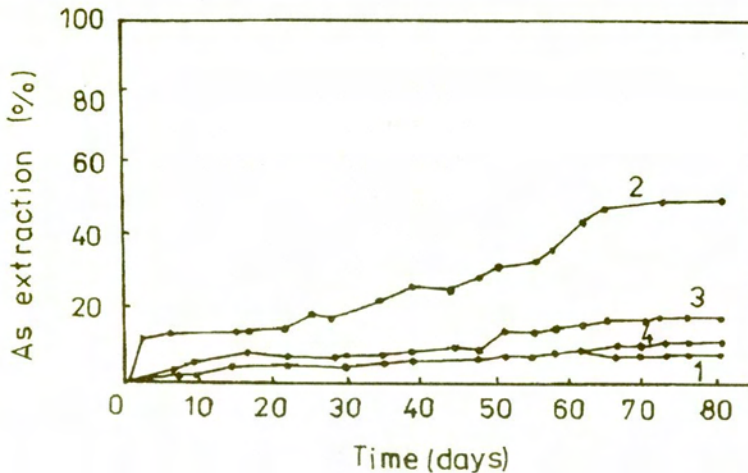

Fig. 1. - Role of bacteria and the influence of S/L ratio on bacterial solubilization of arsenium from pyrite. (In stationary conditions and solution without initial $FeSO_4$). Test 1: Control. Test 2: S/L ratio of 1/100. Test 3: S/L ratio of 1/10. Test 4: S/L ratio of 1/5

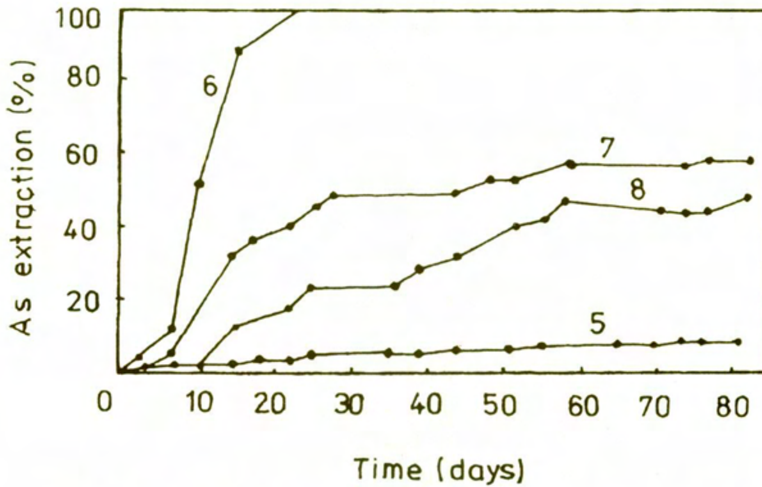

Fig. 2. - Role of bacteria and the influence of S/L ratio on bacterial leaching of arsenium from pyrite. (In stationary conditions and solution with initial $FeSO_4$). Test 5: S/L ratio = 1/10. Control. Test 6: S/L ratio = 1/100. Test 7: S/L ratio = 1/10. Test 8: S/L ratio = 1/5

activate by the direct mechanism of sulphide oxidation with intensive rate of mineral solubilization, the results show a great increase of arsenium solubilization in tests with initial ferrous iron sulphate addition. So, whereas in test 6, 98 % of arsenium was solubilized after 22 days, in the parallel one (test 2) without ferrous iron sulphate addition, after 22 days only 14 % of arsenium was solubilized and a period of 75 days was necessary to achieve 50 % of arsenium dissolution. The same finding is valuable for the other parallel tests in which 1/10 or 1/5 solid/liquid ratios were used. In every case arsenium extraction is larger in the test with initial addition of ferrous iron sulphate.

Though the direct mechanism of mineral sulphide oxidizing has been experimentally found [3], it seems that its contribution in the whole process is quite small at least during the first period of experiments. Grudev considers that *Thiobacillus ferrooxidans* bacteria represent a large scale of different types concerning their activity of iron-oxidizing and sulphur-oxidizing enzymatic systems [5, 6]. By this point of view, our culture being grown on ferrous iron represents a type having an active iron-oxidizing system and a partially inhibited sulphur-oxidizing system. So, bacteria activate mostly by the indirect mechanism of mineral sulphide oxidation intermediated by ferric ions.

3. The solid/liquid ratio influenced greatly the results (figure 1 comparing tests 2, 3 and 4 and figure 2 comparing tests 6, 7, and 8), but its discussion must be approached in a strict connection with the fact that the tests have been made in stationary condition wherein the pyrite drops to the bottom of the flask and the contacting of the liquid and solid phases is deficient.

The use of a solid/liquid ratio of 1/100 resulted in the highest arsenium extraction (98 % in 22 days - test 6), whereas in the same conditions in the test using solid/liquid ratio 1/10 only 40 % of arsenium was solubilised and to

solubilize 50 % of arsenium a period of almost 50 days was necessary. At 1/5 solid/liquid ratio after 22 days only 17 % of arsenium was solubilized and after 50 days solubilizing activity reached 40 %, but 50 % could not be reached even after 81 days.

Using the same solid/liquid ratios as above in tests done without ferrous iron addition showed also differentiated results, the best one being obtained at a 1/100 ratio (50 % arsenium extraction after 81 days).

These differences are, of course, due to the fact that at 1/100 solid/liquid ratio, though pyrite has sedimented on the bottom of the flask, the phase contacting could be achieved at a satisfactory degree because of the small amount of solid material; whereas with the other tests phase contacting could not be achieved at a satisfactory degree. We can conclude that at stationary conditions a solid/liquid ratio of 1/100 (or near this value) must be used in experiments to achieve conclusive results concerning arsenium extraction efficiency. For the higher values of solid/ liquid ratio agitating conditions are required to achieve an adequate phase contacting.

4. Agitation of the pulp accelerates the bacterial arsenium solubilizing process because better phase contacting is ensured. This is shown in figure 3 (test 7 and 11).

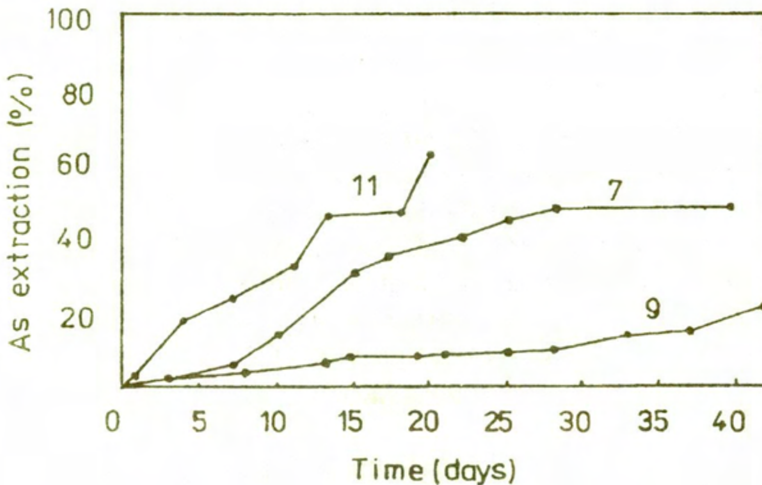

Fig. 3. - The influence of agitation and temperature on bacterial solubilization of arsenium from pyrite. (S/L ratio of 1/10 and solution with initial $FeSO_4$). Test 7: Stationary conditions, T = 35°C. Test 9: Agitating conditions, T = 18 + 25 °C. Test 11: Agitating conditions, T = 35 °C

Under agitating conditions, 61 % of arsenium was solubilized after 20 days in comparison with less than 30 % after same period without agitation.

