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Additional Study on Mg^{2+} Micro Nutrient Phytoplankton Porpyridium Cruentum and Tetraselmis Chuii for Chlorophyll and Protein Production

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ABSTRACT

This study aims to find out : (1) whether the addition of Mg^{2+} can support the nutrient in growth medium in the production of chlorophyll and protein by *phytoplankton P. Cruentum and T. Chuii* (2) in what concentration Mg^{2+} ions can work optimally and (3) what functional group plays a role in the influx of Mg^{2+} into the cells of phytoplankton P. cruentum and T. chuii.

The result reveal that the addition of Mg^{2+} nutrients in *P. Cruentum and T.chuii* can support the nutrients in the growth medium.

The optimum concentration of Mg^{2+} nutrient in the production of chlorophyll and protein by P. Cruentum is 40 ppm (the amount of chlorophyll and protein is 780.000 mg/m^3 and 316.0025 ppm). The optimum concentration of Mg^{2+} nutrient in the production of chlorophyll and protein by T. chuii is at 40 and 20 ppm (the amount of chlorophyll and protein is 27651.04 mg/m^3 and 240.8483 ppm).

The functional groups involved in the process of nutrient influx of Mg^{2+} into P. cruentum and T. chuii are M-S and N-H (for P.cruentum) MX and C-CI (for T. Chuii).

Key Words : Mg^{2+} ion, chlorophyll, protein porphyridium cruentum, tetraselmis chuii

INTRODUCTION

One of microorganisms that grow in water is microalgae. Microalgae are aquatic plants that are able to move passively). Microalgae are photosynthetic microorganisms with varying cell morphology, both single cells and multicellular, small size in waters.[1]

Microalgae or phytoplankton have an important role in aquatic ecosystems which is as a source of food and physical protection for aquatic organisms.[2] Microalgae biomass contains potential chemical composition such as proteins, carbohydrates, pigments (chlorophyll, karatenoid, fikobilin, etc.) of amino acids, lipids and hydrocarbons.[3]

Tetraselmis gracilis species containing amino acids between 94 to 112%.[4] Porpyridium cruentum containing 0.99% fatty acid arachidonic .[5] Generally Tetraselmis Chuii and Porphyridium Cruentum containing functional groups, COOH, NH2, OH-CH2 and CH3 .[6] Tetraselmis chuii was microalgae of green algae group (Chlorophycae).[7]

Tetraselmis Chuii have chlorophyll (green substance) so that bright green and can photosynthesize.[8]. Porpyridium Cruentum is microalgae from the class of red algae (Rhodophyceae) .[3,5]. Both types of species (Tetraselmis chuii and Prophyridium Cruentum) is a class of phytoplankton dinoflagellate microalgae.[1] In the process of growing microalgae require nutrients are taken from the environment.

Among the various elements of inorganic nutrients are needed as Mg^{2+} ions that play a role in the formation of chlorophyll, protein and photosynthesis.[9]

Mg^{2+} ion is a cofactor for hundreds of enzymes. Mg^{2+} ion is an important factor for the transfer of phosphate biochemistry, many enzymatic reactions involving ATP as a phosphate donor, an active

form complex compounds Mg^{2+} and Mg^{2+} ADP ATP. Mg^{2+} plays a role in cell division (cleavage of nucleic acids through the core or ribozom) and a light collection system so that it can absorb a lot of sunlight, thus speeding up the process of photosynthesis.[10]

MATERIALS AND METHODS

Materials

Materials used in this study were cultured phytoplankton Porphyridium cruentum and Tetraselmis chuii MgSO47H2O nutrient solution with each concentration (mg / ml) 0.0: 5: 10: 20: 30: 40: 50: 75: 100 double distilled water, buffer solution, and the medium conwy table. 1

Table 1 Composition of Medium Conwy

Materials	Amount
Stock A	
$FeCI_2$ 6 H_2O	1,3
$MnCI_2$ 4 H_2O	0,36
H_3BO_3	33,6
EDTA (Na-Salt)	45
NaH_2PO_4 2 H_2O	20
$NaNO_3$	100
Double distilled water	1 L
Stock B	
$ZnCI_2$	2,1
$CoCI_2$ 6 H_2O	2
$(NH_4)_6MoO_{24}$ 4 H_2O	0,9
$CuSO_4$ 5 H_2O	2
Double distilled water	100 ml
Stock C	
Vitamin B_{12}	10
Vitamin B_1	200
Double distilled water	100 ml
Stock D	
Na_2SiO_3 5 H_2O	4,00 g
Double distilled water	100 ml

Source : Fogg, 1975[11]

Equipment

The tools used in this study include: the tools glass (pyrex) which is commonly used in laboratories, UV-2900 PC, Microscope Nikon models, models SPNISOSFD oven, digital balance Ohauss NO models AP110 and FT-IR Shimadzu Model 820 1 PCs.

