BIOLOGICALLY ACTIVE COMPOUNDS IN EXTRACTS OF SOME HALOPHILIC AND HALOTOLERANT FUNGI FROM THE ORDO *Dothideales* AND SELECTED YEASTS

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EXTREME HABITATS

AIMS OF THE WORK

- searching for novel biologically active compounds with hemolytic, antibacterial, hemagglutinating and anti-acetylcholinesterase activities

- Investigation of the effect of lowered water activity (a_w) and temperature on synthesis of these compounds

TESTED FUNGI (I)

Organism	Strain	Label	Habitat
Alternaria tenuissimum grupa C	EXF-2329	AltS	salterns
Alternaria tenuissimum grupa C	EXF - 2318	AltS	salterns
Alternaria tenuissimum grupa X	EXF-2338	AltS	salterns
Alternaria infectoria	EXF-2332	AliS	salterns
Alternaria aborescens	EXF-2340	AlaS	salterns
Aureobasidium pullulans	EXF-150	ApS	salterns
Aureobasidium sp. skupina 5	EXF-922	AspA	Arctic
Aureobasidium pullulans var. melanogenum	EXF-3233	ApG	Japanese sea, 4000 m depth
Cladosporium cladosporioides	EXF-381	CcS	salterns
Cladosporium cladosporioides?	EXF-2246	CcA	Arctic
Cladosporium sphaeorospermum	EXF-385	CcS	salterns
Cladosporium sphaeorospermum?	AR 297	CsA	Arctic
Cladosporium dominicanum	EXF-732	CdS	salterns
Cladosporium velox	EXF-466	CvS	salterns
Cladosporium halotolerans	EXF-572	ChS	salterns
Cladosporium salinae	EXF-335	CsaS	salterns
Cladosporium langeronii	EXF-1000	ClS	nails
Cladosporium psychrotolerans	EXF-391	Cps	salterns
Cladosporium fusiforme	EXF-449	CfS	salterns
Cladosporium spinulosum	EXF-334	CsS	salterns

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Hortaea werneckii	EXF-225 / MZKI B736	HwS	salterns
Phaeotheca triangularis	MZKI 206	PtS	salterns
Trimmatostroma salinum	EXF-295 / MZKI B734	TsS	salterns
Fusarium sp. 1	EXF-2254	F1S	salterns
Fusarium sp. 2	EXF-2276	F2S	salterns
Fusarium sp. 3	EXF-2275	F3S	salterns
Fusarium sp. 4	EXF-2277	F4S	salterns
Wallemia ichthyophaga	EXF-994	WiS	salterns
Wallemia muriae	EXF-951	Wm	salterns
Wallemia sebi	EXF-958	Ws	sunflower seeds
Cryptococcus albidus	MZKI K528	CaA	Arctic
Cryptococcus liquefaciens	MZKI K428	ClA	Arctic
Filobasidium floriforme	MZKI K560	FfA	Arctic
Pichia guilliermondii	EXF-518	PgS	salterns
Pichia guilliermondii	EXF-1496	PgA	Arctic
Rhodosporidium babjavae	EXF-513	RbS	salterns
Rhodosporidium diobovatum	MZKI K650	RdA	Arctic
Rhodotorula mucilaginosa	EXF-1630	RmA	Arctic
Trichosporon mucoides	EXF-1444	TmS	salterns
Trichosporon Mucoides	EXF-3366	TmA	Arctic
Candida parapsilosis	EXF-1574	CpS	salterns
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Saccharomyces cerevisiae	MZKI K86	ScK	control

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Rhodosporidium diobovatum	MZKI K650	RdA	Arctic
Rhodotorula mucilaginosa	EXF-1630	RmA	Arctic
Trichosporon mucoides	EXF-1444	TmS	salterns
Trichosporon Mucoides	EXF-3366	TmA	Arctic
Candida parapsilosis	EXF-1574	CpS	salterns
Candida parapsilosis	EXF-517	CpA	Arctic
Saccharomyces cerevisiae	MZKI K86	ScK	control

TESTED FUNGI (II)

