LAPORAN PENELITIAN
HIBAH PENELITIAN STRATEGIS NASIONAL
Tahun Anggaran 2009

JUDUL :

Nitrospira sp SEBAGAI KANDIDAT BIOREMIDIATOR TAHAN SALINITAS PADA BUDIDAYA UDANG SECARA INTENSIF

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UNIVERSITAS BRAWIJAYA
NOPEMBER 2009

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HIBAH PENELITIAN STATEGIS NASIONAL

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RINGKASAN

Latar Belakang. Limbah budidaya yang terbuang ke perairan apabila melebihi kemampuan asimilasi perairan dapat mencemari perairan dan menimbulkan penyuburan berlebihan (eutrofikasi) yang disebabkan oleh akumulasi bahan organic dari sisa pakan yang membusuk. Gejala ini akan menyebabkan menurunnya kadar oksigen terlarut akibat meledaknya populasi organisme tertentu sehingga dapat menimbulkan kematian beberapa organisme perairan. Perombakan nitrogen secara biologi melalui proses nitrifikasi dan denitrifikasi merupakan metoda yang dianggap paling efisien, dibandingkan dengan metoda fisika atau kimia. Permasalahan utama pada pengolahan limbah nitrogen secara biologis adalah bakteri autotropik nitrifikasi, pelaku proses nitrifikasi, selain sangat sensitif terhadap faktor lingkungan, bakteri ini tumbuh lebih lambat dibandingkan dengan bakteri heterotropik yang ada dalam lumpur aktif sehingga efisiensi nitrifikasi dari sistem biologis menjadi sangat rendah. Dalam proses nitrifikasi terjadi dua tahapan reaksi, yaitu oksidasi amonium menjadi nitrit, pada umumnya dilakukan oleh kelompok Ammonia Oxydizer Bacteria (AOB) dan oksidasi nitrit menjadi nitrat oleh kelompok Nitrite Oxydizer Bacteria (NOB).

Akhir-akhir ini penggunaan bioteknologi yang dinamakan "bioremidiasi" atau "bacterial augmentation" mendapat perhatian yang tinggi karena merupakan pendekatan yang ramah lingkungan untuk meminimalkan degradasi lingkungan. Pengembangan produk probiotik (bioremidiator) unggul untuk budidaya udang yang berasal dari isolat dari tambak perlu dilakukan. *Nitrospira* sebagai NOB sangat melimpah diberbagai habitat mulai dari darat sampai laut bahkan pada limbah. Hal ini mengindikasikan bahwa *Nitrospira* sangat kompetitif dan mudah beradaptasi serta memegang peranan penting dalam siklus nitrogen diberbagai ekosistem. Mengingat pentingnya *Nitrospira* sebagai NOB untuk mengontrol aktifitas oksidasi nitrit pada lingkungan budidaya udang maka studi tentang karakteristik *Nitrospira* perlu dilakukan

Tujuan dan manfaat. Penelitian ini dirancang untuk: (1) mengisolasi dan identifikasi *Nitrospira* yang berasal dari sedimen tambak udang intensif, (2) mendapatkan media tumbuh yang sesuai untuk bakteri *Nitrospira,* (3) menguji ketahanan bakteri *Nitrospira* terhadap fluktuasi salinitas dan (4) mempelajari aktifitas *Nitrospira* sebagai NOB secara in-vitro. Sedangkan manfaat dari penelitian ini dapat sebagai penunjang pengembangan ilmu dan informasi bagi pengelolaan udang yang berkelanjutan.

Materi dan Metoda. Sampel sedimen pada petakan tambak dilakukan pada tiga titik mulai dari tepi sampai ke tengah dengan menggunakan soil bottle sampler pada kedalaman 1-5 cm. Tekstur sedmen ditentukan berdasarkan fraksinasi, sedangkan untuk bahan organik dilakukan berdasarkan metode Weende dan Walkey & Black dengan mencampur semua titik sampling.