These experiments have not been intended to optimise the pulp agitating conditions, but such experiments will be necessary in order to elaborate technologies for bacterial treatment of arsenious gold bearing concentrates. A series of researches carried out for this purpose are known [1, 4, 7, 10, 14].

5. Temperature has been found to be a factor greatly influencing the rate of bacterial solubilization of arsenium as we can observe in figure 4

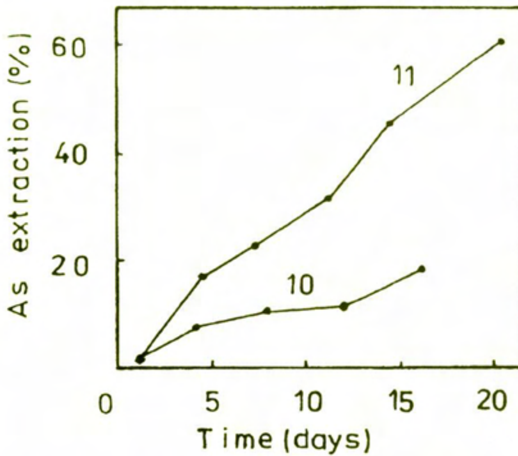

Fig. 4. - Role of bacteria adaptation on arsenium solubilization rate from the pyrite. (In conditions of agitation; solution with $FeSO_4$, S/L ratio = 1/10). Test 10: unadapted bacteria. Test 11: adapted bacteria

(tests 9 and 11). Thus, in conditions of optimal temperature for *Thiobacillus ferrooxidans* (35 °C) the arsenium solubilizing rate was 7-8 times as high as at the ambiental temperature (18-25 °C). Arsenium extraction was 61 % at the test 11 versus only 9 % at the test 9 after a same long period of 20 days. However the process of bacterial solubilization of arsenium continues also at low temperature but its rate is drastically diminished. Therefore to reach an arsenium extraction near 50 % a period of 129 days was necessary.

6. In 1982 Polkin et al. have found the importance of previous adaptation of bacteria on the substrate which has to be treated, such adaptation leading them to obtain higher results in experiments done on different metal concentrates [14]. Our researches for adapting certain cultures of *Thiobacillus ferrooxidans* on the pyrite concentrate Suior have shown that the bacteria cultivated just once on a medium with concentrate as sole energy source are adapting to the particular conditions during a complete cycle of growth of 20-30 days. At the following cycle the duration of "lag" phase is reduced at a half and the solubilization processes are more intensive [12].

Tests 10 and 11 in figure 4 illustrate the influence of the previous adaptation of bacteria that led to an arsenium extraction increasing over 3 times in comparison with the test using unadapted bacteria. However the influence of bacteria adaptation is really considerable only in the first period of the experiment. In prolonged experiments bacteria become adapted and the differences in results begin to diminish. Consequently, it can be stipulated for technological experiments, either to use from the beginning a culture of adapted bacteria, or to spend a period of about 20 days of working with low results after which the bacteria will become adapted. These predictions have been confirmed in our further technological experiments [13].

CONCLUSIONS

1. The results have shown that the culture of *Thiobacillus ferrooxidans* possesses a great capacity to solubilize arsenium from arsenious pyrite concentrate.

2. Addition of ferrous iron sulphate has a positive influence on the process of arsenium bacterial leaching. It increases arsenium extraction about 6 times in comparison with the test without ferrous iron sulphate addition.

3. Under stationary conditions, using a solid/liquid ratio of 1/100 maximal arsenium extraction results (98 % after 22 days). Use of 1/10 or 1/5 ratios results in diminished arsenium extraction because of unsatisfactory phase contact but such ratios must be considered for reasons of industrial practice.

4. Experiments done under agitation resulted in an increase of arsenium extractions degree up to 61 % after 20 days even in conditions of solid/liquid ratio of 1/10, but it is necessary to optimise the agitation conditions in order to achieve maximal extraction.

5. Temperature is a particularly important factor in the process of bacterial leaching of arsenium from the pyrite concentrate. Optimal temperature for bacterial growth (35 °C) ensures the obtaining of high extractions of arsenium (61 % after 20 days), whereas decreasing temperature to 18-25 °C diminished arsenium extraction to merely 9 % in the same period.

6. A previous adaptation of bacteria by cultivating them over a period of 20 days on the same substrate which has to be treated results in intensifying bacterial leaching of arsenium more than 3 times as compared with unadapted bacteria. However this is true only for the initial period of experiments, as prolonging the duration of experiments leads to a bacterial adaptation within the process.

7. The experiments have proved that there are technical and biological possibilities for using the procedure of bacterial tank leaching of the arsenious gold-bearing pyrite concentrate for arsenium removal in order to recover gold. In order to achieve this, technological researches to establish the optimal parameters particular to each type of concentrate are necessary.

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DIVISION AND REGENERATION OF *BACILLUS SUBTILIS* PROTOPLASTS

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Ultrastructural analysis revealed that only a small proportion of *B. subtilis* protoplasts, incubated in nutritive medium DM_3, divide and regenerate. In the absence of cellular wall, as a main structure of initiation of division signals, the protoplasts are dividing after a mechanism similar to the division of Gram negative bacterial cells. In the absence of old cellular wall remnants, the process of regeneration takes place to the final stage of a whole wall. Ultrastructural images point out the dynamics of these processes.

Enzymic protoplasts release and specific conditions for the development of this process are known for numerous types of bacteria (Fitz-James 1964; Popkin et al. 1971; Weiss 1976; Cocking 1979; Gasson 1980; Allock et al. 1982; Hopwood 1981; Schupp and Divers 1986).

In *B. subtilis* cells, wall sensitivity to the lysozyme action is not uniform (Ionescu et al. 1991).

The bacterial protoplasts are viable in media that confer osmotic protection (medium with sucrose 0.3 M). In protoplasts, the synthesis and growth processes, as well as those of exoenzymes secretion (amylase, proteases) occur at a lower rate (Sanders et al. 1975).

The L. forms of *B. subtilis*, induced by antibiotics inhibiting murein biosynthesis, grow and divide in a nutritive liquid medium. They are stable and do not return to bacillary forms (Gilpin et al. 1973).

B. subtilis protoplasts released under lysozyme action preserve competence to regenerate cell wall (Landman et al. 1969, 1976).

Cell wall regeneration does not take place in usual growth media, but only in special media, which confer certain physical and chemical properties and which primarily promote protein synthesis. Regeneration is, too, dependent on protoplasts density, optimum value being 10^6 - 10^7 protoplasts/ml.

The most used regeneration media include in their composition gelatine (20 - 30 %) or agar (2 %).

It is well known that cell wall regeneration is a rapid process, if the protoplast retains yet a small fragment from the original cell wall. This has the primary role for the synthesis of a new wall. In the total absence of cellular wall, protoplasts regeneration is a rare event.

The term "reversion" signifies resynthesis of cell wall and recovery of original cell morphology. The term "reversion" defines the process of cell wall resynthesis after the protoplasts have covered at least one division cycle or after the fusion. The reversion is an obligatory condition for obtaining some new bacterial strains through the protoplasts fusion method.

The aim of this paper is the study of division and regeneration processes in their dynamics in protoplasts of *B. subtilis*, ICA 1-65 strain.

MATERIALS AND METHODS

Young cultures of *B. subtilis*, I C A - 1.65 strain, grown in nutritive broth under stirring conditions, were used. The cells were harvested by centrifugation and twice washed in phosphate buffer 0.2 M, pH 7.2. The last cell pellet was resuspended in the same buffer, supplemented with 0.3 M sucrose, to a density of 10^6 - 10^7 cells/ml and treated with lysozyme at a concentration of 5 mg/ml for 20 min., at 37 °C, for protoplasts release.

