ISOLATION AND ANTIBACTERIAL TEST OF GARLIC OIL

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ABSTRACT

Garlic oil is known to have medicinal effect on hypertension, heart desease, anemia and various infections. The active principles are reported to be allicin, diallil disulfide, allilpropyl disulfide, scordinin, selenium and germanium in addition to the presence of anticoagulant, antihaemolytic and antithrombotic agents. The methods applied for isolation and other conditions during the isolation affect the yield or the compositions of the oil, for instance with steam distillation the allicin will decompose whereas extraction at room temperature will yield oil with allicin as the main component. In the present study isolation was conducted by extraction with ethylacetate or ethanol and the oil obtained was tested for their antibacterial capacity. Tests against ***Staphylococcus aureus, Bacillus cereus*** *and* ***Escherichia coli*** *indicated that the isolated oil were active. On the other hand, commercial garlic extract (KGE) and garlic oil capsule (GOC) gave negative test. This may have been due to either insufficient concentration of the biologically active component present in the commercial drugs or different method of extraction process. Investigation with thin layer chromatography (TLC) of the drugs on silica gel plate using n-hexane and ethyl acetate as eluents showed six components in the GOC and none in KGE whereas for the isolated oil 13 components were identified with iodine vapour. Although the oils indicated antibacterial activity, it is somehow less active compared with oxytetracycline which is used as reference.*

INTISARI

Minyak bawang putih diketahui mempunyai pengaruh sebagai obat penyakit hipertensi, penyakit jantung, anemia dan berbagai penyakit infeksi. Zat aktif yang memegang peranan dilaporkan sebagai allisin, diallil disulfida, allilpropil disulfida, skordinin, selenium dan germanium, disamping adanya senyawa-senyawa bersifat sebagai antikoagulan, antihemolisa dan antitrombosis. Metoda-metoda yang dipakai untuk isolasi serta kondisi lain selama proses isolasi mempengaruhi hasil serta komposisi dari minyaknya, misalnya cara destilasi uap akan menyebabkan allisin terurai, sedangkan dengan cara ekstraksi pada suhu kamar akan menghasilkan minyak dengan allisin sebagai komponen utama. Pada penelitian yang dilaporkan di sini, isolasi dilakukan dengan cara ekstraksi menggunakan etilasetat atau etanol dan minyak yang diperoleh selanjutnya diuji terhadap kemampuan sifat antibakteri. Pengujian terhadap ***Staphylococcus aureus, Bacillus cereus*** *dan* ***Escherichia coli*** *menunjukkan adanya aktifitas antibakteri pada minyak yang diisolasi. Uji coba yang dilakukan menggunakan ekstrak bawang putih komersial (KGE) dan kapsul minyak bawang putih (GOC) memberikan hasil negatif. Hasil negatif ini terjadi mungkin akibat rendahnya konsentrasi zat yang mempunyai sifat aktif dalam sediaan komersial tersebut atau cara ekstraksinya berbeda. Pemisahan dengan teknik kromatografi lapis tipis (TLC) dari minyak bawang putih pada plat Silika Gel dengan eluen n-heksana dan etilasetat menunjukkan adanya 6 komponen pada GOC, komponen tunggal pada KGE dan 13 komponen pada minyak hasil isolasi, setelah diidentifikasi dengan uap yodium. Meskipun minyak hasil isolasi menunjukkan ada sifat antibakteri namun kemampuan ini lebih rendah bila dibandingkan dengan oksitetrasiklin.*