Methods

1. Porphyridium cruentum cultured marine phytoplankton and tetraselmis chuii.
   To obtain the desired density of phytoplankton, use dilution formula:
   $V_1 \times N_1 = V_2 \times N_2$ ……(1)
   Dengan,
   V_1 = Stock volume
   V_2 = Culture volume
   N_1 = Stock phytoplankton cell density
   N_2 = Culture phytoplankton cell density
2. Phytoplankton density calculation formula is used:

$$\text{Amount } \frac{\text{cell}}{\text{mL}} = \frac{\text{number of cells in 4 boxes}}{\text{number of block (=4)}} \times 10000 \ldots\ldots (2)$$

If the cell density is too high, the calculation using the formula :

Amount cell = number of cells in 4 parts x 4 x 10000 … … (3)
mL

3. Determination of the specific growth rate (μ) and the marine phytoplankton Tetraselmis chuii Pophyridium cruentum.
   Determination of the specific growth rate (μ) for each additional Mg^{2+} concentration of nutrient use the formula:

$$\mu = \frac{1nN_1 - 1nN_0}{t} = \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

N_t = Cell population density at time t (cells / mL)
N_o = The population density of cells at the beginning (cells / mL)
μ = Specific growth rate (hr-1)
t = Time (hour)

4. Determination of the amount of chlorophyll by using a UV spectrophotometer.
   For the determination of the amount of chlorophyll is done in two ways, namely the treatment of the sample:
   4.1. For the sample without the addition of nutrients to use the formula:

$$chlorophyll - a\ (mg/m^3) = \frac{\{(11{,}85\ E_{664}) - (1{,}54\ x\ E_{647}) - (0{,}06\ x\ E_{630})\}x\ Ve}{Vs\ x\ d}$$

$$chlorophyll - b\ (mg/m^3) = \frac{\{(21{,}03\ E_{647}) - (5{,}43\ x\ E_{664}) - (2{,}66\ x\ E_{630})\}x\ Ve}{Vs\ x\ d}$$

$$chlorophyll - c\ (mg/m^3) = \frac{\{(24{,}52\ E_{630}) - (1{,}67\ x\ E_{664}) - (7{,}60\ x\ E_{647})\}x\ Ve}{Vs\ x\ d}$$

E_{664} = absorbance 664 nm – absorbance 750 nm : Ve = volume of acetone extract (mL)
E_{647} = absorbance 647 nm – absorbance 750 nm : Vs = volume (mL)
E_{630} = absorbance 630 nm – absorbance 750 nm : d = wide of cuvette diameter (1 cm)

   4.2 For samples with the same procedure with the addition of nutrient treatment 4.1
5. Determination of protein content using a UV spectrophotometer
6. Identification of Functional Groups in Porpyridium Cruentum and Tetraselmis Chuii that play a role in the production of chlorophyll and protein (FT-IR Spectra)

RESULTS AND DISCUSSION

Patterns of marine phytoplankton growth Porpyridium cruentum and Tetraselmis chuii

Phytoplankton growth patterns Porpyridium cruentum and Tetraselmis chuii observed every 24 hours during the growth period. Data and chart growth pattern is presented in figure 1.