Organism	Strain	Label	Habitat
Hortaea werneckii	EXF-225 / MZKI B736	HwS	salterns
Phaeotheca triangularis	MZKI 206	PtS	salterns
Trimmatostroma salinum	EXF-295 / MZKI B734	TsS	salterns
Fusarium sp. 1	EXF-2254	F1S	salterns
Fusarium sp. 2	EXF-2276	F2S	salterns
Fusarium sp. 3	EXF-2275	F3S	salterns
Fusarium sp. 4	EXF-2277	F4S	salterns
Wallemia ichthyophaga	EXF-994	WiS	salterns
Wallemia muriae	EXF-951	Wm	salterns
Wallemia sebi	EXF-958	Ws	sunflower seeds
Cryptococcus albidus	MZKI K528	CaA	Arctic
Cryptococcus liquefaciens	MZKI K428	ClA	Arctic
Filobasidium floriforme	MZKI K560	FfA	Arctic
Pichia guilliermondii	EXF-518	PgS	salterns
Pichia guilliermondii	EXF-1496	PgA	Arctic
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Saccharomyces cerevisiae	MZKI K86	ScK	control

PREPARATION OF EXTRACTS

- cultivation in the laboratory:

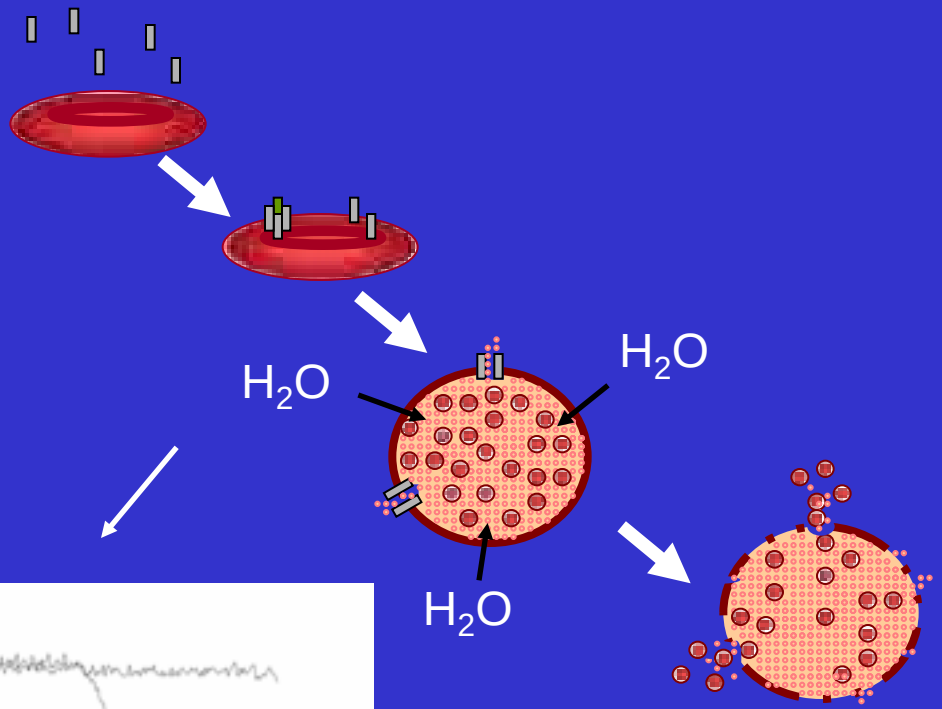
	composition of medium (1l)	temperature
control conditions	YNB (1,7g), $(NH_4)_2SO_3$ (5g), glukoza (20g), agar (13g)	30°C
low temperature	YNB (1,7g), $(NH_4)_2SO_3$ (5g), glukoza (20g), agar (13g)	4°C/10°C
high salt (10%) concentration	YNB (1,7g), $(NH_4)_2SO_3$ (5g), NaCl (50g), agar (13g)	30°C
high glucose (20%) concentration	YNB (1,7g), $(NH_4)_2SO_3$ (5g), glucose (400g), agar (13g)	30°C

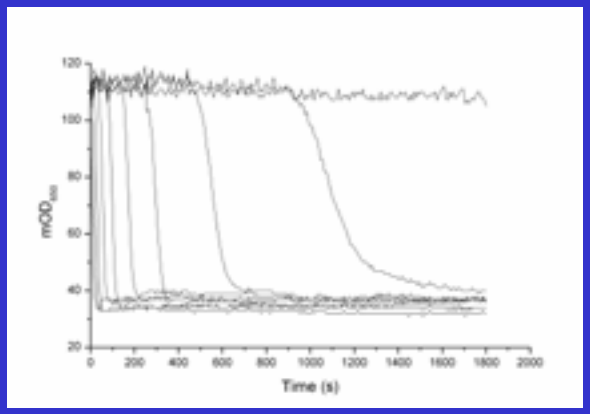
- extraction (acetone, methanol, water): extracts 5 mg/ml

