

Bioconversion of Heavy Hydrocarbon Cuts Containing High Amounts of Resins by Microbial Consortia

Ghollami M^{1*}, Roayaei M¹, Ghavipankeh F² and Rasekh B³

¹Biology Department, Shahid Chamran University, Ahwaz, Iran

²Environment Group, Energy Department, Materials and Energy Research Center, Alborz, Iran

³Microbiology and Biotechnology Group, Research Institute of Petroleum Industry, Tehran, Iran

Abstract

Microbial conversion of petroleum resins existing in a heavy fraction of crude oil was investigated by native isolated consortia. The heavy oil fraction was taken from an end cut of distillation tower of Abadan refinery in Iran. Several Soil samples were taken from different oil contaminated sites of Abadan oil refinery in Khuzestan province. Seven bacterial consortia were then prepared by enrichment method and were examined for their ability to convert resins. Amongst them, consortium AB6 reduced 37.3% of resins while, aliphatic and aromatic compounds increased by 86.8% and 6.7%, respectively. Three pure cultures were isolated from consortium AB6 which were identified as *Serratia* sp., *Raoultella* sp. and *Ochrobactrum* sp.

Keywords: Biological conversion; Resins; *Serratia*; *Raoultella*; *Ochrobactrum*

Introduction

Quality Improvement of crude oils has been one of the important problems in the recent 60 years. There are some chemical and physical methods for crude oil upgrading. But, due to high cost of the current oil processes together with their safety and environmental problems, attention to affordable and environmentally acceptable methods has gained significant importance [1,2]. In this regard, biotechnology as well as special ability of microorganisms for production of a wide range of biochemical materials has been an important technique in overcoming the defects of the other methods [3,4].

Crude oil is a mixture of hydrocarbons containing hydrogen, carbon and lesser amounts of oxygen, sulphur and nitrogen plus some trace metals such as vanadium, nickel, copper and iron [5]. The hydrocarbons can be divided into aliphatic or saturates (including paraffins, isoparaffins and cycloparaffins), aromatics, resins and asphaltenes. Short chain paraffins are more degradable substrates for microorganisms compared with long chain paraffins, isoparaffins, cycloparaffins, aromatics, resins and asphaltenes. Resins and asphaltenes contain several heteroatoms per molecules and typically account for 30-60% of the total sulphur, 70-90% of total nitrogen and 80-90% of the total vanadium and nickel present in a crude oil [6,7]. Resins are large molecules containing polar groups with molecular weights substantially lower than those of asphaltenes. Their percentage in heavy oil fractions is higher than that of asphaltenes which is more in bitumen.

Resins and asphaltenes are less biodegradable and insufficient literatures are presented in this field [8]. Venkateswaran et al. [9] found a consortium consisted of *pseudomonas* sp and several un-named strains, which could degrade 35% of resins fractionated from crude oil, presenting the first results on biological degradation of resins as the authors claimed. In 2006, Ghavipankeh et al. [10] investigated the biological conversion of a heavy hydrocarbon cut named as Isomax feed (a heavy end of vacuum distillation column) by mixed cultures. Their results showed a reduction of more than 10% of the resin fraction of the feed [10]. Heavy fractions of crude oil and distillation residues contain more amounts of resins and asphaltenes [11]. However, there is a trend towards the use of heavier feedstock, which requires additional

processes based on the destruction of heavy petroleum compounds and lead to an increase in the portion of light fractions in feedstock [12].

Middle East region makes up only 1% of Earth's surface. However, it contains 65.7% of the world's oil content. Iran is the first oil-rich country in this region with production capacity of 4 million barrels per day of crude oil and diesel fuel [13]. Khuzestan province contains massive oil and gas reservoirs and so is the most important oil region in Iran. However, most of the crude oils of the region contain a high level of resins. This makes process difficulties, clogging of transportation pipelines and hard environmental deterioration problems when spilled out.

Different reports during recent decades have shown that microbial consortia are more effective on degradation and conversion of hydrocarbons, compared with individual strains [9,14,15]. In this research, different soil samples were taken in order to investigate the effect of native consortia on bioconversion of petroleum resins. This could lead to biological upgrading of heavy petroleum fractions and help in removal of environmental oil spills. Resin Compounds are adhesive hard solids that precipitate in the bottom of distillation tower or in oil wells leading to oil recovery decreases. This paper investigates the biological conversion of resin using native mixed cultures and identifying the types of the bacteria involved in the process.