INTRODUCTION

Garlic (*Allium sativum* L.) belongs to the family of Amaryllidaceae (Liliaceae) which can grow on the altitude between 600 and 1000 metres above sea level at any month of the year and can be harvested within around four months (6). Garlic has been used since many years ago as food and as medicine (1). This plant has recently attracted business sectors including pharmaceutical industries by producing garlic extract or garlic oil for use as traditional medicaments to heal a variety of ailments such as high blood pressure, atherosclerosis, tuberculosis, diabetes, arthritis, cancer, hypoglycemia, bronchitis, asthma, whooping cough, pneumonia, common cold, allergies, intestinal worms, intestinal putrefaction and gas, parasitic diarrhea, dysentry and also insomnia. The active components in garlic had been reported being allicin, diallil disulfide, allilpropyl disulfide, allithiamine and many factors such as antihemolytic, antiarthritic, sugar-regulating, antioxidant and anticoagulant (1,4). Garlic is also an excellent source of biologically active selenium and it is believed that garlic's anti atherosclerotic property is due to its high selenium content (1). Regarding atherosclerosis, experiments on the effect of garlic supplementation to cholesterol rich diet, indicated that garlic could decrease the cholesterol content in the blood of rabbits (8). Many other health disorders that have been reported to be favourably affected by garlic treatment. These examples are eye burns, lip and mouth diseases as leukoplakia, hyperkera-tosis, fissures and ulcer of the lip, upset stomach, hyperemia, grippe, sciatica, chronic colitis, gastritis, pimples and emphysema (1, 3, 9, 10). It was also reported that among the Allium species, garlic showed the greatest antibacterial activity against pathogenic aerobic and anaerobic bacteria. Garlic-antibiotic combinations were synergistic against *Actinobacter calcoaceticus* (5).

Garlic contains alliin (around 0.2%) and when the bulbs are squeezed, alliin will be converted into allicin, which is the typical garlic aroma, by allinase activity. Crystalline alliin (melting point 163-165°C), is easily soluble in water whereas allicin is yellow oil with around 2.5% solubility in water, thermo and alkaline labil (6).

Methods and conditions applied in the isolation of garlic oil affect markedly the composition of the oil. The diagram in Figure 1 describes the anticipated products (1,2).

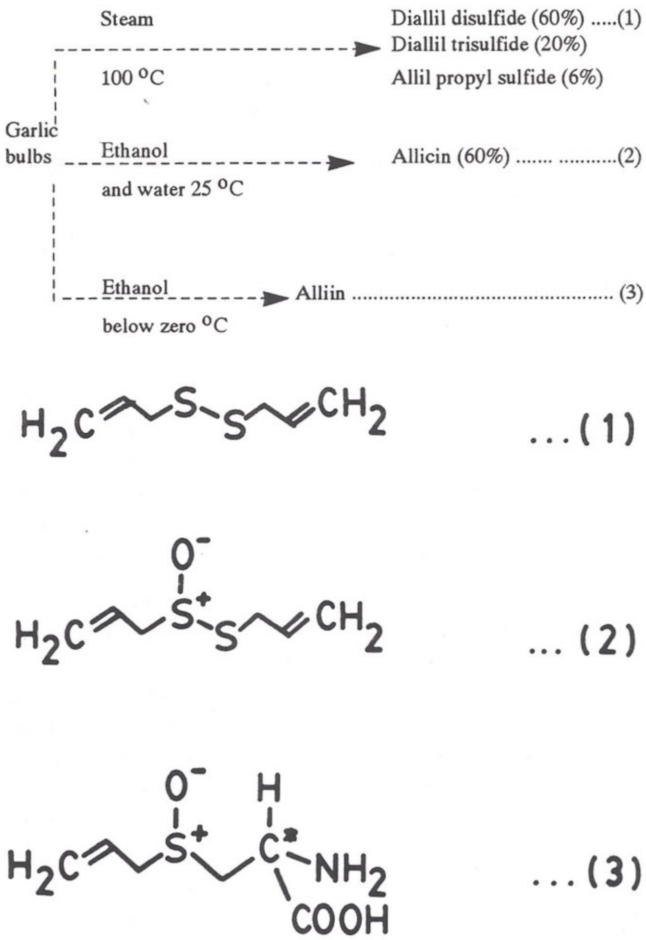

Figure 1. Products of extraction of garlic bulbs at various temperatures(1).

The physical properties of garlic oil have been reported (5) as follows: specific gravity (15°C) 1.055 – 1.098, refractive index (20°C) 1.5578 – 1.5545, optical rotation zero and brownish yellow colour with typical garlic aroma.

Since garlic oil has been widely used in traditional medicinal treatments, studies was carried out in this investigation on its biological activity against gastrointestinal and respiratory tract infections. The garlic oil was obtained by hydrolic pressure and extraction at room temperature. The oils obtained were checked against pathogenic bacteria, that is *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli*, to find out its antibacterial capacity. The antibacterial activity of the oil was compared against oxytetracycline standard.