BIOLOGICAL TESTS (I)

1. estimation of hemolytic activity

(final conc. of extract = 0.5 mg/ml)



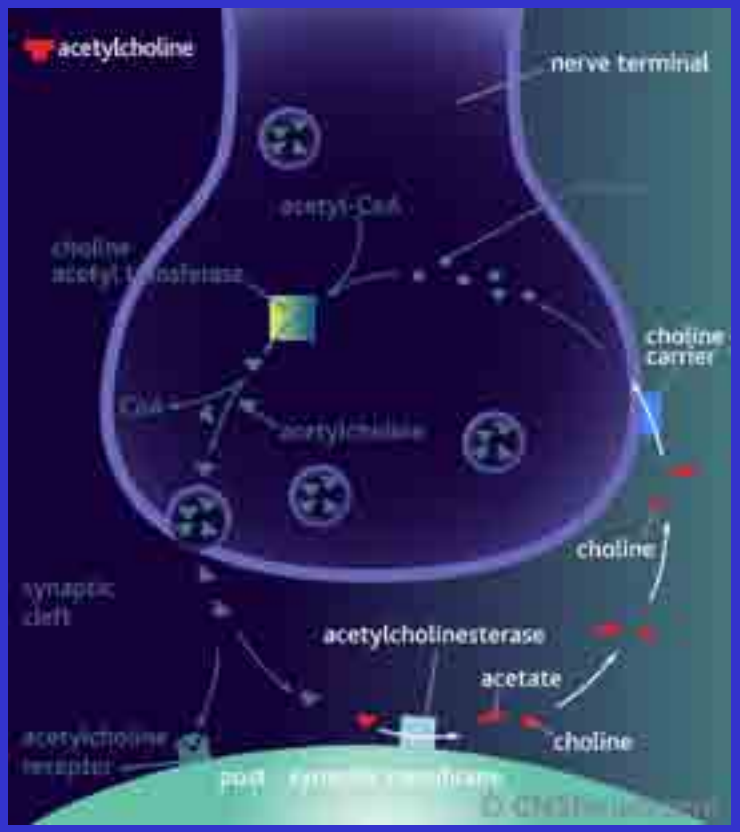


2. hemagglutination test

BIOLOGICAL TESTS (II)

3. estimation of antibacterial activity (*Bacillus subtilis*, *Escherichia coli*)

