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ВОЗНИКНОВЕНИЕ НОВЫХ ШТАММОВ *STREPTOCOCCUS INIAE* ПРОИЗВОДЯЩИХ ЭКЗОПОЛИСАХАРИД ПОСЛЕ ВАКЦИНАЦИИ ШТАММАМИ БАКТЕРИЙ БЕЗ ЭКЗОПОЛИСАХАРИДА.

М.А. Эйнгор, доктор биологических наук, Лаборатория по болязням рыб, Ветеринарный институт им. Кимрона, Israel.

Тел. 972 3 9681643, marinae@int.gov.i

Авшалом Гурвиц, доктор биологических наук, Кибуц Дан,

Верхняя Галилея, Israel.

Тел. 972 50 5674089

Ави Эльдар, доктор биологических наук, Лаборатория по болязням рыб, Ветеринарный институт им. Кимрона, Israel.

Тел. 972 3 9681760,

Ключевые слова: *Стрептококкус иние (Streptococcus iniae), рыба, экзополисахарид (ЭПС), вакцины.*

Стрептококкус иние (Streptococcus iniae) является основной причиной смертности среди видов рыб, живущих в самых разнообразных условиях. В последнее время возобновление вспышек заболеваний были зарегистрированы у радужной форели (Oncorhynchus mykiss) в хозяйствах, где все поголовье рыбы обычно вакцинируется. Новые штаммы отличаются от предыдущих штаммов по их способности производить большое количество внеклеточного полисахарида, который выделяется в питательную среду. Полученные результаты показывают, что внеклеточный полисахарид является одним из основных антигенных факторов который определяет эволюционный отбор в пользу штаммов способных производить внеклеточный полисахарид.

Introduction.

Recently, reoccurrences of disease outbreaks were recorded in fish farms where the entire fish population was routinely vaccinated with the modified vaccine with bacteria described as *S.iniae* type II (ADH-negative strains). Diseased fish showed major pathological changes in all internal organs, and pure colonies, phenotypically undistinguishable from the previously strans were isolated on blood-agar plates from the viscera of the diseased fish; PCR analysis revealed that all current isolates are *S.iniae*.

Batch culture fermentation of vaccine-escape isolates revealed that, contrary to previously described strains new strains gave rise to a viscous culture.

Methods.

Fermentation. Screening for EPS-production by S.iniae strains was performed by batch culture fermentation.

Isolation and purification of S.iniae extracellular and capsular polysaccharide.

Characterization of S.iniae extracellular and capsular polysaccharide be gas chromatograph (GC).

Vaccination of fish and protection assays.

Measerement of Ig titers by enzyme-linked immunosorbent assay (ELISA).

Results.

GC profile of monosaccharides derived from EPS polysaccharide (top panel) and CPS (bottom panel) harvested from growth medium and cell membranes of S.iniae 477, respectively.

The composition of EPS and CPS are qualitatively identical.

The old strains produced a small amount of EPS – 3,9-5,1 μg/liter, i.e. -5 logarithmic orders less than the new strains.

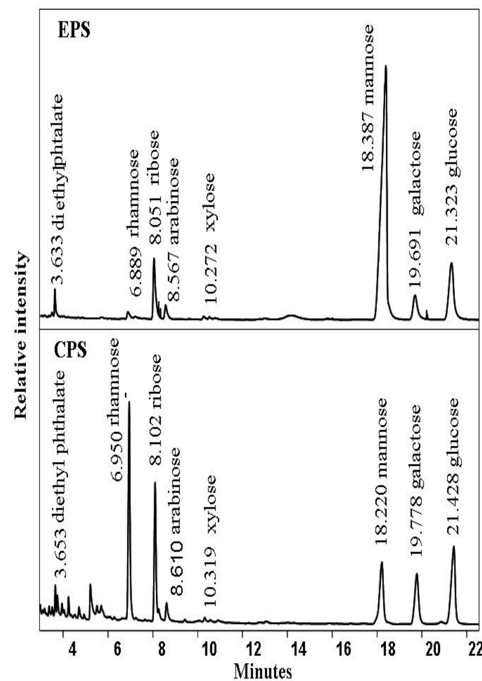

FIG. 1. GC profile of monosaccharide derived from EPS polysaccharide (top panel) and CPS (bottom panel) harvested from growth medium and cell membranes of S. iniae 477, respectively. Retention time shows sugars after hydrolysis of polysaccharide and after conversion into respective alditol acetates. Results are compared to standards to achieve semi-quantitation of the various monosaccharides constructing the polysaccharide. Main sugars are indicated with respective retention times.

EPS concentration in sera.

EPS concentration in the sera of diseased fish 65 ng/ml, while the background (non specific binding to sera of healthy fish) was of only 5ng/ml ($p<0.005$).