Materials and Methods

Samples and feed

Seven soil samples were collected from oil contaminated sites of Abadan oil refinery in Khuzestan province and named as AB1, AB2,

***Corresponding author:** Mahboobeh Ghollami, Biology Department, Shahid Chamran University, Ahwaz, Iran, Tel: 021- 44350761; Fax: 021- 44216661; E-mail: m.ghollami@yahoo.com

Received March 16, 2012; Accepted April 19, 2013; Published April 26, 2013

Citation: Ghollami M, Roayaei M, Ghavipankeh F, Rasekh B (2013) Bioconversion of Heavy Hydrocarbon Cuts Containing High Amounts of Resins by Microbial Consortia. J Pet Environ Biotechnol 4: 139. doi:10.4172/2157-7463.1000139

Copyright: © 2013 Ghollami M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

AB3, AB4, AB5, AB6 and AB7. A Heavy oil fraction containing a high percentage of resin compounds was taken from an end cut of distillation tower of Abadan oil refinery and used as the hydrocarbon feed for enrichment of microorganisms. The characteristics of the heavy end feed are shown in Table 1.

Enrichment and bacterial growth

Batch cultures were conducted in 250 ml flasks containing 5% (w/v) hydrocarbon feed, 10 ml of supernatant prepared from each soil sample (10% (w/v) soil in distilled water) and a mineral salt medium (MSM) consisting of K_2HPO_4 2.75 g/l, KH_2PO_4 2.25 g/l, $(NH_4)_2SO_4$ 1 g/l, $MgSO_4 \cdot 7H_2O$ 0.2 g/l, NaCl 0.1 g/l and $FeCl_3 \cdot 6H_2O$ 0.02 g/l. The pH of the medium was adjusted at 6.8 ± 2 . The flasks were kept in a shaker incubator at 30°C and 150 rpm for 10 days. The inoculation procedure from the previous flasks was repeated three times for preparation of the enrichment cultures. The enrichment cultures were named as AB1, AB2, AB3, AB4, AB5, AB6 and AB7.

Duplicate batch cultures were conducted from the last enrichment cultures in contact with the heavy oil feed as the same conditions as above, in order to investigate the biological conversion of the feed. Also, a flask was prepared without microorganisms as the control sample. All the flasks were incubated at 30°C and 150 rpm for 10 days. The optical densities of the aqueous phases of the cultures were measured along time in order to investigate the growth of the microorganisms. At the end of the incubation, the samples were used for further investigations as follows.

Sample selection and bacteria isolation

Best sample selection was made upon bacterial growth and visual observations of the organic phases compared to that of the control sample. Strains of the consortia were isolated considering their bioconversion capacity. Selected isolates were purified by pour plate method and kept in refrigerator for further experiments.

Hydrocarbon analysis

After bio-treatment of the feed, the oil phases were separated from the aqueous phases in order to analyze the bioconversion of the organics especially the resins by the enriched cultures [16]. In this method, normal pentene was used as the solvent of the organic phase because of its ability to solve the organic phase and different molecular weight with water and its evaporation point is also low. So, it could be easily separated from the organic phase. 15 ml of pentane was added to sample in a decanter funnel and shake vigorously and then let to settle for 45 minute. The oil and aqueous phases were then separated. The procedure was repeated twice. The extracted organic phases were then dehydrated by passing through dehydrated $MgSO_4$ and evaporated in a water bath for the separation of the solvent. The separated bio-treated organic phases plus the control sample were divided into four defined

fractions: Saturates (aliphatics), Aromatics, Resins and Asphaltenes (SARA fractions). The asphaltenes fraction was determined by precipitation with n-pentane according to ASTM method D-893. The remained organic phases after asphaltene separation were conveyed on adsorption chromatography to separate aliphatic, aromatic and resin fractions of the oils. The procedure was made according to ASTM D2549 with some modifications [10,17,18]. This was achieved by using n-pentane solvent (for elution of aliphatics), diethyl ether (for elution of aromatics) and, chloroform and ethanol (for elution of resins). An optical test of TLC paper under UV light was done at the same time for identification of hydrocarbon fractions during elution. A spot of the eluted on TLC paper would turn to purple under UV light by the aromatics while making no color for the aliphatics. The fractions were then evaporated to remove the solvents and weighed. The aliphatic, aromatic and resin fractions of the bio-treated oils were compared to that of the control sample.