Isolasi dan identifikasi bakteri: Teknik most probable number (MPN) yang digunakan untuk analisis NOB pada sampel sedimen dengan media modifikasi Winogradsky. Tabung inokulasi diinkubasi selama 5-15 hari pada suhu 30 ± 2°C. Warna dan ukuran morfologi dan sifat gram koloni yang tumbuh pada PCA diamati. Koloni-koloni tersebut kemudian dimurnikan melalui Sub Culture Technique (SCT) dalam tabung reaksi, dan disimpan pada suhu refrigerator hingga dilakukan analisis lebih lanjut. Pengujian sifat bikimia dilakukan dengan menggunakan Microbact. Untuk PCR DNA, isolasi dilakukan

berdasarkan Ehrich *et al*. (1995) dengan menggunakan 16S rRNA primer (5' CCTGCTTTCAGTTGCTACCG 3') dan (5' GTTTGCAGCGCTTTGTACCG 3'). Aplifikasi PCR dilakukan berdasarkan Coskuner & Curtis (2002) dan Dionisi et al. (2002)

Media kultur: Medium yang mengandung campuran senyawa anorganik digunakan sebagai dasar untuk pengkayaan khusus untuk media tumbuh Nitrospira. Sedangkan untuk uji ketahanan terhadap salinitas dilakukan cara meningkatkan konsentrasi salinitas 2 ppt per hari dari 10 ppt sampai dengan 30 ppt

Profil protein : Protein dideterminasi dengan cara colorimetri (Biuret), Protein dikarakterisasi menggunakan SDS-PAGE (Sundermeyer-Klinger *et al.*. 1984) untuk mendapatkan profil protein berdasarkan berat molekul.

Pengujian aktifitas nitrifikasi: Untuk menguji aktifitas nitrifikasi dilakukan secara in vitro dengan media buatan yang sudah diketahui konsentrasi nitritnya dan diinokulasi dengan bakteri Nitrosomonas dan Nitrospira sebagai bakteri nitrifikasi.

Hasil Penelitian: Tekstur sedimen yang diamati menunjukkan kelas lempung, lempung berpasir dan liat berdebu. Tekstur tanah sangat dipengaruhi oleh komposisi dari fraksi liat, debu dan pasir serta kesuburan tanah sangat ditentukan oleh mineralnya. Berdasarkan tekstur ini tergolong layak untuk lahan budidaya tambak. Kandungan bahan organik melebihi 12,5% dapat berdampak pada penuruan pertumbuhan udang sehubungan dengan pembentukan CH4. Dari uji katalase Nitrospira tergolong bakteri aerob dengan bentuk sel koma dan spiral, warna putih dengan sifat non motile. Kepadatan bakteri yang terisolasi sebesar 2,1 x 10^4 CFU /g. Sedangkan dari uji PCR ditunjukkan mempunyai pasang basa (base par) sebesar 151 dan 119 bp. Media tumbuh yang cocok untuk Nitrospira yang mengandung mineral nitrit yang diperkaya.

7.25 mmol sodium nitrite, 0.07 mmol calcium carbonate,8.56 mmol sodium chloride,0.25 magnesium chloride, 0.86 mmol potassium phosphate, 0.15 µmol magnesium sulfate, 0.79 µmol boric acid, 0.15 µmol zinc sulfate, 0.03 µmol molybdic acid, 0.10 µmol cupric sulfate and 3.50 µmol ferrous sulfate yang dilarutkan dalam campuran air destilasi dan laut.

Total bakteri aerobik dipengaruhi oleh total nitrit dalam substrat. Ada korelasi positif antara nitrit dalam substrat dan total bakteri. Hal ini menunjukkan bahwa melimpahnya bakteri nitrifikasi pada lingkungan sangat tergantung pada substrat.

Total bakteri menurun secara eksponensial dengan meningkatnya salinitas dalam media, Bakteri menurun sebesar 0,21 unit dengan meningkatnya satu unit salinitas. Walaupun pada salinitas yang tinggi bakteri tersebut masih mampu untuk bertahan.

Berdasarkan hasil elektroforesis dengan menggunakan marker protein fermentan didapatkan profil protein dari Nitropira dapat ditunjukkan dengan berat molekul pita yang terbentuk dengan nilai 45,09; 48,37; 52.51; 67,94; 72,88 dan 92,12 kDa.