MATERIALS AND METHODS

Garlic oils

The garlic oils tested were of laboratory prepared ones following the isolation scheme illustrated in Figure 2. The fractions which demonstrated antibacterial activity were garlic oil from juice fraction (GOJF) and garlic oil from pressed cake fraction (GOPCF). Two commercial garlic based medicament samples, Kyolic aged garlic extract (KGE) and garlic oil capsule (GOC) of Wakunaga of America Company and Wakunaga Pharmaceutical Company, Osaka-Japan, respectively, were directly tested for antibacterial activity.

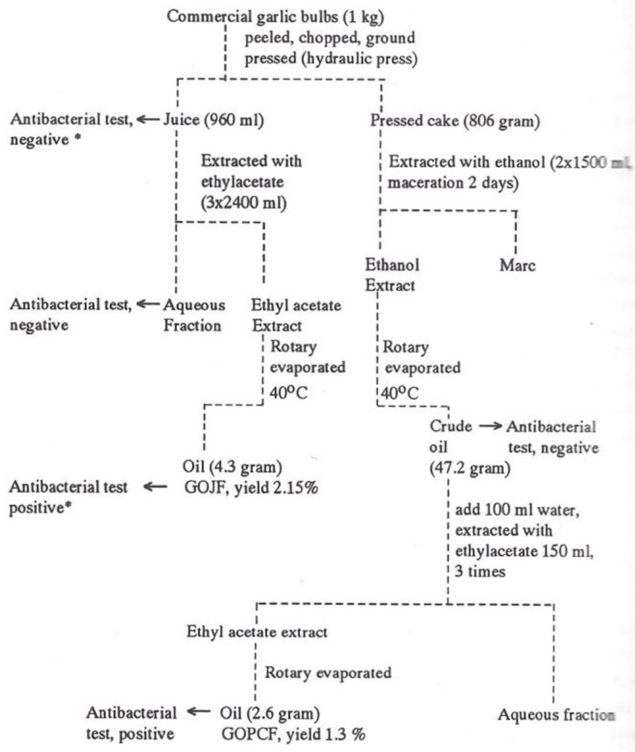

Figure 2. Isolation scheme of garlic oils. GOJF = Garlic oil from juice fraction; GOPCF = Garlic oil from pressed cake fraction.
* Negative if diameter of inhibition < 4 mm
Positive if diameter of inhibition > 4 mm

Antibacterial capacity

The antibacterial capacity of the garlic oils was estimated using *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli* as test bacteria. For reference, activity of standard oxytetracyclin (SIGMA) against *Bacillus cereus* was determined. The inhibition diameter was measured to estimate the inhibition ring area.

Chemicals

Distilled technical grade solvents were used for extraction whereas for thin layer chromatography (TLC) experiments,various solvents used were of pure grade.

TLC experiments

Silica gel plates of 0.5 mm thickness were prepared on glass plate support. Various eluent compositions were used and after elution the samples were visualised by exposing the developed plates to iodine vapour or spraying with phosphomolybdic acid reagent.

RESULTS AND DISCUSSION

Antibacterial tests of the garlic juice, crude extract obtained from ethanol maceration of the garlic pressed cake and aqueous fraction (see Figure 2) gave negative results. Surprisingly both commercial garlic extract (KGE) and garlic oil capsules (GOC) also gave negative results whereas the isolated garlic oils, GOJF and GOPCF demonstrated antibacterial capacity. Negative results in KGE, GOC and mother liquors of GOJF and GOPCF might be due to either insufficient concentration of the biologically active component present or antagonistic effects. Different method of KGE and GOC extraction might also give negative results. The effect of the oil dilution on their activity is shown in Table 1 while graphical presentations of the logarithmic concentration model are shown in Figure 3 and Figure 4. A dilution rate of 1:1200 of the GOJF and of 1:480 of the GOPCF still demonstrated antibacterial activity.

Table 1. Relation between antibacterial capacity expressed as inhibition zone and concentration of garlic oils as compared with oxytetracyclin (SIGMA).