The protoplasts suspension was twice centrifugated in buffered sucrose solution and examined in the electron microscope, in order to control the degree of cell wall digestion.

The protoplasts pellet after the last centrifugation were incubated in DM_3 regeneration medium:

Na succinate	0.70 g
Casaminoacids	0.05 g
Tryptophan	0.25 mg
Yeast extract	0.05 g
K_2HPO_4	0.035 g
KH_2PO_4	0.015 g
Glucose	0.04 g
$MgCl_2.6H_2O$	0.04 g
Agar	0.20 g
Distilled water	10.0 ml

After 15 hours incubation at 37 °C, the harvested protoplasts in 3 % glutaraldehyde for 2 hours and 1 % osmium tetraoxide for 4 hours at 4 °C in 0.15 M, pH 7.2 phosphate buffer. Thin sections were cut on a Porter-Blum ultramicrotome MT-1, stained with uranyl acetate and lead citrate and observed under Philips 201 model electron microscope.

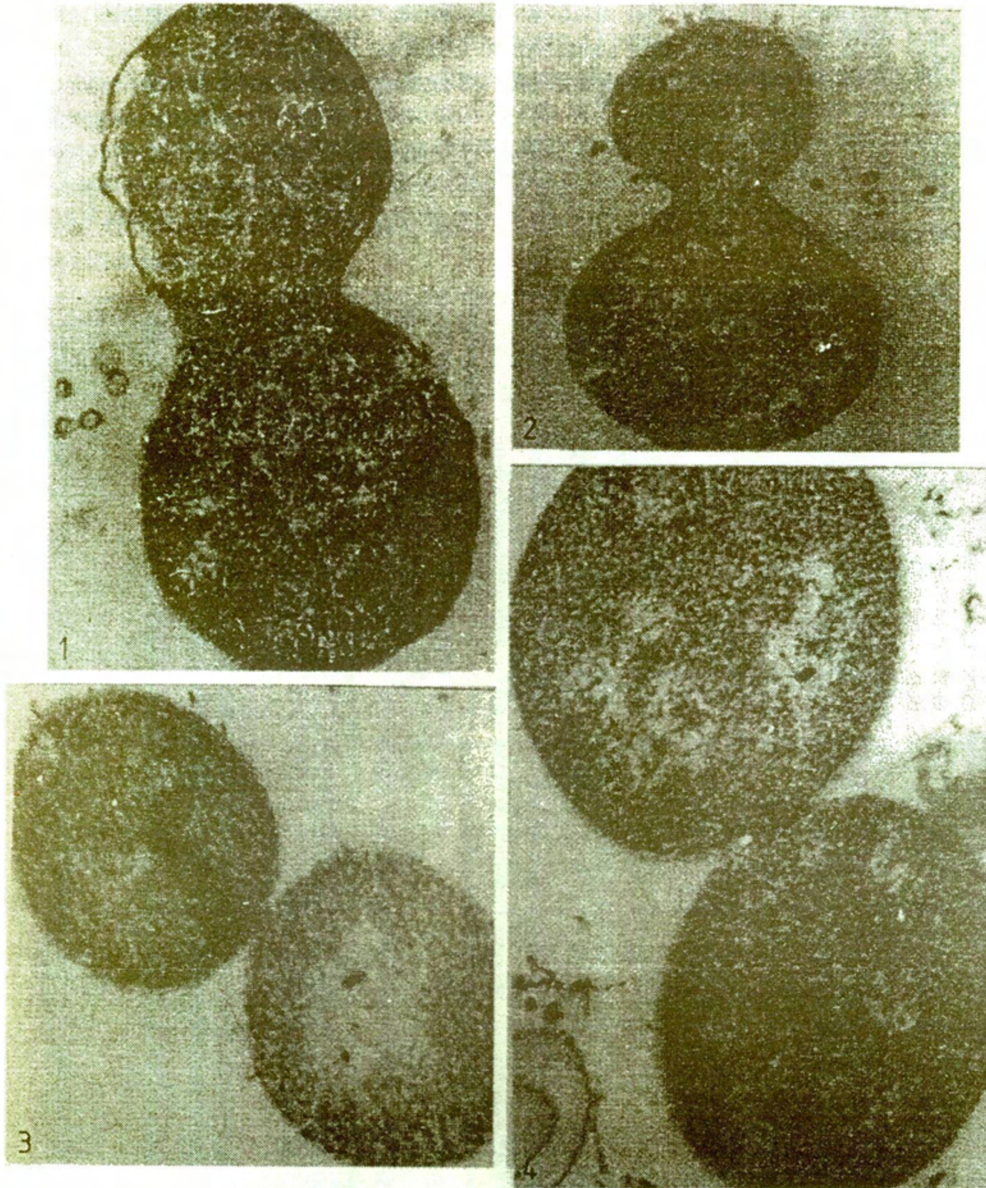
RESULTS AND DISCUSSIONS

In osmotic stabilized solutions, the protoplasts volume increases considerably in time, because their membrane gradually becomes permeable to the molecules of the stabilizing solution (Marquis and Corner, 1976).

Sodium succinate is an osmotic stabilizer, which preserves protoplasts viability and promotes cellular growth.

The protoplasts division in nutritive medium DM_3 (Chang and Cohen, 1979) is a rare event. The dynamics of the division process is similar to the division of Gram negative bacterial cells: centripetal growth of membrane in the equatorial region of protoplast, which progress to the complete separation, of equal sizes protoplasts (Figs. 1-5).

Division is the consequence of biosynthesis and growth processes, which continually take place in protoplasts. In the absence of cell wall, as a main structure



Figs. 1–4. Successive stages of protoplasts division, through centripetal growth of cytoplasmic membrane in equatorial region of the cell (figs. 1, 2 × 50,000, fig. 3 × 80,000, fig. 4 × 50,000).