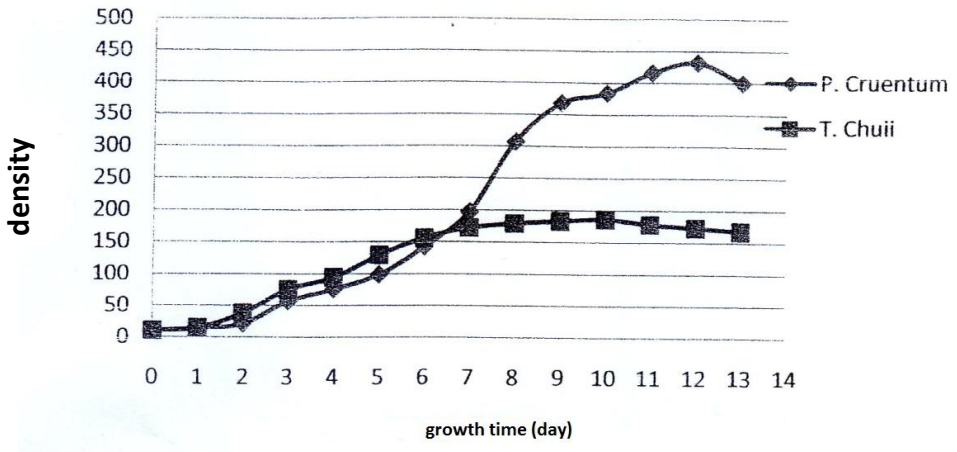

Figure 1. The pattern of cell growth and Tetraselmis phytoplankton Porpyridium cruentum chuii during the growth period of 13 days.

Observations of phytoplankton growth and Tetraselmis chuii Porpyridium cruentum in the medium conwy done every 24 hours for 14 days .

Phytoplankton growth pattern through four phases of growth , namely the adaptation phase , exponential and stationary phases of death.[12]

Based on (Figure 1) appears when needed by phytoplankton growth Porpyridium cruentum and Tetraselmis chuii to adapt to the culture medium to medium conwy relatively shorter (about 2 days) . Exponential phase takes place starting on day 2 to day 12 (for porphyridium cruentum) and day 10 (for tetraselmis chuii) subsequently undergo stationary phase .

In the culture medium , each type of phytoplankton with the initial density of 100,000 cells medium has increased the number of cells is about 14 times higher than the initial density for 12 days to porphyridium cruentum culture and to tetraselmis chuii increased 19 -fold from the initial density culture for 10 days .

Effect of Mg^{2+} nutrients on cell growth of phytoplankton nutrient porphyridium cruentum. The addition of Mg 2 + on the nutrient concentration of 40 ppm showed the highest growth pattern until day 12..

Effect of concentration (ppm) Mg^{2+} nutrients to phytoplankton growth porphyridium cruentum cell population are presented in Table 2 .

Table 2 The density of phytoplankton cells P.Cruentum + Mg^{2+} Nutrients

	Cruentum porphyridium cell density (x 10.000 cell / mL)								
	Mg^{2+} concentration (PPM)								
Day	0	5	10	20	30	40	50	75	100
0	10	10	10	10	10	10	10	10	10
1	14.25	11.25	11.75	14.25	15.5	17	17.75	9	10.25
2	20.75	28	17.75	28	26.75	26.5	51.75	40.5	21.75
3	67.25	26.5	30.25	33.25	36.75	43.5	40	29	27.25
4	56.25	55	70.25	85	93	94	145.75	76	88
5	98.5	114	76	113	128.5	137	175	148	107.25
6	142	215	124	132	194	269	227	172	169
7	197	215	142	160	288	400	325	208	192
8	307	275	263	194	391	425	297	233	224
9	368	328	304	273	336	448	352	256	240
10	384	352	258	224	400	480	393	304	320
11	416	464	334	432	512	554	480	464	384
12	432	400	385	352	624	784	464	672	560
13	400	512	304	416	560	800	576	624	544

From the data of this study (Table 2) shows that with the addition of Mg^{2+} nutrient effect on the number of cell density . At the time of addition of nutrient concentrations of Mg^{2+} with 5,10 and 20 ppm tend to decrease the number of cells . The decrease in the number of cells indicated that P. Cruentum has high sensitivity to changes in the culture medium condition caused by a very small cell size , about 4 to 9 µm . Subsequent addition of Mg^{2+} on the nutrient concentration of 30 and 40 ppm increased the number of cells . This fact indicates that nutrient Mg^{2+} function in the process of cell division . In addition nutrient concentrations of Mg^{2+} with 50,75 , and 100 ppm , up and down through a phase of growth . This indicates the occurrence of Phytochelatin production by phytoplankton cells to detoxify Mg^{2+} . Phytochelatin formed when phytoplankton gets high exposure to Mg^{2+} . After Phytochelatin produced , the phytoplankton can maintain itself visible from the increased number of

cells (at a concentration of 75 ppm) when Phytochelatin are no longer able to detoxify the phytoplankton of nutrient Mg^{2+} . Further decline in phytoplankton growth will enter the death phase is at a concentration of 100 ppm .