Oil sample	Test bacterium		Model of regression equation*	Squared of R
GOJF	*S.aureus*	1D	y = 4.34 (0.36) + 0.00080 (0.00003)x	0.99
		2RA	y = –57.2 (65) + 0.0526 (0.0060)x	0.96
		3DLC	y = –20.5 (2.05) + 8.23 (2.14) log x	0.83
		4DRALC	y = –1627 (175) + 524 (183) log x	0.73
	B.cereus	1D	y = 4.96 (0.41) + 0.00061 (0.00004) x	0.99
		2RA	y = –5.5 (17) + 0.0364 (0.0016) x	0.99
		3DLC	y = –15.5 (1.1) + 6.72 (1.12) log x	0.92
		4DRALC	y = 1163 (92) + 383 (96) log x	0.84
	E.coli	1D	y = 2.45 (2.34) + 0.00076 (0.00021) x	0.81
		2RA	y = –27.5 (25.5) + 0.0326 (0.0023) x	0.98
		3DLC	y = –26.8 (1.22) + 9.41 (1.28) log x	0.95
		4DRALC	y = –1068 (82.7) + 344 (86) log x	0.84
GOPCF	*S.aureus*	1D	y = 5.54 (0.84) + 0.00006 (0.00001) x	0.80
		2RA	y = 45.2 (29.3) + 0.0026 (0.0005) x	0.87
		3DLC	y = –5.2 (0.37) + 2.95 (0.29) log x	0.96
		4DRALC	y = –416 (19) + 127 (15) log x	0.95
	B.cereus	1D	y = 5.76 (0.76) + 0.00004 (0.00001) x	0.77
		2RA	y = 53.8 (28) + 0.0020 (0.00047) x	0.82
		3DLC	y =- –3.0 (0.36) + 2.40 (0.28) log x	0.95
		4DRALC	y = –319 (15) + 103 (12) log x	0.95
	E.coli	1D	y = 5.72 (0.95) + 0.00005 (0.000015) x	0.73
		2RA	y = 53.7 (38.2) + 0.0023 (0.0006) x	0.77
		3DLC	y = –4.72 (0.34) + 2.85 (0.27) log x	0.97
		4DRALC	y = 398 (15) + 123 (12) log x	0.96
Oxytetracyclin	*B.cereus*	1D	y = 7.97 (0.26) + 0.04224 (0.0046) x	0.98
		2RA	y = 134 (13) + 2.78 (0.23) x	0.99
		3DLC	y = 1.43 (0.18) + 5.26 (0.39) log x	0.99
		4DRALC	y = 290 (16) + 432 (36) log x	0.98

Figures between brackets are standard error of Y estimate and standard error of coefficient. R = coefficient of correlation. * Remark for model of regression equations :

1D : y = diameter of inhibition zone (mm); x = concentration (ppm)

2RA : y = ring area (mm^2); x = concentration (ppm)

3DLC : y = diameter of inhibition zone (mm); x = concentration in ppm

4DRALC : y = ring area (mm^2); x = concentration in ppm

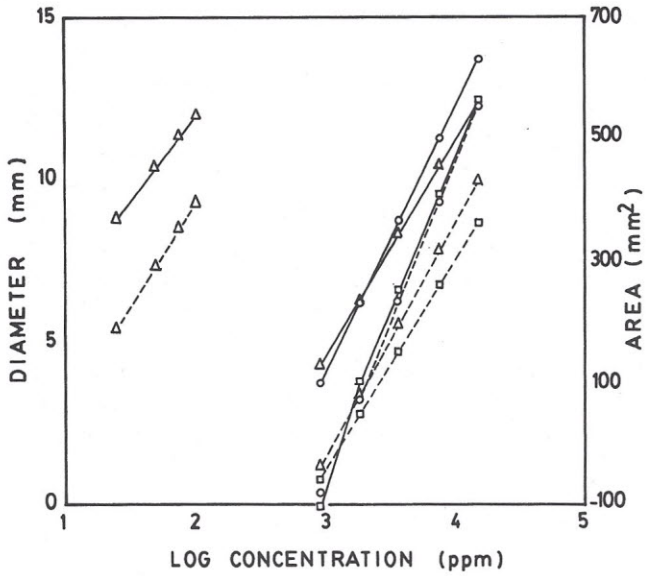

Figure 3. The effect of various concentrations of garlic oil obtained from garlic juice, on the diameter and ring area of inhibition zone against the growth of *Staphylococcus aureus* (○), *Bacillus cereus* (Δ) and *Eschericia coli* (□). Oxytetracycline standard (most-left) was used for comparison using *Bacillus cereus* as test organism. Solid lines refer to diameter of growth inhibition. Dotted lines refer to ring area of inhibition.