Resin analysis

In order to further inspection of the effect of native cultures on the resin contents of the heavy end feed, the separated resin fractions were divided into soft resins and hard resins. The resin fractions were dissolved in 15 ml of a mixture of isobutyl alcohol and cyclohexane (80:20) in which the hard resins were precipitated. The soft resins were then precipitated in 12 ml isobutyl alcohol [6].

GC-MASS analysis

The separated bio-treated organic phases of the selected consortia as well as the control sample were analyzed for the compositional changes using gas chromatography mass spectroscopy (GC-MASS). Here, Agilent instrument model 7890A equipped with a mass selective detector model 5975C and column, BP-5 (50m×0.25mm) was used with initial temperature 50°C, final temperature 280°C, ramp rate 5°C per min and helium as the carrier gas at 20 ml/min.

Identification of the isolates

The pure cultures isolated from the selected consortium were identified by several morphological, biochemical and phylogenetic methods [13,19-21]. The biochemical tests were include Gram stain, Methyl Red test, Voges-Proskauer test, Citrate test, Acid production (TSI), Urease, Indole production, Motility, producing hydrogen sulfide, and lysine decarboxylase. The molecular identification of bacteria was done by amplification of gene 16s rRNA by universal primers. The sequences (5'-3') of the forward and reverse primers were: AAGAGTTTGATCATGGCTCAG and AGGAGGTGATCCAACCGCA, respectively [22]. The PCR program for amplification of gene was 94°C the temperature of denaturation for 1 min, 62°C the temperature of bonding primers for 40 second and 150 second at 72°C. The number of amplification cycles was 30 [23]. After that the DNA sequence data were analyzed using BLAST-N (Local alignment search tool) [24].

Results and Discussion

Enrichment and bacterial growth

After 3 times sub-culturing of the samples at 30°C and 150 rpm, the visual observations were made on the bio-treated samples compared to the control sample. Amongst the seven samples, four samples had imposed obvious changes on the organic phases, while causing turbidity in the aqueous phases. These samples included AB4, AB5,

Parameter	Value
Initial boiling point (°C)	384
Final Boiling Point (°C)	575
Sulfur (ppm)	18863
Carbon (Wt %)	65
Iron (ppm)	3.5
Nickel (ppm)	5.2
Resin (%)	48.95
Asphaltene (%)	7.37
Viscosity (g/cm ³)	0.597

Table 1: The characteristics of the heavy end feed.

AB6, and AB7 Figure 1 shows the growth curves of the two selected consortia (AB4 and AB6) in the presence of the heavy end cuts.

Bacterial isolation

The isolated bacteria of the selected consortia were: 4 strains from AB4, 2 strains from AB5, 3 strains from AB6 and 3 strains from AB7.

Hydrocarbons analysis

The results of the gravimetric measurement of SARA fractions of the selected bio-treated samples are shown in Table 2. According to the table, the aliphatic fractions of the all bio-treated samples are increased compared with the control sample. Also, the aromatic fractions of AB4 and AB6 are increased compared with the control sample. The resin fractions of all bio-treated samples are decreased compared with the control sample but are more significant in AB4 and AB6. This emphasizes the ability of consortia AB4 and AB6 in declining the resin contents of the heavy end cuts as we expected. Also, the increase of aliphatics and aromatics are also a good sign. Considering the resin compounds reduction, we can say the enhancement in aliphatics and aromatics fractions amount that are lighter compounds than resins is probably due to conversion and changes of resin compounds.

Resin analysis

The resin compounds fractionated from the control and the superior bio-treated samples (ABR4 and ABR6) were separated into soft and hard resins. The results are shown in Table 3, which can be seen that the hard resins are entirely removed after bio-treatment of the feed. Though, 14.4% of total resins of the control sample are the hard resins. This indicates that larger molecules are converted to lighter ones via biological process.