4. Estimation of anti-cholinesterase activity



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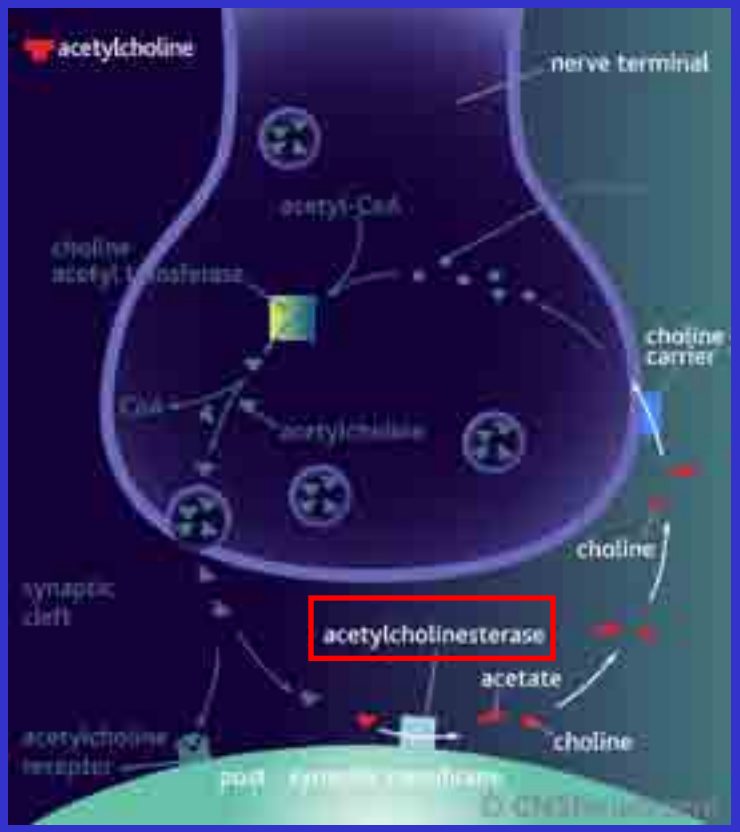
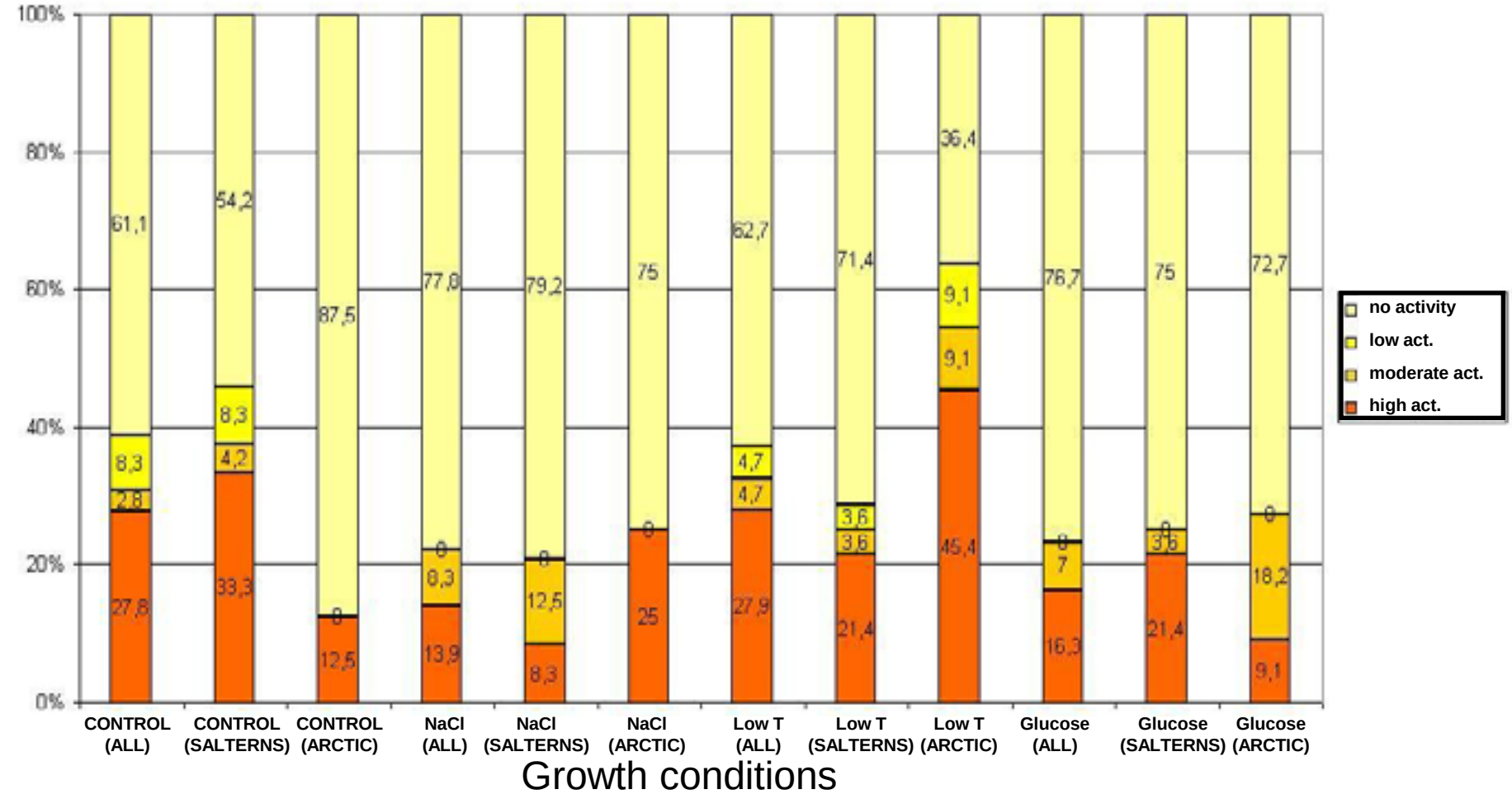
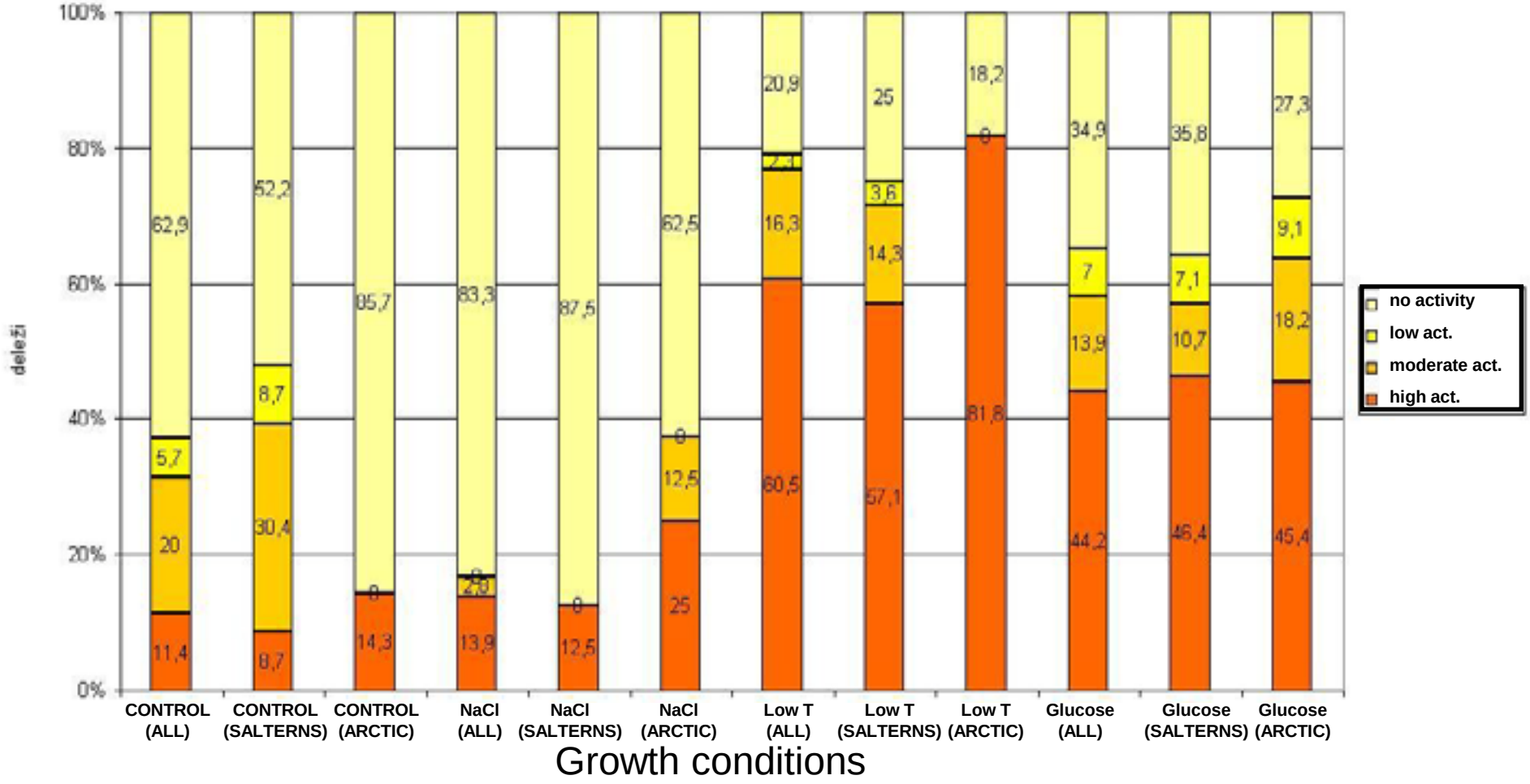


