Catalase activity among leptospires

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Summary. Catalase activity was measured by a quantitative method as an additional screening for discriminating between saprophytic and pathogenic leptospires. Results indicate that water-leptospires have little or no catalase activity.

Catalase has been reported to be present in leptospires[1], and differences between a group of saprophytic and a group of pathogenic leptospires with respect to catalase production have recently been shown[2], although the activity of this enzyme did not correlate either with virulence or with the antigenic pattern of the strains studied.

We investigated the possibility of using catalase activity as an additional marker, as well as 8-azaguanine resistance and growth at 13 °C[3], in the distinction between the saprophytic species *Leptospira biflexa* and the pathogenic *L. interrogans*. With this aim we employed a sensitive and quantitative assay method for catalase activity.

Leptospiral strains and cultural media. As representatives of *L. biflexa*, 19 strains of different serovars were used. Each of them was representative of a different serovar that represents a different serogroup of the species *L. biflexa*. In addition 14 new, not yet classified water-isolates were used. They are listed in tables 1 and 2. As representatives of *L. interrogans*, 18 strains of different serovars were used (table 1). In addition strain 3055 serovar *illini* and strain CH 11 serovar *andamana* were investigated; these 2 strains are considered to occupy an intermediate position, as they were isolated from mammals but behave like saprophytic forms. Strains were grown in Ellinghausen liquid medium[4], at 30 °C.

Assay of catalase activity. Leptospires were grown in 100 ml Erlenmeyer flasks containing 20 ml of medium, without shaking. When growth reached approximately 10^8 cells $\cdot$ ml^{-1}, the bacteria were harvested by centrifugation and washed in saline solution, pH 7.4. Each pellet was resuspended in saline solution in approximately $^1/_{10}$ of the initial volume and the bacterial cell density was again determined. Catalase activity was measured spectrophotometrically, essentially as described by Bellavite et al.[5], following the decrease in optical density with a Perkin-Elmer 576 spectrophotometer, at 230 nm, at 20 °C, using an extinction coefficient of 62.2 $M^{-1}cm^{-1}$,[6]. The assay medium contained: 50 mM sodium phosphate buffer, pH 7.0; 0.2% cetyltrimethylammonium bromide (CTAB) and 20 mM H_2O_2 (Merck-Darmstadt) in a final volume of 3 ml. Controls of the catalase activity were performed with a strain of *Staphylococcus aureus*.

Results and conclusions. Catalase activity of *St. aureus* was found to be 237.7 µmoles $H_2O_2 \cdot min^{-1} \cdot 10^9$cells. Table 1 reports the results of the assay of catalase activity in the strains tested. Numbers are mean values of 3 different assays. The triplicate measurements did not differ significantly. In saprophytic leptospires, *L. biflexa*, the catalase activity is very low or absent, ranging from 0 to 4.6 µmoles $H_2O_2 \cdot min^{-1} \cdot 10^9$ cells. Conversely in pathogenic leptospires, *L. interrogans*, the catalase activity is higher, ranging from 16 to 4733 µmoles $H_2O_2 \cdot min^{-1} \cdot 10^9$ cells, with the only exception of strain RGA. The enzymatic activity was totally

Table 1. Catalase activity of leptospires

Strain	Serovar	Catalase activity*
Leptospira biflexa		
AM 6	roma	1.38
AM 8	lazio	4.6
Ancona Porto	ancona	0
AR 18	tevere	0.045
Aurisina	aurisina	0
Basovizza	basovizza	2.5
Biflexa CDC	codice	0
Bulgaria 4	thracia	1.14
Cau	cau	1.05
CH 11	andamana	1.9
Dindio	dindio	9.048
Farneti	farneti	0
Isola Sacra 1	isolasacra	0
Khoshamian	khoshamian	0
Nomentano	nomentano	0
Patoc 1	patoc	0
RPE	rupino	0
Sobradinho	sobradinho	0.24
Tororo	tororo	0.117
Waz Holland	holland	1.46
Leptospira biflexa illini		
3055	illini	5.4
Leptospira interrogans		
Ballico	australis	37.62
Celledoni	celledoni	151.0
CZ 214 K	panama	49.4
Hardijoprajitno	hardijo	16.02
Hebdomadis I. Pasteur	hebdomadis	108.0
Hond Utrecht IV	canicola	72.0
Jalnà	australis	600.0
LT 821	shermani	233.1
M 20	copenhageni	54.0
Moskva V	grippothyphosa	292.8
Mus 127	ballum	323.5
PB-3	copenhageni	42.8
Perepelizin	tarassovi	1673.0
Pomona	pomona	962.86
RGA	icterohaemorrhagiae	0
Salinem	pyrogenes	630.5
Veldrat Bataviae 46	javanica	1264.7
3522 C	cynopteri	4733.7

* µmoles $H_2O_2 \cdot min^{-1} \cdot 10^9$ cells.