GC-MASS analysis

The GC-MASS analyses of the mixtures of aliphatic and aromatic fractions of AB6 and the control sample are shown in Figure 1. As reveals from the figures, the differences between the chromatograms are not clearly obvious due to high complexity of the compounds. Some of the compounds are shown in the figures, as could be seen the peaks heights are changes.

The analysis of the GC-MASS digital library data revealed some differences as indicated in Table 4. The table shows that some compounds are disappeared from the control sample, while some others are appeared in the bio-treated samples. For example, toluene

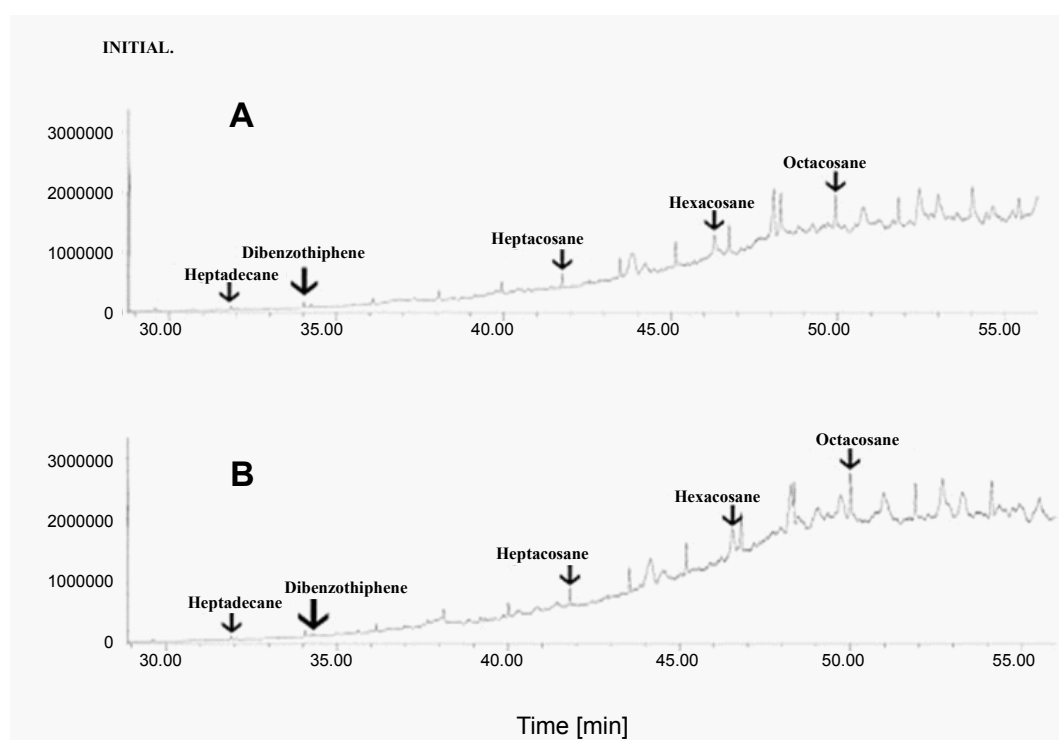


Figure 1: A: GC-MASS chromatogram of the mixture of aliphatic and aromatic fractions of the control sample. B: GC-MASS chromatogram of the mixture of aliphatic and aromatic fractions of AB6 sample.

	Hydrocarbon fractions						
	Saturates		Aromatics		Resins		Asphaltenes
Sample name	% in sample	% of changes	% in sample	% of changes	% in sample	% of changes	% in sample
AB4	26.85	49.75	30.09	18.09	34.58	-27.93	8.48
AB5	26.6	48.35	19.59	-23.12	45.21	-5.77	8.60
AB6	33.51	86.89	27.20	6.75	30.07	-37.3	8.51
AB7	23.99	33.8	23.15	-9.14	44.41	-7.44	8.45
Control	17.93	-	25.48	-	47.98	-	8.61

Table 2: The amounts of SARA fractions in the biotreated oil samples and the percent of changes compare to the control.

Sample name	Hard resin (%)	Soft resin (%)
Control	14.4	85.6
ABR4	0	100
ABR6	0	100

Table 3: The amounts of hard resins and soft resin of the resin fractions.