HEMOLYTIC ACTIVITY – - ACETONE EXTRACTS



HEMOLYTIC ACTIVITY – - METHANOLIC EXTRACTS



ESTIMATION OF HEMOLYTIC ACTIVITY - SUMMARY

Only organic extracts exert hemolytic activity

The synthesis of hemolytically active compounds in stress conditions was increased in 43% of acetone and 73% of methanolic extracts, especially in diferent strains of *Alternaria tenuissima*, *Hortaea werneckii*, *Aureobasidium* and *Fusarium*

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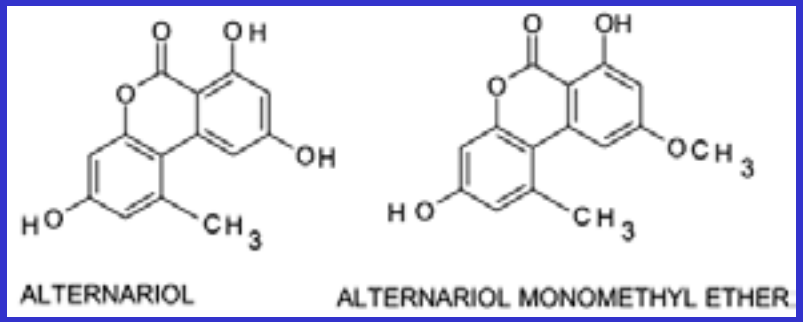
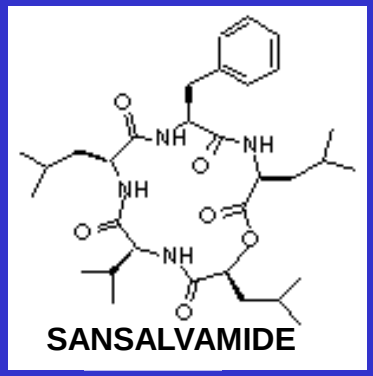
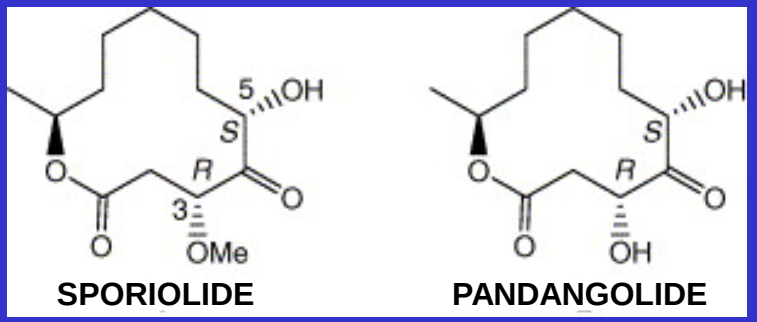
Alternaria tenuissima



Fusarium sp.

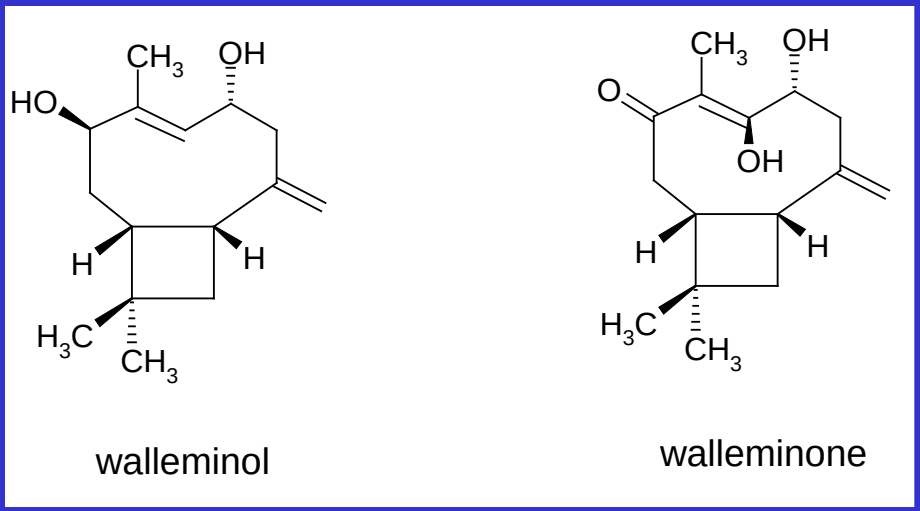


Cladosporium



SPORIOLIDE **PANDANGOLIDE**

Wallemia sp.



walleminol walleminone

Hortaea werneckii

Candida parapsilosis

Trimmatostroma salinum

Aureobasidium pullulans

Rhodosporidium diobovatum

Cryptococcus sp.