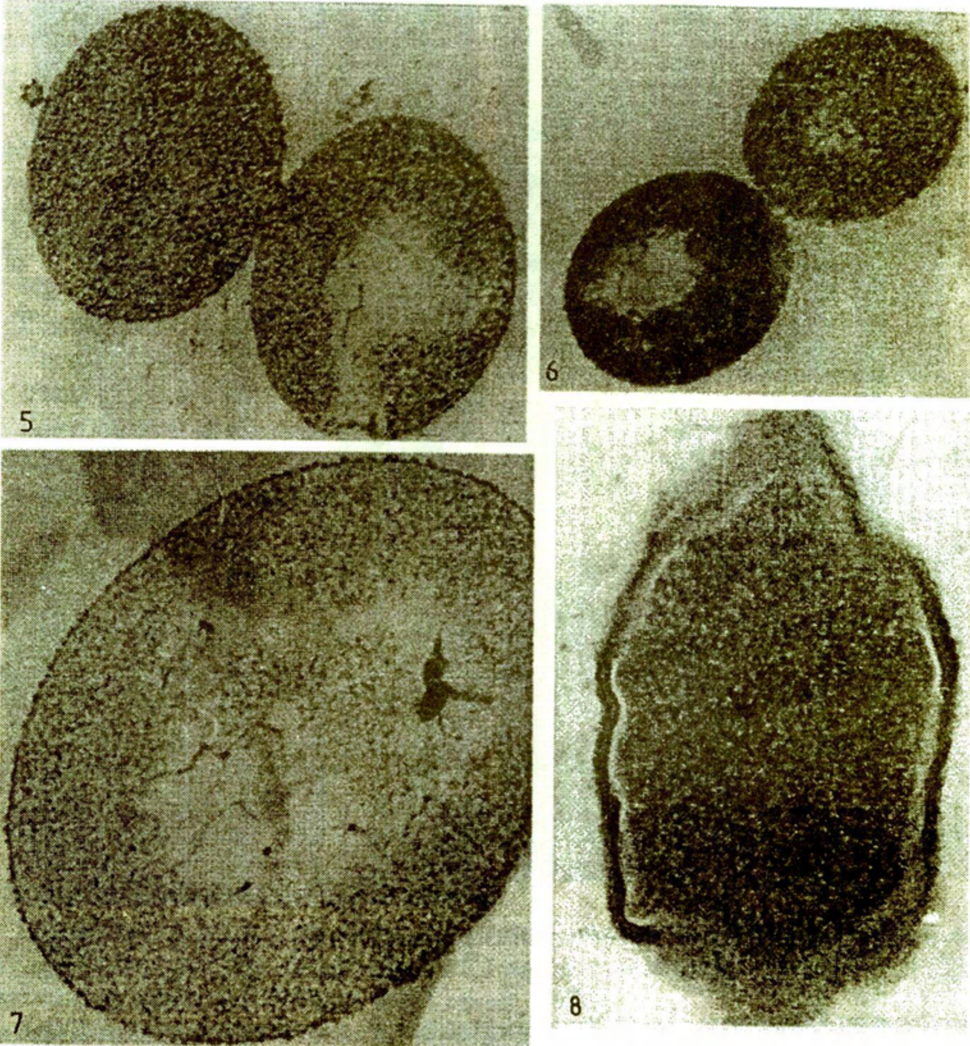
in initiation and dynamics of septum formation, *B. subtilis* protoplasts division is similar to the division process of Gram negative bacterial cells. Nucleoid of dividing protoplasts is sooner in a dispersed state. The images suggest the dynamics of a symmetrical division.

REGENERATION OF *B. SUBTILIS* PROTOPLASTS

The investigation of protoplasts regeneration "in situ" affords the possibility to emphasize directly cellular modifications and successive steps of cell

wall resynthesis. The process of regeneration takes place in nutritive media solidified with agar or gelatine (Landman et al. 1969, 1976).

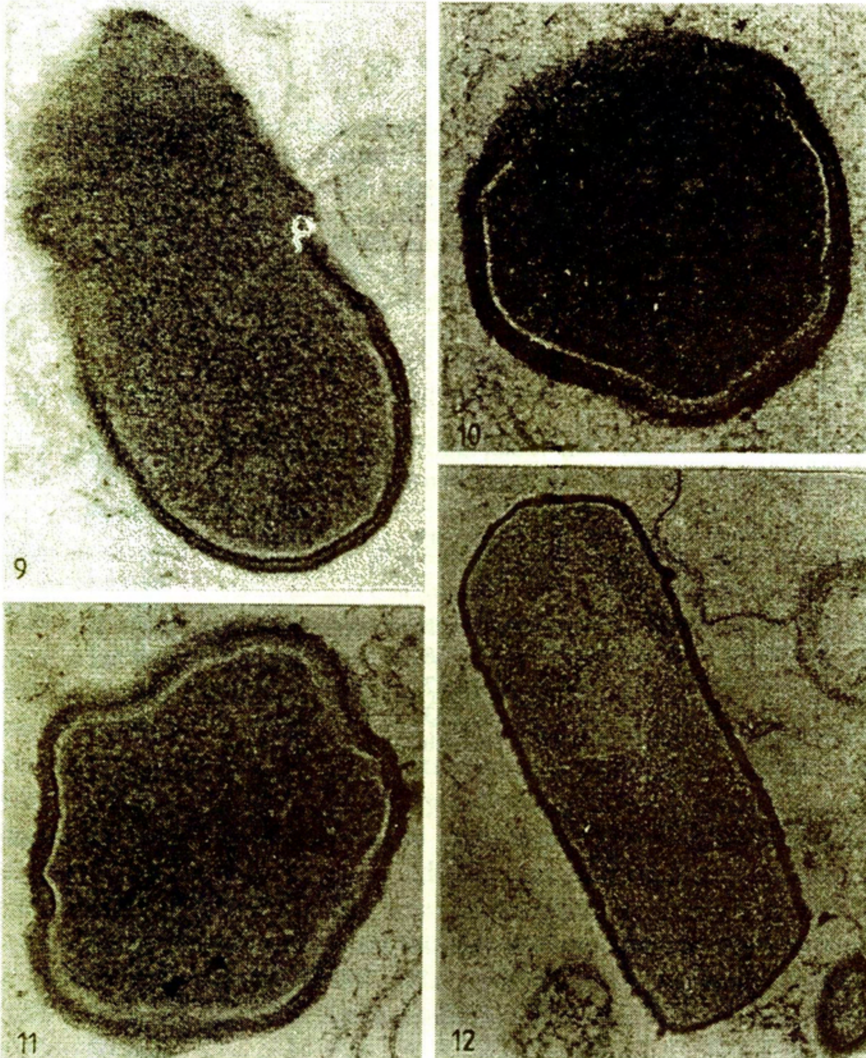
The electron-optical protoplasts investigation, after a 15 hour incubation in DM_3 nutritive medium suggests the development of resynthesis and regeneration processes of the cellular wall. The regeneration process is preceded by the synthesis of an electron-dense material with a lax distribution on protoplast membrane (Fig. 6). The following successive stages after this first step of regeneration, to the final stage were not observed.



Figs. 5-6. Successive stages of protoplasts division, through centripetal growth of cytoplasmic membrane in equatorial region of the cell (fig, 5 × 50,000, fig. 6 × 32,000). Fig. 7. -Cell wall regeneration is preceded by synthesis and deposition of an electron-dense material, with granular texture, at the almost whole periphery of protoplast membrane, × 80,000. Fig. 8 × 115,000. Successive steps of cell wall regeneration. The dynamics of proper wall synthesis is not uniform on the whole cell outer surface. In fig. 8, the wall is regenerated in cylindrical zone, but in the polar ones, the wall material is not definitely structured in parietal network.

The assembling of new wall structure does not uniformly progress on the whole protoplast surface. The process is initiated in multiple spatially separated regions. The affirmation is suggested by the images of cells with incomplete wall (Figs. 7 – 12).

The investigation of *B. subtilis* protoplasts regeneration "in situ" has emphasized that process takes place with a very low frequency, much lower than numerical estimations after protoplasts cultivation on the surface of a nutritive solidified medium.



Figs. 9-11 × 115,000. Successive steps of cell wall regeneration. The dynamics of proper wall synthesis is not uniform on the whole cell outer surface. In fig. 9, the regeneration takes place on half of the cell length. In fig. 10, in a cross section of a cell, the wall is missing in the region marked by arrows. In fig. 11, a regenerated cell, with contour irregularities, in cross section, proper of regeneration process.
Fig. 12. A completely regenerated bacillus, × 50,000.

CONCLUSIONS

1. *B. subtilis* protoplasts preserved the ability to grow and divide in regeneration medium DM_3.

2. In the absence of the celular wall, the mechanism division of *B. subtilis* protoplasts is similar to the division of Gram negative bacterial cells.

3. Protoplasts regeneration in nutritive DM_3 regeneration medium develops with a very low frequency. The synthesis of the wall proper is preceded by an electron-dense material, uniformly distributed on the protoplast membrane.