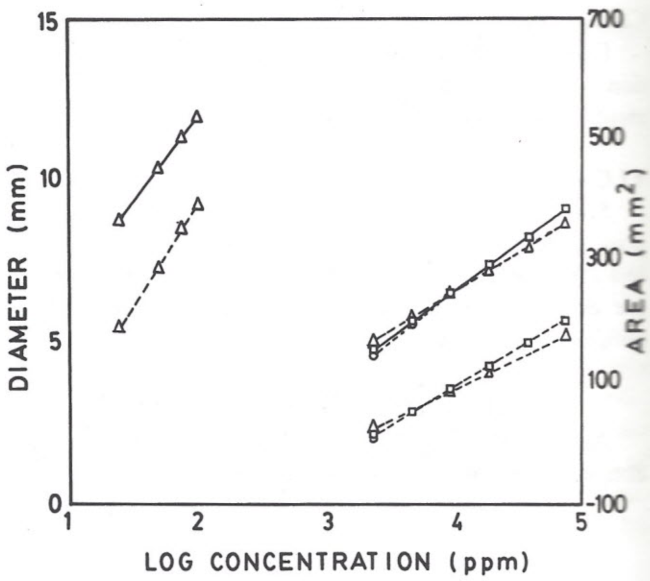

Figure 4. The effect of various concentrations of garlic oil obtained by extraction of garlic pressed residue with ethanol on the diameter and ring area of inhibition zone against the growth of *Staphylococcus aureus* (○), *Bacillus cereus* (Δ) and *Escherichia coli* (□). Oxytetracycline standard (most-left) was used for comparison using *Bacillus cereus* as test organism. Solid lines refer to diameter of growth inhibition. Dotted lines refer to ring area of inhibition.

Quantitatively the capacity could be judged from the slopes of each regression equation listed in Table 1. It is clear that GOJF is more active than GOPCF. However when one compares the activity with that of oxytetracyclin, it is obvious that the isolated oils are much inferor, that is 1.42% and 0.09% of oxytetracyclin, respectively for GOJF and GOPCF. It has been reported that allicin possessed antibacterial activity as much as 1% compared to penicillin (4). The inferiority of GOJF and GOPCF might have been due to low allicin concentration in the oils which further associated with its decomposition to sulfides and its condensates.

TLC of the oils with n-hexane:ethyl acetate (7:3) used as solvent and iodine vapour as visualiser indicated the presence of at least 13 components in both GOJF and GOPCF while KGE gave only one component with Rf 0.53 and GOC produced six spots (see Table 2). The TLC experiment with eluent n-hexane alone showed seven components in the isolated oils while that of GOC separated into three spots and KGE was not eluted at all. The components with Rf value of 0.50 and 0.27 had been reported and identified as dialliltrisulfide and 2-vinyl-(4-H)-1,3-dithiin respectively (9). Nonpolar components such as allicin, allil methyltrisulfide, 3-vinyl (4-H)-1,2-dithiin, diallildisulfide and dialliltetrasulfide had also been reported and identified present in garlic oil (7).

Table 2: TLC separation of garlic oils on silica gel plate using n-hexane:ethyl acetate (7:3), solvent-1 or n-hexane only, solvent-2 as eluents.

Solvent used	Rf values of the separated garlic oil sample			
	GOJF	GOPCF	KEG	GOC
Solvent-1	(1) 0.02	0.02		
	(2) 0.06	0.06		
	(3) 0.19	0.19		(1) 0.18
	(4) 0.25	0.24		
	(5) 0.29	0.29		
	(6) 0.53	0.52	(1) 0.53	(2) 0.49
	(7) 0.58	0.58		
	(8) 0.69	0.68		(3) 0.64
	(9) 0.73	0.73		(4) 0.69
	(10) 0.86	0.86		(5) 0.77
	(11) 0.91	0.90		(6) 0.89
	(12) 0.95	0.94		
	(13) 0.97	0.97		
Solvent-2	(1) 0.05	0.05	0.00	(1) 0.07
	(2) 0.13	0.12		(2) 0.15
	(3) 0.27	0.27		
	(4) 0.50	0.49		
	(5) 0.55	0.53		
	(6) 0.80	0.78		(3) 0.73