Pichia guilliermondii

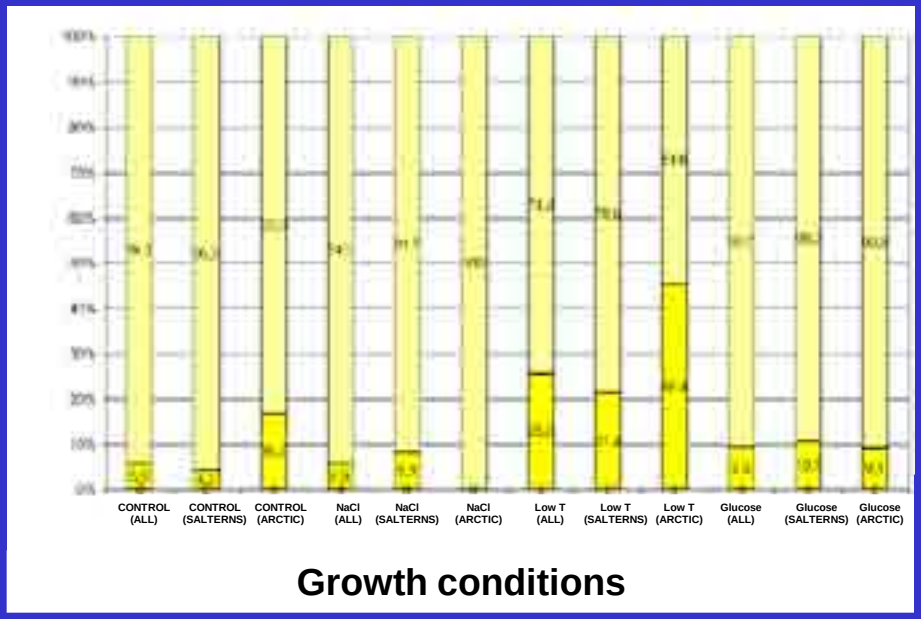
Rhodotorula mucilaginosa

Rhodosporidium babjevae

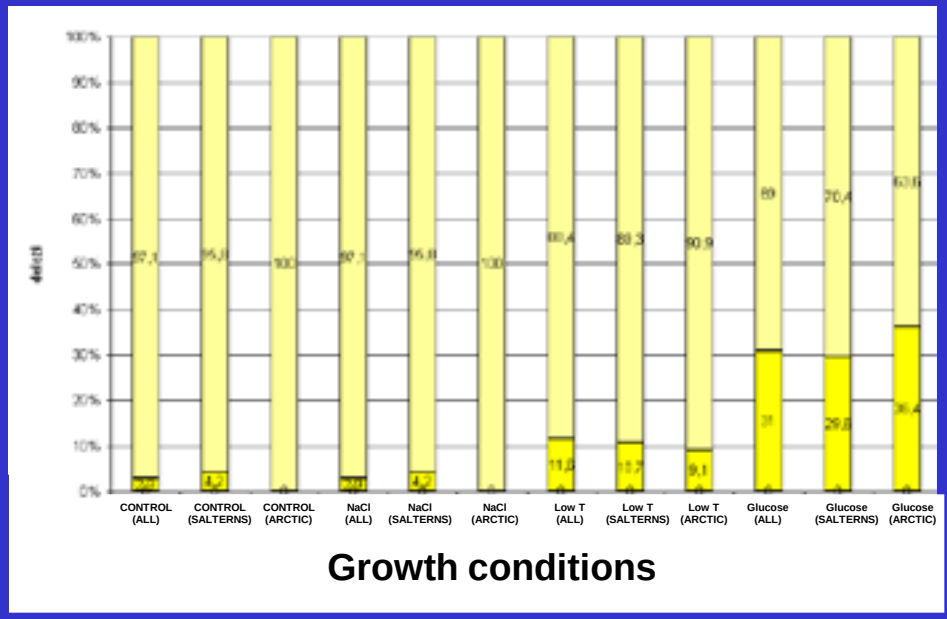
UNDESCRIBED HEMOLYTICALLY ACTIVE COMPOUNDS