Time	Compounds	% in control sample	% in treated sample
4.014	1-propanol, 2- methyl	—	0.22
4.025	Propene, 2-methyl-3-Propoxy	9.59	—
9.856	Pentadecane	0.95	—
9.879	Toluene	—	0.54
28.630	Tetradecane	—	0.20
28.933	Dibromotetradecane-1-01acetate	0.26	—
36.028	Heptacosane, 1-chloro-	—	0.35
36.097	Dibenzothipene, 4-methyl-	0.54	—
36.795	Imidazo(4,5-f)quinolone,2-amino-3-methyl-	2.68	—
36.961	Anthracene, 9, butyl tetradecahydro	—	0.34
39.856	Octadecane	—	0.37
39.856	Cyclotriacontane	—	—
39.953	Phenanthrene, 3,6-dimethyl	1.25	—
41.756	Heptacosane, 1-chloro-	1.35	—
41.807	Triacotane	—	3.04
45.149	Hexacosane	2.86	—
45.206	Octacosane	—	5.63
52.439	9,19-cyclolanost-23-ene-3, 25-diol,	15.00	—
52.679	3-acetate	—	12.32

Table 4: The compositional changes of the mixture of aliphatic and aromatic fractions of the sample treated by AB6 compared with that of control, according to GC-MASS Digital library.

Strains			
Characteristics	AB6S1	AB6S2	AB6S3
Morphology	Bacilli	Cocobacill	Cocobacill
Gram stain	-	-	-
Urea hydrolysis	-	-	-
Citrate	+	+	+
MR	+	+	-
VP	-	-	+
TSI	Alk-Alk	Alk-Alk	Acid-Acid
Lysine decarboxylase	+	+	+
Indole	-	+	-
Hydrogen sulfide	-	-	-
Motility	+	-	-

Table 5: Biochemical characteristics of the isolated strains of AB6 consortium.

and tetradecane did not exist in the control but appeared in the bio-treated sample by AB6. Also, the amounts of short chain alkanes were decreased compared to the control. However, the amounts of alcoholic compounds, alkanes and polycyclic compounds were increased compared to the control.

Identification of the isolates

Three pure cultures were isolated from consortium AB6 as the best native consortium on conversion of resins. The biochemical characteristics of the isolated organisms were shown in Table 5. The strain AB6S1 produced red pigments on nutrient agar plates with a weak elevation after 24 hours of incubation. They were rod shaped, aerobic, gram negative and motile. They were also citrate positive, decarboxylases lysine, indole negative and hydrogen sulfide negative. Morphologically, the colonies of AB6S2 were isolated were pigmented in shades of green and cells were also round and small. The bacteria were citrate positive, decarboxylases lysine, indole positive, hydrogen sulfide negative, non- motile, aerobic, and gram negative. Another

isolate, AB6S3 produced colonies, which were cream, circular, flat and rough edges. Microscopically, they were seen as gram negative and non-motile. The bacteria were citrate positive, indole negative, hydrogen sulfide negative and decarboxylases lysine. The results revealed the genus of the bacteria.

Conclusions

This research investigated the biological conversion of a heavy end cut containing high amounts of resins by means of native bacterial consortia. Seven consortia were adapted from which consortium AB6 had the highest activity on the feed. The analytical results on the bio-treated oil showed that the amounts of aliphatics and aromatics were increased by 86.9% and 6.7%, respectively. Also, the amounts of resins were decreased by 37.3%. This could be due to elimination of heteroatoms in molecules of resins by the bacteria that breaks the large molecule. Also, further analysis of the resins content of the feed showed that all the hard resins were eliminated after bio-treatment and the remained resins were all in the class of soft resins. This reinforces the idea of cleavage of hard resins into smaller molecules by bacteria, which again could be due to elimination of heteroatoms from the molecules.

The isolates of consortium AB6 were identified as *Serratia sp.*, strain PBCC12 (Gen bank Accession No, JN566121.1), *Raoultella sp.*, strain PBCC13 (Gen bank Accession No, JN566122.1) and strain PBCC14 (Gen bank Accession No, JN566123.1) *Ochrobactrum sp.*

Acknowledgements

This work was supported by Materials and Energy Research Center under a M.Sc. Thesis. The authors are so thankful to Mr. Shahab Aldin Zarekhafr for him numerous helps during the whole states of experiments.