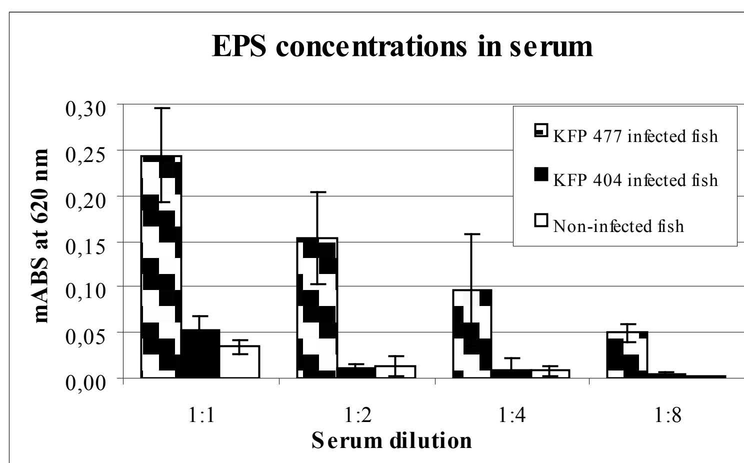

FIG. 2. EPS concentration in sera. Biotin-linked lectinsorbent assay applied to wells of microtiter wells coated with EPS purified from pooled sera obtained from four diseased and four healthy fish. Polysaccharides concentrations were determined through the Ascent software (version 2,6). Bars represent standard deviation of the mean of four separate experiments. P<0.005 (EPS obtained from sera of KFP 404 infected fish and KFP 477 infected fish versus healthy fish).

Protection after vaccination.

The experimental groups:

KFP- 404 – formalin-killed *S.iniae* type II (EPS non-producers strains) – 34% protection;

KFP- 477 – formalin-killed cells (novel strain EPS producers strains) -78% protection;

KFP- 404 – formalin-killed cells plus EPS – 82% protection;

EPC - 72% protection.

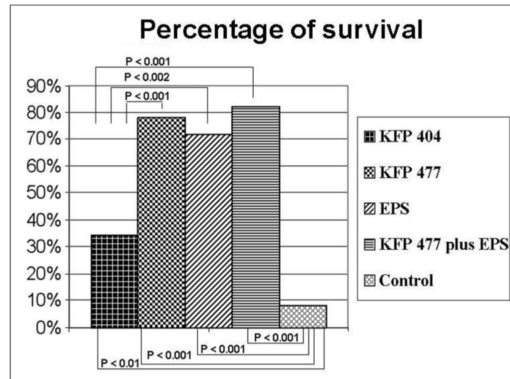

FIG. 3. Protection after vaccination. Fish (in groups of 30) were vaccinated with KFP 404 formalin killed-cells, KFP 477 formalin-killed cells, KFP 477 formalin-killed cells plus purified EPS and purified KFP 477 EPS. Challenges were carried out 8 weeks post-vaccination by intraperitoneal infection with 1.5 x10^{6} CFU (5 LD$_{50}$s) of the virulent S. iniae KFP 477 strain. Mortalities were monitored on a daily basis, for 21 days. Data are presented as the survival average from four experiments. Significance levels among all groups, including the control fish, are shown in the figure.

Development of trout antibodies to S.iniae bacterial cells (panel A),
to S.iniae extracellular polysaccharide (panel B) and to both components (panel C).

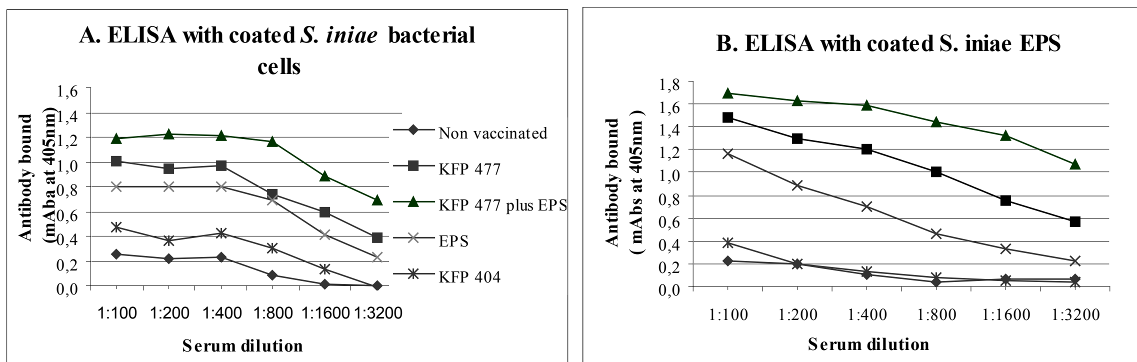

FIG. 3. Development of trout antibodies to S. iniae KFP 477 bacterial cells (panel A), to S. iniae extracellular polysaccharide (panel B) and to both components (panel C). The left y axis show the optical density in milliabsorbance versus the dilution of sera (x axis). The antibodies used for this assay were obtained from fish immunized with S. iniae KFP 477 (¾), EPS obtained from S. iniae KFP 477 culture (Ð), S. iniae KFP 477 cells plus EPS (▲), heterologous (previous vaccine) KFP 404 strain (Ú), and non vaccinated fish ().

Conclusions.

Protection rates related with the level of specific anribodies against new isolate, quantified be the ELISA assay;

The extracellular polysaccharide of new *S iniae* strains is not only a major virulence factor, but also core targets for protective immunity.