Uji bakteri Nitrospira terhadap suhu dan pH dilakukan untuk mendapatkan suhu dan pH optimum. Aktivitas bakteri tertinggi ditemukan pada suhu 33-35°C. Pengaruh pH juga menunjukkan bahwa aktifitas tertinggi untuk Nitrospira pada pH 8,0. Dari uji nitrifikasi secara invitro didapatkan bahwa terjadi proses nitrifikasi yang ditandai dengan penurunan TAN sebesar 1,24 mgN/l $hari^{-1}$. Semua TAN yang ditambahkan teroksidasi selama 9 hari. Dilain sisi kadar nitrit kurang dari 1 mg NO2-N l^{-1} .Sementara konsentrasi nitrat meningkat 1,02 mg NO3-N $hari^{-1}$. laju oksidasi amonia telah melampaui oksidasi nitrit dengan hanya margin kecil untuk menghasilkan akumulasi nitrit.

Kesimpulan dan saran: Nitrospira dapat diidentifikasi dari sedimen tambak udang intensif; media yang diperkaya dapat digunakan sebagai media tumbuh untuk Nitrospira; Nitrospira dapat tumbuh pada kisaran salinitas 10-30 ppt, walaupun ada penurunan secara eksponensial dengan meningkatnya salinitas; laju aktivitas nitrospira sebagai NOB dipengaruhi oleh suhu dan pH (33^{o}C dan pH 8); aju penurunan TAN sebesar 1,24 mg TAN/l/hari dan konsentrasi nitrat meningkat sebesar 1,05 mg NO3-N /hari dengan inokulasi bakteri. Dalam penelitian ini dapat disarankan dilakukan sequencing DNA Nitrospira untuk mendapatkan ecophysiologynya dan perlu isolasi dan pengujian aktivitas enzim spesifik untuk menjelaskan peranannya dalam proses nitrifikasi.

SUMMARY

Background. Nitrification is a key process of the biogeochemical nitrogen cycle in natural and engineered habitats. The two steps of aerobic nitrification, the oxidation of ammonia to nitrite and subsequently from nitrite to nitrate, are catalysed by two distinct functional groups of chemolithotrophic prokaryotes: the ammonia-oxidizing bacteria (AOB) and the nitrite-oxidizing bacteria (NOB). So far, most genomic and physiological data originate from a few "model" nitrifiers, in particular AOB related to the genus *Nitrosomonas* and NOB of the genus *Nitrobacter*. Among the fastidious and slow growing nitrifiers these two organisms stand in the forefront of isolated microorganisms. However, cultivation-independent approaches have revealed a huge diversity of poorly characterized AOB and NOB in a variety of ecosystems. Moreover, most of these yet uncultivated nitrifiers seem to represent nitrifying key players in different environments. Hence, our current understanding of nitrification and the biogeochemical nitrogen cycle is far from being complete. Beside these direct toxic effects of several nitrogen-compounds on living organisms, raised deposition of anthropogenic nitrogen compounds into natural habitats causes fatal ecological damages. Nitrogen deposition caused an ecological imbalance into many ecosystems and the effects would have been even more dramatic without the high buffering capacity of nitrogen cycling microorganisms. However, to effectively monitor ecological changes due to anthropogenic pressure a better understanding of the mechanisms behind the global nitrogen cycle is urgently needed. It is important to understand the nitrogen cycle at a range of scales of biological organizations, beginning with the vast microbial diversity involved in nitrogen conversion and their interactions. The following sections will focus on nitrite-oxidizing bacteria (NOB) of the genus *Nitrospira*, one of the aforementioned key players in the nitrogen cycle but yet less intensively studied groups of NOB.

The purpose and benefits. This research is designed to: (1) isolate and identify of indigenous Nitrospira from intensive shrimp pond sediment, (2) a media suitable for growing Nitrospira, (3) to test Nitrospira that resistance to salinity fluctuations and (4) learning activities as NoB Nitrospira in in-vitro. While the benefits of this research are able to support the development of science and information for the sustainable management of shrimp culture.

Materials and Methods. Shrimp pond sediment was taken from three point sampling from the edge to the middle by using the soil bottle sampler at a depth of 1-5 cm. Sediment texture is determined by fractionation, while organic matter content was measured according to Weende and Walkey & Black methods.