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EVIDENCE FOR CORRELATION BETWEEN A CONDITIONALLY DOMINANT LETHAL EFFECT AND MUTATIONS IN THE MOTOR DOMAIN OF Cin8p-A KINESIN-RELATED MOTOR PROTEIN AT *SACCHAROMYCES CEREVISIAE*

LUMINIŢA PARAOAN

The experiments described in this paper are part of the effort to explore the relationship structure-function of Cin8p – a kinesin-related motor protein required for mitotic spindle assembly and function at *Saccharomyces cerevisiae*.
In order to determine in what functional domain the mutations causing a conditionally dominant lethal effect of some ts alleles of CIN8 gene are, new constructs with different sequences of the gene were made. The newly constructed plasmids were transformed into a wild-type yeast strain (with regard to the respective gene) and tested for the phenotype induced. No ts dominant lethal effect could be seen any more. Consequently, one could conclude that the mutations causing the dominant lethal effect of these mutants are located in the region encoding the motor domain of Cin8p.

The motor proteins generate movement by converting the chemical energy stored in ATP into a directional movement of cellular components along the microtubules.

Kinesin was identified as a protein capable of conferring microtubule-dependent motility to small organelles toward the plus end of the microtubules (6). Since 1990 several other kinesin-related genes have been discovered, establishing the existence of a large kinesin-related protein superfamily. Each member of the superfamily shares homology with the motor domain of kinesin, while other parts of proteins are substantially different – this fact, probably, conferring functional specificity. The characteristic motor domain is attached to a coiled-coil α-helical extension. A big effort is currently made in many laboratories to establish the roles of the kinesin-related proteins in mitosis, to determine their functional domains, and to demonstrate how amino-acid sequence determines nucleotide and substrate specificity.

In the case of kinesin, it has been shown that the amino-terminal 340 amino-acids of its heavy chain contain all the elements needed for that conversion (7). A smaller subset of this family (bimC subfamily) shows a sequence similarity of up to 60%; this subfamily includes, among others, CIN8 and KIP1 genes from *Saccharomyces cerevisiae*.

The putative kinesin-related motors encoded by the yeast *Saccharomyces cerevisiae* genes CIN8 and KIP1 were shown to act redundantly, being required for the assembly of the mitotic spindle (1) and essential for maintaining the structural integrity of the spindle (4). The sequence of CIN8 was reported (1); this gene encodes a 1038 amino-acid polypeptide that contains a region (amino-acids 67 through 522)

with strong similarity to the heavy chain of the microtubule-based mechanochemical enzyme kinesin. The sequence of the kinesin-related KIP1 gene was also reported (3). The conserved region of sequence similarity has been found at the N-terminal end of the proteins and corresponds to the 340 amino acid motor domain. The Cin8p motor domain has also a region with a poorly conserved sequence that contains a segment of about 100 amino acids (residues 255-353) not present in other characterized kinesin-related proteins (1). By analogy to kinesin, it is expected that Cin8p interacts with microtubules through the N-terminal motor domain and, possibly, binds an antiparallel microtubule with the C-terminal end.

A critical issue for understanding the function of these proteins is to localize their domains and sites of action and to reveal the details for the correlation of structure and function. The possibility of different regulatory systems and different sites for interaction is taken into account, although the basic structure is shared by different motor elements. At present, there is little known about the structure and/or force-generating mechanisms of these proteins.

One of the most rewarding approaches to the analysis of the cell cycle has been the isolation and characterization of cell cycle mutations in the unicellular yeast *Saccharomyces cerevisiae*. Conditionally dominant-lethal mutants of CIN8 gene have been induced and isolated in a previous screening (results submitted for publication). Cin8p-ts mutants have been phenotypically characterized and five of them were dominant lethal at non-permissive temperatures both on CIN8 and/or KIP1. The only case when this effect was not observed was in cells cin8-Δ kip1-Δ double mutants kept alive by CIN8 on a CEN plasmid. All five mutants were caused by null-mutations, as they could not by themselves support growth of yeast cells (i.e., when no copy of CIN8 or KIP1 wild-type were present in cell).

The purpose of the experiments described in this paper was to identify the domain of Cin8p where the respective mutations were localized, thus characterizing the interesting mutants obtained with respect to their functional domains.

MATERIAL AND METHOD

Five conditional-lethal (ts) cin8 alleles, previously induced by random mutagenesis (i. e., hydroxylamine treatment of plasmid DNA) were tested and proved to be dominant to wild-type CIN8. In order to determine in what domain of the encoded protein the mutations that caused those phenotypes were, the cin8-ts alleles were genetically engineered in the following manner.

The cin8-ts alleles were carried by a CEN plasmid (LEU2 $CYH2^S$) of about 12.8 kb constructed in Dr. M. A. Hoyt laboratory (i.e., a BamHI/SalI fragment of 4135 bp containing CIN8 gene into BamHI/Sal I pRS 318); PstI has a unique site for action on DNA sequence of CIN8 gene. In this paper the plasmid carrying CIN8 wild-type gene is referred as Pl and those with cin8-ts dominant alleles as Pl-1 through Pl-5. The *Saccharomyces cerevisiae* strain used, MAY591, was also provided by the same laboratory (MAY591 : α lys2-801 his3-Δ200 leu2-3112 ura3-52 CIN8 KIP1).

The following constructs were made: the large fragment (F) generated by the double digestion PstI/BamHI of P1 was ligated to the small fragment (f) resulted

from the double digestion with the same enzymes of P1-1 through P1-5. The newly obtained plasmids were tested for dominance.

The restrictions were carried out in a total volume of 30 μl, the F fragment was treated with alkaline phosphatase (New England Biolabs). Both F and f were phenol-chloroform extracted, followed by DNA precipitation, as PstI cannot be inactivated by heat.

The electrophoresis was performed in 1% agarose gels, 2h at 100V. The corresponding DNA bands were excised from the ethidium bromide stained agarose gels and the DNA was extracted by GENECLEAN® proceaure using GLASSMILK® Suspension.

For ligation a 3:1 ratio for insert: vector was used and the total volume was 15 μl.

The *E. coli* transformants were selected on LB medium with 100 μg/ml ampicillin. As a control, the fragment F alone (dephosphorylated) was used. Yeast transformations were performed by the lithium acetate method (2).

Rich (YEPD) and minimal (SD) media were used as described (5).

RESULTS AND DISCUSSIONS

Five interesting dominant lethal mutants of CIN8 gene from *Saccharomyces cerevisiae* were subject to some experiments for localizing the domain of the mutations causing this phenotype. The ts cin8 alleles provided for the new constructs a small fragment of about 2.5 kb corresponding to the segment PstI/BamHI from the respective plasmids Pl-1 through P1-5. With respect to functional domains of Cin8p, this fragment corresponds roughly to the gene segment encoding the tail domain. The large fragment used in construction (F) of the new plasmids (referred here as Pl-1 through Pl-5) was obtained by the double digestion PstI/BamHI of the plasmid P1 and corresponds to the P1 fragment containing besides all markers and sequences essential for plasmid function, the motor domain of CIN8. The F fragment has about 10.3 kb.

The corresponding bands (see Figure 1) were excised from the agarose electrophoresis gels and used, after extraction, for ligation. Although the migration was concludent with regard to the size of DNA fragments resulted in the double digestion, another digestion was also performed – i.e., PstI/BamHI/SphI digestion of P1. SphI has a unique site of action on the sequence of the plasmids used, namely on the sequence of CIN8 gene, between the sites of BamHI and Pst I, at 1533bp far apart from PstI site. Consequently, in the triple digestion the band corresponding to the f fragment could not be seen any more, but a new characteristic band (1533bp) appeared.