CONCLUSIONS

The following conclusions could be drawn from the results of the present experiments. (1). The isolated oils, GOJF and GOPCF possessed antibacterial activity, specifically against *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli.* On the other hand commercial garlic extract (KGE) and garlic oil capsule (GOC) did not demonstrate antibacterial activity against those bacteria tested for GOJF and GOPCF. (2). TLC technique could detect the presence of 13 components in GOJF and GOPCF, six in GOC and one in KGE. (3). The pressed cake fraction of the garlic still contained antibacterial active oil, hence for future experiments, the oil extraction should be carried out from the whole garlic bulbs. (4). Further bioassays and chemical elucidation are needed to confirm the bioactive components present in the oil.

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Penemuan penisilin dan pengembangan industri antibiotik merupakan suatu terobosan besar pada abad-20 dan merupakan penemuan yang paling bermanfaat sepanjang masa. Akibatnya persepsi masyarakat tentang bioteknologi tidak dapat diabaikan. Pada saat ini industri antibiotika menjual produk seharga lebih dari $ 5 milyar yang membuatnya setara dengan nilai jual terbesar produk industri kimia. Antibiotika ini dapat dikatakan telah memberikan dampak yang berarti pada bioteknologi dalam persaingannya dengan industri-industri utama. Sejalan dengan penemuan antibiotika baru serta keberhasilan perdagangan, terjadi peningkatan yang luar biasa pada pengembangan proses dan produktivitas. Saat ini penisilin diproduksi dalam fermentor berukuran 100.000 liter dengan konsentrasi 50 g/l dan dengan biaya lebih rendah dari 1 % biaya pada tahun 1945. Beberapa faktor lain dari pengembangan proses memberikan sumbangan yang amat berarti pada perkembangan ekonomi bioteknologi saat ini. Selama perang dunia ke-2 pemerintah Amerika Serikat memberlakukan pajak keuntungan yang berlebihan untuk membayar usaha perang. Karena banyak industri fermentasi yang harus membayar pajak ini maka penelitian tambahan membebani biaya total. Beberapa usaha penelitian antibiotika dan pengembangan proses dari penisilin dibiayai dengan cara ini.

Meskipun produksi antibiotika dapat menghasilkan keuntungan, pengembangan bioteknologi dinilai amat lambat. Enzim diramalkan akan mengalahkan industri kimia pada awal dekade 1960 tetapi industri enzim berkembang lambat, mencapai nilai penjualan hanya sekitar $ 600 juta, suatu nilai yang amat kecil bila digunakan sebagai nilai penjualan global. Pertumbuhan industri pati dan deterjen biologi tidak secepat seperti yang pernah diramalkan pada dekade 1960, terutama karena ketakutan akan reaksi alergik akibat pemakaiannya, meskipun akhirnya dapat diatasi dengan cara pembuatan pelet. Kesuksesan terbesar dari pengetahuan enzim industri tercerminkan pada keberhasilan produksi sirop jagung dengan kadar fruktosa tinggi (HFCS) menggunakan glukosa isomerase teramobilisasi, yang mencapai nilai penjualan sebesar $ 1,4 milyar. Perkembangan komersial dari enzim pada umumnya lambat karena kendala biaya dan sifat labil dari enzim, disamping masih dibutuhkannya biaya yang tinggi untuk penelitian dan pengembangannya.

Asal mula bioteknologi di Jepang berbeda dengan di Eropa dan Amerika Utara. Orang Jepang secara tradisional menghasilkan bermacam makanan fermentasi, misalnya yang berasal dari kacang kedele. Pada awal abad ke-20 ini mereka menemukan enzim kapang yang berperan pada proses tersebut, misalnya takadiastase, dan kemudian Jepang mengembangkan industri enzim untuk makanan. Mereka juga menemukan bahwa MSG (monosodium glutamate) merupakan salah satu senyawa cita rasa aktif pada beberapa makanan terfermentasi seperti kedele. Melalui pemilihan strain dan pengembangan proses mereka membangun industri yang menghasilkan 400.000 ton MSG

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