Effect of Mg^{2+} addition of nutrients to the cell population growth of phytoplankton Tetraselmis chuii presented in Table 3.

Table 3. The density of phytoplankton cells T.Chuii + Mg^{2+} Nutrients

Day	Tetraselmis chuii cell density (x 10.000 cell / mL)								
	Mg^{2+} Concentration (PPM)								
	0,0	5	10	20	30	40	50	75	100
0	2	2	2	2	2	2	2	2	2
1	4	5	9.25	11	12	9.75	5.25	7.25	5.25
2	8	8.5	10.75	9.5	11.75	13	15.25	9.25	6
3	14.25	15.75	18	19.5	14.75	23.5	11	14	9.75
4	24.25	31.5	33	26.5	34.5	27.75	29.25	26.25	25.5
5	34	37.5	46.75	48	47.5	38.25	36	37.5	36.75
6	24.5	28	27.75	28	26.5	21.5	51.75	49.5	21.75
7	67.75	69	69.75	66.5	74.5	64.25	44.5	59	63.25
8	86.25	110	104	106.25	103.5	125.5	89.5	67	89.25
9	110	139	118.25	168.5	116.5	137.25	94.5	74.25	94.5
10	136	156	124	186	135	155	108	80	109

Based on (Table 3) visible growth pattern chuii Tetraselmis phytoplankton cell populations at various levels of exposure to a concentration of 5 ppm , 10 ppm , 20 ppm , 30 ppm , 40 ppm , 50 ppm , 75 ppm and 100 ppm relative to the same control (0.0 ppm) is constantly rising population density phytoplankton cells from first day addition of nutrients Mg^{2+} until first day, table 3 shows the addition of nutrients Mg^{2+} concentration of 5 ppm , 10 ppm , 20 ppm and 40 ppm in cultured phytoplankton Tetraselmis chuii looked the pattern of growth higher than 0.0 ppm . Concentration of 20 ppm showed the highest growth pattern .

The addition of Mg^{2+} nutrient effect on the number density of the same day i.e. on 10th day which is the maximum cell growth (Table 3) . At the time of nutrient addition of Mg^{2+} at a concentration of 5 and 10 ppm decreased the number of cells . This is because the cells are sensitive to changes in nutrient concentrations were added . At a concentration of 20 ppm the amount of the highest cell density (Table 3). At this concentration of Mg^{2+} nutrients to function well in cell division . At a concentration of 30 , 40 , 50 ppm and so on cell growth has been up and down , the nonlinear growth . This indicates the occurrence of Phytochelatin production by phytoplankton cells . At a concentration of 75 ppm through a phase of death .

Effect of the addition of Mg^{2+} on the nutrient and chlorophyll production by phytoplankton porphyridium cruentum proteins and tetraselmis chuii .

Table 4. Levels of protein and chlorophyll porphyridium cruentum phytoplankton cells by the addition of Mg^{2+} nutrient

nutrient addition Mg^{2+}	Protein content (ppm)	chlorophyll content (ppm)	cell density (10.000 celll/ml)
0	243,05	700,55	432
5	276,34	667,91	400
10	244,25	578,89	385
20	259,35	555,78	352
30	274,31	715,50	624

40	316,00	780,00	784
50	274,65	705,99	464
75	276,00	615,33	672
100	269,36	630,89	560

Based on Table 4 above, with the addition cruentum porphyridium Phytoplankton nutrient Mg 2 +, the amount of protein and chlorophyll contained in the optimal concentration of 40 ppm. The amount of protein and chlorophyll were 316.0025 and 780.0000 ppm mg/m3. This is in accordance with the number.