The newly constructed plasmids were transformed into *E. coli* strain DH5α and the transformants selected on LB+ampicillin plates. No colonies grew on control plates (transformation using the dephosphorylated F fragment alone).

The yeast transformants (into *Saccharomyces cerevisiae* strain MAY591 - wild-type for both CIN8 and KIP1) were tested appropriately by frogging on rich and

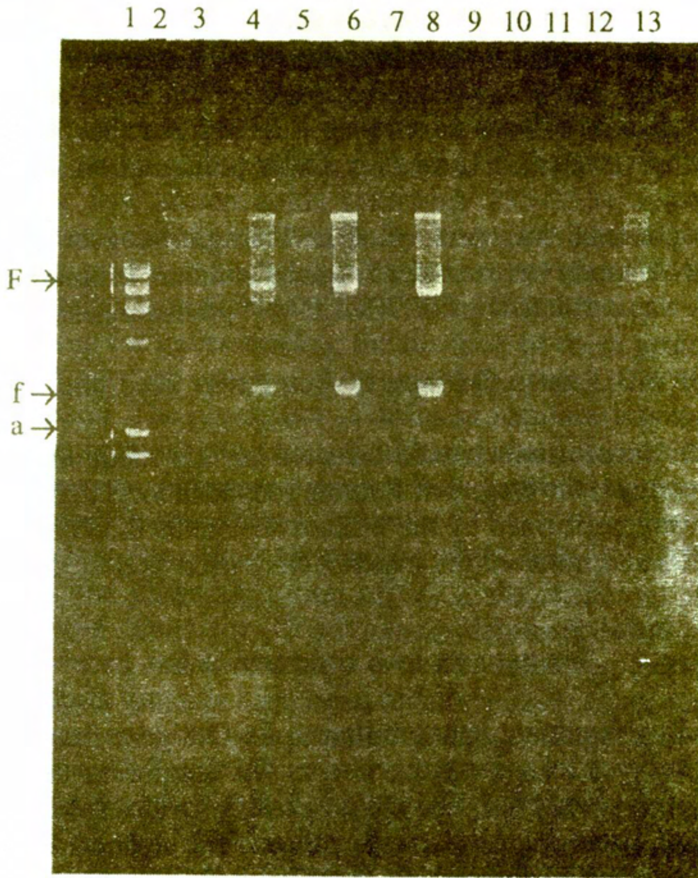

Fig. 1.- Agarose gel electrophoresis for the fragments resulted in plasmid digestion. lane 1 - λ HindIII - size marker, lanes 4,6,8 - PstI/BamHI double digestion of P1, P1-1, P1-2; lanes 2,3,5,7,9,10,11,12- simple different digestions of P1 and P1-1 through P1-5; lane 13 - PstI/BamHI/SphI digestion of P1-1. Arrows indicate: F=10.3kb band; f=2.5kb band ; a= 1533bp band.

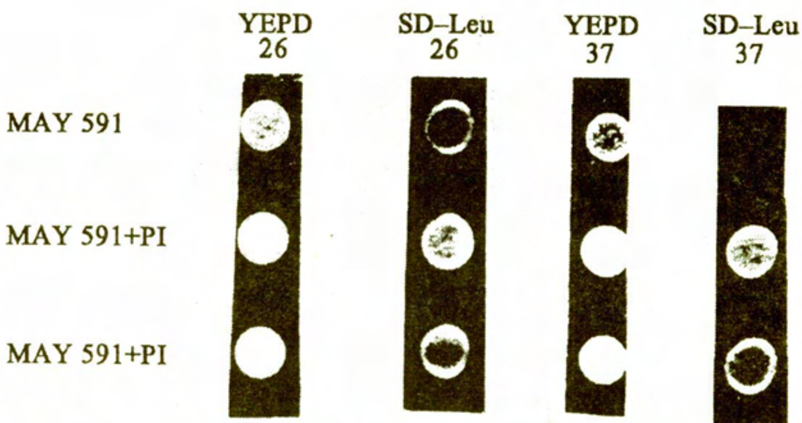

Fig. 2.- Test for ts dominant lethal effect of cin8 alleles. P1- plasmid carrying wild-type CIN8 gene; Pİ- newly constructed plasmid.

selective medium, at temperatures ranging from 26°C to 37°C (see Figure 2). The control used in these tests was MAY591 + P1. The transformants MAY591 + Pl-1 through Pl-5 grew all well at all tested temperatures; no detectable phenotype was observed in comparison with both MAY591 alone and MAY591 + P1. No conditionally dominant lethal effect was observed. So, one could conclude that the newly constructed plasmids contained a cin8 allele (very probably the wild-type CIN8) which no longer shows a ts dominants over CIN8 and KIP1.

Consequently, these experiments are sufficient to conclude that the mutation(s) causing the ts dominant lethal phenotype in the presence of CIN8 and/or KIP1 were localized in the region of CIN8 gene encoding the motor domain of *Saccharomyces cerevisiae* Cin8p.

As the tertiary structure of the motor domain of Cin8p is not known yet, there cannot be formulated more implications with regard to the affected internal domains of the motor domain itself. Instead, the experiments being informative with respect to the activity of Cin8p (and Kip1p), one could make a few suppositions concerning the organization of functional domains and the relation structure-function in Cin 8p.

Dominant mutants are an expected class of mutants for the kinesin relatives. One of the simplest possibilities that could be imagined is that they are caused by the loss of function of one domain, while the remaining domain(s) interferes with the function of the wild-type form. Information about the function of kinesin-like proteins has primarily come from investigations of mutant phenotypes. It is expected that Cin8p, like other kinesin relatives, possesses domains of different function. Mutations of multi-domain proteins could determine different phenotypic properties depending upon the domain affected. In case of CIN8 gene, different mutants have been already isolated. It is interesting to mention that all ts dominant lethal mutants isolated in the screening described had mutations in the region encoding the motor domain. Various classes of mutants in this region could be anticipated : motor domain mutants unable to cross-link the microtubules or unable to support the motor driven sliding, motor domain mutants that bind the microtubules but do not support motility or have a reduced microtubule-stimulated ATPase activity. To explain the mechanisms of action of Cin8p one has to take into consideration the localization of the protein and its requirement to hold the two halves of the bipolar spindle together.

Finally, it should be noticed that since the motor domain sequence is conserved in all superfamily members, the properties of CIN8 motor domain alleles have significance for the structure and function of all kinesin-related proteins. Besides being informative such mutants could be useful for further analysis of spindle function in experiments meant to identify interacting gene-products by suppression analysis.

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EXOPOLYSACCHARIDES PRODUCTION BY STRAINS OF *CEPHALOSPORIUM* AND *SCHIZOPHYLLUM*

IOANA GOMOIU

Two strains of *Cephalosporium* and *Schizophyllum* were screened for exopolysaccharide production. Production of these exopolysaccharides is affected by quality of carbon and nitrogen sources in the medium. The tolerance of *Schizophyllum commune* and its polysaccharide to NaCl and seawater may permit use of seawater as makeup water for its production on sea platforms.

The cell walls of fungi are primarily composed of polysaccharides such as glucans, mannans and chitin. The walls of Ascomycetes and Basidiomycetes contain chitin and branched (1-3)-β-D-glucan. There are glucans with (1-3)-β-links, (1-6)-β–links and with alternating (1-3)-β and (1-6)-β-links. In *Schizophyllum commune*, first of all are synthesized (1-3)-β-glucan chains, while (1-6)-β-linkages appear secondarily subapically in the hyphal walls. A part from these polysaccharides is like a mucilage around hyphae or in culture filtrate. Their use in the food, pharmaceutical, oil and textile industry has attracted interest in science: Sutherland and Ellwood (12), Sandford (8), Gabriel (3), Okamura et al. (17), Yanaki et al. (13), Shinohara et al. (10).