Table 2. Metabolic characteristics of water-isolated leptospires

Strain	Growth at 13 °C	8-AG*	Catalase activity**
Be-Noné	+	+	0
Bernamont	+	+	0.23
Chambray	+	+	0
Etang 3	+	+	4.0
Frout	+	+	0.26
Guerigny	+	+	0
Maintenon	+	+	0
Maroc II	+	+	0.3
Montargis II	+	+	0
Oved-n'Fis	+	+	0
Saada	+	+	0
Tokio	+	+	0
Thu-Duc	+	+	0
Van Lieden	+	+	0

* Growth in the presence of 200 µg/ml 8-azaguanine. ** µmoles $H_2O_2 \cdot min^{-1} \cdot 10^9$ cells.

inhibited by 0.5 mM NaN_3 or KCN (data not shown) which are known inhibitors of heme enzymes including catalase. Table 2 shows the catalase activity of 14 strains of water-isolated leptospires in course of classification in our reference laboratory, together with 8-azaguanine resistance and growth at 13 °C[3]. These strains behave as saprophytic leptospires as regards catalase activity, growth at 13 °C and 8-azaguanine resistance. Statistical analysis[7] stressed the significant difference between the 2 species *L. biflexa* and *L. interrogans* with regard to catalase activity ($F = 17.36^{**}$). In addition our data confirm the saprophytic behaviour of strain CH 11, even though it was isolated from a mammalian host. Likewise strain 3055, serovar *illini*, showed a weak catalase activity of 5.4 µmoles $H_2O_2 \cdot min^{-1} \cdot 10^9$ cells, but this value differs significantly from that for the saprophytic strains ($F = 26.69^{**}$).

It is interesting to recall in this respect that strain 3055 was isolated from a mammalian host and differs in morphological characteristics, G/C content and cultural behaviour from the two leptospiral species. These findings support the proposal to include strain 3055 in the new genus *Leptonema*[8].

According to the data presented, catalase activity can serve as an additional characteristic for the taxonomy of leptospires.

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Effects of dikegulac-sodium on negative geotropic response, endogenous tryptophan and IAA-oxidase activity in *Glycine max* roots

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Summary. Dikegulac-sodium inhibited primary and lateral root growth in *Glycine max*. The roots, curved and showed negative geotropic response after dikegulac-sodium treatment. The tryptophan level decreased and the activity of IAA-oxidase increased with increasing concentrations of dikegulac-sodium.

Dikegulac-sodium (sodium 2,3:4,6-di-O-isopropylidene-α-xylo-2-furanosonate) or Atrinal® affects most of the phases of growth, physiological processes and metabolic steps in plants[2-10]. Negative geotropic responses of radicles in *Helianthus annuus* and *Brassica campestris* have recently been established[6,7]. Negative geotropic responses would be expected if the IAA content of roots remain sub-optimal[9]. No information is yet available on the tryptophan and IAA content of dikegulac-sodium-treated ageotropically growing roots. The present report deals with it for *Glycine max*.

Seeds of *Glycine max*. L (Mill) were soaked in 15 ml distilled water or aqueous solutions of dikegulac-sodium (50–750 mg/l) of pH 7.0 in the dark for 8 h at 28 ± 2 °C. The rest of the procedure adopted has already been described[7] 6 days after the start of the experiment, the roots of half of the control seedlings were decapped by cutting of approximately 3 mm of the tips. All the roots, treated and untreated, were harvested on the 7th day after the start of the experiment. For determination of tryptophan, the root-tissue was extracted after sampling. The fresh weight of the

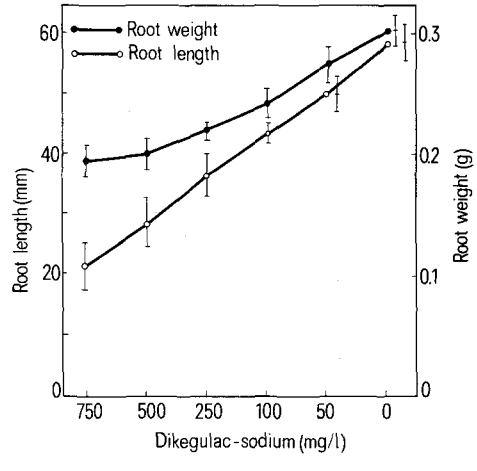

Fig. 1. Weight and length of *Glycine max* roots treated with dikegulac-sodium.

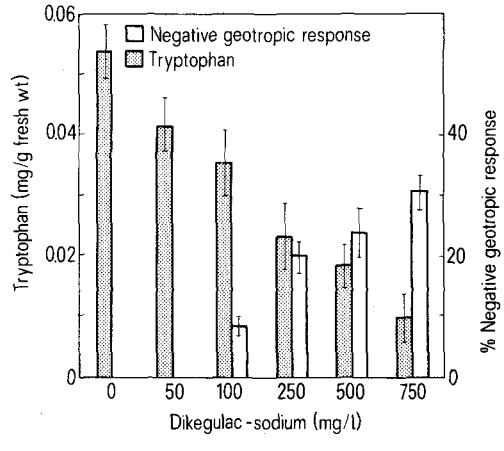

Fig. 2. Tryptophan content and negative geotropic response in *Glycine max* roots treated with dikegulac-sodium.