Table 5 Levels of protein and chlorophyll chuii Tetraselmis phytoplankton cells by the addition of Mg^{2+} nutrient

nutrient addition Mg^{2+}	Protein content (ppm)	chlorophyll content (ppm)	cell density (10.000 cell/ml)
0	224,05	25000,12	136
5	225,75	25660,19	156
10	227,45	25003,15	124
20	240,85	25551,18	186
30	223,75	25899,19	135
40	225,45	27651,04	155
50	218,34	25899,22	108
75	200,64	25901,19	80
100	218,65	22765,11	109

In Table 5 the highest amount of protein present in the nutrient addition of Mg^{2+} at a concentration of 20 ppm , equal to the number of cells . The highest amount of chlorophyll which is not present in a concentration of 20 ppm , but are at a concentration of 40 ppm .

The amount of protein and chlorophyll may also depend on the number of cell density , cell number density can also depend on the medium used for culturing phytoplankton and type of nutrients are added .

The amount of protein is directly proportional to the number of cells in the P. and T. Chuii Cruentum is due to cell division (increase in number) and cell enlargement (increase in size) is a process that requires protein synthesis, therefore the amount of protein and cell is directly proportional . While the amount of chlorophyll is not always proportional to the number of these cells might be due to the rate of photosynthesis of each cell sizes vary.

The addition of Mg^{2+} ions indicates the number of different proteins produced by Porphyridium cruentum and Tetraselmis phytoplankton chuii . This is probably due to the size of phytoplankton cells smaller Porphyridium cruentum (4-9 µm) compared with chuii Tetraselmis phytoplankton cell size (7-12 µm) . the smaller the cell size , the larger the surface area means that the greater the ability to react .

Identification of functional groups in Porphyridium cruentum and Tetraselmis chuii that play a role in the production of chlorophyll and protein .

Analysis of infrared spectra (IR) on biomass blank Chuii T. and T. bimassa Chuii with the addition of Mg^{2+} ions are presented in Table 6 .

Groups of phytoplankton that plays a role in the interaction with Mg^{2+} ions can be observed in Table 6 .

Based on Table 6 looks at biomass blank Tetraselmis chuii there are some peak wave number . Similarly, biomass after the addition of Mg^{2+} appears peak nutrient that can provide guidance in the analysis of functional groups and the metal interaction with phytoplankton .

Cluster -shifted , appear or disappear on Tetraselmis chuii after the addition of Mg^{2+} ions . In the interaction between the metal blank with N wavelengths identified in 339.47 cm - 1 . At the time of the addition of Mg^{2+} ions shift the wavelength be 354.9 cm - 1 . This indicates an interaction between Mg^{2+} with N. This is reinforced by the presence of clusters N - H are identified at a wavelength of

2113.98 cm - 1 (blank) , then shifted into 2106.27 cm - 1 . Because peak was lost , this suggests that the interaction is a covalent bond coordination . Wave number at 362.62 cm - 1 (blank) is the interaction between M by S , after Mg^{2+} ions are added to the wavelength shift into a 370.33 cm - 1 . This indicates that the interaction between Mg^{2+} with S. The existence of this interaction is reinforced by the SS functional groups identified at a wavelength of 478.35 CM1 (blank) , then shifted to 470.63 cm - 1 after the addition of Mg^{2+} ions . Because peak was not lost , this suggests that the interaction is a covalent bond coordination . The interaction of M with X at a wavelength of 509.2 cm - 1 disappeared , termination occurs ionic bonds between metals with X after the addition of Mg^{2+} ions at a wavelength of 609.51 appears functional group C - CI after the addition of Mg^{2+} ions .

Table 6 FT-IR spectra data of biomass Tetraselmis chuii + Mg^{2+} ions

No	TC Blanko	Functional groups/Interactions that occur	TC + Mg^{2+}	Functional groups/Interactions that occur	info
1	324,04	M – O	324,04	M – O	fixed
2	339,47	M – N	354,9	M – N	Small shift / not significant
3	362,62	M – S	370,33	M – S	Small shift / not significant
4	478,35	S – S	470,63	S – S	Small shift / not significant
5	509,2	M – X	-	-	disappear
6	-	-	609,51	C – CI	Appear
7	918,12	N – O	918,12	N – O	Appear
8	1041,56	C – N	1041,56	C – N	Fixed
9	1157,29	S = O	1157,29	S = O	Fixed
10	1234,44	C – O	1234,44	C – O	Fixed
11	1411,89	CH_2	1404,18	CH_2	Small shift / not significant
12	1543,05	-O – C = O -	1543,05	-O – C = O -	Fixed
13	1658,78	C = C	1658,78	C = C	Fixed
14	2113,98	N – H	2106,27	N – H	Small shift / not significant
15	2337,72	C ≅ N	2337,72	C ≅ N	Fixed
16	2862,36	CH_3	2862,36	CH_3	Fixed
17	2924,09	CH_2	2924,09	CH_2	Fixed
18	3309,85	O – H	3309,85	O – H	Fixed
19	3749,62	N – H	3749,62	N – H	Fixed
	∑ 19	∑ 18	∑ 18		