Isolation and identification of bacteria: Technical most probable number (MPN) which is used for analysis of sediment NOB with Winogradsky medium modifications. Inoculation tubes incubated for 5-15 days at a temperature of 30 ± 2 ° C. Morphology, color,size, colonies and gram stanning of PCA was observed. The colonies were then purified through a Sub Culture Technique (SCT) in a test tube, and stored at refrigerator until further analysis carried out. Biokimical test was conducted by using Microbact. For DNA PCR, isolation is based on the Ehrich et al. (1995) by using 16S rRNA primers (5 'CCTGCTTTCAGTTGCTACCG 3') and (5

'GTTTGCAGCGCTTTGTACCG 3'). Amplification of PCR was run based on Coskuner & Curtis (2002) and Dionisi et al. (2002).

Culture media: Medium containing a mixture of inorganic compounds used as the basis for specific enrichment of Nitrospira grow. Fluctuation of salinity was performed by added NaCl for 2 ppt per day until 30 ppt in the media culture.

Profile of protein: Protein determined by colorimetri (Biuret), protein characterized using SDS-PAGE and electrophoresis (Sundermeyer-Klinger et al .. 1984) to obtain protein profiles based on molecular weight.

Nitrification activity: Nitrification activity was done by in vitro test with artificial media of known concentration of nitrite and in the same time nitrification (Nitrosomonas and NItrospira) bacteria was inoculated.

Results: Textures of sediment indicates the clay, sandy clay and clay dusty. Soil texture was influenced by the composition of the clay fraction, dust and sand and soil fertility is determined by the mineral. Based on this texture quite worthy of cultivation pond. Content of organic material exceeds the 12.5% reduction can impact on shrimp growth in relation to the formation of CH4. From the catalyze test, Nitrospira as a aerobic bacteria belonging to the form of commas and spiral cells, white color and non-motile. The isolated bacterial density of Nitrospira is 2.1 x 104 CFU g^{-1}. Whereas the PCR test has shown base pairs for 151 and 119 bp. Media suitable for growing Nitrospira-containing minerals enriched nitric are 7.25 mmol sodium nitrite, 0.07 mmol calcium carbonate,8.56 mmol sodium chloride,0.25 magnesium chloride, 0.86 mmol potassium phosphate, 0.15 µmol magnesium sulfate, 0.79 µmol boric acid, 0.15 µmol zinc sulfate, 0.03 µmol molybdic acid, 0.10 µmol cupric sulfate and 3.50 µmol ferrous sulfate diluted by mixed distillated and salt water. Total aerobic bacteria is influenced by the total nitrite in the substrate. There was a positive correlation between nitrites in the substrate and the total bacteria. The total nitrification bacteria significantly depend on the substrate concentration. On the other hand the total bacteria exponentially decrease by the increases of salinity in the culture media. Total bacteria count reduce by 0.21 units with one unit salinity increase. Despite the high salinity, bacteria are still able to survive.

Based on the results of electrophoresis of proteins using fermentan marker, protein profiles obtained from Nitropira can be shown with molecular weight bands formed with the value of 45.09; 48.37; 52.51; 67.94; 72.88 and 92.12 kDa.

Test of temperature and pH resistances were conducted to obtain optimum temperature and pH in the nitrification prosesses. The highest bacterial activity was found at a temperature of 33-35°C. The influence of pH also showed that the highest activity for Nitrospira at a pH of 8.0. The nitrification process was occurred it can be seen by the reducing of TAN in the media 1.24 mg N $l^{-1}day^{-1}$ All the added TAN oxidized for 9 days. On the other side of the nitrite content of less than 1 mg NO2-N l^{-1}. While nitrate concentration increased 1.02 mg NO3-N $l^{-1}day^{-1}$ day-1. Ammonia oxidation rate has exceeded the oxidation of nitrite with only a small margin to produce the accumulation of nitrite

Conclusions and recommendations: Nitrospira can be identified from the sediments of intensive shrimp ponds; enriched media can be used as a medium culture for Nitrospira; Nitrospira can grow in the range of 10-30 ppt salinity, although there was an exponential decline with increasing salinity; nitrospira activity rate as influenced NOB by temperature and pH (33^{o}C and pH 8); decrease rate of TAN by 1.24 mg $l^{-1}day^{-1}$ and increased

concentrations of nitrate by 1.05 mg NO3-N $l^{-1}day^{-1}$ with bacterial inoculation. In this research can be carried out DNA sequencing is recommended for ecophysiology and isolation of specific enzyme activity of Nitrospira to explain its role in the nitrification process.

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