ANTIBACTERIAL ACTIVITY – - *E. coli*

ACETONE



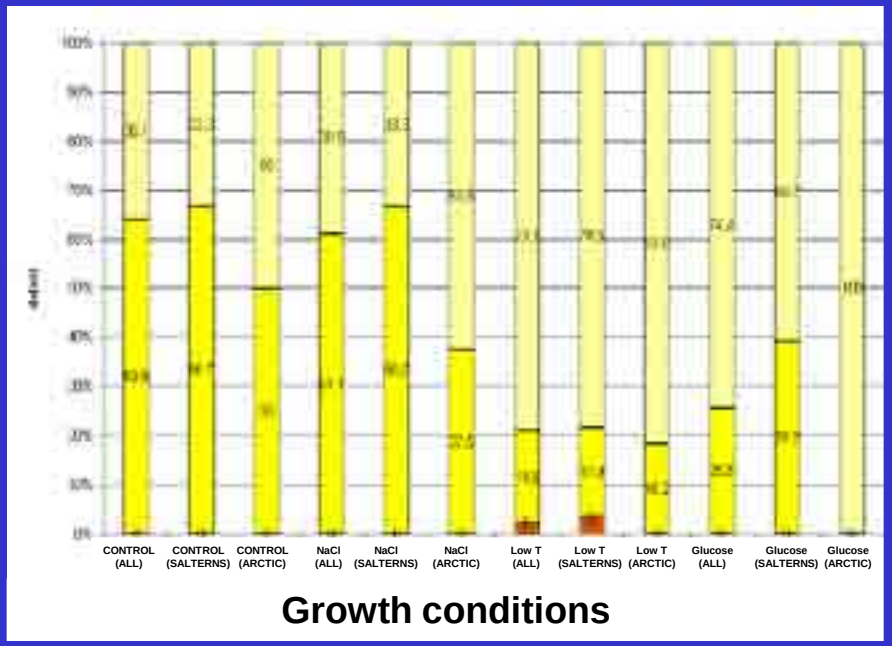
METHANOL



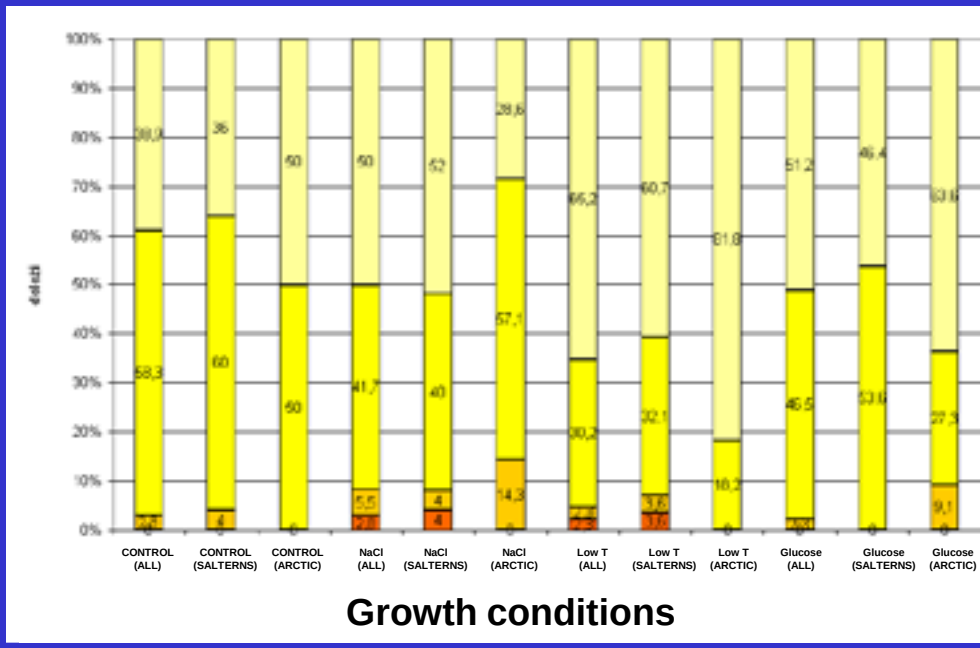
- no activity
- low act. (1-5 mm)
- moderate act. (6-10 mm)
- high act. (11-15 mm)

ANTIBACTERIAL ACTIVITY – - *B. subtilis*

ACETONE



METHANOL



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At low temperature, *Cladosporium spinulosum* synthesizes compounds with potent antibacterial activity against

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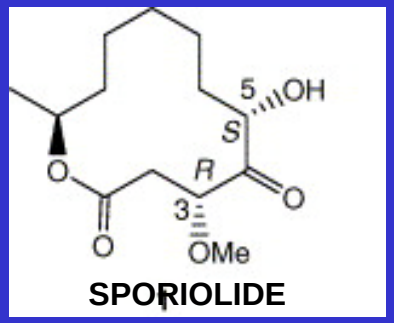
Cladosporium sp.



SPORIOLIDE

Also in:

Fusarium sp.
Wallemia sebi

Alternaria sp.

Cryptococcus sp.

Phaeoteca triangularis

Hortaea werneckii

Candida parapsilosis

Trimmatostroma salinum

Aureobasidium pullulans

Rhodosporidium diobovatum

Cryptococcus sp.

Pichia guilliermondii

UNDESCRIBED COMPOUNDS WITH ANTIBIOTIC ACTIVITY

HEMMAGLUTINATION

Only weakly present in water extracts of *Aureobasidium pullulans* and *Cryptococcus liquefaciens*

INHIBITION OF ACETYLCHOLINESTERASE

Absent in all the tested extracts

CONCLUSIONS

Selected halotolerant and halophilic species synthesize bioactive metabolites under stress-conditions (decreased a_w, low T)

The specialisation of adaptation on the level of the chemical nature of the solute that lowers a_w can be observed, e.g.:

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- several Arctic species: higher activity at low temperatures

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