References

- Simanzhenkov V, Idem R (2003) Crude Oil Chemistry. CRC Press Newyork USA.
- Mashreghi M, Marialigeti K (2005) Characterization of Bacteria Degrading Petroleum Derivatives Isolated from Contaminated Soil and Water. Journal of Sciences, Islamic Republic of Iran 16: 317-320.
- Das N, Chandran P (2011) Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int.
- Obire O, Nwaubeta O (2001) BioDegradation of Refined Petroleum Hydrocarbons in Soil. J Appl Sci Environ Mgt 5: 43-46.
- Grishchenkov VG, Townsend RT, McDonald TJ, Autenrieth RL, Bonner JS, et al. (2000) Degradation of petroleum hydrocarbons by facultative anaerobic bacteria under aerobic and anaerobic conditions. Process Biochem 35: 889-896.
- Speight JG (2002) Handbook of Petroleum Product Analysis. John Wiley & Sons, Inc. USA.
- Gailiute I, Grigiskis S, Zekaite G, Cipinyte V (2011) Selection of Microbes and Conditions That Induced Bio-Cracking of Branched Hydrocarbon Squalane. Environment Technology Resources 1: 46-52.
- Van Hamme JD, Singh A, Ward OP (2003) Recent Advances in Petroleum Microbiology. Microbiol Mol Biol Rev 67: 503-549.
- Venkateswaran K, Hoaki T, Kato M, Maruyama T (1995) Microbial degradation of resins fractionated from Arabian light crude oil. Can J Microbiol 41: 418-424.
- Ghavipanjeh F, Pazouki M, Shayegan J, Hosseinnia A (2006) Evaluation of microorganism utilization in cracking of ISOMAX feed (project phase 1, final report). National Iranian Oil Refining and Distribution Company (NIORDC).
- Bernard O, Michel M (2005) Petroleum Microbiology. American Society for Microbiology.
- Grin'ko AA, Golovko AK (2011) Fractionation of resins and asphaltene and investigation of their composition and structure using heavy oil from the Usa field as an example. Petrol Chem 51: 192-202.

13. Amiryan A, Mazaheri Assadi M, Saggadian VA, Noohi A (2004) Bioemulsan Production by Iranian Oil Reservoirs Microorganisms. *Iranian J Env Health Sci Eng* 1: 28-35.
14. Diaz-Ramirez IJ, Escalante-Espinosa E, Favela-Torres E, Gutierrez-Rojas M, Ramirez-Saad H (2008) Design of bacterial defined mixed cultures for biodegradation of specific crude oil fractions, using population dynamics analysis by DGGE. *Int Biodeter Biodegr* 62: 21-30.
15. Ghazali FM, Abdul Rahman RNZ, Salleh AB, Basri M (2004) Biodegradation of hydrocarbons in soil by microbial consortium. *Int Biodeter Biodegr* 54: 61-67.
16. Korda A, Santas P, Tenente A, Santas R (1997) Petroleum hydrocarbon bioremediation: sampling and analytical techniques, in situ treatments and commercial microorganisms currently used. *Appl Microbiol Biotechnol* 48: 677-686.
17. Premuzic ET, Lin M, Bohenek MS, Zhou WM (1999) Bioconversion Reactions in Asphaltenes and Heavy Crude Oils. *Energy Fuels* 13: 297-304.
18. ASTM committee (1982) Annual Book of ASTM Standards. Part 46.
19. Okoh AI (2006) Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. *Biotechnology and Molecular Biology Review* 1: 38-50.
20. Mittal A, Singh P (2009) Isolation of hydrocarbon degrading bacteria from soils contaminated with crude oil spills. *Indian J Exp Biol* 47: 760-765.
21. Malatova K (2005) Isolation and Characterization of Hydrocarbon Degrading Bacteria from Environmental Habitats in Western New York State. Rochester Institute of Technology New York USA.
22. Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173: 697-703.
23. Benson HJ, Brown AE (2006) Benson's Microbiological Applications: Laboratory Manual in General Microbiology. McGraw-Hill Higher Education USA.
24. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215: 403-410.