The work presents exopolysaccharide production by strains of Cephalosporium and Schizophyllum grown on media with different carbon and nitrogen sources, with different concentrations of NaCl and in nutrient media with seawater.

MATERIALS AND METHODS

Cephalosporium sp. and *Schizophyllum commune* strains isolated from soil or wood were stored on slopes of potato dextrose agar at 4 °C.

The effect of different carbon sources was studied replacing glucose by various sugars at a final concentration of 1 g %. Also we used a mixture of starch potatoes and glucose.

The effects of different nitrogen sources on exopolysaccharide production at the initial concentration of 0.15 gl^{-1}, total nitrogen were studied on media containing starch potatoes and glucose.

The various salinities tested (0, 1, 5, 10, 15 %) were established by adding the appropriate amount of NaCl to media containing starch potatoes and glucose.

The production of exopolysaccharide by *Schizophyllum commune* was studied on nutrient medium containing seawater in different dilutions.

The glucan was estimated gravimetrically after precipitation with acetone or determined as glucose equivalents using the phenyl sulphuric acid method, Dubois et al (2).

Viscosity of 0.8 ml sample placed in a clean Wells-Brookfield LVT-c/p microviscometer chamber (at 25 °C) was read at 2, 25, 4, 50, 11, 25, 22, 5, 45 and 90 s^{-1}.

RESULTS AND DISCUSSIONS

Results obtained for *Cephalosporium sp.* and *Schizophyllum commune* on media with different carbon sources are presented in Table 1.

Table 1

Effect of carbon sources on exopolysaccharide production by *Cephalosporium sp.* and *Schizophyllum commune*

Carbon Source	*Cephalosporium sp.*		*Schizophyllum coomune*	
	Biomass (gl^{-1})	Exopoly-saccharide (gl^{-1})	Biomass (gl^{-1})	Exopoly-saccharide (gl^{-1})
Lactose	2.9	0.60	3.0	1.9
Xylose	5.1	1.03	3.2	3.5
Galactose	4.8	0.90	3.6	1.7
Fructose	5.9	1.20	3.9	2.3
Maltose	4.9	1.0	4.0	3.2
Starch (maize)	6.1	0.63	4.2	3.0
Starch (potatoes)	8.2	0.92	5.1	6.0
Glucose	6.9	0.95	4.3	3.9
Starch (potatoes)+ + glucose	7.2	2.50	6.2	7.4

Nitrogen source was $NaNO_3$ except starch potatoes + glucose where it was missing.

Although both strains produced exopolysaccharides on media with all carbon sources tested the best was the mixture: starch potatoes and glucose.

There is no correlation between high biomass and exopolysaccharide production for *Cephalosporium sp.* The highest biomass was found on media with starch potatoes but the yield of exopolysaccharides was on media with starch-potatoes and glucose.

The lowest polysaccharide yields were obtained on media with lactose. Glucose as sole carbon source is not a good substrate for exopolysaccharide production.

Using various nitrogen sources we found $NaNo_3$ and urea both for exopolysaccharide and biomass production by *Cephalosporium sp.*

In case of *Schizophyllum commune* all nitrogen sources may be used for exopolysaccharide production (Table 2).

According to Niederpruem et al (6), Sarkar et al (9), Stasinopoulos, Seviour (11), Gomoiu (4, 5) biosynthesis of exopolysaccharides depends on the culture condition and fungal strain.

Table 2

Effect of different nitrogen sources on exopolysaccharide production by *Cephalosporium sp.* and *Schizophyllum commune* (0.15 gl^{-1} total N)

Nitrogen source	*Cephalosporium sp.*		*Schizophyllum commune*	
	Biomass (gl^{-1})	Exopoly-saccharide (gl^{-1})	Biomass (gl^{-1})	Exopoly-saccharide (gl^{-1})
NH_4Cl	4.2	0.68	6.0	7.0
NH_4NO_3	5.1	0.92	5.7	7.2
$NaNO_3$	5.3	1.08	5.3	7.0
Uree	5.9	1.00	5.0	6.9

Carbon source was starch potatoes + glucose

High yields of β-glucan in both fungi were associated with pellets. There were no pellets in media with lactose or galactose in case of *Schizophyllum commune* or with lactose and starch maize in case of *Cephalosporium sp.* Exopolysaccharides attached to hypha and freely dispersed in nutrient medium decrease the oxygen supply. Culture filtrate of *Schizophyllum commune* has a high viscosity and the oxygen supply is low. Thus, exopolysaccharide synthesis by *Schizophyllum commune* is stimulated in oxygen limitation conditions. The same results were obtained hy Dosoretz et al. (1), Wecker and Onken (14) working with *Phanerochaete chrysosporium* and *Aureobasidium pullulans*.

Exopolysaccharide production by *Cephalosporium sp.* and *Schizophyllum commune* on media with a different concentration of NaCl 1-25 % is presented in Table 3.

Table 3

Effect of varying NaCl concentration on exopolysaccharide production by *Cephalosporium sp.* and *Schizophyllum commune*

NaCl concentration (g/l)	*Cephalosporium sp.*		*Schizophyllum commune*	
	Biomass (gl^{-1})	Exopoly-saccharide (gl^{-1})	Biomass (gl^{-1})	Exopoly-saccharide (gl^{-1})
1	5.8	1.15	5.6	7.1
5	3.8	0.90	5.8	7.0
10	2.6	0.63	5.9	7.1
15	-	-	6.0	7.0
20	-	-	5.2	6.8
25	-	-	4.9	6.0

Cephalosporium sp. can grow in media with 1-10 % NaCl but can produce exopolysaccharides in media with 1 % NaCl. In case of *S. commune* both growth

are no important differences between yields of exopolysaccharides at different concentrations of NaCl. Viscosity read at 11, 25, sec^{-1} and 25 °C had the same level (165-180 cp).

Exopolysaccharide production on media with seawater (Table 4) takes place at the same level. It appears that the production of exopolysaccharides by *Schizophyllum commune* may take place on media with seawater as makeup water.

Table 4

Yield of exopolysaccharides by *Schizophyllum commune* on nutrient medium containing seawater

Ratio of dilution	*Schizophyllum commune*	
	Biomass (gl^{-1})	Exopolysaccharide (gl^{-1})
Nondilute	6.1	6.9
1/1	6.0	7.0
2/1	6.0	6.8
1/3	6.1	7.0

CONCLUSIONS

1. High biomass is not correlated with high exopolysaccharide production by *Cephalosporium sp.*
2. A mixture of glucose and starch potatoes represents the best source for exopolysaccharide production by *Cephalosporium sp.* and *Schizophyllum commune.*
3. The best N source for exopolysaccharide production by *Cephalosporium sp.* is $NaNO_3$.
4. The tolerance of *Schizophyllum commune* strain and its polysaccharide to NaCl and seawater may permit the use of seawater as makeup water for its production on sea platforms.

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LECTURER DOCTOR EMILIA ILIESCU AT THE 90th ANNIVERSARY

GEOGETA FABIAN

Lecturer dr. Emilia Iliescu (Bucur) was born in commune Cata county Braşov on January 7, 1904. She began her primary school in Bucharest. After she finished Carmen Silva High School she passed an entrance examination at the Faculty of Natural Sciences at the University of Bucharest. After she graduated in 1926 according to her very good results she became an assistant in the Plant Anatomy and Physiology Department led by Professor Emanoil Teodorescu , at the University of Bucharest.