The results of the analysis of infrared spectra (IR) in the form of biomass . P Cruentum and biomass P. Cruentum with the addition of Mg^{2+} ions (Table7) .

Group shifted appeared or disappeared in porphyridium cruentum after the addition of Mg^{2+} ions . In the interaction between the metal blanks with the X identified at a wavelength of 300.9 cm - 1 . At the time of the addition of Mg^{2+} ions are wavelength shifted to 308.61 cm - 1 . This interaction is reinforced by the presence of the C - CI functional groups identified at a wavelength of 601.79 cm - 1 . After the addition of Mg^{2+} ions . In the interaction between the metal blank with N identified at wavelengths 354.9 cm - 1 . At the time of Mg^{2+} ions wavelength shift into a 347.19 cm - 1 . This indicates an interaction between Mg^{2+} with N. This situation is reinforced by the presence of absorption at a wavelength of 1141.86 cm - 1 (blank) , which identified the presence of NH group ,

then shifted into 1118.71 cm - 1 after the addition of Mg^{2+} ions . Because peak was not lost , this suggests that the interaction is a covalent bond coordination . At a wavelength of 362 cm - 1 appears interactions between metals with S. interaction is reinforced by the presence of absorption at a wavelength of 478.35 cm - 1 identified the SS group . At the time of the addition of Mg^{2+} ions wavelength shifted to 493.78 cm - 1 , indicating that the possibility of S from the SS functional group interaction with Mg^{2+} ions . At a wavelength of 3394.72 cm - 1 identified group O - H (blank) after the addition of Mg 2 + ions shifted to 3387.00 cm - 1 . The shift in wavelength due to the van der Waals force.

Table 7 FT-IR spectra data Porphyridium biomass Cruentum + Mg^{2+} ions

No	TC Blanko	Functional groups/Interactions that occur	TC + Mg^{2+}	Functional groups/Interactions that occur	info
1	300,9	M – X	308,61	M – X	Small shift / not significant
2	354,9	M – N	347,19	M – N	Small shift / not significant
3			362	M – S	Appear
4	479,35	S – S	493,78	S – S	Small shift / not significant
5	601,79	C – CI	609,51	C – CI	Small shift / not significant
6	1141,86	N – H	1118,71	N – H	Shift
7	1435,04	CH_2	1435,04	CH_2	Fixed
8	1527,62	CH_3	1543,05	CH_3	Small shift / not significant
9	1635,64	C = C	1651,07	C = C	Small shift / not significant
10	2276,0	C ≅ C	2276,0	C ≅ C	fixed
11	2368,59	C ≅ N	2368,59	C ≅ N	fixed
12	2931,80	CH_3	2931,80	CH_3	fixed
13	3394,72	O – H	3387,00	O – H	Small shift / not significant
	∑ 12		∑ 13		

CONCLUSION

Based on the research and the data obtained, it can be concluded that:
1. The addition of Mg^{2+} on phytoplankton nutrient porphyridium cruentum, tetraselmis chuii can support nutrients in the growth medium.
2. Optimal concentration of Mg^{2+} in the nutrient and chlorophyll production by phytoplankton porphyridium cruentum proteins is 40 ppm. Optimal Mg^{2+} concentration of nutrients in the production of phytoplankton chlorophyll and protein by tetraselmis chuii are 40 and 20 ppm.
3. Interactions of functional groups involved in the process of entry of Mg $^{2+}$ ions on phytoplankton porphyridium cruentum was MS and NH.
Interactions of functional groups involved in the process of entry of Mg $^{2+}$ ions on phytoplankton Tetraselmis chuii is MX and C-CI.

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