She was awarded the Ph. D. degree in November 1936 with the thesis "Cambium and secondary vascular tissue at monocotyledonous plants".

In 1937 she passed an exam for confirmation as an assistant and a researcher.

In 1949 she became a head of practical works and in 1962 she was promoted lecturer. After retirement she was a counsellor lecturer.

In 1937, dr. Emilia Bucur married captain Gheorghe Iliescu. Their marriage was happy but short. Lieutenant colonel Iliescu was decorated with "Mihai Viteazul Order" but he died in battle as a hero on July 9, 1941 at Stoicăneşti. Their daughter was only 3 years old.

Dr. Emilia Iliescu began her didactic activity as a supervisor of the students'practical works, having weekly scientific talking or exams in terms with students. She did her works with calm and competence, doing all she could to help and encourage students.

Dr. Emilia Iliescu organized and chaired The Student Debating Society on Plant Physiology. With patience she taught them to work in this field and inspired them the love for research.

From 1950 Dr. Emilia Iliescu taught Plant Physiology (evening courses). Beginning with 1955 she taught Water regime of plant and Mineral nutrition. Dr. Emilia Iliescu introduced new practical works in the students'programme and delivered original lectures.

From 1956 Dr. Emilia Iliescu delivered lectures on Plant Physiology for the students of the Faculty of Biology and of the Faculty of Geography.

Very appreciated was her activity as a supervisor for students'graduate diploma. A part of her activity was dedicated to High School teachers being an excellent supervisor for their practical works diploma and degree exams. For them, Dr. Emilia Iliescu wrote the work "Plant Physiology without machines".

Dr. Emilia Iliescu carried out her scientific activity on Plant Physiology within the Faculty of Biology. In 1948 she set up the Plant Physiology Group from Romanian Academy till the Institute for Biological Researches was founded including a Plant Physiology Laboratory.

Her favourite field of research was plant stimulation with mineral substances and other stimulants synthesized in laboratory.

Dr. Emilia Iliescu published her scientific results in 29 original works. She also wrote many popularising works as a member of the Natural Sciences Society.

All her life, Dr. Emilia Iliescu devoted herself to Plant Physiology working almost every day. She was loved by her students and co-workers for whom she set high ethical and intellectual standards.

Dr. Emilia Iliescu may have made her greatest contribution to Plant Physiology through the leadership and direction of her graduate students and her encouragement and advice to her faculty colleagues.

Her daughter Rodica Muşeteanu-Iliescu is working in a Tissue Culture Laboratory and her nephew is a student at the Biochemistry Faculty in Germany.

It is a great pleasure for her colleagues, co-workers and many generations of students to congratulate Dr. Emilia Iliescu on the occasion of her 90th birthday and wish her good health and long years of further activity.

WEBER H. (Ed.), 1993, *Allgemeine Mykologie* , Gustav Fischer Verlag Jena - Stuttgart, 541 pp., 206 plates, 66 tables

Drawn up by a team of 12 prestigious authors, widely recognized scientific authorities in the domain, the book *Allegemeine Mykologie*, edited by Professor H. Weber of Jena, fills up a gap in the biological literature in general, and mycological literature, in particular, focusing attention on the significance of this complex botanical science, really topical and unexpectedly linked to and involved in the life of human communities.

This book is divided into 10 chapters containing 541 pages, of which 474 represent the background of this work (Chapters 2 - 10), supplemented with 206 plates and 66 tables, and ended with a list of fungus genera and species, and an index of scientific terms used.

The classical chapters of this book deal with: fungus cytology and morphology (82 pp., E. Jelke, H. Kreisel), their reproduction and propagation (21pp., H. Weber, R. W.Arnold), taxonomy (53 pp., N. Dörfelt) and fungus ecology (33pp., W. Dörfelt); these are completed by updated chapters on molecular biology and fungus genetics (38 pp., H. Weber, W. Künkel), metabolic transport processes (35 pp., W. Ross), etc.

A prominent part of this book (Chapter 8, 143 pp.) is devoted to the significance and practical value of fungi, which is reflected in various and essential activities of man's life, and especially in biotechnologies. This chapter tackles the edible mushroom cropping (R. W. Arnold, H. Dörfelt), fungi in consumption goods industry (W. Hirte), albumins from fungi (G. Straube, H. Weber), products of secondary metabolism of fungi and their conversion (D. Gröger), exchange relationships between fungi and metals (G. Straube), fungi in preservation of materials and their biodeterioration (G. Staube), fungi and their role in the biological control of plant pests and diseases (W. Hirte, R. W. Arnold).

The last but one chapter of this book (Chapter 9) reviews the plant pathogenic fungi (M. Schmiedeknecht) as one of the phytopathological mycology concerns, while the last one (Chapter 10) dwells on the problems of medical mycology – human and veterinary – i.e. fungi causing mycoses in man and animals.

Allgemeine Mycologie is a model for approach a basic botany science – mycology – in its interrelations with the human activity, in its whole variety, translated into practice as food biotechnologies (bread-making, yeast production, wine-making, brewery, production of ethanol, starch, dairy, meat and fishery foods, fermentation of coffee, tea, cocoa and tobacco, preparing juices, glucose, enzymes, vitamins and aromatic products, food products biodeterioration), biosynthesis of antibiotics, enzymes, vitamins, hormones and other compounds of chemical and drug industry, preservation of materials (wood, paper, leather, plastics, museum exhibits, books, etc.) and their biodeterioration, biotechnologies used in plant protection for the biocontrol of diseases and pests of cropped plants, in the plant, medical and veterinary mycology. All these branches are competently and scrupulously analysed by the authors of this book.

The volume also benefits of an exceptional printing skill, which is also extended to its whole iconography.

This book is intented to specialists working in the field of mycological research, as well as in biological, biochemical, biotechnological, agricultural and medical (human and veterinary) high school teaching, and also to students who prepare for one of these careers. Its value recommends this book as an authoritative achievement in plant biology, in general, and in mycology – in particular.

Dr. Tatiana Eugenia Şesan

AVIS AUX AUTEURS

La «Revue Roumaine de biologie - Série de biologie végétale» publie des articles originaux d'un haut niveau scientifique, de tous les domaines de la biologie végétale: morphologie, systématique, géobotanique, physiologie, écologie, génétique, microbiologie, phytopathologie. Les sommaires des revues sont complétés par d'autres rubriques, comme: 1. La vie scientifique, qui traite des manifestations scientifiques du domaine de la biologie: symposiums, conférences, etc. 2. Comptes rendus des livres de spécialité parus en Roumaine. Les auteurs sont priés d'envoyer leurs articles, notes et comptes rendus dactylographiés en deux exemplaires. Les tableaux et l'explication des figures seront dactylographiés sur pages séparées et les diagrammes seront exécutés à l'encre de Chine noire, sur papier calque.

Les tableaux et les illustrations seront numérotés avec des chiffres arabes. La répétition des mêmes données dans le texte, dans les tableaux ou dans les graphiques sera évitée.

Les références bibliographiques, citées par ordre alphabétique, comporteront le nom de l'auteur, l'initiale du prénom, le titre de la revue abrégé conformément aux usances internationales, l'année, le tome le numéro, la page. Les travaux seront accompagnés d'un court résumé de maximum 10 lignes, en anglais. Les textes des travaux ne doivent pas dépasser 7 pages dactylographiées (y compris les tableaux, la bibliographie et l'explication des figures). La responsabilité concernant le contenu des articles revient exclusivement aux auteurs.

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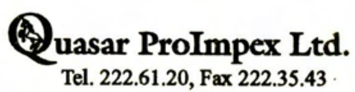

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